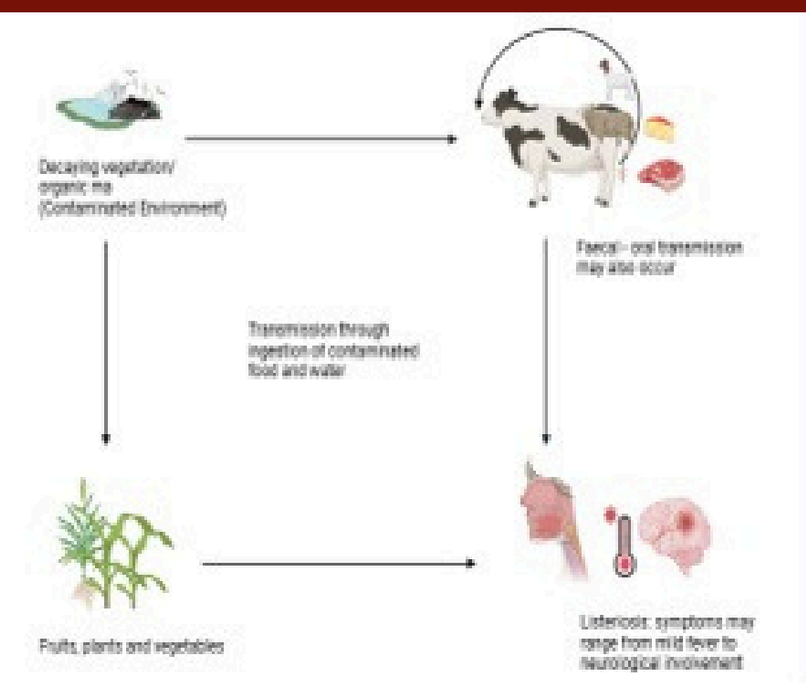
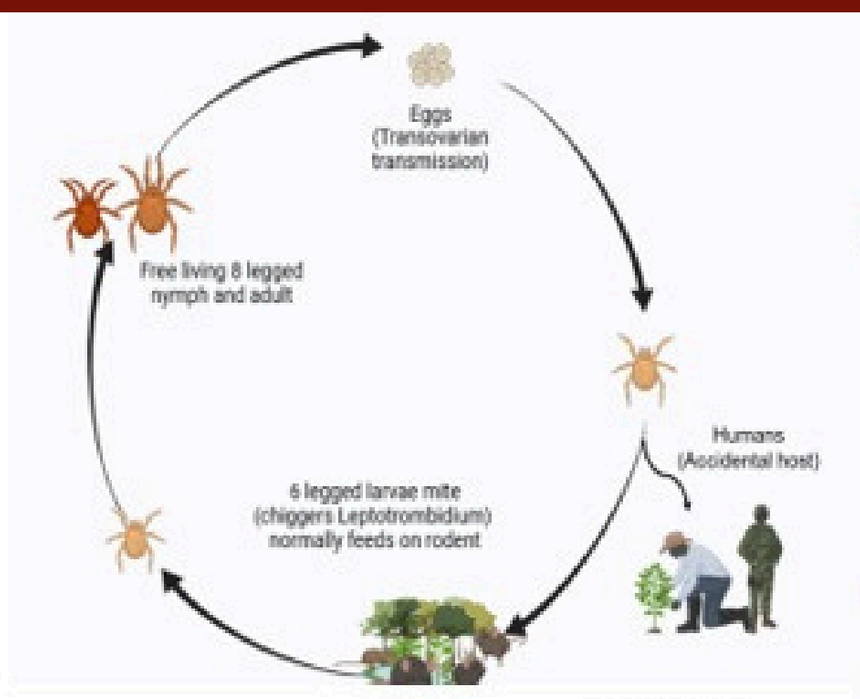
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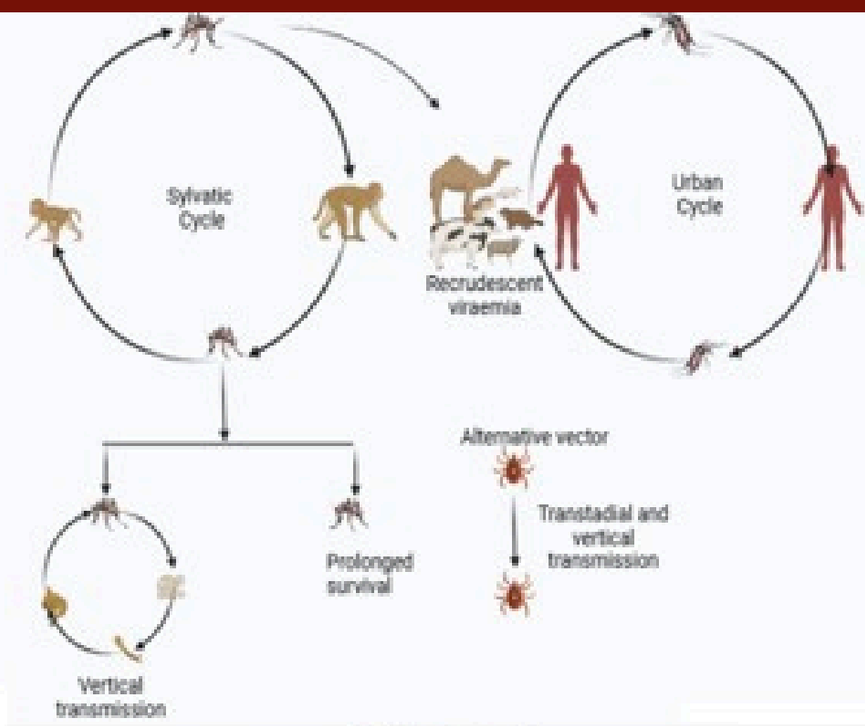
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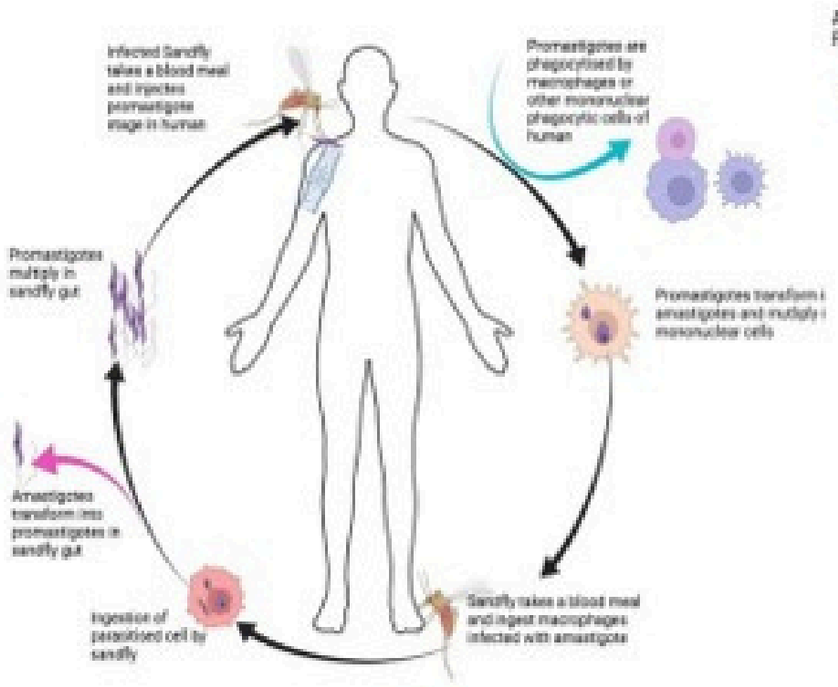
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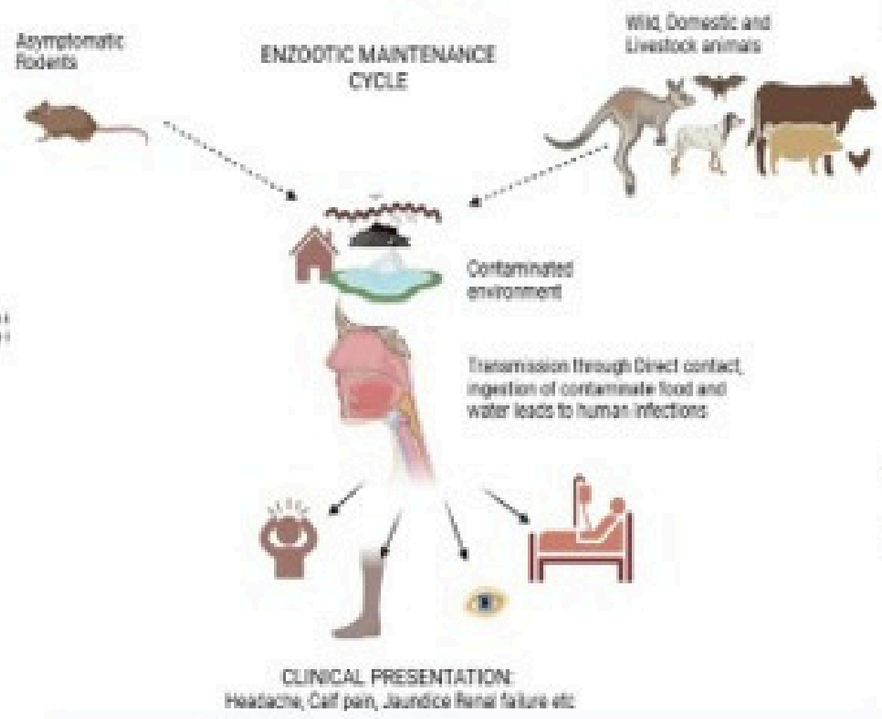
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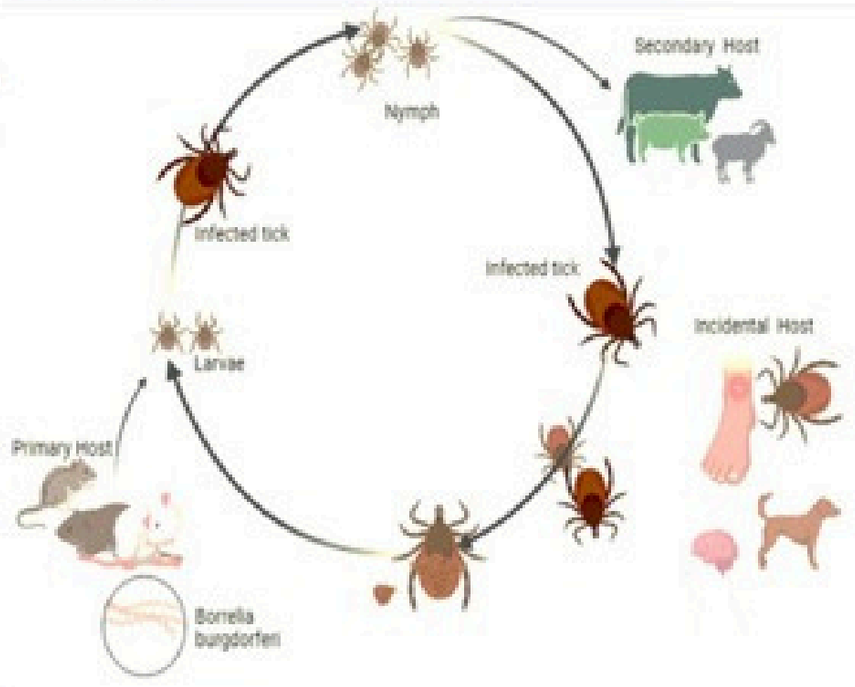


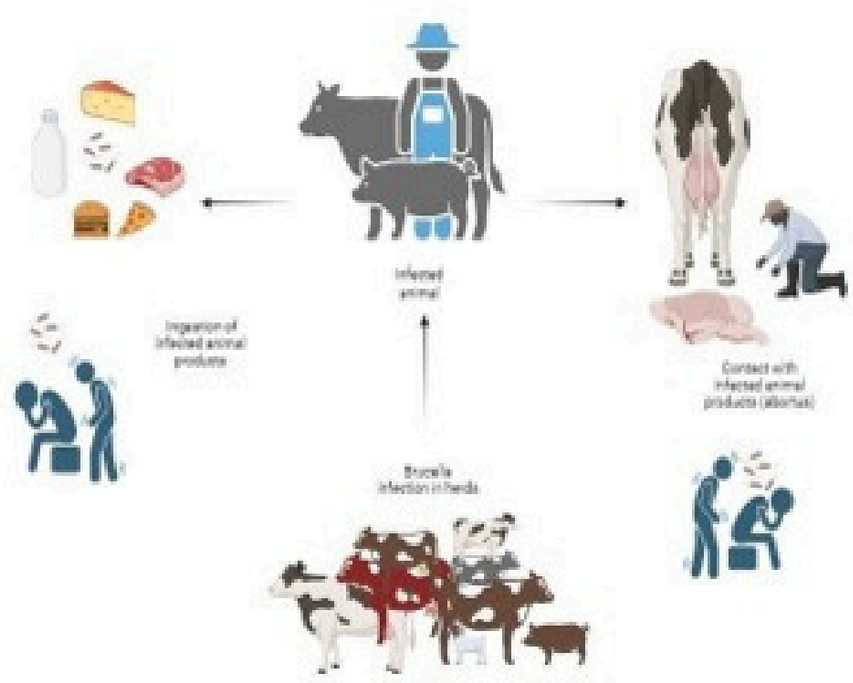


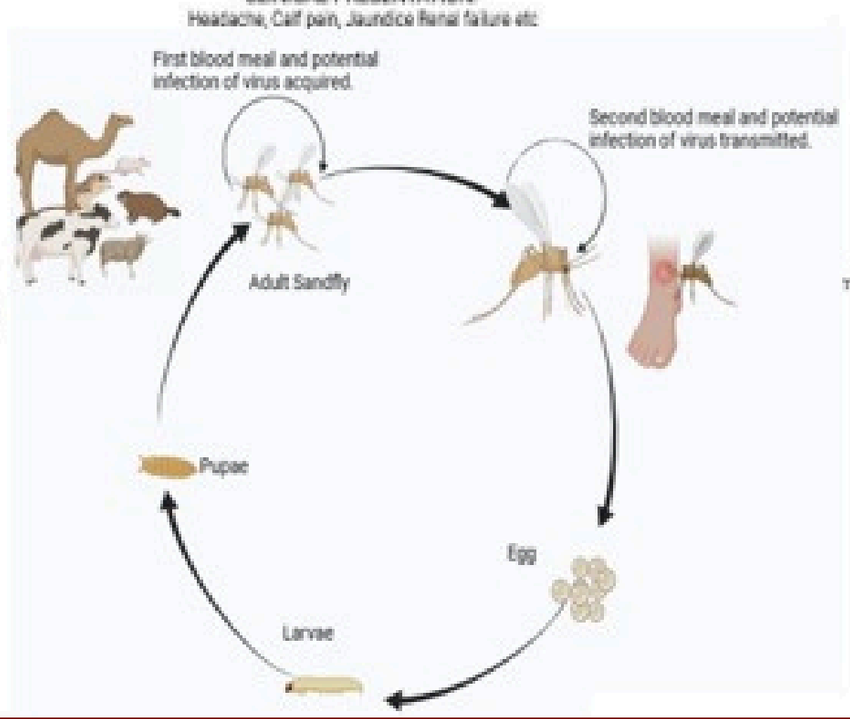


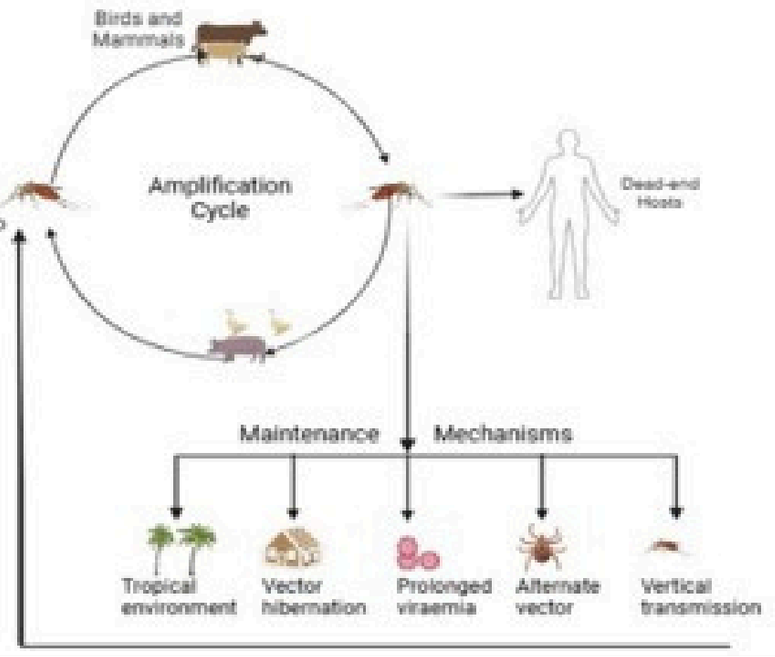












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Message

Zoonotic diseases pose a significant and ongoing threat to public health, both in India and across the globe. These infections are transmitted between animals and humans and encompass a wide range of viral, bacterial, parasitic, and fungal pathogens. While some zoonoses—such as Rabies, Japanese Encephalitis, Plague, and Leptospirosis—have long been endemic in India, others like Nipah virus, Zika, Mpox, and SARS-CoV-2 have emerged in recent years with devastating consequences.

India's vast and diverse ecological landscape, high population density, and close human-animal interface make it particularly vulnerable to zoonotic outbreaks. The persistent and often re-emerging nature of zoonotic pathogens underscores the urgent need for comprehensive surveillance, preventive strategies, and rapid response systems.

The manual on zoonotic diseases was first published in 1985, with subsequent editions released in 2000, 2006, and 2016. Each edition has progressively incorporated evolving epidemiological trends, diagnostic advancements, and prevention and control directions. In the 2025 edition, new chapters have been added on topics of current significance that support the broader *One Health* approach to zoonosis control.

I extend my sincere appreciation to the Director NCDC and officers of NCDC who have devoted their expertise and time to compiling and refining this volume. Their commitment ensures that this manual continues to serve as a key resource for addressing the complex and evolving landscape of zoonotic diseases in India.

(Sunita Sharma)

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No. AS/PS/DDG(AS)/Message/2025
Dated 1st July, 2025

MESSAGE

Zoonotic diseases present an increasingly urgent challenge to public health systems worldwide, impacting communities both nationally and globally. The complex and often close interactions among humans, animals, and the environment-especially amid rapidly shifting ecological landscapes, have contributed to the more frequent and unpredictable emergence and re-emergence of these infections.

In India, zoonotic diseases such as rabies, brucellosis, leptospirosis, along with emerging threats like the Nipah virus and avian influenza, continue to pose serious risks to human and animal health as well as to socio-economic development. The COVID-19 pandemic has further highlighted the critical need for a robust, integrated response to health threats that arise at the human-animal-environment interface.

I applaud the National Centre for Disease Control (NCDC) for its visionary initiative in uniting all relevant stakeholders and developing the comprehensive Manual of Zoonotic Diseases of Public Health Importance. This invaluable resource aims to strengthen inter-sectoral collaboration and will play a pivotal role in training medical, veterinary, and One Health professionals. Moreover, it enhances our national capacity to prevent, detect, and control zoonotic diseases of significant public health concern.

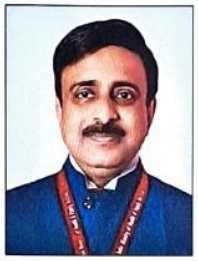
This document marks a crucial milestone in our collective commitment to the One Health approach—an approach that rightly recognizes the intrinsic interconnectedness of human, animal, and environmental health. Such a holistic strategy is indispensable for improving preparedness, enabling early detection, and ensuring effective management of zoonotic threats.

(Raghavendra Bhatta)

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MESSAGE

Zoonotic diseases remain a major public health concern, not only due to their direct impact on human and animal health but also for their potential to spark widespread outbreaks, disrupt livelihoods, and burden health systems. There have been considerable changes in importance of certain zoonotic diseases in many parts of the world, resulting from ecological changes such as urbanization, industrialization, etc. The increasing frequency of zoonotic spillovers underscores the urgent need for integrated, multisectoral strategies rooted in the One Health approach.

The 2025 edition of this manual expanded to cover almost all major zoonotic diseases including epidemic/pandemic prone diseases which are of public health importance. The aim of this manual is to provide a comprehensive understanding the epidemiology, clinical features, laboratory diagnosis and strategic insights for prevention and control of zoonotic pathogens of major public health concern. Additionally, it provides clear, practical information along with illustrated transmission cycles of key zoonotic pathogens, which is a unique feature of this edition. These illustrations aim to enhance understanding of the complex dynamics at the human-animal-environment interface.

I am proud to support this initiative of my team and I am confident that this Manual on Zoonotic Diseases of Public Health Importance will provide an overview and a comprehensive reference to aid to the public health experts, state directorates, laboratory professionals, veterinary experts, medical microbiologists, and researchers. I also commend the efforts of all stakeholders: scientists, public health professionals, microbiologists, veterinarians, and environmental experts, who have contributed to this important document.

It is our hope that this manual will serve as a useful resource for policy-makers, researchers, and practitioners striving to strengthen health systems and build resilience against future outbreaks.

रंजन दास
13/6/25
(Ranjan Das)

Place : Delhi

Dated : 13/06/2025

स्वच्छ भारत
एक कदम स्वच्छता की ओर

Antibiotic resistance Containment Stewardship: Our Role, Our Responsibility
Judicious Use of Antibiotic: Key to Contain Antibiotic Resistance

ANTIBIOTICS USE WITH CARE

NATIONAL HEALTH MISSION
राष्ट्रीय स्वास्थ्य मिशन

FOREWORD

Emerging zoonotic diseases are increasingly recognized as a serious challenge with potentially serious human health and economic impacts. Almost 60% of all human infections and 75% of newly emerging infectious diseases are of zoonotic origin. Of every ten infectious diseases that are identified in humans, six are "zoonotic" diseases – diseases originating in animal populations. With ever-increasing human intrusion into natural ecosystems, the growing demand for animal-based food products, international trade, international travel, and other factors, human exposure to zoonotic diseases has never been higher Increasing numbers and severity of infectious disease outbreaks over the last two decades, such as NIPAH, ZIKA, KFD, Highly Pathogenic Avian Influenza in India and disease like Ebola globally had profound impacts on human health, caused severe burdens on human livelihoods and economies, and posed global security threats.

India, with its rich biodiversity, dense population, and extensive human-animal interface, remains particularly vulnerable. Established zoonoses such as Rabies, Brucellosis, Toxoplasmosis, Leptospirosis, and Scrub Typhus, along with emerging threats like CCHF, H1N1, and Avian Influenza, pose significant challenges to public health and economic stability. Furthermore, the presence of competent vectors, susceptible animal reservoirs, and a conducive environment places the country at risk of outbreaks from high-threat pathogens such as Yellow Fever, Rift Valley Fever, Hantavirus, and Marburg.

Addressing such a complex landscape requires more than isolated action, it demands a One Health approach, recognizing the interconnectedness of human, animal, and environmental health. India has embraced this vision through the National One Health Programme for Prevention and Control of Zoonoses (NOHP-PCZ). This multi-sectoral initiative brings together ministries, academic institutions, research organizations, and international partners to create a cohesive framework for zoonotic disease prevention and control.

In this context, the Manual of Zoonotic Diseases of Public Health Importance has been developed by the Centre for Arboviral and Zoonotic Diseases, National Centre for Disease Control (NCDC). It aims to serve as a practical, comprehensive resource for professionals across sectors such as public health workers, veterinarians, wildlife officials, researchers, and policymakers offering evidence-based information on priority zoonotic diseases, their transmission, and control strategies. This manual reflects India's strong commitment to integrated action against zoonoses. It is designed to support frontline decision-making, build capacity, and strengthen intersectoral coordination across the country. We gratefully acknowledge the contributions of the many experts, institutions, and field professionals whose dedication has made this resource possible.

We hope this manual will be widely utilized across states, and districts and will play a key role in fostering informed action, collaborative response, and a resilient One Health system for India's future.

Dr. Simmi Tiwari
Joint Director
National Centre for Disease Control (NCDC), Delhi

PREFACE

Zoonotic diseases pose significant public health challenges not only in India but across the globe. While some of these diseases have afflicted humanity for centuries, others have emerged as serious threats in recent times. In India, several zoonotic diseases remain a major concern due to their high rates of human morbidity and mortality. These include viral infections such as Japanese Encephalitis, Rabies, and Dengue; bacterial infections like Plague, Leptospirosis, and Brucellosis; as well as parasitic diseases such as Leishmaniasis and Toxoplasmosis. The persistent and emerging nature of these diseases underscores the need for robust surveillance, prevention, and control strategies.

The global emergence and re-emergence of infectious diseases such as Ebola, Zika, Mpox, Nipah, and Crimean-Congo Hemorrhagic Fever (CCHF) in recent years have raised serious concerns for India. These outbreaks highlight the growing threat posed by zoonotic diseases. Among them, the COVID-19 pandemic—caused by the SARS-CoV-2 virus and classified as a zoonotic infection—has had an unprecedented impact, resulting in millions of deaths worldwide since its emergence in December 2019. This underscores the urgent need for heightened preparedness and a coordinated response to zoonotic threats at both national and global levels.

Established in 1963, Centre for Arboviral and Zoonotic Diseases (formerly known as the Zoonosis Division) plays a crucial role in providing laboratory-based evidence through specialized and reference-level diagnostic tests that are typically not available in most medical colleges or institutes across India. This division serves as a backbone for outbreak investigations, surveillance, and formulation of national policy related to zoonotic diseases. Zoonosis Division played an important role in establishment of National Committee of Zoonosis established by Government of India in 1993 which was reformed as Standing Committee of Zoonosis in 2006. One of the important recommendations of this committee is to list zoonotic diseases of priority in the country and collect relevant information about Zoonoses which are important from Indian context and have periodic review of same in view of emerging and re-emerging diseases.

The first edition of manual on zoonoses was published in 1985. The second, third, and fourth editions of the manual on zoonotic diseases of public health importance were published in 2000, 2006, and 2016 respectively. In the last edition, chapters on Ebola virus disease, Zika virus disease, and CCHF were added with detailed information about newer epidemiological aspects.

In 2025 edition, new chapters on Arthropod Vectors of Public Health Importance, IPC with special reference to zoonotic diseases, Research Priorities in Zoonosis, Nation Health Programme under MoHFW and Department of Animal Husbandry & Dairying, Chandipura, West Nile virus, Avian Influenza, Coronavirus, Fungal Zoonosis, Hantavirus, Lyme Disease, Melioidosis, Mpox, Nipah Virus, Zoonotic tuberculosis have been added. No attempt has been made to make the chapters exhaustive; however, illustrated transmission cycles of various zoonotic pathogens/diseases of public health importance have been introduced, which is the highlight of the 2025 issue.

The new edition of "Manual of Zoonotic Diseases of Public Health Importance (2025)" has been revised with a view to bring awareness, new insights and impart uniform training to medical, veterinary, wildlife professionals, environmental specialists etc. on arboviral and zoonotic diseases.

Sincere acknowledgement is due to authors of various chapters who have spent their valuable time in writing the manuscripts and also revising in light of comments provided by editorial team.

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Joint Director
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(On behalf of Editorial Board)

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For further improvement, comments/suggestions on the manual are invited at Centre for Arboviral and Zoonotic Diseases (monil.singhai@gov.in)

The life cycles of vectors and transmission cycles of various zoonotic diseases used in chapters, created with BioRender (https://www.biorender.com/)

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PART- I

ZOONOSES - GENERAL ASPECTS
CHAPTER I

1. Definition

The word 'Zoonosis' (Pleural: Zoonoses) was introduced by Rudolf Virchow in 1880 to include collectively the diseases shared in nature by man and animals. Later WHO in 1959 defined that Zoonoses are "those diseases and infections which are naturally transmitted between vertebrate animals and man". Zoonoses include only those infections where there is either a proof or a strong circumstantial evidence for transmission between animals and man.

2. Zoonoses - An International Problem

Historically, zoonotic diseases had a tremendous impact on the evolution of man, especially those cultures and societies that domesticated and bred animals for food and clothing. Zoonoses are among the most frequent and dreaded risks to which mankind is exposed. Zoonoses occur throughout the world transcending the natural boundaries. Their important effect on global economy and health is well known, extending from the international movement of animals and importation of diseases to bans on importation of all animal products and restrictions on other international trade practices. So, zoonoses no longer are solely a national problem. For effective control of zoonoses global surveillance is necessary.

With recognition of inter-relationships between countries, the internationalization of control efforts has become more relevant to technical, economic, and social fields. The control of zoonoses retains its prominent place among the actions of international agencies according to the health and economic problems specific to each region.

3. Zoonoses- An Emerging Problem

Over the last few decades, there has been considerable change in the importance of certain zoonotic diseases in many parts of the world, resulting from various risk factors such asfood production practices, antimicrobial resistance and ecological changes such as urbanization, industrialization, climate change, deforestation etc.

The classical zoonotic diseases known since centuries and still require focused attention are such as anthrax, plague, brucellosis, influenza Bovine tuberculosis, leptospirosis, salmonellosis, spotted fever caused by Rickettsiae, rabies, several common athropod borne viral infections (arboviral infection), certain parasitic diseases, especially cysticercosis, hydatid disease, trypanosomiasis and toxoplasmosis.

Further in recent decade the emergence of new zoonotic diseases and reemergence of certain zoonotic diseases such as SARS COV-2, Nipah virus, Monkey pox, Ebola virus, Marburg Virus, Crimean Congo Haemorrhagic fever, Kysanur Forest Disease etc have stirred the public health machinery.

4. Classification

With the advanced laboratory techniques and increased awareness among medical and veterinary scientists, ecologists and biologists, more than 300 zoonoses of diverse etiology are now recognised. Thus, a very large number of zoonoses calls for classification, especially for easy understanding.

These are classified as follows:

4.1 According to the etiological agents

Bacterial Zoonoses	Anthrax, Brucellosis, Plague, Leptospirosis, Salmonellosis, Lyme disease.
Viral Zoonoses	Rabies, Arbovirus infections, KFD, Yellow fever, Influenza, CCHF.
Rickettsial zoonoses	Murine typhus, Tick typhus, Scrub typhus, Q-fever
Protozoal zoonoses	Toxoplasmosis, Trypanosomiasis, Leishmaniasis.
Helminthic zoonoses	Echinococcosis (hydatid disease), Taeniasis, Schistosomiasis, Dracunculiasis.
Fungal zoonoses	Deep mycosis- Histoplasmosis, Cryptococcosis, Superficial dermatophytes.

4.2 According to the mode of transmission

4.2.1 Direct zoonoses

These are transmitted from an infected vertebrate host to a susceptible host (man) by direct contact, by contact with a fomite or by a mechanical vector. The agent itself undergoes little or no propagative or developmental changes during transmission, e.g. rabies, anthrax, brucellosis, leptospirosis, toxoplasmosis.

4.2.2 Cyclozoonoses

These require more than one vertebrate host species, but no invertebrate host for the completion of the life cycle of the agent, e.g. echinococcosis, taeniasis.

4.2.3 Metazoonoses

These are transmitted biologically by invertebrate vectors, in which the agent multiplies and/or develops and there is always an extrinsic incubation (prepatent) period before transmission to another vertebrate host e.g., plague, arbovirus infections, schistosomiasis, leishmaniasis.

4.2.4 Saprozoonoses

These require a vertebrate host and a non-animal developmental site like soil, plant material, pigeon dropping, etc. for the development of the infectious agent e.g. aspergillosis, coccidioidomycosis, cryptococcosis, histoplasmosis, zygomycosis.

4.3 According to the reservoir host

4.3.1 Anthropozoonoses

Infections transmitted to man from lower vertebrate animals e.g. rabies, leptospirosis, plague, arboviral infections, brucellosis, and Q-fever.

4.3.2 Zooanthroponoses

Infections transmitted from man to lower vertebrate animals e.g. streptococci, staphylococci, diphtheria, enterobacteriaceae, human tuberculosis in cattle and parrots.

4.3.3 Amphixenoses

Infections maintained in both man and lower vertebrate animals and transmitted in either direction e.g. salmonellosis, staphylococcosis.

5 Factors Influencing Prevalence of Zoonoses

5.1 Ecological changes in man's environment

With the expansion of human population, man is forced to exploit the virgin territories and natural resources like harnessing the power of rivers, constructing roads and pipelines through virgin or thinly populated areas, clearing, irrigating and cultivating new

land, and deforestation. All this would lead to entering of humans in the unaccustomed ecosystem in which potential pathogens form part of the biotic community (natural focus).

Large scale expansion of agricultural and engineering resources, construction of dams, artificial lakes, irrigation schemes, clearing of forests -all these lead to changing of the biting habits of the blood sucking vectors and alteration in the population of reservoir animals, which has led to the spread of leptospira, tuleraemia, helminthic infections, etc.

5.2 Handling animal by-products and wastes (occupational hazards)

There are significantly higher attack rates in workers during the course of their occupation than the rest of the population, e.g. anthrax in carpet weavers, live stock raisers and workers with animal hair in the textile industry, leptospirosis in rice field workers, listeriosis in agricultural workers, erysipeloid in butchers and fish merchants, tularemia and trypanosomiasis in hunters, creeping eruptions in plumbers, trench diggers etc. Other examples of zoonoses as occupational hazards are Q-fever in abattoir and rendering plant workers, jungle yellow fever and tick borne diseases in wood cutters, salmonellosis in food processors, bovine tuberculosis in farmersetc.

5.3 Increased movements of man

Land development, engineering project work, pilgrimages, tourism, etc. expose the people to contaminated food and water leading to diseases like amoebiasis, colibacilliosis, giardiasis, salmonellosis, shigellosis, etc.

5.4 Increased trade in animal products

Countries which import hides, wool, bone meal, meat, etc. from an area where some of the zoonoses are endemic, are likely to introduce the disease into their territories, e.g. salmonellosis, foot and mouth disease, anthrax, Newcastle disease, etc.

5.5 Increased density of animal population

Animals may carry potential risk of increased frequency of zoonotic agents in man e.g. dermatophytosis, tuberculosis, brucellosis etc.

5.6 Transportation of virus infected mosquitoes

Aircraft, ship, train, motor and other vehicles bring the viruses in to a new area, e.g. yellow fever, Chikungunya fever, dengue fever etc.

5.7 Cultural anthropological norms

In Kenya, people allow the dogs and hyenas to eat human dead bodies infected with hydatidosis. This leads to the perpetuation of the transmission cycle of the disease.

6. Zoonoses as A Public Health Problem

In the past decade (2015–2025), India has experienced a rise in zoonotic outbreaks, both endemic (scrub typhus, leptospirosis) and new/emerging (Nipah, Chandipura, KFD, Monkeypox, SARS CoV-2, etc). Although no major plague outbreaks have occurred in India since 1994, continued surveillance remains essential due to the risk of sudden re-emergence, persistent natural reservoirs, ecological and climatic triggers, and potential bioterrorism threats. Given its historical impact and potential for rapid spread, plague surveillance is a strategic imperative for strengthening India's public health resilience, ensuring timely detection, and maintaining outbreak preparedness.

While rabies and brucellosis remain important in terms of most fatal and most common neglected tropical diseases, officially reported human cases are relatively fewer. Strengthened surveillance efforts, especially under the One Health framework and IDSP, are improving detection and reporting. Geographical hotspots may vary for endemic zoonoses such as JE, leptospirosis, leishmaniasis, however, they continue to carry a chronic burden with seasonal and regional variations. Anthrax still remains an active, neglected zoonosis,

particularly among rural and tribal communities, with risk intertwined with environmental and socio-cultural practices. Prevention and control of zoonoses in both animals and humans is essential—not only to reduce the burden of morbidity and mortality, but also to mitigate the significant economic costs associated with outbreaks, treatment, and loss of productivity.

Further Reading

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1. Introduction

Arboviruses are viruses, which are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by hematophagus arthropods; they multiply and produce viremia in the vertebrates, multiply in the tissues of arthropods and are passed on to new vertebrates by the bites of arthropod after a period of extrinsic incubation period. The amplifying vertebrate host develops viremia of sufficient titre and duration to infect subsequently feeding vectors. Infection of the vector is typically life long, with no apparent ill effect. Certain arboviruses may be transmitted transovarially and venereally by their arthropod vectors. In addition to biological transmission, some arboviruses occasionally may be transmitted mechanically by arthropods and other may be transmitted via throat secretions, milk or other excreta of the vertebrate host.

2. Epidemiology

2.1 Causative Agent

More than 530 viruses have been listed as arboviruses. Of these a few are not arthropod-borne but are antigenically related to known arboviruses. Vectors mainly responsible for transmission are mosquitoes, ticks, sandflies, midges and mites. Nearly 41 viruses have been isolated in India of which a few viruses of public health importance are Japanese Encephalitis (JE), Dengue, West Nile and Kyasanur forest disease (KFD) viruses etc.

2.2 Mode of Transmission

Arboviruses induce high titers of viremia in susceptible vertebrates 1-2 days after parenteral inoculation or following bites by infected arthropods; viremia persists for several days and serves as a source of infective blood meals for other biting arthropods. The primary site of virus replication is not known but is likely to be in the reticuloendothelial cells in lymph node, liver and spleen or endothelial cells of blood vessels. Release of virus from these sites may be associated with non-specific 'flu like' symptoms. During this period of viremia, usually at about 5–7 days after exposure, virus enters the target sites of the central nervous system, skin, etc. There may be a brief period of relief after the initial symptoms, i.e., before the onset of specific features, followed by serious manifestations such as encephalitis, hemorrhagic fever syndrome in some cases. Thus, the presentation is a biphasic illness.

Symptoms of encephalitis begin 7-10 days after exposure to infection and persist 1 week or more, followed either by remission or by death. Wild birds and mammals regularly exhibit viremia without symptoms. Antibodies are first detected when the fever subsides, sometimes as early as 2 days after the onset of encephalitis and persist for many years. Antibodies of the IgM class may persist 1–7 weeks after infection; subsequently, they are of IgG class.

It is important to note that although many subjects become infected with encephalitis viruses, relatively few develop illness manifested as meningitis or encephalitis. There is little information about the role of the immune system, although it may have a role in the pathogenesis of the dengue haemorrhagic shock syndrome, which is seen in young children who experience a second dengue virus infection. Antigen-antibody complex formation has been thought to underlie the syndrome, which is associated with increased capillary permeability.

2.3 Current Situation

Arboviruses are most prevalent in the tropical rainforest areas of different continents. It is due to the favourable climatic conditions and of the abundance in kind as

well as in number of animal and arthropod species. As the arboviruses are maintained by cycles involving arthropods as well as vertebrates, the tropics offer the most favourable conditions for these complex biological cycles.

In India, out of the mosquito-borne infections, Japanese encephalitis has been responsible for outbreaks in most of the states, with a case fatality rate of 6-60%. An antibody to JE has been detected in different species of animals. Dengue viruses have been responsible for many outbreaks in many cities and some rural areas in many parts of the country.

3. Clinical Features

As might be expected with such a heterogeneous group of agents, clinical manifestations in humans are diverse & can be divided into fever (including fever with rash), encephalitis, and haemorrhagic fever.

a) Fever: Little is known about the pathology of mild, undifferentiated fevers, because mortality is rare. The most common symptoms usually benign are characterized principally by mild, undifferentiated fevers with duration of 3 – 7 days. The onset is usually abrupt with fever, headache, and general malaise with vomiting or nausea and pain on moving the eyes-muscle or joint pains may be conspicuous; a macular or maculopapular rash or a series of rashes may be present. Leucopenia sometimes very marked, is quite common. For arboviruses like Chikungunya, Dengue and West Nile, fever is the major clinical symptom.
b) Encephalitis: The second most frequent symptom is encephalitis or meningoencephalitis. It typically comprises of the sudden onset of fever and headache, followed by neck stiffness, nausea or vomiting, drowsiness, and disorientation, frequently advancing into stupor or coma after an incubation period of 4 – 14 days. Convulsions may be an important feature of the acute illness. Rigidity or weakness of the limbs may occur, together with absent or irregular deep tendon reflexes and upgoing plantar reflexes. Symptoms are most severe 2 – 10 days after exposure, subsequently the patient may die or the fever and other symptoms may regress slowly during the next 2 – 3 weeks. During most outbreaks of arboviral encephalitis, a proportion of case develops aseptic meningitis only, without significant involvement.
c) Haemorrhagic Fever: A general and frequently massive erythrocytic diapedesis is seen in many skin lesions and other tissues. Edema and haemorrhage are usually conspicuous. A mild or severe thrombocytopenia is common. Most infections with hemorrhagic fever viruses result in mild fever rather than severe disease. Arboviruses that may cause hemorrhagic fever include Dengue, KFD, and Yellow Fever etc.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Isolation of arboviruses from clinical or field-collected specimens is difficult and frequently unproductive, even when the appropriate specimens are obtained. Due to the lability of arboviruses, proper collection, storage, shipment, and processing of specimens to preserve virus infectivity are essential.

Arboviruses are infrequently isolated from patient specimens mostly; the infectious virus typically is no longer detectable by the time the patient seeks care. Viremia in most arboviral infections ceases 2-3 days after onset of symptoms. Antibody becomes demonstrable 5-7 days post-onset of symptoms. Therefore, an acute-phase blood specimen

for isolation should be collected immediately when arboviral aetiology is suspected to avoid the inhibitory effects of antibodies. Prompt specimen collection increases the probability for positive isolation.

Mosquito borne encephalitis viruses such as JE are rarely isolated from blood of patients. In contrast, dengue viruses are frequently isolated from blood of patients during the first two days of illness and sometimes for a longer duration. In general, isolates can be recovered from blood commonly after onset of symptoms for those viruses for which humans are the principal vertebrate host such as YF, dengue and chikungunya.

Humans

Blood/serum (Paired samples) – one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.

Cerebrospinal fluid

Sometimes other organ tissues like spleen, lungs, liver, etc.

Animals

Blood

Tissues

4.2 Storage and Transport

Specimen should be shipped and processed promptly after collection. The specimen must be transported to laboratory at 2-8 ˚C if reaching the laboratory within 48 hours, -20 ˚C if reaching after 48 hours upto 7 days and thereafter stored at -80 ˚C.

4.3 Laboratory Procedures

a) Isolation and Identification of viruses: Arboviruses can be demonstrated by the use of animals, cell cultures or vector inoculation

Animal inoculation

- Suckling mice – by intracerebral and intraperitoneal routes
- Other animals: Hamster, Chick embryo, etc.
- Check for sickness in mice and confirm by immunofluorescence or haemagglutination (HA) & haemagglutination inhibition (HAI) test etc. or check for presence of neutralizing antibodies in surviving mice.

Cell Cultures

- Vertebrate cell lines – Vero, BHK-21, LLC-MK2
- Primary cells-Hamster kidney, chick embryo, duck embryo, etc.
- Invertebrate cell lines-C6/36
- Check for cytopathic effect, plaque formation, plaque reduction neutralization test (PRNT), and viral antigen by ELISA/ immunofluorescence, etc.

Vectors

- Mosquito inoculation by intracerebral and intrathoracic route.
- Check and confirm viral antigen by ELISA/Immunofluorescence/HAI and Strain confirmation by use of monoclonal antibodies or Polymerase Chain Reaction in mosquito pool i.e., Dengue.

b) Serological Tests: Presence of antibodies to arboviruses can be detected by the following techniques:

- Haemagglutination and Haemagglutination Inhibition Test: Arboviruses have the property of agglutinating goose erythrocytes at different pH. This property is used in identification of an isolate. Antibodies to a particular virus inhibit the HA. This can be a monospecific reaction or group specific depending on the days post infection for collection of sample or cross reactivity in viruses with close antigenic relationship. The

sera used in HI test are treated to remove non-specific antibodies using Kaolin or acetone. A four-fold or more rise in paired sera is considered a recent infection due to the virus for which the antigen has been used in the test. Secondary response i.e. high titres may be present in a patient with previous exposure to antigenically related viruses, e.g., JE, West Nile or Dengue.

- Complement Fixation Test: The CF Test may be used to identify an isolate by testing the unknown agent against a number of specific antisera or to establish a presumptive diagnosis in the absence of virus isolation by screening acute and convalescent specimens for rise in antibody against several viruses considered to be likely etiological agents. CF test is less specific than the neutralization test and is frequently group specific. A fourfold or greater difference in titre between acute and convalescent phase sera is considered diagnostic.
- Neutralization test: Antibodies against a particular virus/or arboviruses present in a person coming in contact with such viruses can neutralize the virus in the laboratory. The test is useful in demonstration of antibodies to a particular virus when virus used is a known virus or for identification of virus when antisera are known. The neutralization test can be utilized using different assay systems e.g. suckling mice /cell cultures.
- Immunofluorescence test: Detection of specific antibodies to arboviral infections is done by indirect immunofluorescence using viral antigens fixed on slides & fluorescein-labelled anti-human antibodies. The tissues taken from patients or inoculated animals can be tested for the presence of antigen by using specific conjugates labelled with specific antibodies.
- Enzyme immunoassays: Presence of IgG & IgM antibodies or antigens can be detected by immobilizing a particular antigen/ antibody on a solid phase and then capturing them. The reaction is made visible by use of an enzyme and its specific substrate. The commonly employed test for establishing an infection is IgM capture ELISA used for Japanese Encephalitis, West Nile virus and Dengue viruses, which can detect IgM antibodies in blood/CSF.

c) Molecular test: PCR can be used to detect many arboviruses, provided at least part of the gene sequence is known for the virus under consideration. PCR is used to select a segment of the viral gene sequence, reverse transcribe it with reverse transcriptase, amplify the resulting complementary DNA (cDNA) and detect the amplified product.

5. Treatment

There are a few effective specific antiviral treatments for arbovirus infections. Treatment is often symptomatic and systemic management such as administration of analgesics to reduce discomfort and antipyretics to reduce fever.

6. Prevention and Control

Integrated vector management is a key for prevention and control of vector borne diseases. The details for vector control can be accessed from chapter on Arthropod vectors of zoonotic importance in this manual.

Table 1*:* Arboviruses and Rodent-borne viruses of Public Health Importance

Family	Genus	Important Arbovirus and Rodent-borne virus	
Arenaviridae	Arenavirus	Lassa and lymphocytic choriomeningitis viruses	
Togaviridae	Alphavirus	a) Encephalitis causing virus ➢ Eastern Equine encephalitis ➢ Western Equine encephalitis ➢ Venezuelan Equine encephalitis	b) Fever causing virus ➢ Chikungunya ➢ Onyong-nyong ➢ Mayaro ➢ Sindbis ➢ Ross River
Flaviviridae	Flavivirus	Mosquito Borne a) Encephalitis virus ➢ St. Louis encephalitis ➢ West Nile ➢ Japanese encephalitis ➢ Murray Valley encephalitis b) Yellow fever virus c) Dengue virus types 1,2,3,4 d) Zika Virus	Tick Borne a) Encephalitis virus ➢ Russian spring summer encephalitis complex ➢ Louping III b) Haemorrhagic Fever ➢ Kyasanur Forest Disease ➢ Omsk Haemorrhagic Fever
Bunyaviridae	Bunyavirus	➢ Bunyamwera Group ➢ Bwamba Group ➢ California Group ➢ Simbu Group ➢ Turlock Group	
	Hantavirus	➢ Hantaan virus (Korean hemorrhagic fever) ➢ Seoul virus (hemorrhagic fever with renal syndrome) ➢ Sin Nombre virus (hantavirus pulmonary syndrome)	
	Nairovirus	➢ Crimean Congo Haemorrhagic Fever Group ● CCHF ● Hazara	➢ Nairobi Sheep Disease Group ● Nairobi Sheep Disease ● Ganjam
	Phlebovirus	➢ Rift Valley Fever ➢ Phlebotomas Fever Group ● Sandfly fever ● Karimabad	
Filoviridae	Marburg-like	➢ Marburg viruses	
	Ebola-like	➢ Ebola viruses	
Reoviridae	Orbovirus Coltivirus	➢ African Horse Sickness ➢ Colorado tick fever ➢ Palyam ➢ Vellore	
Rhabdoviridae	Vesiculovirus	➢ Chandipura Virus	

Excellent vaccines are available for YF (live attenuated) and JE (inactivated) but there are no available approved vaccines for most other arboviruses in India. Although clinical trials for some candidate vaccines are ongoing.

Further Reading

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1. Introduction

Arthropod-borne zoonoses are a major group of diseases of public health significance. Ecological changes, increased human mobility, density of animals and global trade along with certain occupational and cultural factors have led to the emergence/resurgence of these diseases over the past two decades. This chapter describes the important arthropod vectors of zoonotic importance such as ticks, mites, fleas in Table 1.

Table 1: Major zoonotic diseases transmitted by Arthropod vectors. (*No case reported in India)

Vectors	Diseases	Pathogen	Important Vector Species	Reservoir
Ticks	Kyasanur Forest Disease (KFD)	KFD virus (Family Flaviviridae)	*Haemaphysalis spinigera*	Monkey/ birds
	Crimean Congo Hemorrhagic Fever (CCHF)	CCHF virus (Family Bunyaviridae)	*Hyalomma anatolicum anatolicum* *H. marginatum*	Tick, cattle, goats, sheep, and hares
	Indian Tick typhus	*Rickettsia conorii*	*Rhipicephalus sanguineus*	Dog
	Lyme disease	*Borrelia burgdorferi sensu lato*	*Ixodes ricinus* *I. persulcatus* *I. scapularis*	White-footed mouse
	Anaplasmosis	*Anaplasma phagocytophilum*	Deer ticks (Blacklegged ticks) *Ixodes scapularis* *I. pacificus*	Deer, mouse
	Babesiosis	*Babesia microti*	*Ixodes scapularis* *I. ricinus*	Cattle, roe deer and rodents
	Tick-borne Relapsing Fever	*Borrelia hermsii*	*Ornithodoro shermsi*	Rats
		B. parkeri	*O. parkeri*	
		B. turicatae	*O. turicata*	
	Tularemia	*Francisella tularensis*	*Dermacentor variabilis* *Dermacentor andersoni* *Ambylomma americanum*	Rabbits, rodents, cattle
	Ehrlichiosis	*Ehrlichia chaffeensis* *E. ewingii* *E. muris eauclairensis*	*Ambylomma americanum* *Ixodes scapularis*	Deer
	*Rocky Mountain Spotted Fever (RMSF)	*Rickettsia rickettsia*	*Dermacentor andersoni* *D. variabilis* *Amblyomma cajennense sensu lato* *A. aureolatum* *Rhipicephalus sanguineus*	Small mammals (rodents and rabbits, and dogs)
	*Colorado Tick Fever	Coltivirus	*Dermacentor andersoni*	Small rodents (squirrels, chipmunks, and mice)
	*Tick-borne Encephalitis	Flavivirus family Flaviviridae	*Ixodes ricinus* *I. persulcatus* *I. scapularis*	Rodents

Mites	Scrub typhus	*Orientia tsutsugamushi*	*Leptotrombidium deliense Schoengastiella ligula*	Mites
	Scabies		*Sarcoptes scabiei*	Human
	Rickettsial pox	*R. akari*	*Liponyssoides sanguineus*	Rodents
Fleas	Plague	*Yersinia pestis*	*Xenopsylla cheopis*	Rodents
	Murine typhus or Endemic typhus	*Rickettsia typhi*	*Ctenocephalides felis Xenopsylla cheopis*	Cats, wild opossums, rats, mice, and other rodents
	Cat-scratch fever	*Bartonella henselae*	*Ctenocephalides felis*	Cats
Louse	Epidemic typhus	*Rickettsia prowazekii*	*Pediculus humanus humanus*	Human
Sandfly	Kalazar	*Leishmania donovani*	*Phlebotomus argentipes*	Human
	Chandipura virus disease	Chandipura Virus	*Phlebotomus papatasi*	

The major zoonotic diseases transmitted by mosquitoes are listed in Table 2

Table 2: Zoonotic diseases transmitted by mosquitoes.

Mosquitoes	Zoonotic Disease	Caused by	Primary transmission Vector species
Aedes	Dengue	Flaviviridae family, Genus Flavivirus	*Ae. aegypti,* *Ae. albopictus*
	Chikungunya	Togaviridae family, Genus Alphavirus	
	Zika Virus disease	Flaviviridae family, Genus Flavivirus	
	Yellow fever	Flaviviridae family, Genus Flavivirus	*Ae. aegypti*
Culex	Japanese Encephalitis	Flaviviridae family, Genus Flavivirus	*Cx. tritaeniorhynchus*
	West Nile Virus disease	Flaviviridae family, Genus Flavivirus	*Cx. tarsalis,* *Cx. quinquefasciatus,* *Cx. stigmatosoma,* *Cx. thriambus, C. pipiens,* *Cx. nigripalpus*
Anopheles	Knowlesi malaria	*Plasmodium knowlesi*	*An. dirus,* *An. balabacensis*
	Inui malaria	*Plasmodium inui*	*An. cracens*
	Cynomolgi malaria	*Plasmodium cynomolgi*	*An. freeborni,* *An. stephensi*

Several indices have been described and are currently used to monitor vector population. The important indices to measure vector burden are listed in Table 3.

Table 3: Important indices to measure vector burden.

Vectors	Indices	Definition
Ticks	Tick Prevalence	Measures the proportion of hosts or individuals within a population that are infested with ticks.
	Tick Density	Total Number of Ticks / Unit Area
	Intensity of tick infestation	The intensity of infestation is the total number of ticks observed divided by the number of infested hosts
Mites	Rodent Infestation Rate	If 50 rodents are caught and 5 are infested with mites, this index would be 5/50=10%
	Rodent trap rates	Number of rodents collected/number of trap nights
	Chigger Index	Mean number of chiggers collected per rodent.
Fleas	General Flea Index	Average number of fleas (all species included) per rodent. e.g. if 100 fleas are recovered from a total of 10 rodents examined, the index would be 100/10 = 10.
	Specific Flea Index/ *X. cheopis* index	This is same as general flea index but calculated exclusively for *X. cheopis*. e.g. in above example, if a total of 10 of the 100 fleas are *X. cheopis*, this index would be 10 /100 = 0.1
	Percentage incidence of flea species	This is the percentage of each species of fleas, out of the total fleas sampled per rodent.
	Rodent infestation rate	If 50 rodents are caught and 5 are infested with fleas, this index would be 5/50=10%
Mosquitoes	House Index (HI)	The percentage of houses infested with larvae or pupae
	Container Index (CI)	The percentage of water-holding containers infested with larvae and pupae out of those inspected.
	Breteau Index (BI)	Number of positive containers per 100 houses inspected (Aedes immatures).
	Pupal Index (PI)	Number of pupae per 100 houses inspected.

2. Vectors of Public Health Importance

There is ample evidence that arthropods play important roles as mechanical vectors of pathogens, causing important diseases of humans and domestic animals. Arthropods transmit diseases via their ability to function as hematophagous vectors which is characterized by their ability to feed on blood at some or all stages of their life cycles. Arthropods transmit parasites either by injection into the blood stream of the host directly via their salivary glands, or by forcing parasites into a pool of blood which develops when chewing the skin.

2.1 Ticks

Ticks are obligate parasitic arthropods of the class Arachnida with a vast global distribution, especially in warm and humid regions. There are two major families of ticks – Ixodidae, the hard ticks and Argasidae, the soft ticks, which transmit many diseases. Ticks have gained significant attention in the past two decades as a serious threat to both humans and animals.

Vector Ecology

Ticks are haematophagous ectoparasitic arthropods that feed on animals, including mammals, birds, and reptiles. Various environmental factors, such as temperature, humidity, vegetation, and host availability influence their survival and reproduction. Ixodidae contains over 700 species and has a scutum or hard shield, while Argasidae has about 200 species and lacks scutum. All the stages in the life cycle (larva, nymph & adult) of ticks are parasitic. Ticks acquire pathogens by feeding on infected hosts and subsequently transmit them to other hosts during subsequent feeding. Ticks are extremely tough, hardy, and resilient.

Hard ticks exhibit 'Questing', a phenomenon in which the various tick stages climb on vegetation & actively seek a host. The soft ticks in contrast behave like bedbugs, hiding during daytime in cracks and crevices.

The understanding of interactions between ticks, hosts, and pathogens, including their spatial distribution, host preferences, feeding behaviour, and disease transmission dynamics is crucial for effective surveillance, prevention, and management of tick-borne diseases.

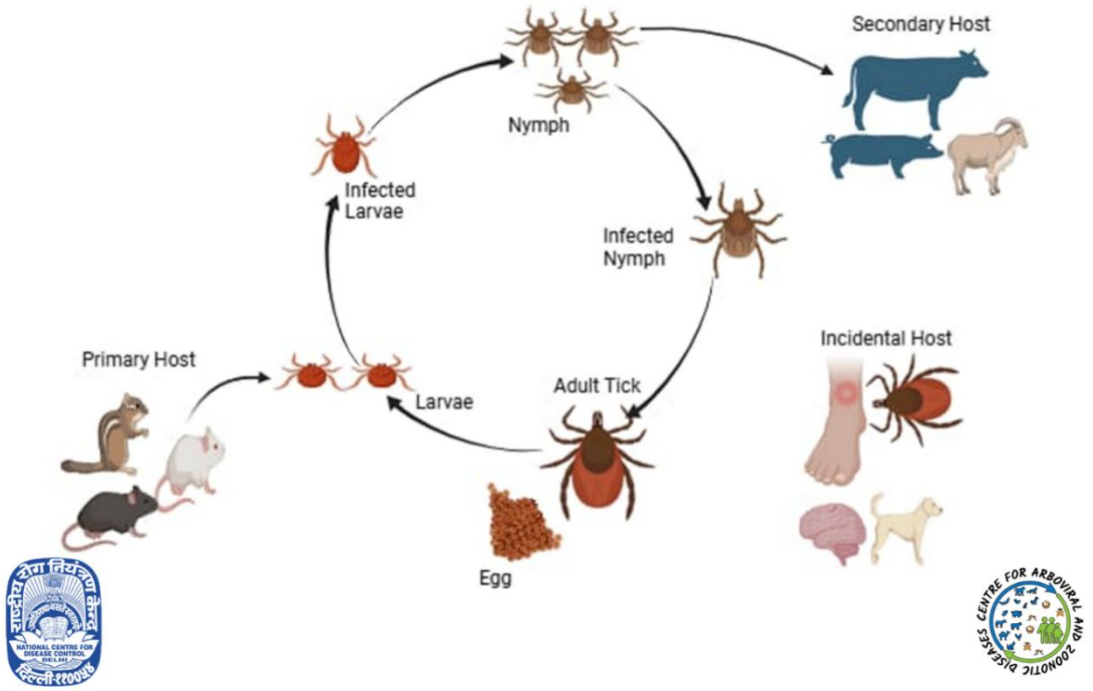


Figure 1: Life cycle of Tick and transmission cycle of Tick-borne diseases.

Survey Methods

Various survey methods are used to assess the presence, abundance, species composition, potential hotspots and infection rates of ticks with pathogens. Survey methods, often used in combination, contribute to a better understanding of tick ecology, geographic

distribution, and disease transmission patterns Some commonly employed survey methods are:

a) Dragging: This method involves dragging a piece of cloth or flannel over vegetation while walking in a designated area. Ticks that attach to the fabric are collected and later identified and counted.
b) Flagging: It uses a white or light-coloured cloth or flag attached to a pole. The cloth is swiped over vegetation, and ticks clinging to it are collected and studied but prolonged dragging over distances results in the loss of ticks. Thus, the operator must stop frequently enough (e.g., every 10 m) to remove ticks from the flag or drag.
c) Host Animal Sampling: Ticks are often collected directly from host animals, such as mammals or birds, by physically inspecting their fur or feathers or by trapping them. This method provides insights into tick infestation rates and species distribution among various hosts. Most data sets consist of samples from domestic animals in relatively uncontrolled circumstances, from trapped rodents or birds.
d) Vegetation Surveys: Ticks frequently reside on vegetation, especially in grassy and wooded areas. Collecting ticks directly from vegetation using forceps or other tools is used
e) Trapping: Specialized tick traps, such as CO_2-baited traps or dry ice traps, can attract ticks, allowing for their collection.

Sampling must be conducted over homogeneous patches of vegetation, but a stratified sampling approach may be adopted to obtain a reliable representation of questing ticks in an area. A survey should involve at least two or three consecutive years of sampling at relatively short intervals of time to be meaningful, preferably at 7–10 day intervals as relatively infrequent sampling (e.g., monthly intervals) fails to capture the seasonality of most tick species as well as the influence of abiotic factors on questing.

Control Measures

Controlling tick populations and reducing the risk of tick-borne diseases involves a combination of personal, environmental, and targeted intervention measures. Some commonly employed control measures for ticks are:

a) Personal Protective Measures: Individuals should wear long-sleeved shirts, long pants, and closed-toe shoes when venturing into tick-infested areas. Applying insect repellents containing N,N-Diethyl-meta-toluamide (DEET) or picaridin to exposed skin, treating clothing with permethrin, full body check and taking a shower after returning from outdoor activities reduces risk of tick-borne diseases.
b) Habitat Modification: Modifying the environment to reduce tick habitat like keeping lawns and vegetation trimmed short, removing leaf litter and brush piles, and creating a barrier between wooded areas and living spaces using gravel or wood chips. Implementing acaricide treatments, such as sprays or granules, in residential yards mainly areas around shrubs, tall grass, and perimeter areas can help reduce tick populations.
c) Pet/animal treatment: Use of anti-tick vaccines such as Bm86 protein based vaccine amongst animals and pet treatment with acaricides like Formamidines (Amitraz), Pyrethroids (Cypermethrin, Alphacypermethrin), OPs (Chlorpyrifos, Chlorfenvinphos) and combination products of OP and Pyrethroids are effective strategies. The vaccines lack the “knock-out effect” associated with acaricides.

d) Wildlife Management: Implementing wildlife management strategies, such as deer fencing, repellents, and targeted hunting programs to manage deer populations, and habitat modification can limit the number of ticks that feed on them.
e) Information, Education and Communication: For effective tick-borne diseases management, it is important to educate people staying in endemic areas about gross tick identification, and methods of tick prevention with a focus on body examination for tick detachment before 48 hours to prevent transmission of the pathogen.

A multi-faceted approach (Integrated tick management) is most effective in preventing tick-borne diseases and includes a combination of personal protection measures, environmental modifications, targeted pesticide application, anti-tick vaccines for animals and regular surveillance to monitor tick populations and disease prevalence. Biological control agents like *Metarhizium anisopliae* fungal spores are also being evaluated for tick population control.

2.2 Mites

Mites are small arachnids and belong to the order Acarina and the family Trombiculidae, which comprises many species of worldwide distribution. Fossil evidence suggests that mites have existed since the early Devonian period, making them one of the oldest arthropod groups on Earth. Mites causing zoonotic infections are the scabies mites which infest humans and cause skin irritation/discomfort, the house mouse mite which transmits rickettsial pox, and most importantly the chiggers which cause scrub typhus, which is a remerging disease.

Vector Ecology

The distribution and abundance of mites are influenced by factors such as climate, vegetation type, host availability, and environmental conditions. It takes 6-12 weeks for development from the egg to the adult stage. The larvae are the only parasitic stage in the life cycle of trombiculid mites and parasitize a vast range of animals from cold-blooded to warm. The larvae are characterized by the presence of 6 legs and unlike other haematophagous arthropods, they feed on lymph & tissue fluids for 2-3 days and then drop off to the ground concealing themselves in loose soil where they develop into nymphs and finally into adults which have 8 legs. The chiggers are distributed in areas called 'Mite islands' which are patches of ground characterized by thick vegetation cover mainly shrubs, relative humidity of 80-100% at ground level and temperature of 27±5 C. Mites are most active during the rainy season and go deeper into the soil during dry weather. *Suncus murinus, Rattus blanfordi, Mus booduga, Rattus r. satarae* and *R. r. rufescens*, all play a role in the maintenance of *O. tsutsugamushi*. It therefore appears that in order to have an active focus of Scrub typhus essential factors are presence of *O. tsutsugamushi*, availability of chiggers of the Leptotromibidium species and wild forms of the subgenus Rattus along with the presence of transitional vegetation. This has been referred to as the "zoonotic tetrad."

Survey Methods

These small arthropods are found in a wide range of habitats, including soil, vegetation, and animal hosts. Some of the commonly used survey methods are:

a) Rodent trapping: Baited Sherman traps are placed in suspected mite islands at dusk and collected at dawn and the trapped rodent is examined (ear, eyes, anal area, and rump) for any larval attachment.
b) Sentinel animal collection: Laboratory animals can be placed in mite islands and examined for larval presence.

c) Pitfall Traps: These traps consist of containers partially buried in the ground. Mites crawling on the soil surface fall into the trap, allowing for the collection and subsequent identification. Dry ice aids in the enhanced attraction of larvae to these traps.
d) Berlese Funnel Extraction: This technique involves placing soil or litter samples in a funnel, which is heated from above. The heat and desiccation drive mites deeper into the funnel, where they eventually fall into a collection vial. This method is commonly used to sample mites from soil and leaf litter.

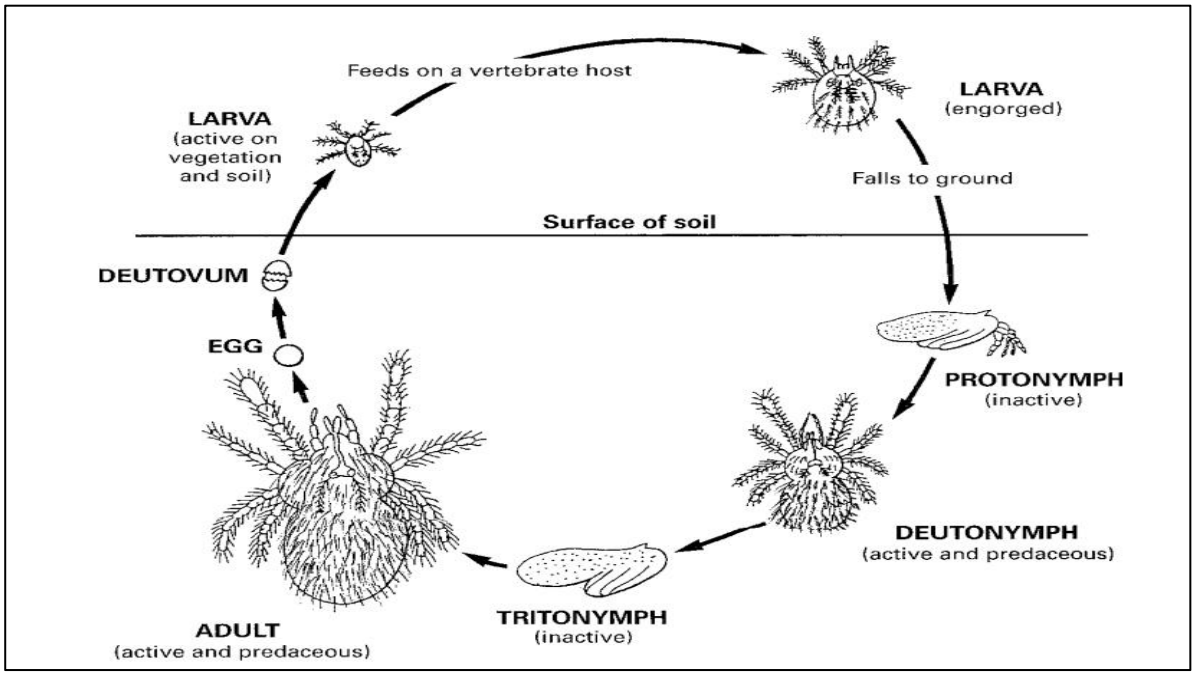


Figure 2: Life Cycle of a Leptotrombidium mite (Ref: Medical Entomology for students, Service 2012).

e) Sweeping and Beating: In ecological studies, mites on vegetation can be collected by sweeping a net through the plant foliage or by beating branches over a white sheet. The dislodged mites can then be collected and identified.
f) Sticky Traps: These traps consist of adhesive surfaces placed on plants or in mite habitats. Mites moving across these surfaces get stuck, allowing for their subsequent identification.
g) Tent method: Small black cardboard tents measuring not more than 2 inches can be placed in suspected mite islands for sampling mite larval stages.
h) Soil sampling: Soil from suspected mite islands or from near rodent burrows is collected and placed in large enamel bowls with water overnight. The next day, the clear supernatant water is examined with a magnascope for any floating figure of eight (nymph and adult forms).

Survey methods for mites vary depending on the target habitat, mite species of interest, and research objectives.

Control Measures

Some common control measures used to manage mite populations:

a) Proper sanitation and personal protective measures: Regularly removing plant debris, and maintaining clean living spaces for animals can help reduce mite populations. Personal hygiene and cleanliness practices are essential for managing mites that affect

human health. Regular washing of bedding, vacuuming carpets, and maintaining low humidity levels can help reduce mite populations indoors. Use of topical repellents, barrier clothing, repellent patches, and insecticide-treated clothing/camping essentials while outdoors, etc. are other methods of prevention.

b) Crop Rotation: Rotating crops can disrupt mite life cycles by reducing their preferred host plants. This practice helps prevent mite build-up and spread in agricultural fields.
c) Biological Control: Predatory mites, insects, and beneficial nematodes can be used as natural enemies to control mite populations. These organisms feed on mites or their eggs, providing a sustainable and environmentally friendly approach to mite management.
d) Chemical Control: In cases where mite infestations are severe or other control methods have proven ineffective, pesticides (bifenthrin, cyfluthrin, permethrin, malathion, propoxur etc) may be used. Acaricides specifically formulated to target mites can be applied to plants or animals.
e) Integrated Pest Management (IPM): IPM combines multiple control strategies to manage mite populations effectively. It involves monitoring mite populations, using cultural practices, employing biological control agents, and judiciously applying chemical control methods only when necessary.

It is important to note that the choice of control measures should be based on the specific mite species, the severity of the infestation, and the potential impacts on the environment and non-target organisms. Integrated approaches that prioritize non-chemical control methods are generally preferred to minimize negative consequences and promote sustainable mite management.

2.3 Fleas

Fleas are small wingless insects that feed on the blood of various warm-blooded animals, depending on the species, and can transmit diseases to their host. Fleas have plagued humans and animals throughout history, causing discomfort and transmitting diseases. In ancient times, fleas thrived in unsanitary living conditions, particularly in overcrowded areas. The Middle ages saw devastating outbreaks of flea-borne diseases like the bubonic plague, resulting in millions of deaths. Over the centuries, efforts were made to control fleas using herbal remedies, insecticidal powders, and fumigation. In the late 19th century, the discovery of insecticides improved flea control. Throughout the 20th century, advancements in hygiene, housing, and the use of insecticides reduced flea prevalence in developed countries. However, fleas remained a concern in regions with poor sanitation and close human-animal contact.

Vector Ecology

Fleas are ectoparasites of primarily mammals and birds. Fleas especially rat, cat and dog fleas, play a significant role as disease vectors transmitting diseases like plague, endemic typhus, bartonellosis and dog tapeworms. The fleas of major public health importance, however, are the rat fleas, *Xenopsylla cheopis* and the cat flea *Ctenocephalides felis*. The flea life cycle consists of four stages: egg, larva, pupa, and adult. Flea eggs are typically laid on the host or in their environment, such as rodent burrows, bedding, or carpets. The eggs hatch into larvae, which feed on organic debris and flea faeces containing semi-digested blood. Larvae thereafter spin cocoons and enter the pupal stage. Host movement, heat or any other pressure triggers the emergence of adults from the pupal stage.

Fleas have remarkable jumping abilities and can leap several times their body length, allowing them to move between hosts or from the environment onto a host. Fleas exhibit host specificity, preferring certain hosts over others, but they can opportunistically infest different species if their preferred hosts are not available. Their distribution is influenced by various factors, including host availability, environmental conditions, and human activities.

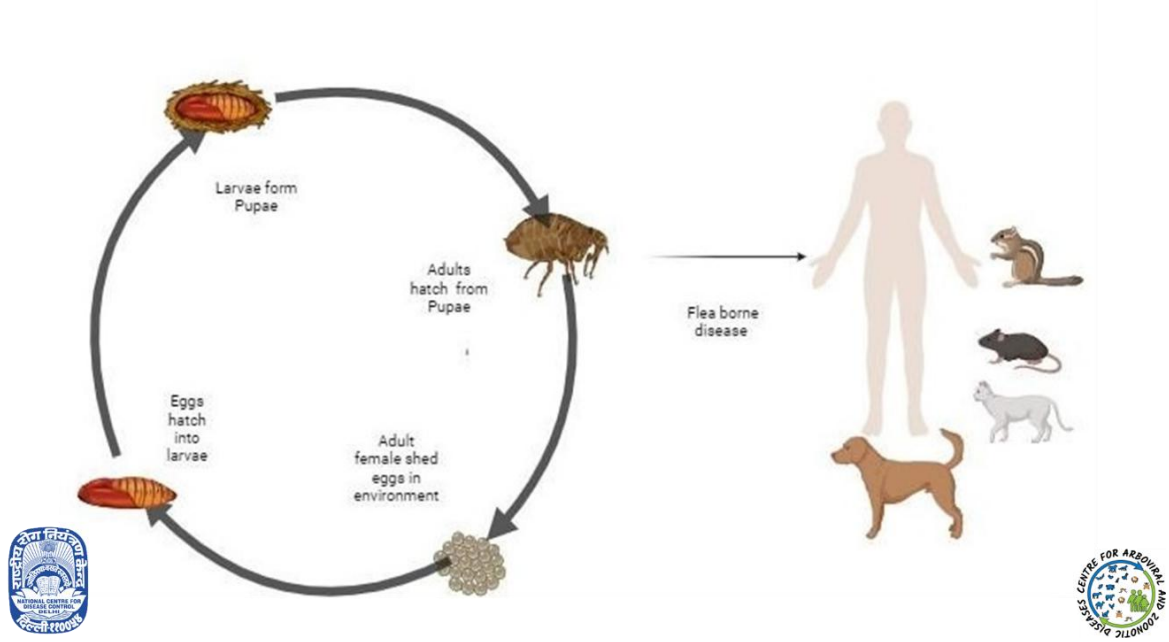


Figure 3: Life cycle of flea and transmission of flea-borne diseases.

Survey Methods

The common survey methods used in studying fleas are:

a) Visual inspection: Direct visual inspection of animals, such as pets or wildlife, is a straight forward method to identify adult fleas. Careful examination of the fur or feathers, particularly in areas where fleas tend to hide, can provide insights into their presence and abundance.
b) Flea combing: This method involves using a fine-toothed comb to comb through the fur or feathers of animals. As fleas move around, they can be trapped in the comb, allowing for their identification and collection for further analysis.
c) Trapping: Flea traps can be set up in infested areas to attract and capture fleas. These traps often use light, heat, or chemical attractants to lure fleas into a container or sticky surface, making it easier to collect and study them.
d) Environmental sampling: Flea eggs, larvae, and pupae are commonly found in the environments where infested animals reside. Collecting samples from pet bedding, carpets, or outdoor areas can provide information about flea populations and breeding sites.
e) Host surveys: Surveys involving the collection and examination of various host species (rodents etc.) can be conducted to determine the prevalence and diversity of fleas. This approach is particularly useful in understanding the host range and host preferences of different flea species.

These survey findings guide the development of effective flea control strategies and help mitigate the impact of fleas on human and animal health.

Control Measures

Controlling fleas involves a combination of integrated pest management strategies aimed at reducing flea populations and preventing infestations. Some common control measures used against fleas are as under:

a) Area Treatment: Insecticidal sprays, powders, or fogging with synthetic pyrethroids and Insect Growth Regulators (IGR) are used for the treatment of infested areas. It is essential to focus on areas where fleas are likely to reside, such as pet resting areas, carpets, cracks in floors or furniture and outdoor areas like yards or kennels and along rat runs.
b) Pet Treatments: Regularly treating pets with flea preventive products, such as synthetic pyrethroids (cyphenothrin, deltamethrin, flumethrin, and permethrin), topical treatments or oral medications with Insect growth regulators (IGRs) (lufenuron, methoprene, and pyriproxyfen) or flea collars, is crucial in preventing flea infestations. These treatments kill fleas on the animals and can also disrupt the flea life cycle.
c) Environmental Sanitation: Vacuuming carpets, rugs, and upholstery regularly can help remove flea eggs, larvae, and pupae from the environment. Washing pet bedding and using hot water can kill flea stages present on these items.
d) Prevention: Regular grooming of pets, checking for fleas after outdoor activities, avoiding contact with infested animals, keeping the surroundings clean, maintaining good hygiene, and minimizing contact with wildlife can help reduce the risk of flea infestations.

Implementing a comprehensive approach that combines pet treatments, environmental management, and targeted insecticide use can effectively control fleas and minimize their impact on both human and animal health.

2.4 Sandfly

Sandflies, the vectors of Leishmaniasis, belong to the order Diptera, family Psychodidae and subfamily Phlebotominae. The sandflies are tiny, blood-feeding insects measuring 1.5–3.5 mm and are characterized by hairy bodies, very long legs, large black eyes and lanceolate wings.

Vector Ecology

In India, sandflies are primarily found in tropical and subtropical regions, particularly in states like Bihar, Jharkhand, West Bengal, Uttar Pradesh, and Rajasthan, where leishmaniasis is endemic. The adult sandflies are weak fliers and prefer dark, humid environments such as cracks in walls, caves, tree bark, burrows, and damp areas to rest. The lifecycle comprises egg, larva, pupa, and adult stages, with the immature stages being very difficult to survey. Female sandflies require blood meals for egg development, making them efficient vectors of diseases, notably visceral lesihmaniasis or kala-azar (caused by *Leishmania donovani* in India), cutaneous and mucocutaneous leishmaniasis. Sandflies also transmit diseases such as bartonellosis (Carrion's disease) and phleboviral infections like sandfly fever (papatasi fever). Seasonal changes, deforestation, urbanization, and climate change affect population dynamics of sandflies.

Survey Methods & Control Measures

Survey methods for studying sandfly adult populations include light traps, sticky traps, illuminated sticky traps, funnel traps, aspirators, animal-baited traps, and human landing

collections, aiding in understanding their distribution and vector potential. Effective integrated vector management measures viz. indoor residual spraying (IRS) with approved insecticides, the use of insecticide-treated bed nets or LLIN, microenvironment management with focus on elimination of breeding sites such as waste and burrows, and personal protective measures are key to vector control and the prevention of sandfly borne diseases. Additionally, public health awareness and community participation will play a crucial role in effective vector management and disease prevention, helping India achieve its target of eliminating lymphatic filariasis by 2027—three years ahead of the global goal set by the WHO for 2030.

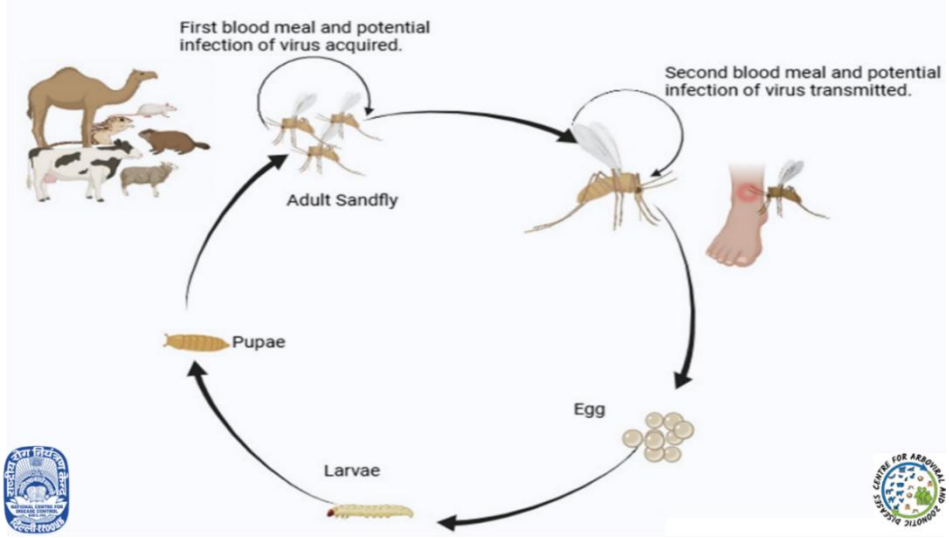


Figure 4: Life cycle of Sandfly and mode of transmission of sandfly borne diseases.

2.4 Mosquitoes

Mosquitoes are one of the most important vectors for transmission of emerging zoonotic diseases like dengue, yellow fever, Zika, chikungunya, West Nile disease and knowlesi, inui, cynomolgi malaria. Mosquito control measures are essential to reduce morbidity and mortality due to various emerging and re-emerging zoonotic diseases. Integrated vector management is a rational decision-making process to optimize the use of resources for vector control and is the cornerstone of preventive & control measures today.

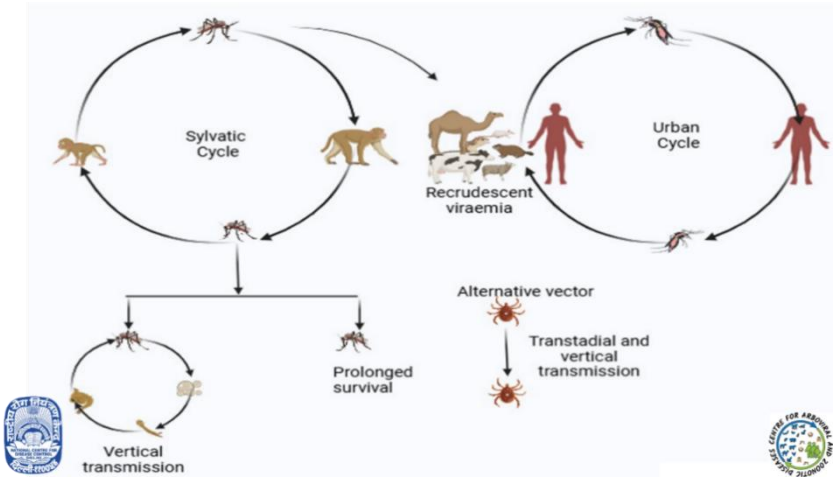


Figure 5: Life cycle of mosquitoe and transmission cycle of mosquito-borne diseases.

Table 4: Various Mosquito Control Measures

Mosquitoes control measures	Description
Anti-Larval Measures	Environmental control: Reducing breeding places, called "Source Reduction," e.g., filling up ditches, managing stored water in coolers, and vases, and avoiding water collection in waste tins and tyres
	Chemical control: use of larvicides - Temephos, Diflubenzuron, Pyriproxyfen and Bti
	Biological control: Larvivorous fish e.g., Gambusia, Poecilia (5-10 fish per linear metre to 20 fish if larval density high)
Anti-Adult Measures	Indoor Residual spray: 2 to 3 rounds/year spraying on the walls with DDT, Malathion or Synthetic pyrethroids as per NCVBDC guidelines
	Knockdown spraying in the space of rooms/barracks
	Outdoor fogging: Indicated in case of high mosquito density
Personal Protection	Mosquito net: Insecticide-treated net (ITNs) / Long lasting insecticide net (LLINs)
	Repellent creams and lotions: From dusk to dawn on exposed parts every 4 hours e.g., DEET or DEPA-based repellents against Anopheles and Culex-borne diseases and dawn to dusk against Aedes-borne diseases
	Mosquito proofing of accommodation
	Proper clothing
Other Measures	Health education, Legislative Measures, Research & Innovation, and Surveillance
Note: Refer to NCVBDC guidelines for updated approved insecticide formulations, dosage, and survey methods	

3. Integrated Vector Management (IVM)

The insecticides and pesticides have been used to control vector borne diseases rampantly over the years. Excessive and inadvertent use and exposure to pesticides have however been documented to impact the environment by negatively affecting the wildlife by reducing biodiversity, killing bees, affecting soil, water and air, polluting water sources as well as causing adverse health outcomes ranging from neurodevelopmental disorder, birth defects, fetal death, neurologic illness like Parkinson's disease to causing many types of cancer etc. in addition to contributing to the development of insecticide resistance amongst a large number of vector arthropods. It is thus important to emphasize that an integrated vector management (IVM) approach is crucial in the prevention and control of these emerging /re-emerging vector-borne zoonoses especially because as of date there is no effective vaccine against these diseases. IVM seeks to optimize the synergy between a diverse set of pest management tools (biological, chemical, cultural, and mechanical).

4. Conclusion

Arthropod-borne zoonotic diseases are emerging and re-emerging as a major global public health challenge. The adoption of the "One Health" concept in letter and spirit

coupled with a sound understanding of the vector ecology, impact of climate, and associated sociocultural factors, coupled with the complex host-pathogen-vector dynamics, are crucial in preventing these diseases from spiralling out of control. Implementation of Integrated Vector Management strategies would go a long way in ensuring effective management of these vector-mediated zoonoses.

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INFECTION PREVENTION AND CONTROL WITH SPECIAL REFERENCE TO ZOONOTIC DISEASES

CHAPTER IV

1. Introduction

Infection control is essential in managing and preventing the spread of zoonotic diseases, which are infectious diseases that can be transmitted from animals to humans. Zoonotic diseases pose significant public health risks, and proper measures must be implemented to protect both human and animal populations. Zoonotic diseases are shared between animals and humans. A person may become infected with an animal disease indirectly (from the environment or through flies, mosquitoes, ticks, and fleas) or directly (through close contact between animals and people). Infection prevention and control in zoonotic infections has a slight tangent, but overall follows similar rules of hospital infection prevention and control with the basic premise to consider all biological material, live or dead, animal or human as a potential biohazard. Human exposure can be orally through food and water, recreational or accidental exposure to animal/human source, occupational. The emergence and re-emergence of zoonotic pathogens, highlight the need for robust infection prevention and control (IPC) measures to mitigate their impact. Recently, new zoonotic entities with pandemic/ outbreak potential in humans such as Monkey pox (2022) and SARS COV-2 (2019 onwards), Crimean Congo Hemorrhagic fever (2011 onwards), Nipah virus infection (2001 onwards), Ebola virus (2014 onwards), Avian Influenza (2006 onwards) & H1N1 Influenza (2009 onwards) have stirred the public health machinery. Apart from these, country is threatened by import of exotic zoonotic infections like Yellow Fever, Hantavirus infection, Rift Valley fever, etc. Further, few high-risk pathogens such as CCHF, Nipah virus, Ebolavirus also require special transmission-based precautions to be followed based on risk assessment

This chapter aims to provide an overview of the current state of IPC strategies in zoonotic diseases, focusing on surveillance, risk assessment, PPE, hygiene practices, and vaccination. Additionally, the importance of interdisciplinary collaboration, community engagement, and global cooperation in ensuring effective IPC is emphasized.

2. Risk Mitigation

Risk Assessment and mitigation are guiding principles for defining appropriate IPC strategies required for prevention and control of zoonotic diseases. Risk assessment encompasses identifying high-risk animal-human interfaces, understanding transmission dynamics, and evaluating socio-economic factors that contribute to disease spread. Quantitative modelling techniques, such as agent-based modelling and network analysis help predict disease transmission patterns and evaluate the effectiveness of intervention measures. Risk management involves implementing targeted interventions such as culling infected animals, restricting movement, and enhancing biosecurity measures in high-risk areas.

Risk associated with any biohazard cannot be totally eliminated but only reduced by having safety features such as PPE, Good laboratory practices, Safety equipment, Infection control practices, human competency and training. The barrier precautions for handling high threat pathogens must be selected after appropriate risk assessment.

2.1 Personal Protective Equipment (PPE)

Proper utilization of PPE is crucial in minimizing the risk of zoonotic disease transmission and is considered the the primary barrier precaution.

Healthcare workers, veterinary personnel, and individuals in close contact with animals are particularly vulnerable. PPE includes gloves, masks, gowns, and eye protection.

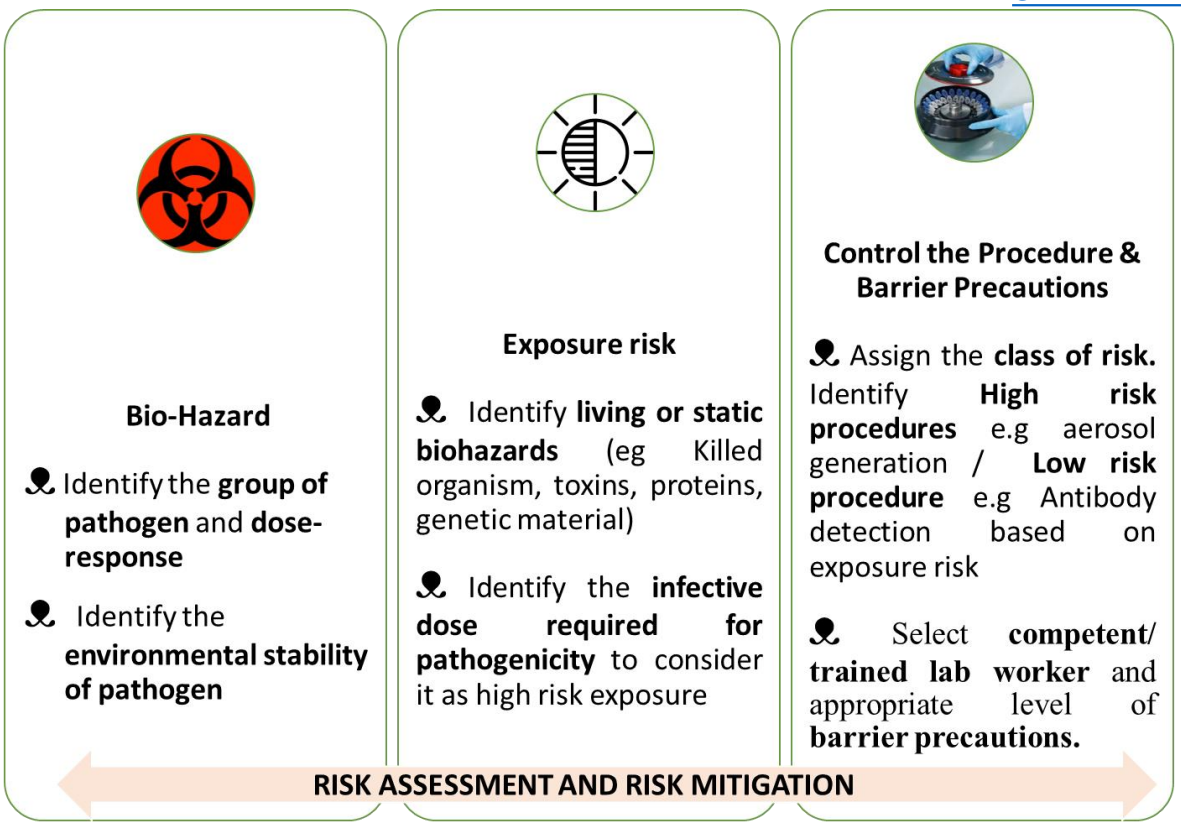


Figure 1: Risk assessment and mitigation for biological hazards.

Ensuring correct usage, appropriate disposal, and regular training on PPE protocols are vital components of IPC. Correct and effective selection and usage of PPE based on work activity evaluation [who is the user (age, size and sex), mode of transmission, risk group, nature of procedure] at all times of exposure is the most crucial component of IPC. It should be made sure that PPE fits and does not add more risk (ill fitting, slippery material, overheating, limiting visibility or dexterity etc). Recent advancements in PPE design and materials have enhanced comfort, durability, and protection, thereby improving compliance and reducing transmission risks.

Table 1: Barrier Precautions for handling High-threat pathogens

PRIMARY BARRIER • Personal Protective Equipment (PPE)
SECONDARY BARRIER • SAFE PRACTICES: Good Laboratory practices, Good microbiological practices • SAFETY EQUIPMENT: Biosafety cabinets/Centrifuge • INFECTION CONTROLS: Engineering controls (Quality by Design Facility, Ventilation System, Autoclaves)/ Administrative controls (Training, Procedure, Policy, or shift designs), Effluent Treatment Plan/Laboratory Waste Management Plan

2.2 Hygiene Practices and Environmental Management

Hygiene practices, both in healthcare and community settings, are integral to IPC in zoonotic diseases. Effective hand hygiene, disinfection, and waste management minimize the risk of contamination. Behavioural change communication campaigns promote proper hygiene practices among communities, emphasizing the importance of hand washing and safe food handling. Additionally, environmental management, such as controlling vector populations and ensuring safe water sources, contributes to reducing disease transmission.

The livestock farms & fairs are common sites for acquiring zoonotic infections and prevention protocols need to be strategized in such conglomerates. Hand hygiene must be done immediately when exiting animal areas, after removing contaminated clothing or shoes, and before eating or drinking. One must ensure sufficient numbers of hand washing stations are available during such fairs/farms and that secure boxes or step-stools are provided to make the hand washing station accessible to children. Number of washing areas should be one hand washing station for every 225 square feet of area accessible to people. This does not include the area of the barn that animals occupy. The animal waste generated from such farms/fairs should be properly disposed.

Table 2: Before and After Moments of Hand Hygiene with Soap and Water, as an effective tool for IPC in Zoonotic Diseases:

Before and After	After
Preparing meals and handling uncooked meat, fish, and eggs	Using the restroom or after changing a diaper
Eating and drinking	Cleaning animal pens and cages
Handling and caring for animals	Laundering soiled laundry& caring for animals who are ill

Table 3: Infection Prevention and Control Measures for Zoonotic Infections:

IPC Measures	Remarks
Hand Hygiene with Soap and Water and Not Alcohol Based Handrubs	Proper hand washing with soap and water after handling animals or their products, and before eating, is essential. Avoid direct contact with sick animals or their bodily fluids.
Personal Protective Equipment (PPE)	When working with animals known or suspected to carry zoonotic diseases, individuals should use appropriate personal protective equipment, such as gloves, masks, goggles, and gowns
Pre-emptive Detection Through Surveillance and Monitoring in Medical and Veterinary Diagnostic Facilities	Regular surveillance and monitoring of zoonotic diseases are crucial for early detection and response. This will create alerts by localizing and identifying new endemic zones. This shall help identify potential outbreaks and understand the transmission patterns by generating early warning signals
One Health Approach	This involves collaboration among human health, animal health, and environmental experts. This approach recognizes the interconnectedness of human, animal, and environmental health and aims to address zoonotic diseases holistically
Quarantine and Isolation	Suspected or confirmed cases of zoonotic diseases should be isolated to prevent further transmission. Additionally, quarantine measures may be necessary for exposed individuals or animals to prevent the spread of the disease especially for exotic diseases such as Ebola virus and Yellow fever

Vaccination	Vaccination of both animals and/or humans can be an effective preventive measure in some of the zoonotic diseases like Rabies, Zoonotic influenza, Brucellosis, Leptospirosis, Anthrax etc and can help achieve elimination/control targets too.
Vector Control	Zoonotic diseases which are transmitted by vectors such as mosquitoes, ticks, or fleas, can be reduced by controlling vectors. E.g. Dengue, Chikungunya, KFD, Plague etc
Proper Food Handling	Zoonotic diseases like Brucellosis, Salmonellosis etc can be transmitted through contaminated food. Dairy farms /Slaughter houses etc should be closely monitored by health authorities and enforced to comply with FSSAI regulations; ensuring safe food handling practices, including proper cooking temperatures and avoiding cross-contamination, is crucial to reduce the risk of transmission of food borne Zoonosis
Teaching, Education and Awareness (IEC)	Education about zoonotic diseases to Animal handlers, Laboratory staff and other healthcare professionals about their transmission, and preventive measures is essential.
Animal Population Management	A proper strategy and management planning will reduce the incidence of the disease in the affected areas if animal population is brought under control e.g Dog Population management for Rabies, Rodent population management for Leptospirosis etc
Health impact assessment and Environment impact assessment of developmental projects	Health impact assessment should be made mandatory for all developmental projects along with environmental assessment e.g. Developmental activities like canal network, roads and railway lines obstruct natural drainage of rain water causing its accumulation for longer periods leading to surge in Leptospira cases.

2.3 Vaccination Strategies

Vaccination plays a pivotal role in preventing zoonotic disease transmission. Developing vaccines for zoonotic pathogens presents unique challenges, including antigenic diversity and cross-species barriers. Advances in vaccine technologies, such as recombinant DNA and nanoparticle-based vaccines, have facilitated the development of innovative vaccine candidates. Vaccination campaigns targeting both humans and animals, as seen in rabies control programs, have successfully reduced zoonotic disease burden. However, ensuring vaccine coverage, addressing vaccine hesitancy, and maintaining cold chains remain critical challenges.

Interdisciplinary Collaboration and Community Engagement: Effective IPC in zoonotic diseases necessitates interdisciplinary collaboration across multiple sectors, including human health, veterinary medicine, ecology, and social sciences. The One Health approach recognizes the interconnectedness of human, animal, and environmental health and emphasizes on the importance of joint efforts in disease prevention and control. Community engagement fosters local ownership and empowers communities to participate in surveillance, risk assessment, and implementation of IPC measures. Building trust, fostering dialogue, and incorporating local knowledge enhance the success of IPC interventions.

3. Global Cooperation and Preparedness

Zoonotic diseases transcend national borders, necessitating global cooperation in IPC efforts. International organizations, such as the World Health Organization (WHO) and the

World Organisation for Animal Health (OIE), play essential roles in coordinating surveillance, response, and capacity-building initiatives. The one health mechanisms during the times of Outbreak management when human to human transmission after zoonotic spillover event occurs are usually well coordinated with the multidisciplinary team working with shared goal to control the outbreak. However, during the non-outbreak period, routine surveillance/ prevention and control activities for diseases in animal population is often neglected.

Classical infectious diseases like rabies and plague, well known for centuries, are zoonotic infectious diseases which have not been eradicated despite major efforts from human and veterinary health sectors. The zoonotic diseases of major public health importance in India are Dengue, Japanese encephalitis, leptospirosis, plague, rabies, anthrax, Kala azar, Kyasanur Forest Disease, Rickettsial diseases, cysticercosis, hydatid disease, trypanosomiasis and toxoplasmosis, some of which cause outbreaks at a great frequency.

Preparedness planning involves establishing rapid response teams, stockpiling medical supplies, and conducting simulation exercises. The COVID-19 pandemic has underscored the need for robust global cooperation and knowledge sharing in combating zoonotic diseases.

Recently, initiatives have been taken for intersectoral collaboration and coordination, Prevention, Control and Surveillance of zoonotic diseases of public health importance by establishing One health Mission. To complement the mission and vision of One Health Framework, multiple stakeholders under Ministry of Health and family welfare, Indian Council of Agricultural Research (ICAR), Indian Council of Medical Research (ICMR) and Department of Animal Husbandry and Dairying etc are working in the area of surveillance and epidemiology of zoonotic/trans-boundary diseases through various national programmes/ Networks/Consortium Platforms and even setting up independent institutes to monitor and control such diseases and prevent its further dissemination.

4. Conclusion

Infection prevention and control are critical components in managing the risks posed by zoonotic diseases. Effective IPC strategies encompass surveillance, risk assessment, PPE, hygiene practices, vaccination, interdisciplinary collaboration, community engagement, and global cooperation. As zoonotic disease threats persist, continuous research, innovation, and investment in IPC measures are essential to safeguard public health and mitigate the impact of future outbreaks.

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NATIONAL ZOONOTIC DISEASE CONTROL PROGRAMS: HUMAN & ANIMAL SECTORS

CHAPTER V

1. Problem Statement

Combating Zoonoses requires multidisciplinary, multisectoral collaborative approach popularly known as One Health Approach. Although the "One Health" term is relatively a new but the multisectoral collaboration, communication and coordination the core principle of One Health Approach has been long been recognized to effectively address the complex public health issues including zoonoses of endemic as well as pandemic potential, Anti-microbial Resistance, Food Safety and Security and Climate related health issues. The One Health approach has gained significant momentum in recent years, particularly following influenza and the COVID-19 pandemic, highlighting the interconnectedness between human, animal, and environmental health, leading to a greater focus on collaborative strategies across disciplines to address these issues. One Health approach emphasizes early detection, surveillance, and rapid response to disease outbreaks. Collaborative efforts between human and veterinary health sectors enable better monitoring and control of zoonotic diseases, reducing the likelihood of pandemics and improving public health outcomes. One Health also recognizes the overuse and misuse of antibiotics in both human and veterinary medicine as a significant concern and thereby fostering collaboration, it strongly advocates for responsible antimicrobial stewardship, promoting the judicious use of antibiotics in both human and animal health settings, thus combating the growing threat of antimicrobial resistance. The One Health approach compel to underscores the importance of sustainable environmental practices and justifies appropriate and optimum investment in Research and Innovation by encouraging interdisciplinary research for the development of novel diagnostics, vaccines, and treatments for both human and animal, improving health outcomes for all species.

Definition of One Health Approach-

One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent-(WHO definition)

2. Economic impact of Zoonoses

The zoonotic diseases lead to significant economic impact on local, regional and global scale. The impact is both Direct (healthcare expenses, loss of productivity and Indirect Cost (Trade restrictions, tourism losses, food security etc.)

As per World Bank Report, the economic losses from six major outbreaks of highly fatal zoonoses between 1997 and 2009 (Nipah Virus in Malaysia, West Nile Fever in USA), SARS in Asia, Canada & other, HPAI in Asia & Europe), BSE in US & UK, Rift Valley Fever in Tanzania, Kenya & Somalia) amounted to at least US$80 billion.1 If these outbreaks had been prevented, the benefits of the avoided losses would have averaged $6.7 billion per year. Fortunately, none of those outbreaks developed into a pandemic.

3. Conclusion

One Health approach is crucial in addressing the growing threat of zoonoses, AMR, and climate-related health issues. These various national programs under MoHFW & Ministry of Fisheries reflect India's commitment to improving human and animal health, strengthening disease surveillance, and integrating the One Health approach into disease

prevention strategies. Investments in capacity building, technological innovation, and policy integration will further enhance India's preparedness and resilience against future zoonotic threats, ensuring better health outcomes for humans, animals, and the environment.

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NATIONAL HEALTH PROGRAMS FOR ZOONOSES IN INDIA BY MINISTRY OF HEALTH & FAMILY WELFARE

CHAPTER VI

1. Introduction

India has implemented several national programs to address zoonotic diseases through the One Health framework, integrating human, animal, and environmental health approaches. These programs aim to improve surveillance, diagnosis, prevention, and control of zoonotic diseases while fostering intersectoral collaboration.

A brief about all the National Health Programs for Zoonoses is as under

2.1 National Rabies Control Program

A. Background

Rabies is an acute viral disease that causes fatal encephalomyelitis in virtually all the warm-blooded animals including humans. The disease is caused by neurotropic viruses in the family Rhabdoviridae, genus Lyssavirus. The major source of infection is the domestic stray dogs as 95 % of the cases of human rabies are following dog bites. Wild animals such as cats, monkeys, raccoons skunks, jackals and bats serve as a major reservoir of rabies virus. The number of human deaths globally due to dog-mediated rabies is estimated to be 59,000 annually, with an associated loss of 3.7 million DALYs. The annual cost of rabies (as per WHO) is to the extent of US $6 billion per year including an estimated US $1.6 billion for meeting the cost of Post Exposure Prophylaxis. The majority of deaths are estimated to have occurred in Asia (59.6%) and Africa (36.4%).

In Indian context, as per WHO –APCRI 2004 Study, there were estimated 17.4 Million Animal bites and 20000 deaths /year due to Rabies in India. The Million Deaths Study 2012, India has an estimated 12700 deaths due to furious rabies. In India, Rabies is endemic in all States and UT except UT of Andaman & Nicobar and Lakshadweep.

ICMR has undertaken a community-based nationwide cross-sectional survey during 2022-23 with a multistage cluster-sampling design covering 60 districts in 15 Indian states. The study revealed annual incidence of animal bite was 6·6 per 1000 population, translating into 9·1 million bites nationally (weighted and adjusted with 95% CI 5·7–7·6). Annual dog-bite incidence was 5·6 (4·8–6·6) per 1000. The study has used probability decision-tree model to analyse the information from survey and animal laboratories and estimated 5,726 human rabies deaths per year, i.e., nearly a 75% decrease in the number of deaths in the past 20 years. However, the average number of animal bites reported on IDSP portal during 2012-2023 are 3-4 million per year (Range 1.7- 7.5 million per year).

To achieve rabies control and eventual elimination of the disease from a defined geographic area One Health Approach is extremely essential.

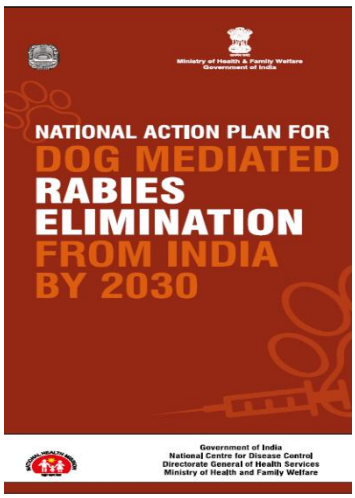
Rabies is a zoonotic disease. The major source of infection is the domestic stray dogs. By adopting One Health approach, dog-mediated rabies has been eliminated from western Europe, Canada, the USA, and Japan. 28 of the 35 Latin American countries report no human deaths from dog transmitted rabies. The neighbouring countries of India such as Bangladesh, the Philippines, Sri Lanka, Tanzania, Vietnam, and South Africa have also made great strides in reducing rabies deaths during recent years.

B. India's strategic approach to Rabies: Zero by Thirty

Rabies is categorised under "Neglected Tropical Disease, and in the SDG Goal -3, Target 3.3 is envisaged to end the epidemics of AIDS, tuberculosis, malaria and all Neglected Tropical Diseases by 2030 and in line with this there is Global and regional Call to eliminate Canine Mediated Rabies deaths in Humans by 2030.

Although Rabies is almost 100 % fatal disease yet it is fully vaccine-preventable with a timely institution of appropriate post-exposure prophylaxis (PEP) to the animal bite victims. Since no drug is available to effectively treat a patient of rabies, primary prevention through vaccination is the most appropriate strategy.

A Pilot project on Prevention and Control of Human Rabies was carried out as a 'New Initiative' under 11th Five-Year Plan from 2008 till 2012 by National Centre for Disease Control, Ministry of Health and Family Welfare, GOI, for prevention of rabies deaths in humans. Subsequently, in the 2012 National Rabies Control Programme (NRCP) was approved by MoH&FW to be implemented under the Umbrella of NHM and the program was expanded in all states and UTs. Govt. of India recognised Rabies a priority zoonosis and it is a policy thrust area in the National Health Policy- 2017, wherein it emphasizes to strengthen the National Rabies Control Program.



Subsequently National Rabies Control Program conceptualised and formulated "National Action Plan for Dog Mediated Rabies Elimination from India by 2030 (NAPRE)" in coordination and collaboration with all stakeholders with One Health Approach. The NAPRE was launched in 2021 jointly by Ministry of Health and Ministry of Fisheries, Animal Husbandry and Dairying.

The National Action Plan for dog mediated Rabies Elimination in India identifies the role of various stakeholder to achieve the Global Target of Rabies Elimination by 2030. The two key components envisaged under NAPRE are Human Health Component and Animal Health Component.

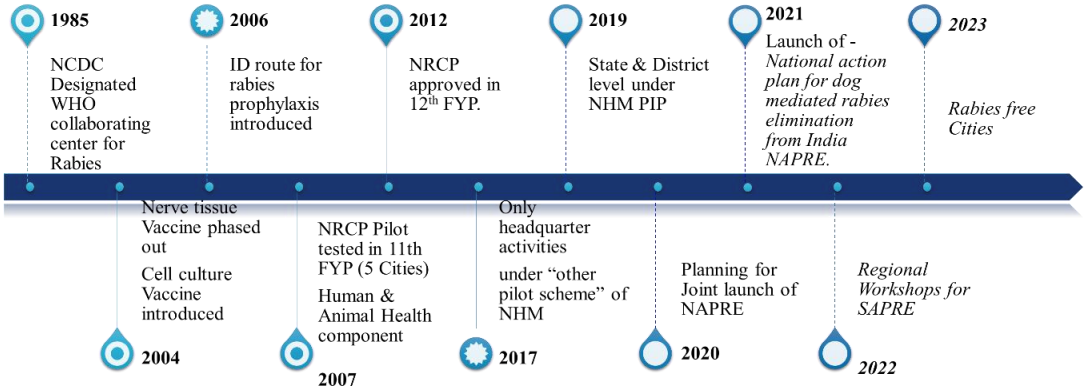


Figure 1: Rabies: A journey from Neglected Tropical Disease to priority Zoonoses.

C. Key Components

I. Human Health Component-

The human health component is envisaged to be implemented by the nodal agency for rolling out the activities in NRCP Program management unit under Centre for One Health at NCDC through State/UT Government and in coordination and collaboration with other key stakeholders. The State and below state level activities of human health component are integrated under NHM. The key activities are under human health components

- To ensure availability of Anti Rabies Vaccine (ARV) and Anti Rabies Serum (ARS) to all animal bite victims at all levels of health facilities. As ARV & ARS are included in essential drug list, all States/UTs are advocated to utilize the funds available under National Free Drug Initiative.
- To undertake capacity building of professionals - Training of target professionals is undertaken on Rabies pre-& post-exposure prophylaxis, program management's aspects, Joint training of medical and veterinary professionals for Intersectoral Coordination, rabies diagnostics, Rabies case investigations and Notification.
- To encourage pre-exposure prophylaxis for high-risk groups through sensitization and implementation of protocols for safety of health workers/ professionals exposed to environment conducive for rabies virus transmission.
- Strengthening surveillance of animal bites and rabies cases in human is undertaken by advocating necessary implementation of rabies notification by reporting animal bite cases/rabies confirmed cases on IDSP.
- Operational research in the Rabies, strengthening inter-sectoral coordination mechanism between veterinary and medical sectors for regular sharing of reports/ data on animal rabies, targeted IEC and strategic BCC and fostering public private partnership through involvement of NGO and community organizations are important activities under Human Health component

II. Animal Health Component

Animal health component is envisaged to be implemented by Department of Animal Husbandry and Dairying under Ministry of Fisheries, GOI through state/UT veterinary departments and urban and rural local Governments. The key activities under animal health component are –

- Estimation of canine population and identification of rabies risk zone to calculate the logistics requirement
- Planning & implementing strategic mass dog vaccination programme –to achieve anti rabies vaccination in at least 70 % of dog population, annually for three consecutive years and maintain the 70 % vaccination status in a defined geographic area to provide adequate protective 'herd immunity 'in canine population.

- Dog population management is undertaken as per animal birth control (ABC) rule to limit the man-dog conflict and to reduce the numbers of stray dogs to an acceptable level through Animal Birth Control.
- To promote responsible dog ownership by community involvement and to undertake solid waste management.
- Confinement and containment of suspected rabid dog is to be undertaken by competent authority as per Prevention and Control of Infectious Disease Act, 2006.

In the Indian context, the large stray dog population, limited logistics, and prevailing community beliefs about compassion towards stray dogs have posed significant challenges in meeting the targets of Zero by Thirty. The launch of the National Action Plan for Dog-Mediated Rabies Elimination by 2030 has provided a strategic framework with clearly defined roles and responsibilities, integrating the One Health approach into rabies elimination efforts in India. Additionally, the formulation of State Action Plans for Dog-Mediated Rabies Elimination, alongside the Rabies-Free City Initiative targeting tier 1 and tier 2 cities, has successfully brought together all stakeholders on a shared platform. This unified approach fosters a common vision and goal of rabies control and elimination, with heightened commitment to progressively achieve rabies-free status in their respective jurisdictions.

2.2 National One Health Program for Prevention and Control of Zoonoses

A. Background

The National One Health Program for Prevention and Control of Zoonoses was initiated as a Central Sector Scheme of "Ministry of Health and Family Welfare approved in 12th Five-year Plan with the initial name as "**Strengthening Inter-Sectoral Coordination for Prevention and Control of Zoonotic Diseases"** Over the years the program has made significant achievements in sensitization of stakeholders and bringing together multidisciplinary experts on a common platform to build a consensus to have a structured, 'One Health' approach to respond to the Zoonotic threats in the country.



Figure 2: Key Stakeholders for One Health in India.

Currently, major stakeholders in India for One Health include Ministries of Health and Family Welfare, Agriculture, Animal Husbandry, Environment and Forest, Science and Biotechnology, Water and Sanitation Human Resource, etc. and organization such as NDMA, DCGI, FSSAI and Remount Veterinary Corps, ICAR, and ICMR institutes etc.

B. Vision, Mission & Goal

a. **Vision:** To institutionalize structural mechanism for one health in the country at each level i.e., national, state, district, block and village level.
b. **Mission:** To bring all stakeholders from policymakers till front line workers on One Health Platform with shared vision and common goals.
c. **Goal:** To protect communities and minimizing socio-economic losses due to emerging and re-emerging zoonotic threats.

C. Objectives of the Program

The key objective of the "National One Health Program for Prevention and Control of Zoonoses" is to operationalize "One Health" mechanisms for prevention and control of zoonoses through strengthening inter-sectoral coordination among all Stakeholders at the national, state, and district and up to grass root level. The programs envisage following 6 components

- **Component I:** Institutionalization of One Health Structure for Zoonoses at National, State, and District Level-

  This component envisages to have structured and systematic continuum of collaborations among the stakeholders for functional arrangements not only for prevention of zoonoses but also to cover other core domains of one health, such as AMR, Climate sensitive emerging and remerging infectious diseases and Food safety and security. The component envisages to formulate the relevant guidelines and SOPs and to bring stakeholders together to deliberate upon any policy and legislative gaps and accordingly seeking appropriate solutions with consensus for One Health operationalization at all levels. At National level, the program is being executed through National Program Management Unit (NPMU) at Centre for One health of NCDC. The NPMU is overall responsible for planning, execution, coordination and monitoring of all activities envisaged under the program. The program at present is being executed as a vertical program through a network of Regional Coordinators and Sentinel Surveillance sites. Under the program State Level Zoonoses Committee (SLZC) and District Level Zoonoses Committee (DLZC) have been constituted with representation of all concerned stakeholders of One Health such as medical, veterinary, wildlife sectors along with relevant stakeholders i.e., panchayat raj institutions, urban local bodies, agriculture department, education department, etc.

  Program has various subject specific Technical Advisory Groups, Task force and Roster of disease specific expert working groups for development of national guidelines and SOPs to address priority Zoonoses in the country.

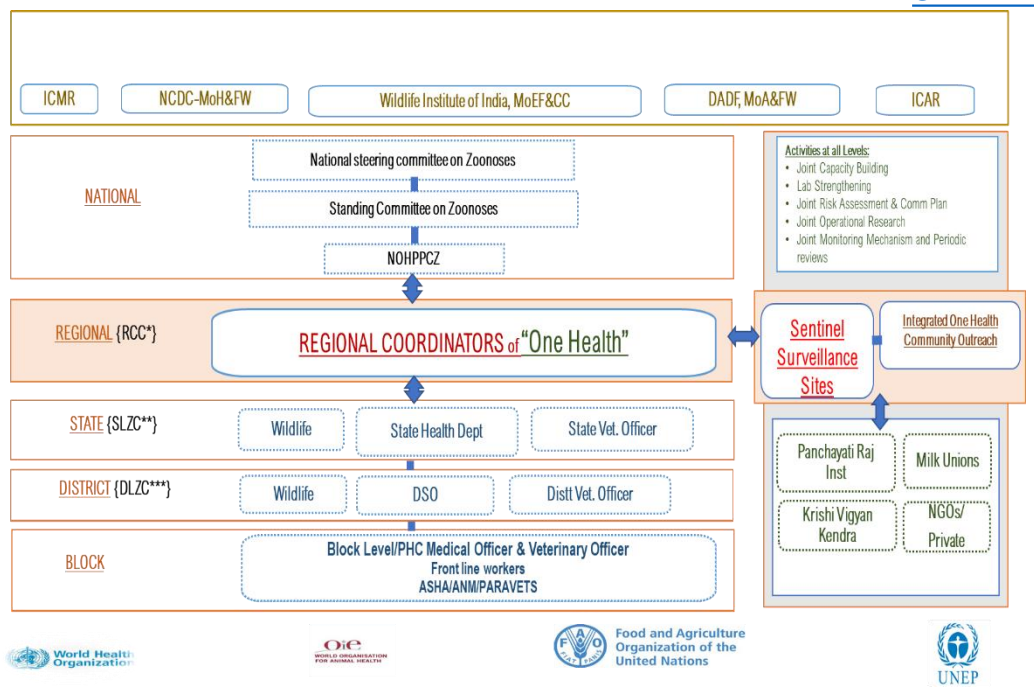


Figure 3: Institutional Mechanism of One Health.

- **Component II:** Integrated Capacity building program on Zoonoses through multidisciplinary regional network of One Health Institutes and partner organizations. The capacity building activities are undertaken as Joint training of Medical, Veterinary, Wild life, Food authorities and Local Governing Bodies on various aspects of Zoonoses such as Laboratory Diagnosis (Hands on Training), Joint Risk Assessment and Joint Outbreak Investigation etc. The institutes designated as regional coordinators belong to all core disciplines such as medical sector, veterinary sector, wild life sector etc. As of now, there are 17 Regional coordinators across the country.

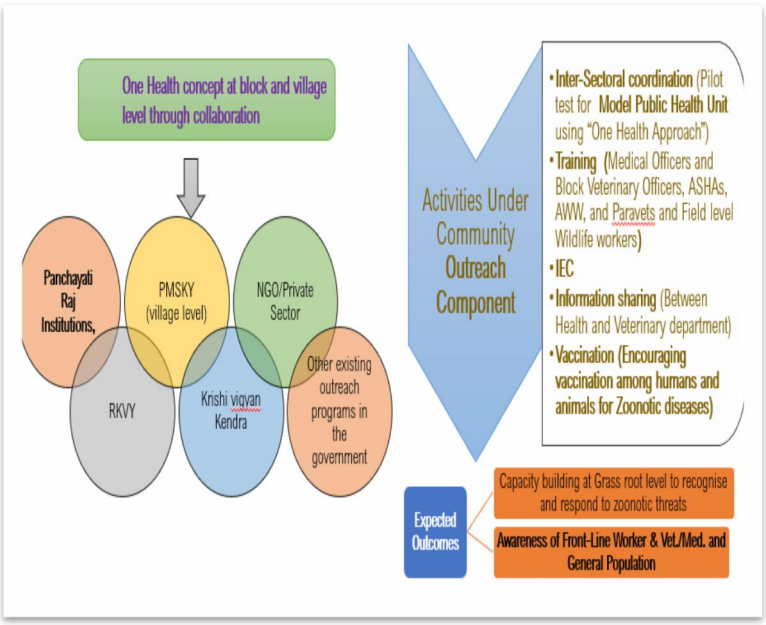


Figure 4: Integrated Community Outreach Activity.

- **Component III:** Integrated surveillance program on Zoonoses. Under this component, it is envisaged to create a network of 120 Sentinel Surveillance for Zoonoses in selected institutes across the country. The sentinel sites are strengthened to

undertake diagnosis on priority zoonoses. The National Reference Laboratory at Centre for Arboviral and Zoonotic Diseases, regional coordinator labs and sentinel surveillance sites acts as hub and spoke model to address the public health need of priority zoonoses in the country. Systematic data sharing mechanism among the key stakeholders on agreed minimal essential parameters for the zoonotic pathogens of public health importance is also undertaken.

- **Component IV:** Integrated community outreach program on Zoonoses with One Health approach at grass root level. The community is at the helm of One Health operationalization. As more than 60 % of the rural population is still dependent on farming and livestock, this is a most vulnerable section who needs to be educated and informed about the significance of prevention, early diagnoses, and control of zoonoses for not only to promote and restore health but also for better care of their livestock and economic security. Research studies have shown that farmers, animal handlers such as milkers, paravets, artificial inseminators, pashu sakhis, pahshu mitra, and forest workers and wild life professionals are classified as high-risk groups for being exposed to zoonotic diseases. A 2019, KAP study done in Punjab about awareness of zoonotic diseases, it was observed that 85% of livestock farmers had heard the term 'zoonoses' but only 40%, 31% and 25% farmers were aware of the zoonotic diseases such as tuberculosis, japanese encephalitis and taeniasis, respectively and yet 23% farmers reported consumption of raw milk and only 10% and 8% livestock farmers ever got their animals tested for brucellosis and tuberculosis, respectively.

  The objective of integrated community outreach program on Zoonosis is to foster the One Health concept at block and village level through collaboration with panchayati raj institutions, rashtriya krishi vikas yojana, pradhan mantri krishi sinchayee yojana, krishi vigyan kendra's and other existing outreach programs in the government and private sector.

- **Component V:** Advocacy and Risk Communication activities. This will include sensitization of government policy makers at various levels and partners on One Health approach for advocacy & support. The program envisages strategic IEC activities for improving knowledge, attitude, behaviour and practice of community on health threats or risks at human, animal, and environment interface.

- **Component VI:** The complex nature of zoonotic pathogens and diseases needs a multidisciplinary approach to completely understand its epidemiology, diagnostic requirements, clinical and prevention and control aspects. Significant gaps in knowledge, attitude and practices about zoonoses do exists in general physicians, veterinarians, wild life handlers and community. The operational research is undertaken in close collaboration with ICMR, ICAR and various academia and universities across various disciplines to identify & address the gaps.

- The National one health programs for prevention and control of zoonoses has been able to sensitize the stakeholders to mobilize the resources and prioritize the activities requited to address the zoonotic diseases. Over a period of time One Health approach has gained significant momentum and various initiatives are being undertaken by different stakeholders to address Zoonoses, AMR and Climate and Health related issues. However sectoral priorities, limited resources, limited infrastructures, and legislative gaps are the challenges to realize the One Health on ground in India.

2.3. Program for Prevention and Control of Leptospirosis

A. Background

India has one of the most important coastal, agro-ecosystems in the world, with an 8,129-kilometer coastline and abundant natural resources. India has one of the highest humans and domestic animal population with close interaction between them which is responsible for emergence of Zoonotic disease. Many new zoonotic diseases have emerged as a result of rapid ecological changes in the area over the last decade, resulting in epidemics that have caused considerable morbidity and mortality in humans. One of them is leptospirosis.

Leptospirosis is an important public health problem associated with significant morbidity and mortality. The magnitude of the problem in the coastal states of India is largely attributed to climatic and environmental conditions but in view of changing agro economic conditions, Leptospirosis is increasingly being reported from the different parts of the country especially in post monsoon period.

Pilot project on Control of Leptospirosis was undertaken from 2008 -2011. The project was carried out in selected districts of Gujarat, Tamil Nadu, Maharashtra and Karnataka. The strategy for prevention and control of Leptospirosis was found to be feasible and implementable and later Government of India (GOI) approved programme on Prevention and Control of leptospirosis in 2012 for rolling out in 6 endemic states/UTs viz. Maharashtra, Gujarat, Karnataka, Kerala, Tamil Nadu and Andaman and Nicobar. The Program at present is being implemented in 181 districts of 12 States and 2 UT (**Punjab, Odisha, Andhra Pradesh, West Bengal, Gujarat, Kerala, Tamil Nadu, Maharashtra, Karnataka, UT of Andaman & Nicobar Island, Uttar Pradesh,**



Figure 5: Programme States under PPCL.

Assam, Goa, Dadra and Nagar Haveli, Daman and Diu).

B. Key Objective:

Reduction of morbidity and mortality due to Leptospirosis.

C. Key component under the programs-

I. Trainings on Diagnosis & Case Management of Leptospirosis

Training programs for healthcare professionals should follow the National Guidelines on Diagnosis, Case Management, Prevention & Control of Leptospirosis issued by the Ministry of Health & Family Welfare (MoHFW). These trainings are being focused on clinical identification and laboratory diagnostics (IgM ELISA, PCR, MAT). Healthcare workers are also being trained in surveillance, outbreak investigation. Training is being conducted at state and district levels.

II. Strengthening Diagnostic facility

As diagnostic facilities strengthening affected districts of the programme States/ UT are vital for disease detection and further management of patients, it is envisaged under the programme to provide financial support for the procurement of equipment's and consumables for proposed lab for institutes which will serve as Regional Reference Centre for Leptospirosis. For States, funds are being given as a GIA for strengthening the laboratory capacity in program States.

Under the program 5 Regional laboratories are strengthened to undertake hands-on training of health care professionals on laboratory diagnosis of leptospirosis.

III. Strengthening case management facility

Prompt case management is crucial in Leptospirosis. Adequate supply of drugs for treatment of Leptospirosis has to be ensured (Doxycycline) at all PHCs/CHCs/ Districts/ Medical colleges of the State. For high-risk groups (severe leptospirosis, leptospirosis in pregnancy, organ dysfunction) the specific drugs and management facilities should be made available to referral centers (CHCs/District/ Medical colleges). National Guidelines for Diagnosis, Case Management Prevention and Control of Leptospirosis (Year 2015) have been published under the programme and disseminated to States. Advisory has issued from NCDC to States from time to time to undertake preventive measures especially during monsoon season and flood events. States have also been provided guidelines on measures to be taken for chemoprophylaxis during outbreaks. It is envisaged under the programme that States/UTs would be advocated though regular review meetings, field visits and during monitoring of programme to strengthen the patient management facilities especially for complicated cases of Leptospirosis as ICU facilities with ventilator support, dialysis etc. States may strengthen the facilities through tertiary health component of NHM or state funds as per state needs.

IV. Information, Education and Communication (IEC)

Given the high morbidity and mortality associated with leptospirosis, mass awareness campaigns play a crucial role in prevention and control. The program emphasizes community participation and the use of standard IEC messages to educate the public on risk factors, symptoms, early medical intervention, and preventive measures. Under the programmes, Radio spot and radio jingles have been prepared by the programme division and are available on the NCDC website. https://ncdc.gov.in/index1.php?lang=1&level=3&sublinkid=503&lid=161

V. Strengthening Surveillance for Leptospirosis

Under the program it is envisaged to strengthen the surveillance of Leptospirosis. The recommended minimum data elements need to be recorded and reported on IDSP. Case-based record to be maintained at all the health centers with facility to treat and diagnose the cases of leptospirosis.

Further Reading:

1. National guideline for Rabies Prophylaxis, 2019, NRCP. https://ncdc.mohfw.gov.in/wp-content/uploads/2024/04/National-Guidelines-for-Rabies-Prophylaxis.pdf
2. National Action Plan for Dog Mediated Rabies Elimination from India by 2030 (NAPRE)- https://ncdc.mohfw.gov.in/national-rabies-control-programme/
3. Training Module for Medical Officers. NRCP https://ncdc.mohfw.gov.in/wp-content/uploads/2024/04/National-Guidelines-for-Rabies-Prophylaxis.pdf
4. Rabies: General Aspects & Laboratory Diagnostic Techniques, 2022. https://ncdc.mohfw.gov.in/wp-content/uploads/2024/04/RabiesGeneralAspectsLaboratoryDiagnosticTechniques2022.pdf
5. National Guidelines on Diagnosis Case Management and Prevention and Control of Leptospirosis https://ncdc.mohfw.gov.in/wp-content/uploads/2024/04/File558.pdf

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NATIONAL PROGRAMMES UNDER DEPARTMENT OF ANIMAL HUSBANDRY AND DAIRYING, MINISTRY OF FISHERIES

CHAPTER VII

1. Introduction

The Department of Animal Husbandry & Dairying (DAHD), Government of India, has launched several key programs aimed at improving animal health, disease surveillance, and livestock productivity. These programs play a crucial role in preventing zoonotic diseases, ensuring food security, and supporting farmers by reducing livestock mortality and disease burden. Below are the major initiatives in detail:

2.1. National Animal Disease Control Programme (NADCP)

A. Background

The National Animal Disease Control Programme (NADCP) is a flagship initiative launched in 2019. The program primarily focuses on controlling and eradicating two major livestock diseases i.e., Foot and Mouth Disease (FMD) and Brucellosis, which significantly impact animal health and productivity. The program aims for 100% vaccination coverage of cattle, buffaloes, sheep, goats, and pigs against FMD, and of female bovines (cattle and buffaloes) against Brucellosis. Regular disease monitoring and nationwide vaccination drives ensure that animals remain disease-free, ultimately improving milk production and farmer income. The program also includes tagging and registration of animals to track vaccination status and disease prevalence. By preventing these economically devastating diseases, NADCP contributes to better livestock trade, export potential, and rural livelihood security.

B. Objectives of the Programme

The overall aim of the National Animal Disease Control Programme for FMD and Brucellosis (NADCP) is to control FMD by 2025 with vaccination and its eventual eradication by 2030.

C. Major Activities under NADCP for FMD and Brucellosis

- Vaccinating the entire susceptible population of bovines, small ruminants (sheep and goats) and pigs at six-monthly intervals (mass vaccination against FMD)
- Primary vaccination of bovine calves (4-5 months of age)
- Deworming one month prior to vaccination
- Publicity and mass awareness campaigns at national, state, block and village level including orientation of the state functionaries for implementation of the programme
- Identification of target animals by ear-tagging, registration and uploading the data in the animal health module of Information Network for Animal Productivity and Health (INAPH)
- Maintaining record of vaccination through animal health cards
- Serosurveillance/seromonitoring of animal population
- Procurement of cold cabinets (ice liners, refrigerators, etc.) and FMD vaccine
- Outbreak investigation, pathogen isolation and typing
- Recording/regulation of animal movement through temporary quarantine/ checkposts
- Testing of pre-vaccination and post-vaccination samples

- Generation of data and regular monitoring including evaluation of impact of the programme
- Providing remuneration to vaccinator which should not be less than Rs. 3/- per vaccination dose and Rs. 2/- per animal for ear-tagging including animal data entry

2.2 Livestock Health & Disease Control (LHDC) Programme

A. Background

The Livestock Health & Disease Control (LHDC) Programme aims to improve veterinary services, disease diagnostics, and outbreak preparedness in India. The program supports the establishment of regional disease diagnostic laboratories and mobile veterinary units to ensure quick response to disease outbreaks. It focuses on disease mapping, surveillance, and control programs for high-risk livestock diseases. Under this initiative, veterinary hospitals and dispensaries are strengthened with modern diagnostic tools and trained manpower. The program also promotes the use of rapid diagnostic kits and AI-based disease prediction models, making disease detection more efficient. By enhancing veterinary infrastructure, LHDC contributes to early disease detection, timely treatment, and better overall animal health outcomes.

B. Objective of the programme

a. To implement critical animal disease control programme to eradicate Peste des Petits Ruminants (PPR) by 2030 by vaccinating all sheep and goats and to control Classical Swine Fever (CSF) by vaccinating the entire pig population
b. To provide veterinary services at the farmers doorstep through mobile veterinary units (MVUs)
c. To assist States/UTs for control of animal disease by prevention & control of important livestock and poultry diseases prevalent in different States / UTs as per the State /UT's priorities.

C. Critical Animal Disease Control Programme component

a. Peste des Petits Ruminants Eradication Programme (PPR-EP)- This component covers the entire sheep, goat population & small ruminant in the country for carpet vaccination against Peste des Petits Ruminants (PPR), for 100% effective coverage. Migrants' flocks/animals will also be covered under the vaccination programme. The sole objective of having the eradication programme for 4 years is to establish adequate herd immunity and ensure that the country becomes free from PPR.
b. Classical Swine Fever Control Programme (CSF-CP)- CSF is enzootic in most of the pig producing States. The best possible remedial measure is vaccination of eligible animals. In view of the importance of the disease, causing huge loss to the piggery industry and small farmers, as well as the fact that piggery production has extended well beyond the North East Region, the dedicated control programme for control of CSF is being implemented as a national control programme to include all States / UTs for 100% effective coverage of the entire pig population.

c. The Assistance to States for Control of Animal Diseases (ASCAD) program provides financial and technical support to state governments for controlling major livestock diseases. It covers vaccination, surveillance, disease investigation, and strengthening of veterinary services. The program primarily targets diseases such as hemorrhagic septicemia, black quarter, classical swine fever, peste des petits ruminants (PPR), and Rabies, which have a high economic impact on farmers. ASCAD funds are utilized for disease diagnostics, procurement of vaccines, and capacity-building programs for veterinary staff. Additionally, ASCAD focuses on public awareness campaigns, educating farmers on biosecurity measures and the importance of vaccination. The program ensures that states are better equipped to prevent and control livestock diseases, thus reducing economic losses in the dairy and meat sectors.

2.3. National Digital Livestock Mission (NDLM)

A. Background

The National Digital Livestock Mission (NDLM) is a technology-driven initiative designed to create a comprehensive digital database for the livestock sector. It launched in FY 2014-15 and aims to provide real-time data on animal health, breeding, and movement, enabling better disease surveillance and livestock management. The NDLM includes a unique digital identification system for animals, which helps in tracking disease outbreaks, vaccination records, and productivity. Farmers can access digital records through mobile applications, improving their ability to manage livestock health efficiently. The mission also integrates data with veterinary services, ensuring that disease outbreaks are detected early and addressed promptly. Additionally, it provides a platform for farmers to access government schemes, insurance, and veterinary telemedicine services. NDLM is a major step toward data-driven decision-making in the animal husbandry sector, aligning with the broader Digital India initiative.

B. Goals of National Digital Livestock Mission (NDLM)

- To create a 'farmer-centric system' where modern information infrastructure and applications enable farmers to seamlessly access services and information through an intentionally designed national digital architecture.
- To build a mechanism for Direct Benefit Transfer (DBT) programmes.
- To enable improved participation of the private sector so that both the farmer and market can seamlessly access each other regardless of the location or size of the farmer's holdings, resulting in a connected livestock market for India.
- To create robust closed-loop breeding systems, disease surveillance/control programmes and traceability programmes for the livestock sector.
- To promote linkage between the R&D systems to the field so that best quality science improves the functioning of various national and state programmes in service of the farmers.
- To better alignment between various national and state programmes, and build an architecture that enables states to create and manage their own programmes.

NATIONAL PROGRAMMES UNDER DEPARTMENT OF ANIMAL HUSBANDRY AND DAIRYING, MINISTRY OF FISHERIES

CHAPTER VII

NDLM has taken into consideration the learnings from the various state and national programmes that have provided input from the field, not only in the animal husbandry sector, but also in other nation-scale programmes such as Aadhaar, National Digital Health Mission and innovations in the financial sectors in India. Extensive consultations with individuals and groups involved in these efforts as well as key stakeholders such as states, the private sector and subject matter experts have led to this first draft that articulates both the vision and the proposed approach. In addition to the discussions that have taken place as part of the NDLM design process, it builds on the learnings of other major efforts that have been launched over the last few years.

Further Reading:

1. National Animal Disease Control Programme https://www.dahd.gov.in/schemes/programmes/nadcp.
2. National Digital Livestock Mission, https://megahvt.gov.in/notification/National%20Digital%20Livestock%20Mission.pdf
3. Operational Guidelines for Livestock Health Control Programme. https://www.dahd.gov.in/sites/default/files/2024-11/2853-LHDC-10-08-2022-F-E.pdf
4. IEC Materials- https://ncdc.mohfw.gov.in/iec-material-on-zoonotic-disease/\

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RESEARCH PRIORITIES ON ZOONOTIC DISEASES OF PUBLIC HEALTH IMPORTANCE IN INDIA

CHAPTER VIII

1. Introduction

Zoonoses have been defined as infectious diseases that are naturally transmitted between animals and humans. Zoonotic diseases are caused by either bacterial, viral or parasitic pathogens and pose a significant threat in terms of their impact and burden on human and animal health and on economies and livelihoods. Zoonotic diseases are estimated to constitute 60% of all emerging infectious diseases (EIDs), of which more than 75% are reported to be associated with or originating from wildlife. Majority of EIDs and all known human pandemics in the past century [including influenza, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), human immunodeficiency virus (HIV) and Middle East respiratory syndrome (MERS)]have been linked to zoonotic spillover, frequently involving wildlife reservoirs.. Globally, it is estimated that zoonotic infections cause about 2.5 billion cases of human diseases and 2.7 million fatalities annually. It is reported that the pace of spread of EIDs has accelerated at an annual rate of 6.7% from 1980, with the number of outbreaks growing to several hundred every year since 2000. Emerging and re-emerging zoonotic diseases not only pose a critical threat to the health of humans and animals but also represent a significant burden on global economies. There are over 200 known types of zoonoses and they can be categorised based on various criteria, including the type of pathogen, mode of transmission, and animal reservoirs.Over the years, the incidence of zoonotic diseases worldwide has shown an overall increase, influenced by a range of factors including rising human population density, global population growth, expanded travel and trade, urbanization, climate change, and the degradation of natural animal habitats..

South East Asia has recently been described as one of the major hotspots for the future emergence of zoonoses. Within this region, India is considered a global hotspot for zoonotic emerging disease risk, owing to various factors such as high rates of deforestation, changes in land-use and agricultural practices, rapid human population growth and density, urbanization, rich biodiversity with significant spatial overlap between wildlife and human populations, dense livestock population, and challenges associated with the underperforming health systems. Identifying the drivers of zoonotic disease risk factors, and setting research priorities are essential for advancing understanding of disease aetiology , and for developing improved diagnostic tools as well as preventive and management strategies. According to the report published by World Bank, it is reported that the global economy in 2020 contracted by 4.4 % amounting to about US$3.6 trillion worth of lost goods, services, and other outputs from the impacts of the COVID-19 economic shutdown.

Along with the global trend of increasing zoonotic disease transmission and emergence, India has also witnessed a rise in such diseases over the last few decades. The unique combination of factors such as the large human population, biodiversity hotspots, and high density of tropical livestock, makes India vulnerable to the transmission of emerging and re-emerging zoonotic diseases. Over the years, several major zoonotic diseases of public health concern have been reported in the country, including Brucellosis, Cysticercosis, Crimean-Congo hemorrhagic fever, Echinococcosis, Japanese Encephalitis (JE), Kyasanur forest disease (KFD), Leptospirosis, Nipah, Plague, Rabies, Scrub typhus, Toxoplasmosis, Chandipura viral encephalitis, Lyme Disease, and Trypanosomiasis . A recent study by Kaviprawin et al. (2025), which analyzed zoonotic disease outbreaks notified under the Integrated Disease Surveillance Program (IDSP) in India between 2018 and 2023 to assess temporal trends and spatial variation, reported that of the 6,948 outbreaks recorded during this period, approximately 8.3% (583) were of zoonotic origin. As per national programme data for 2024, zoonotic diseases continued to impose a significant public health burden in India. Japanese

Encephalitis Virus (JEV) accounted for 1,472 cases and 105 deaths (NCVBDC), there were 3,717,336 dog bite cases with 54 suspected human rabies deaths (NCDC), and Seasonal Influenza A (H1N1) caused 20,414 cases with 347 deaths (NCDC), highlighting the diverse and persistent challenges of zoonotic disease control in the country. These zoonotic diseases have a multifaceted and wide-ranging impact on not only on public health, but also livestock, wildlife, food and nutrition security and the economic security.

2. Research priorities to address the gaps and challenges

There is a need to address the gaps and challenges across multiple facets of our understanding of zoonotic diseases and their management. Setting research priorities for zoonotic research in India requires a comprehensive approach that considers the country's specific challenges, ecological diversity, and public health needs. Some research priorities for zoonotic research in India include:

2.1 Disease surveillance and early warning systems

Disease surveillance and early warning systems are of paramount importance as a preventive and control tool for any infectious diseases especially zoonotic diseases where animal and environment systems are involved in the transmission chain. This includes creating mechanisms for jointly reporting and tracking outbreaks, as well as establishing early warning systems to detect and respond to potential outbreak of a known/unknown or new infectious condition. Some specific aspects that could be addressed in research on disease surveillance and early warning systems include, (a) enhancing surveillance networks: creating synergies between different surveillance networks for both human and animal health to enable timely and effective data sharing. (b) integration of data: integration of data from various sources, such as health facilities, veterinary clinics, wildlife monitoring programs, environmental monitoring stations combined with systematic analysis, can help towards early identification of potential spillovers and help establish links between environmental factors, animal health, and human health.. (c) adoption of emerging technologies: exploring the use of technologies like artificial intelligence, machine learning, and big data analytics can help in identifying disease patterns and detect potential outbreaks at an early stage. (d) sentinel surveillance: prioritizing sentinel surveillance can aid in providing a higher level of detailed and useful epidemiological data and risk factor information on diseases. This data can help enabling the identification of trends over time and timely responding to potential disease outbreaks. (e) risk mapping: conducting risk mapping exercises not only can help identify hotspots but also can aid in resource allocation for a focused and improved disease control and prevention efforts. (f) predictive modeling: developing predictive models using prior epidemiological data and environmental factors can assist in forecasting potential disease outbreaks.

2.2 Wildlife and livestock interaction studies:

Understanding the interactions between wildlife and livestock is essential for identifying potential sources of infection, assessing transmission routes, and thus implementing effective preventive measures. This research can also help identifying high-risk areas and potential spillover events. Some important aspects in this area include identifying reservoir hosts, understanding transmission dynamics, mapping high-risk zones, role of domestic animals as disease amplifiers, behavioral studies, cross-species pathogen sharing pattern and risk mitigation strategies. Prioritizing research on wildlife, livestock interactions and human behavior and practices in the context of zoonotic diseases is crucial for developing evidence-based strategies for disease prevention, surveillance, and control. It requires collaboration between experts in wildlife ecology, veterinary

sciences, social and public health, and other relevant fields to comprehensively address the complexities of zoonotic disease dynamics.

2.3 One Health Approach

One Health approach is regarded a pragmatic strategy for effectively addressing the challenges posed by endemic, emerging, and re-emerging zoonotic diseases.. It is a collaborative and multidisciplinary approach that recognizes the interconnectedness of human, animal, and environmental health and aims to understand and mitigate the complex factors that contribute to zoonotic disease emergence, transmission, and spread. The stakeholders in One Health include the ultimate beneficiaries (*i.e*. animals, people and the environment) and collaboration across sectors such as health professionals, public health agencies, veterinary services, environmental experts, agriculture and food industry, wildlife experts, Government agencies, research institutions, non-Governmental organizations,policy makers, international organizations and community and the public. However, identifying these stakeholders and establishing inter-sectoral collaboration between stakeholders is indeed challenging. Prioritizing research under the One Health framework for zoonotic diseases can yield numerous benefits such as (a). early detection of diseases: integrating data from human health, animal health, and environmental monitoring systems can help in early detection of zoonotic diseases and thereby timely implementation of response measures preventing outbreaks/limiting their impact. (b) understanding disease transmission dynamics: One Health research perspective allows for a comprehensive understanding of disease transmission between animals, humans, and the environment as is thereby essential for designing targeted interventions. (c). identifying reservoirs and amplifiers: One Health approach enables in the identification of reservoir hosts and amplifiers which is critical for devising effective control strategies. (d) assessing environmental determinants: environmental determinants, such as deforestation, climate change, and urbanization, can influence zoonotic disease dynamics. Research within the one health framework can help in assessment of the contribution of these factors towards disease emergence and spread. (e). cross-sectoral policy development: one health research approach provides a framework for the development of cross-sectoral policies and regulations to address zoonotic diseases. One Health approach is an indispensable research priority for zoonotic diseases as it emphasizes collaboration, data integration, and a holistic understanding of disease dynamics. It lays the foundation for evidence-based and comprehensive strategies to prevent, detect, and control zoonotic diseases, ultimately safeguarding human and animal health and promoting environmental sustainability.

2.4 Pathogen discovery and characterization for development of robust diagnostics

Since zoonotic diseases are caused by pathogens that can be transmitted between animals and humans, understanding the nature of these pathogens, their reservoirs, and transmission routes is crucial for effective disease prevention, diagnosis, and control. Prioritizing research in pathogen discovery and characterization such as identifying new and emerging pathogens, understanding the transmission dynamics, assessing host range and reservoirs, evaluating virulence factors can help towards gaining a deeper understanding of the agents responsible for these diseases and developing evidence-based strategies to prevent and control them effectively. The broad and non-specific clinical manifestations of zoonotic diseases often lead to misidentification or delayed diagnosis. Therefore, development of standard clinical algorithms can play a crucial role towards guiding clinicians and healthcare personnel through a systematic approach to accurately and timely diagnose

zoonotic diseases. Syndromic approaches, through the development of symptom-based algorithms, may also enable early detection and management of zoonotic diseases in the absence of immediate laboratory confirmation. Research aimed at enhancing diagnostic capabilities for zoonotic diseases can significantly strengthen preparedness and response to emerging threats. This includes investment in the research and development of innovative diagnostic technologies, strengthening laboratory infrastructure and expertise, and fostering collaboration between human and animal health sectors. Some of the important research areas which can be looked into includes (a) advancing diagnostic capabilities for identification of emerging pathogens (b) enhancing diagnostic tools for differentiation of clinically similar diseases (c) developing diagnostic capabilities for monitoring of antimicrobial resistance (d) developing reliable point-of-care testing (e) validating and improving the performance of new diagnostic technologies (f) implementing genomic surveillance using next-generation sequencing (NGS) for the detection of unknown pathogens

2.5 Antimicrobial resistance (AMR) in zoonotic pathogens

Emergence and spread of AMR in humans or animals poses a significant public health threat especially in the in low and middle-income countries. It is now recognized as an urgent concern across multiple sectors, including animal husbandry, agriculture and food production, fisheries and poultry. Prioritizing research in this area is essential to investigate the prevalence and mechanisms of antimicrobial resistance in zoonotic health conditions, devising targeted interventions to prevent the spread of resistant strains, advocating the responsible use of antibiotics thereby ensuring the availability of effective treatment options for zoonotic diseases. The priority areas requiring essential research in antimicrobial resistance include (a) assessment of treatment/drug efficacy studies, (b) monitoring the transmission of resistant strains, (c) understanding the reservoirs and routes of transmission of AMR, (d) impact on food Safety (e) AMR in environmental reservoirs (f) investigating alternate treatment options. This research is essential for safeguarding public health, promoting responsible antimicrobial use, and mitigating the impact of AMR on global health security.

2.6 Risk factors and behavioral studies

Zoonotic diseases are influenced by various human behaviors, interactions with animals, and environmental factors. Understanding these risk factors and behaviors is crucial and essential for designing effective prevention and control strategies. Analyzing the several social, cultural, and behavioral factors influencing the transmission of zoonotic diseases between animals and humans can help towards identifying/developing targeted interventions and awareness campaigns. Some of the areas that can be studied include the identification of high-risk activities, assessment of cultural practices, evaluationof livestock farming practices, impact of urbanization and deforestation, travel and migration patterns, as well as risk perception and preventive measures. .

2.7 Development of vaccines and therapeutics

Many zoonotic diseases lack specific and effective treatments unlike other infectious diseases and often present unique challenges due to their diverse pathogens and complex transmission patterns. Developing targeted therapeutics and investing in drug development is crucial to enhance preparedness for future outbreaks and ensure effective response to emerging zoonotic threats. The key priority towards newer drug development include identification of potential targets, drug screening, investigating combination therapies,

developing novel drug delivery systems, monitoring antimicrobial resistance and drug efficacy studies. Developing new/improved vaccines and implementing effective vaccination strategies for zoonotic diseases in both animals and humans plays a crucial role in disease prevention, reducing zoonotic transmission, cross-species protection reducing the likelihood of spillover and the control of disease outbreaks. Research priorities in the area of vaccines and vaccination strategies for zoonotic diseases include the development of innovative and versatile vaccines/vaccine technologies, optimizing vaccine schedules, evaluating vaccine safety and adverse effects for successful implementation of vaccination programs. In addition, understanding public attitudes and perceptions about vaccines is also essential. Furthermore, collaboration between researchers, healthcare professionals, veterinarians, and policymakers is also crucial for advancing research related and ensuring effective vaccine development and implementation.

2.8 Capacity Building

Capacity building is a critical gap in the effective response to zoonotic diseases, particularly in countries like India. Prioritizing research in capacity building can have several significant impacts such as focusing on improving disease surveillance and reporting systems, identifying gaps in knowledge and training needs among healthcare professionals and researchers, developing expertise in field epidemiology and outbreak responses, establishing and strengthening laboratory networks, improving risk communication and public awareness campaigns, antimicrobial stewardship for addressing the responsible use of antimicrobials and developing evidence-based policies and implementation. Prioritizing research and investing in capacity building can help in fostering long-term improvements in zoonotic disease management and contribute to a safer and healthier society. Developing comprehensive training modules for clinicians and laboratory staff is indeed critical in imparting essential knowledge and skills, providing regular, up-to-date, and tailored training modules will enhance healthcare workers' preparedness and response capabilities towards handling zoonotic diseases effectively and safely.

2.9 Public awareness and education

Research to develop and identify the best model to enhance public awareness and education for zoonotic diseases can go a long way to help empowering communities for proactive measures in timely prevention and management zoonotic infections. Research in this area requires collaboration with public health experts, educators, social scientists, and community leaders for developing evidence-based strategies which can resonate to the target audiences. Research priority includes (a) research in knowledge and understanding can help in identifying gaps and misconceptions, perception of risk, cultural relevance influencing zoonotic disease transmission and prevention, Identifying high risk groups (b) research to explore the factors influencing behavioral changes in response to zoonotic disease awareness campaigns (c) development of innovative and effective communication channels/mediums (e) measuring impact of public awareness and education initiatives /interventions (f) developing strategies for outreaching the vulnerable populations and reporting of illness

The Indian Council of Medical Research has adopted a research prioritization strategy based on the ‘4Ds’ framework, which encompasses Discovery, Development, Descriptive, and Delivery research. This approach aims to guide and focus research efforts towards addressing key health challenges and optimizing the impact of research outcomes. By adopting the 4Ds framework, ICMR aims to streamline its research efforts to address

zoonotic diseases comprehensively. This approach recognizes the importance of integrating different aspects of research, from fundamental discoveries to practical implementation. It aligns with a holistic and evidence-based approach to tackling zoonotic diseases, which require multidisciplinary efforts and collaboration between various stakeholders, including researchers, policymakers, healthcare professionals, and the public. By prioritizing research based on the 4Ds framework, ICMR aims to encourage researchers across the country to make significant strides in zoonotic disease prevention, control, and management in India.

3. Conclusion

Addressing research priorities on zoonotic diseases in India is crucial to safeguard both the health of its vast population and the integrity of its diverse ecosystems. Given the country's significant reliance on agriculture, close human-animal interactions, and extensive biodiversity, it is necessary that comprehensive investigations are made into emergence, transmission, and risk factors/etiologies of zoonotic diseases. Prioritizing research in this field, can help in enhancing the understanding of zoonotic disease dynamics, developing effective surveillance and early warning systems, and implementing proactive measures to mitigate potential outbreaks. Additionally, promoting interdisciplinary collaboration among scientists, veterinarians, ecologists, public health experts and policymakers is vital towards fostering a holistic One Health approach. Ultimately, prioritizing and investing in research on zoonotic diseases will strengthen s the country's preparedness and response mechanisms to respond rapidly and effectively to emerging threats, thereby minimizing the impact of zoonotic diseases on human health, animal health, agriculture, and biodiversity within its borders and beyond.

Further Reading

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PART- II

ANTHRAX
CHAPTER 1

1. Introduction

'Anthrax' originated from a Greek word 'anthrakis' meaning 'coal' (due to 'black coal' like skin lesions on affected humans) is also synonymously known around the world as 'Charbon', 'Wool sorters disease', 'Malignant carbuncle', 'Malignant pustule' 'Rag pickers disease', as well as various regional linguistic nomenclatures across India. Anthrax, an acute and potentially fatal bacterial disease, is primarily known to affect herbivores, domestic and wild animals, which is transmissible to humans. The disease has been one of the foremost causes of uncontrolled mortality in herbivores (cattle, buffalo, sheep, goats) in India despite the availability of effective vaccine along with efficient antibiotics. India, being a home to the largest livestock population in the world with high intensification of livestock farms along with urbanization across various zones, could pose a greater risk for zoonotic diseases transmission to humans.

In addition, bacillus spores could potentially act as source of biological warfare and bioterrorism, and hence get exceptional global attention and represent a serious public health concern.

2. Epidemiology

2.1 Causative Agent

Bacillus anthracis, a Gram-positive, rod-shaped, 3-5 µm X 1 µm, non-motile, endospore forming bacterium, is the only obligate pathogen within the genus *Bacillus*. The genome of *B. anthracis*, comprises of a single circular chromosome and two circular virulence plasmids, namely pXO1(182 kb) and pXO2 (96 kb). The pXO1 plasmid genes mainly encode for exotoxins which include *pagA* [protective antigen (PA, 83 kDa)], l*ef* [lethal factor (LF, 87 kDa)] and *cyaA* [Oedema Factor (EF, 89 kDa)]. The pXO2 plasmid encodes capsule biosynthesis genes (capBCADE) which are found in a cluster and contribute to capsule formation.

The capsule and the toxin complex are considered as major virulence factors of *B. anthracis*. The capsule (poly-D-glutamic acid) is known to protect the bacterium from phagocytosis. LF and EF in combination with PA act as a lethal toxin and oedema toxin, respectively, which are responsible for the characteristic signs and symptoms of anthrax.

Spores are reckoned as resting form of vegetative cells shed from infected herbivore animals. Spores are highly resistant to biological extremes like temperatures (heat, cold), pH, desiccation, chemicals, irradiation and other such adverse conditions, leading to persistence for long periods in the environment and acting as a source of infection for susceptible animals. The spores germinate to produce vegetative forms within the infected host

2.2 Mode of Transmission

The transmission of anthrax in animals is cyclical in enzootic places. Subsequent to the death of an anthrax infected animal, the growth and multiplication of the vegetative form of the bacteria ceases due to exposure to free oxygen leading to spore formation. The spores remain viable in the contaminated soil, skin, hair, hides, wool, bones etc of infected dead animal for several years as these are resistant to adverse environmental conditions. Thus, the environmental cycle is maintained resulting in regular outbreaks in enzootic regions. The spores in soil/vegetation can act as a source of infection to other animals. Once the spores are harboured by the host, they are converted into vegetative form and hence cause the disease.

The humans get infection by coming into contact with anthrax infected animal either by inhalation, ingestion or cutaneous routes. Cutaneous anthrax occurs when spores enter

the skin, usually through a cut or scrape. Pulmonary anthrax results from the inhalation of spores in particles less than 5 µm in diameter that may reach the terminal alveoli of the lungs. Aerosols of such particles may be created by the agitation of the hair or wool in the industry settings. Accidental infection may occur among laboratory workers. The possibility of direct person to person transmission of anthrax is extremely rare.

Intestinal and oropharyngeal anthrax results from ingestion of contaminated meat. There is no evidence that milk from infected animal transmits anthrax.

The disease spreads among omnivores and carnivores through contaminated meat, bonemeal and other feeds and among wild life from feeding on anthrax carcasses. Vultures have been reported to spread the organism from one area to another.

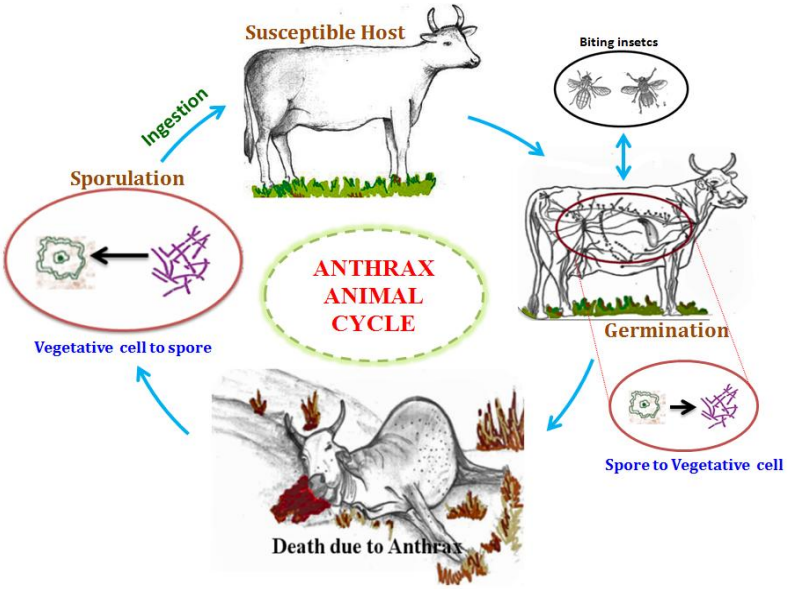


Figure 1: Epidemiological cycle of anthrax in endemic areas.

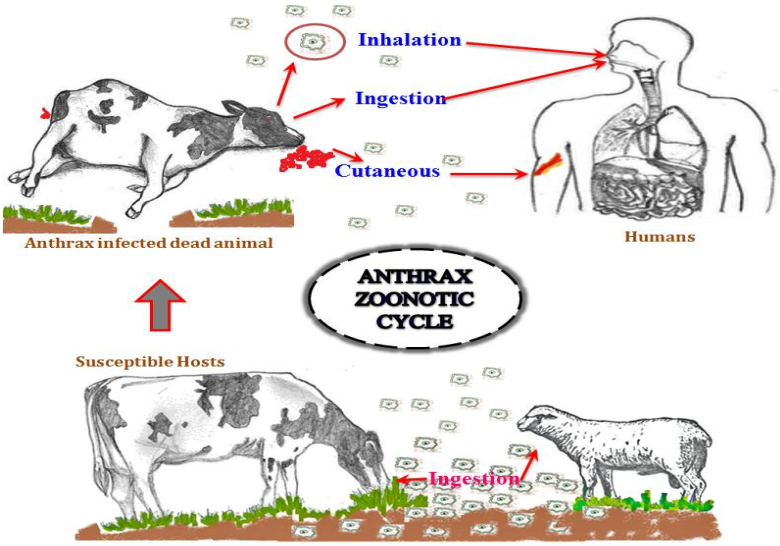


Figure 2: Different modes of transmission of Anthrax in humans.

2.3 Current Situation

Anthrax is highly endemic in states of Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Kerala, Odisha, Jharkhand, Chhattisgarh and West Bengal. The disease has also been occasionally reported from Madhya Pradesh, Gujarat, Rajasthan, Punjab and Bihar. Anthrax is enzootic in India but is less frequent to absent in Northern India. The reported human anthrax outbreaks in India have been predominantly from four states namely, Odisha, Andhra Pradesh, West Bengal and Jharkhand.

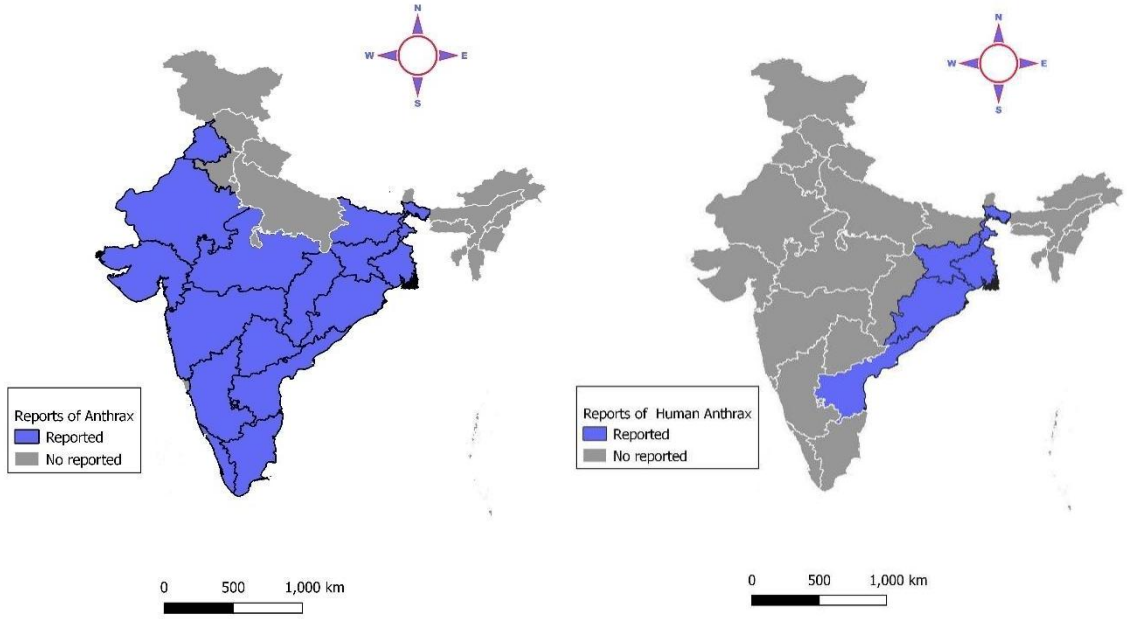


Figure 3: Anthrax in animals (left map) and humans (right map) in India.

3. Clinical Features

The clinical manifestations can be toxin-dependent and/or toxin independent in different host (animal and humans) species which is known to vary greatly with different forms.

Humans

➢ On the basis of exposure to spores, human anthrax infections are divided into two types:

a) Non-industrial anthrax [Occupational anthrax]: It affects veterinarians, farmers, butchers and animal handlers. Infection occurs following accidental exposure to aerosol or contaminated materials. It usually manifests as the cutaneous form of anthrax.

b) Industrial anthrax: It affects in individuals who are employed in the industrial processing of bones, hides, wool and other animal products. The contaminated animal products act as a source of infection.

➢ On the basis of the route of infection, human anthrax infections are divided clinically in to three types:

a) Cutaneous anthrax: It is a non-invasive form acquired through skin following an insect bite or a small cut, abrasion or other lesion (bite, ulcer etc.,) following contact with spore contaminated animal products. Lesions are seen on exposed regions of the body, mostly on the face, neck, hands and wrists. Skin lesions often start as a small pimple or papule appearing in a 1-2 days followed by a ring of vesicles developing around the papule by 3 to 4 days and then, vesicular fluid may be exuded. By 5-7 days, the original papule ulcerates to form the characteristic ‘eschar’.

Clinical symptoms may be more severe if the lesion is located in the face, neck or chest. Although, the 'eschar' begins to resolve by 10th day, complete resolution takes almost six weeks. If untreated, a small proportion of cases develop systemic anthrax and finally lead to high fever, toxaemia, oedema; shock and death.

Cutaneous form is most common and account for 95% or more of human cases. Fatality rate ranges from 10-20% in untreated humans in comparison to other two lethal forms (inhalation/ gastro-intestinal). If timely treated by antibiotics it does not progress to lethal forms.

b) Gastro-intestinal tract anthrax: It is contracted through ingestion of either insufficiently cooked contaminated food, especially meat of an infected/dead animal, or contaminated water. It occurs in two clinical forms.

- Intestinal anthrax: Symptoms include nausea, vomiting, fever, severe abdominal pain, haematemesis, bloody diarrhoea and massive ascites. Unless treatment commences early enough, toxaemia and shock develop followed by death.
- Oropharyngeal anthrax: Clinical features are sore throat, dysphagia, fever, regional lymphadenopathy in the neck and toxaemia. The mortality is about 50% if untreated. Since, it is difficult to diagnose at early stage and death usually occurs 2-5 days following symptoms in affected individual.

c) Inhalation (Pulmonary) anthrax: It occurs following inhalation of airborne *B. anthracis* spores or spore laden dust. The chances of inhaled spores inducing anthrax depends greatly on the size of the particles (1 to 5 micron) which lead to respiratory infection. Symptoms are nonspecific and flu-like with mild upper respiratory tract involvement at initial stage. Generally, illnesses begin insidiously with mild fever, fatigue and malaise lasting up to several days. The mild initial phase is followed by sudden development of dyspnoea, cyanosis, disorientation with coma and death within 24 hr following the onset of hyperacute phase. About 90 % of untreated cases result in death of a person.

Animals

It greatly varies with the animal host species affected and its immune status. In most cases, the incubation period ranges from 36 to 72 hours. Upon the entry of spore/bacteria, cells grows rapidly in the blood depending on the host species. Clinical features of hyperacute (Disease progression is rapid, and often animals are found dead without premonitory clinical signs) or acute symptoms usually with a fatal outcome are observed.

The first signs of an anthrax outbreak in animals are one or more 'sudden deaths' in the affected livestock population with oozing of unclotted tarry colored blood from natural orifices. The action of the toxin complex on the endothelial cell lining of the blood vessels results in their breakdown, internal bleeding and the characteristic 'bleeding to the exterior' from natural orifices.

Before death, signs of distress, off feed, disorientation, difficulty in breathing, reduced milk production, subcutaneous haemorrhage, swellings in the submandibular fossa, with/without rise in body temperature are noticed in acute presentations. Animal dies with massive septicaemia, toxaemia, coma and shock. In wild animals, local oedemas and swelling of the face and neck or of lymph nodes, particularly mandibular and pharyngeal and/or mesenteric are noticed. Carnivores are resistant, however, they can get infected following ingestion of contaminated meat. Some transient symptoms such as fever, restlessness, dyspnoea or agitation may be apparent

4. Laboratory Diagnosis

Laboratory diagnosis for anthrax should be attempted only by laboratory well trained to do so. High index of suspicion of the disease is important. Collection and transportation should be carried out under strict aseptic condition. Diagnosis of anthrax from suspected clinical samples of humans and either dead/live animals (domestic/wild) generally follow the standard microbiological methods such as staining and subsequent attempt for isolation of causative agent and confirmation by molecular assays. BSL-3 laboratories are required while handling suspected anthrax samples for activities with high potential for droplet or aerosol production from *B. anthracis*.

4.1 Collection of Specimen

Humans

a) Cutaneous Anthrax

Swabs from the lesion- Collect two swabs as follows:

- In early stage, vesicular exudate from the lesions by sterile swab can be collected
- In later stage, material to be taken from underneath of eschar after lifting up of eschar with sterile forcep.

The swab should be put in Carry-Blair transport medium and with another swab, smear on microscopic slide may be prepared and heat fixed.

b) Intestinal Anthrax

- If patient is not severely ill, a faecal specimen can be collected.
- If patient is severely ill ascitic fluid (peritoneal fluid) can be collected.

c) Pulmonary Anthrax

- If patient is not severely ill, sputum can be collected.
- If patient is severely ill, bronchial lavage can be collected.

Animals

Anthrax should be considered as possible cause of death in herbivore that has died suddenly with haemorrhages from nose, mouth or anal orifice. As a standard approach post-mortem examination of dead animals should be avoided. Samples to be collected are as follows in exceptional circumstances

a) Caracass 1 to 2 days old

- Unclotted blood from nasal, buccal or anal orifice may be collected.
- If autopsy done; body fluid, spleen and/or unclotted blood may be collected.
- It is advisable to take a peripheral blood sample/oozed unclotted blood from natural orifices of dead animals for staining of smears and bacterial isolation by culture.

b) Old putrefying carcasses

- Any blood-stained material may be collected.
- If autopsy done, then body fluid, spleen may be collected.
- If buried soil or other material from burial site may be collected.

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the guidelines for handling specimens from suspected, probable, or confirmed cases/contacts of high-threat pathogens.

4.3 Laboratory Procedures

a) Staining: Grams's staining method is primarily used to identify purplish colored long rod-shaped Gram-positive bacilli having a 'boxcar (square ended)' appearance in the smears for presumptive diagnosis. Additionally, polychrome methylene blue (Mc'Fadyean reaction) staining is used for demonstration of capsulated bacillus used wherein capsule stains pink and the bacillus cells stain dark blue. Further, malachite green stain is used for demonstration of endospore in the bacilli especially for aged cultures or vegetative cells under sporulation process.
b) Bacterial culture: The bacterium readily grows on nutrient agar. However, horse or sheep blood (5-7%) agar is the medium of choice. For pure bacterial isolation, sheep/horse blood (5-7%) agar is commonly used for processing of clinical materials. The bacterial colonies on blood agar plate appear grey-white to grey, non-haemolytic, 0.3-0.5 mm in diameter with a ground glass moist surface, and appear very tacky upon teasing with a loop. The colonies are characteristically described as a 'medusa head' or 'curled hair' as they grow with prominent wisps trailing back toward the parent colony, which is indicative of *B. anthracis*. For environmental samples and tissues heavily contaminated with invading bacteria, selective medium such as PLET (Polymyxin B-Lysozyme-EDTA-Thallous acetate Agar) has been recommended. Further, motility test can also be performed to observe non-motile *B. anthracis*.
c) In the event of difficulty in isolation of causative bacteria by growth media, laboratory animals such as mice or guinea pigs could be inoculated either subcutaneously or intramuscularly with broth cultures of suspected clinical materials and re-isolation of bacteria from blood of dead mice. It also ascertains the pathogenicity of bacteria in animal model, however rarely employed technique nowadays. Additionally, the susceptibility of *B. anthracis* organisms to Gamma bacteriophage could be performed on agar plate in like disc diffusion method wherein zone of inhibition/lysis is observed. Similarly, Penicillin antibiotic susceptibility test could also be employed for confirmatory diagnosis of suspected bacterial culture.
d) Serological assays: Indirect-ELISAs are employed for detecting exotoxins (PA, LF, EF) specific antibodies in serum of affected animal's serum samples. These assays are useful in screening of carrier/latent/ subclinical infection in animals. These assays could be useful in identifying of carnivores/omnivores domestic as well as wild animals for routine sero-surveillance for identifying anthrax cases in surrounding areas as well as indirectly understanding the prevalence/hotspots of anthrax in endemic regions.
e) Molecular Assays: Polymerase chain reaction (PCR) assays (uniplex/multiplex) along with chromosomal marker as internal control have been extensively used for rapid and highly specific identification of *B. anthracis* from suspected clinical specimen by amplifying toxin genes such as pX01 (pag gene for PA antigen) and pX02 (cap gene for capsule antigen) plasmids. Additionally, a number of nucleic-acid based typing tools such as multiple-locus variable-number tandem-repeat analysis (MLVA) or VNTR, pagA gene sequencing and/or whole genome sequencing have been effectively used to determine the genetic diversity of circulating *B. anthracis* in epidemiological studies.

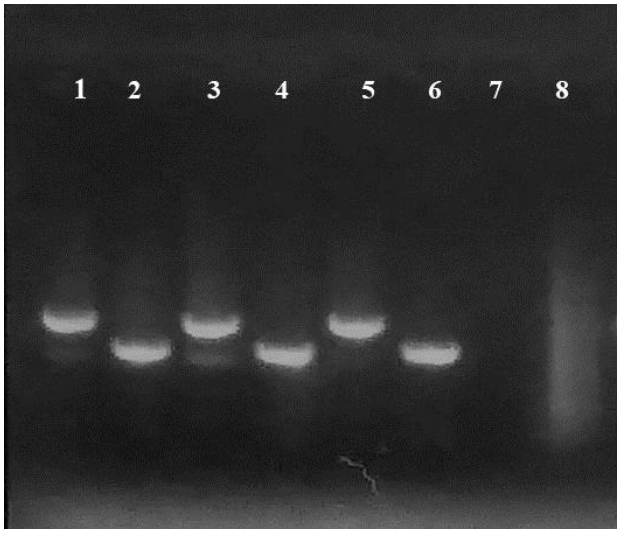


Figure 4: Lanes:1,3,5 - Cap gene (846 bp), Lanes:2,4,6- PA gene (639 bp), Lane:7- Negative control, Lane:8- 1Kb DNA Ladder.

5. Treatment

Antibiotics are effective if the disease is recognized early and the full recommended dose and course of the antibiotic is completed. If left untreated or if the antibiotic treatment is discontinued early, the disease can be fatal. General measures for treatment of shock are also necessary. Penicillin is the drug of choice. In severely affected patients or when pulmonary or gastrointestinal anthrax is suspected, Penicillin G, 2 million units per day by infusion or by slow intravenous injection should be administered until the temperature returns to normal. After that, treatment should be continued in the form of intramuscular procaine penicillin, 1 million units every 12-24 hours. Streptomycin, 1-2 grams per day intramuscularly, may act synergically with penicillin.

In case of allergy to penicillin, erythromycin, tetracycline, chloramphenicol, ciprofloxacin or gentamycin can be used. Oral antibiotics such as penicillin V (500 mg 6 hourly) or procaine penicillin (1 million units 12- 24 hourly by intramuscular route) can be prescribed for mild forms of cutaneous anthrax. Animal suspected for ingestion of large volume of spore, the antibiotic therapy should be continued for 10 or more days.

6. Prevention and Control

An ideal and best way to prevent anthrax is by regular vaccination of susceptible herbivores animals in endemic areas. A capsular live spore vaccine (Sterne strain) is being used to vaccinate all herbivores animals for the prevention of anthrax outbreaks in most parts of India. However, the anthrax vaccine is not commonly recommended for human vaccination for the general public. In the recent past, several advanced vaccine approaches intended for use in humans and animals have been attempted which are at various stages of development. A standard biosafety and biosecurity measures should be employed to control the disease and prevent further spread to other susceptible animals and humans.

a. Disposal of sick/infected animals: A quarantine of suspected live infected animals followed by antibiotic therapy is recommended. All confirmed and sudden dead animal carcasses and contaminated materials need to be disposed either by burning or deep burial. Decontamination of premises/farm, and disposal of all the contaminated feed stuffs should be followed. As flies are considered to be potential agents in the mechanical transmission of anthrax, fly control in animal farms need to be considered.

b. Biosafety and Biosecurity measures: Appropriate biosafety measures and containment measure should be employed. Any known or suspected areas of contamination should be disinfected. Freshly prepared 10% sodium hydroxide, 5% formaldehyde, and 2% glutaraldehyde are among the better disinfectants for *B. anthracis*. Personal protective equipment (PPE) with or without respirator needs to be worn by veterinarian/laboratory technicians as well as animal handler's during disease outbreak investigations and handling of clinical materials. In integrated livestock farming, strict biosecurity measures need to be implemented along with advanced animal husbandry practices in order to reduce the risk of human anthrax cases. Further, farmers/public and healthy animal's access to contaminated area/materials should be restricted.

c. One Health approach: There is a need to strengthen the collaborative integrated disease (active) surveillance, laboratory diagnosis, and monitoring methodologies/outbreak investigation between allied health departments by involving veterinary and medical professionals to reduce the cases of anthrax in animals as well as humans. Further, a stringent law and its implementation are required for preventing the use of contaminated herbivores animal meat/byproducts in animal feed and environmental contamination by the effluent of tanneries or wool processing factories in the vicinity of animal farms. Additionally, awareness programmes for the general public/farmers need to be implemented. Cutaneous anthrax, the most common form, could be greatly reduced in the high-risk occupations by appropriate clothing and gloves, dressing wounds and other hygienic practices. The gastrointestinal form could be avoided by not consuming the infected meat/meat products.

   A resource-poor farming system need to be upgraded with improved management of the various livestock species in backyards and development of appropriate package of practices for multi-species farms. Livestock farmers, animal handlers, meat shop owners as well as general public need to be made aware about disease and consequences of handling/consuming dead meat/mutton.

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1. Introduction

Chandipura virus (CHPV) is an arbovirus of emerging importance in India that causes acute encephalitis, associated with high case fatality rate, particularly in the pediatric age group. The virus has caused large scale outbreaks in India, especially in Central and Western Indian states. The virus transmitted by sandflies causes a rapidly progressing, acute encephalitis/encephalopathy, leading to death within 48-72 hours of hospitalization. Genomic analysis has shown that CHPV is more closely related to other old world vesiculo viruses including the Isfahan virus and Piry virus, than to New World vesiculo viruses.

2. Epidemiology

2.1 Causative Agent

CHPV belongs to genus Vesiculovirus, family Rhabdoviridae, and order Mononegavirales. The virus has a typical bullet shaped morphology, 150–165 nm long and 50–65 nm wide, as determined by transmission electron microscopy. It is an enveloped virus with a helical ribonucleoparticle (RNP) surrounded by an outer bilayer lipid membrane. The viral genome is a linear, negative sense, single stranded, non-segmented RNA of about 11kb, that comprised of a leader sequence (l) followed by five transcriptional units coding for viral polypeptides- the glycoprotein (G), matrix protein (M), nucleoprotein (N), phosphoprotein (P), large polymerase protein (L) and a trailer sequence (t), arranged in the order 3' l-N-P-M-G-L-t 5'.

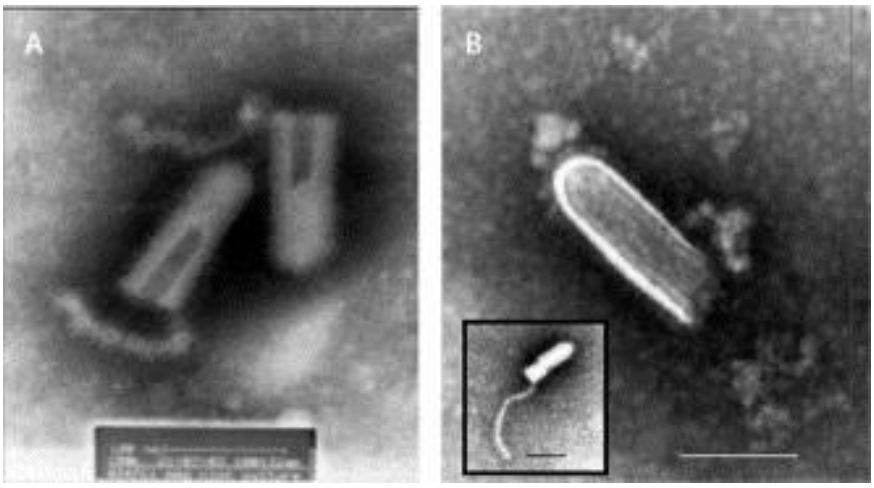


Figure 1: Two negatively CHPV particles showing stain filled canals and basal attachments (A) and negatively stained CHPV particle showing a typical vesiculovirus morphology. Reprinted from Rao et al. (2004).

Glycoprotein (G) is a trimeric transmembrane protein that enables receptor recognition and membrane fusion. Being the sole spike protein, G acts as the major antigenic determinant and elicits neutralizing antibody response. G in coordination with the M protein, plays a major role in virus assembly and budding. M also suppresses the host interferon response and contributes to viral pathogenicity. The L protein of vesiculoviruses has RNA-dependent RNA polymerase (RdRp) activity necessary for transcription and replication, capping and methylation of the 5' ends of transcripts. Entry of the RdRp into the template is modulated by the leader sequence (l). Following transcription, accumulation of nucleocapsid (N) protein induces a switch in the function of RdRp, from transcription to

replication. Replication requires interactions between L and P proteins where P serves as a co-factor during the RdRp activity of L. The template of replication in vesiculoviruses is the ribonucleoprotein complex (RNP), wherein genomic RNA is encapsidated by the N. Nucleation signals from the leader sequence lead to unwinding of RNP and initiation of viral replication. The RNP of CHPV is by itself infectious.

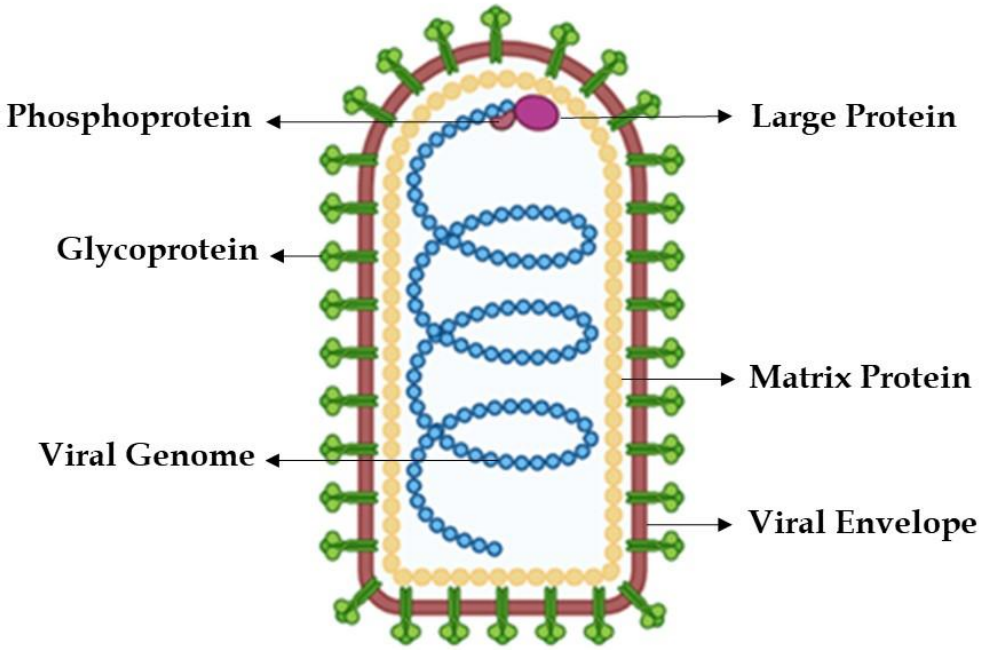


Figure 2: Structure of Chandipura virus.

CHPV was first time identified during an outbreak of acute febrile illness among residents of Chandipur village of Maharashtra in 1965. Though the virus was associated with neurological complications, it garnered significant attention only after a large outbreak of acute viral encephalitis associated with high mortality rates occurred in Andhra Pradesh. Since then, multiple outbreaks have been reported from Gujarat, Maharashtra, Telangana, and Andhra Pradesh. Retrospective analysis of archived human samples (collected during 1955-67) at National Institute of Virology, Pune, India, has shown a wide distribution of CHPV, with the prevalence of neutralising antibodies (NAbs) ranging from 6% in Kerala to 89% in Uttar Pradesh. NAbs have also been detected from apparently normal children in Andhra Pradesh (70%), Maharashtra (64%), and Gujarat (65%). NAbs have also been demonstrated in rhesus monkeys, cows, goats, sheep, buffaloes, dogs, camels, pigs, and other domestic animals in India as well as wild macaques in Sri Lanka. CHPV endemicity have been confined to Central and Western India, although occasional cases have been reported from Bihar, Madhya Pradesh, Odisha, and Chhattisgarh. Detection of the virus has also been reported from Nigeria, Senegal, Nepal, and Bhutan.

2.2 Mode of Transmission

Phlebotomus and *Sergentomyia* genus of sandflies are incriminated as the natural transmission arthropod vector of CHPV. Though several species of mosquitoes (esp *Aedes*) have been shown to be able to transmit and replicate the virus under experimental conditions, their role in the natural cycle of CHPV is still undetermined.

For further details on vector, please refer to chapter “Arthropod Vectors of Zoonotic Importance”.

CHPV can remain dormant for prolonged periods and cause explosive outbreaks when environmental factors allow due to its maintenance in Phlebotomus sand-flies. This, coupled with the lack of virus isolations from domestic animals, necessitates investigations into the possible role of peri-domestic or wild animals as potential reservoir hosts

CHPV infects and replicates in multiple cell lines, including cancer cell lines. This pantropism of the virus may be due to interactions between viral glycoprotein (G) and LDL receptors in cells. A short incubation period coupled with significant viremia following infection suggests a haematogenous route for the spread of virus to the CNS. This can either be through a Trojan horse mechanism involving the B cells or by infection of brain microvascular endothelial cells and disruption of the blood-brain barrier.

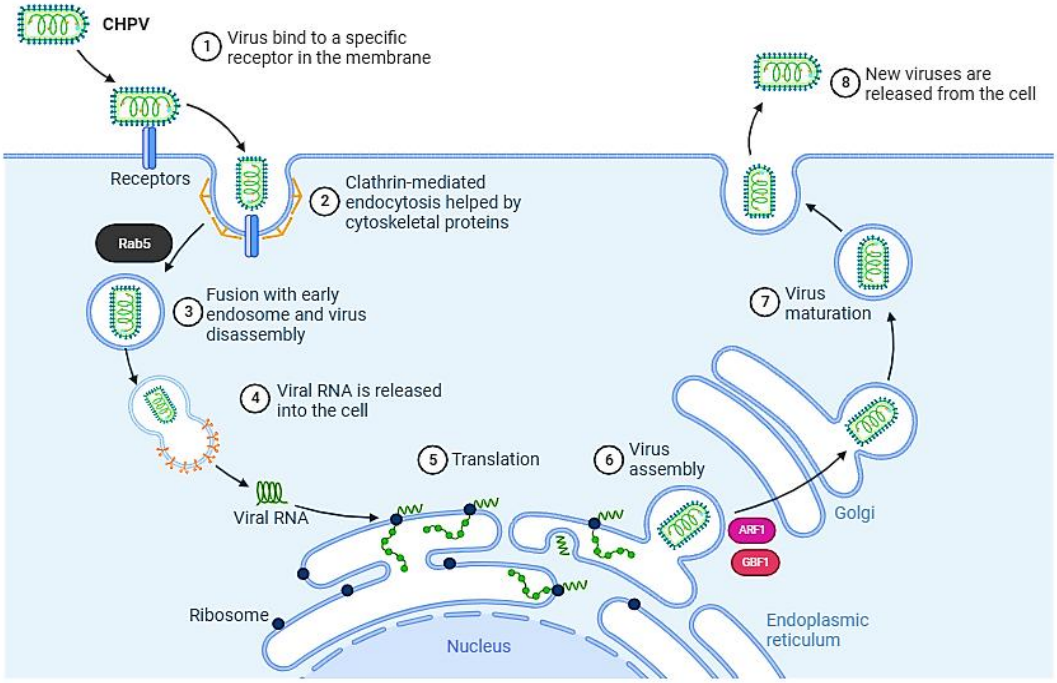


Figure 3: Events in the cell entry and replication of Chandipura virus.

2.3 Current Situation

Currently, CHPV is endemic in Central and Western India, with sporadic cases reported from parts of Gujarat and Maharashtra. The outbreaks of Chandipura encephalitis have occured after every 4-5 years in India. However, a large-scale vector surveillance has failed to detect the virus.

Recently between early June and 15 August 2024, the Ministry of Health and Family Welfare of the Government of India reported 245 cases of acute encephalitis syndrome (AES) including 82 deaths (CFR 33%). Of these, 64 are confirmed cases of Chandipura virus (CHPV) infection. This outbreak was the largest in the past 20 years.

3. Clinical Features

Humans

Chandipura infection with the virus may remain asymptomatic or lead to fever with/without encephalitis as suggested by sporadic reports. The disease typically affects children, especially those below the age of 15 years. Clinically, the disease is characterized by sudden onset of low grade fever (duration of fever less than 3 days), headache, vomiting, diarrhoea, and mild influenza like illness with cough, which then proceeds to involve the

CNS, causing altered sensorium and convulsions followed by motor weakness, spells of unconsciousness before becoming comatose and/or death. Case fatality rate of the disease ranges from 45-70%, with death occurring within 24-48 hours of hospitalization.

Rarely, the disease has also been found to affect adults. Atypical presentations including cranial nerve palsies, vesicular eruptions which heal with hyperpigmentation, and bleeding manifestations have also been reported. The patients may also develop disseminated intravascular coagulation (DIC) or shock.

Clinically tachycardia, tachypnoea or dyspnoea, irregular breathing patterns, and crepitations on auscultation are observed. Nervous system examination may show hypo or hypertonia, decreased power, sluggish pupillary reflexes, papilledema, absent or sluggish deep tendon reflexes, abnormal plantar reflexes, and decerebrate posturing among others. Elevated levels of IL-2, IL-6, TNF-α, and IFN-γ, mild elevations in liver enzyme levels, leucocytosis, and elevated blood urea levels may be detected. Brain imaging shows diffuse swelling of brain parenchyma with dilated ventricles. In patients with neurological symptoms, the average duration of hospital stay has been estimated to be 2-21 days (average 2-9 days). Complete recovery is seen among survivors, with no neurological sequelae.

Animals

Naturally occurring CHPV infections in animals have not yet been reported. Experimental inoculation of mice shows age related susceptibility, with adult mice showing survival from infection. Symptoms in infected mice include ruffled fur, hunched posture, rapid running movements, followed by convulsions, paralysis of front or hind limbs, and death.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood/serum (Paired samples)- one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.

CSF-Acute phase (Disease with CNS manifestations)

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedures

a) Isolation of virus: Virus isolation can be done by inoculation of clinical sample in mice and tissue culture (BS-C-1) and further detection is performed using neutrilization test.

b) Serological diagnosis: A highly sensitive and specific IgM capture ELISA has been developed and validated by research laboratories. Although plaque reduction neutralization test (PRNT) is considered to be the gold standard test to detect neutralizing antibodies, although it is labour and time intensive. Further, a micro-neutralisation ELISA has been developed recently for sero-surveillance.

Though other tests like Hemagglutination assay, complement fixation test, and immunofluorescence assays have been described, these are currently not used due to less sensitivity and specificity.

c) Molecular tests: Conventional RT-PCR assays targeting the P, N, and G genes and real time RT PCR assays targeting the P gene of CHPV have been developed and validated, with both assays showing 100% specificity.

5. Treatment

No specific antiviral drugs are currently approved for the treatment of CHPV infections. Hence, management of CHPV encephalitis hinges on supportive care i.e. anti-epileptic drugs, broad-spectrum antibiotics, acyclovir, empirical anti-malarials and/or anti-rickettsials, and anti-edema measures.

Antivirals with broad spectrum activity- Favipiravir (RdRp inhibitor) and Ribavirin (guanosine analogue) have been shown to inhibit growth and replication of CHPV, using in-vitro and in-vivo systems. MicroRNA-155 and nitrosporeusines have been shown to have anti-CHPV activity.

6. Prevention and Control

As of now, there are no approved vaccines against CHPV. Vaccine candidates making use of an inactivated virus and the recombinant glycoprotein of the virus, have been developed and have shown promising results in animal models, but human trials are pending.

Control of the disease currently therefore hinges on control of the vectors. Sandfly control can be achieved with the use of repellents and insecticides. This needs precise mapping of sand fly habitats and breeding areas with continuous spraying and baseline data regarding insecticide resistance. Newer approaches include the use of pheromone bait traps and plants which provide noxious stimuli to sand flies.

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1. Introduction

Chikungunya fever is a re-emerging viral disease caused by chikungunya virus (CHIKV). The disease is almost always self-limiting and is rarely fatal. Molecular characterization has demonstrated 2 distinct strain lineages that cause epidemics in Africa and Asia. These geographical genotypes exhibit differences in transmission cycles: a sylvatic cycle in Africa is maintained between monkeys and wild mosquitoes while in Asia the cycle exists between humans and *Aedes aegypti* mosquitoes. Chikungunya virus was first isolated from the serum of febrile human by RW Ross in Liteho, Newala District Tanzania in WN Mice in 1953. The notes recorded in the Indian Medical Gazette on eruptive fever descriptions, to which attention of medical practitioners in India has been drawn, dating back to the 18th century which closely resemble the present-day clinical manifestation of Chikungunya.

2. Epidemiology

2.1 Causative Agent

Chikungunya virus is an RNA virus belonging to the family Togaviridae and genus Alphavirus. Chikungunya is a positive-sense, single-stranded RNA virus (~11.8 kb genome) that has gained global focus due to its inclusion in Coalition for Epidemic Preparedness Innovations (CEPI's) priority pathogens for vaccine development. The virus can be classified into four genotypes based on genomic diversity: West African (WA), East/Central/South African (ECSA), Asian and Indian Ocean lineage (IOL). The Asian and ECSA genotypes are the most prevalent globally. The Asian lineage and the IOL sublineage are the most important clades in terms of public health impact.

2.2 Mode of Transmission

Chikungunya virus is most commonly transmitted to humans through the bite of infected *Aedes* mosquitoes, although vertical transmission is also possible. High viremia is typical during the first 2 days of illness, declines at days 3 and 4, and usually disappears by day 5. "Silent" CHIKV infections may occur in children. Chikungunya virus infection, clinically evident or silent, is thought to confer lifelong immunity.

For further details on vector, please refer to chapter "Arthropod Vectors of Zoonotic Importance".

2.3 Current Situation

In 2005, an outbreak occurred on the French islands of La Reunion, Mayotee, Mauritius, and Seychelles. In the same year, CHIKV reappeared in India after nearly 3 decades of quiescence, with 13 lakhs suspected cases in 12 states and has been reported by all the states thereafter.

The state-wise current Chikungunya situation in India can be accessed from the following link. https://ncvbdc.mohfw.gov.in/index4.php?lang=1&level=0&linkid=486&lid=3765

3. Clinical Features

Chikungunya fever should be suspected when the characteristic triad of fever, rash, and joint pain occurs. Incubation following mosquito bites averages 48 hours but may be as long as 12 days. Symptom onset is abrupt and heralded by fever and severe arthralgia, followed by constitutional symptoms and rash lasting for a period of 1 to 7 days. Fever rise can be dramatic, often reaching high temperatures and accompanied by intermittent shaking chills. Arthralgias are polyarticular and migratory, and predominantly affect the

small joints of hands, wrists, ankles, and feet, with lesser involvement of larger joints. Patients in the acute stage may complain bitterly of pain when asked to ambulate and they characteristically lie still. Joint swelling may occur, but fluid accumulation is uncommon. Mild articular manifestations usually resolve within a few weeks, but more severe cases may remain symptomatic for months. Generalized myalgias, back pain, and shoulder discomfort are common.

Cutaneous manifestations begin with flushing over the face and trunk and evolve to an erythematous dermatitis. The trunk and limbs are most frequently involved, but lesions may also appear over the face, palms, and soles. The rash eventually simply fades or desquamates. Infection can infrequently result in meningoencephalitis, particularly in newborns and those with preexisting medical conditions. Chikungunya outbreaks typically result in several hundreds or thousands of cases, but deaths are rare.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood/serum (Paired samples)- one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.

CSF-Acute phase (Disease with CNS manifestations)

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedure

a. Isolation: Chikungunya virus isolation from blood is accomplished by either in vivo (mice or mosquito) or in vitro techniques in insect cell lines.
b. Serological diagnosis: Virus-specific IgM antibodies can be helpful with diagnosis.
c. Molecular Detection: Chikungunya virus detection is also possible in early stages using traditional polymerase chain reaction (PCR) methods. A specific and sensitive 1-step RT-qPCR assay has recently been developed as a rapid indicator of active infection by quantifying viral load in clinical samples or cell culture supernatant.

Duration of illness	Recommended Test	Utility
Less than 7 days	Nucleic acid amplification test, Virus isolation*	Active infection
More than 7 days	Immunoassay (IgM and IgG antibodies)	Active and Past infection

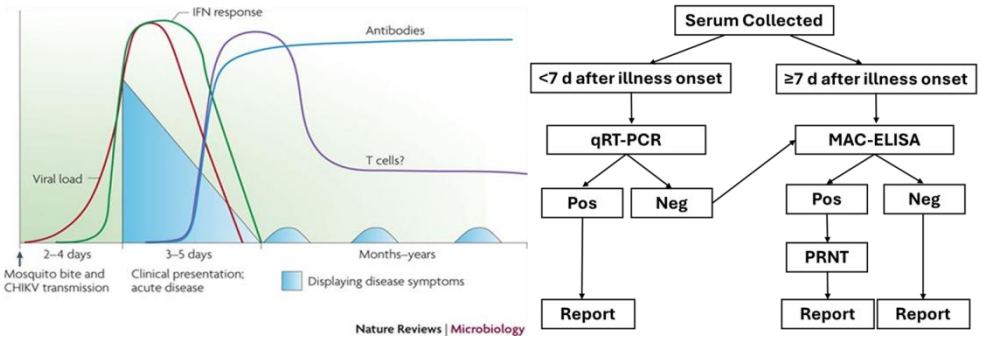


Figure 1: Recommended test based on the immune response post CHIKV transmission and duration of illness. (*PRNT precisely helps to differentiate cross reacting antibody)

5. Treatment

Chikungunya fever is usually self-limiting, and no specific treatment exists. Rest and supportive care are indicated during acute joint symptoms. Movement and mild exercise tend to improve stiffness and morning arthralgia, but heavy exercise may exacerbate rheumatic symptoms. Nonsteroidal anti-inflammatory drugs may be beneficial, and chloroquine has been used in severe cases. Infective persons should be protected from mosquito exposure so that they do not contribute to further transmission.

6. Prevention and Control

No vaccine is available, and preventive efforts are focused on vector control. Elimination of breeding sites or source reduction is important. Larvivorous fish (eg, gambusia, guppy), which eat mosquito larvae, may be introduced into local endemic areas. Protection from mosquito bites is achieved by insect repellent and use of insecticide-treated mosquito nets, especially during the daytime when the mosquitoes feed. Well-planned fogging operations are sometimes recommended in high-risk villages, where clustering of cases has been reported. Active epidemiological surveillance for CHIKV is crucial for promoting effective community education and transmission control.

Further Reading

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1. Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a viral haemorrhagic fever caused by tick-borne virus of the nairovirus group. The disease was first described in the Crimea (former USSR) in 1944 and given the name Crimean haemorrhagic fever. In 1969, it was recognized that the pathogen causing Crimean haemorrhagic fever was the same as that responsible for an illness identified in 1956 in the Congo, and linkage of the two first outbreak place names resulted in the current name for the disease and the virus.

CCHF outbreaks constitute a threat to public health services because of its epidemic potential, its high case fatality ratio (10-40%), its potential for nosocomial (hospital acquired infection) outbreaks and the difficulties in its treatment and prevention.

2. Epidemiology

2.1 Causative Agent

CCHF virus belongs to the family Bunyaviridae, genus Nairovirus. It is an enveloped, single stranded negative-sense RNA virus with a tripartite genome. Since it is an enveloped virus, it can be readily inactivated. CCHF virus is stable for up to 10 days in blood kept at 40^0C. The virus is clustered in seven genotypes, which are Africa-1, Africa-2, Africa-3, Europe-1, Europe-2, Asia-1 and Asia-2.

2.2 Mode of Transmission

Ticks are arthropods which suck blood from animals and humans. A number of tick genera are capable of becoming infected with CCHF virus, but the most efficient and common vector of CCHF are the members of *Hyalomma* genus, the family Ixodidae.

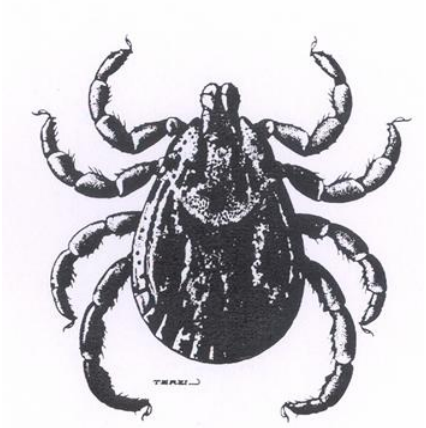


Figure 1: Adult hard tick, *Dermacentor andersoni*, family *Ixodidae* (from Lane and Crosskey 1993).

The ticks are also natural reservoir of CCHF virus. The Hyalomma ticks are hard ticks, and can be easily distinguished by four pairs of legs in adults and lack of clear segmentation of the body. The adult ticks are flat and oval in shape. The ticks have four life stages viz. eggs, larvae, nymph and adults. Larvae, nymph and adults need blood meals for their maturation. Larvae and nymph remain on the same host. The engorged nymph drops to the soil where it moults to adult form which seeks a new host. Both larvae and nymph largely feed on lower vertebrates (such as rodents, rabbits, hare etc.) while the adults feed on higher vertebrates (such as cattle, goat, sheep etc.). Male and female ticks suck blood. Both male and female can act as a vector for disease transmission. Transovarial transmission (transmission of the virus from infected female ticks to offspring via eggs) and trans-stadial (ie, from larvae to nymph to adult) transmissions have been demonstrated amongst some vector species,

indicating a mechanism which may contribute to maintaining the circulation of the virus in nature.

For further details on vector, please refer to the chapter "Arthropod Vectors of Zoonotic Importance".

Hard ticks are the reservoir and the vector for CCHF virus. In addition, infected animals may also act as reservoir during the period of viremia. The CCHF virus may infect a wide range of wild animals and domestic ruminant animals such as hares, rats, camel, cattle, sheep and goats. Many birds are resistant to infection, but ostriches are susceptible and may show a high prevalence of infection in endemic areas. Animals become infected with CCHF from the bite of infected ticks. The most important source for acquisition of the virus by ticks is believed to be infected small vertebrates on which immature Hyalomma ticks feed. Domestic ruminant animals, such as cattle, sheep and goats, are viremic for around one week after becoming infected.

Ecological changes, poverty, social instability, poor health services, and absence of standard infection control practices have contributed to increased transmission of the CCHF virus and two main modes of transmission are as follows:

a) Animal to Human Transmission: Human beings may acquire the CCHF virus by direct contact with blood or other tissues of infected livestock or they may become infected through a tick bite or crushing of infected tick, however, a zoonotic spillover event is rare and difficult to document. Meat itself is not a risk because the virus is inactivated by post-slaughter acidification of the tissues and would not survive cooking.
b) Human to Human Transmission: Humans can become infected mainly if blood, body fluids and wastes from patients with the disease comes into contact with broken skin or mucous membranes, as occurs when medical care personnel sustain accidental needle stick injury. In advanced stages of the disease, aerosol contact of blood of the patient can also lead to transmission of the virus.

CCHF virus circulates in an enzootic tick–vertebrate–tick cycle, and there is no evidence that the virus causes disease in animals.

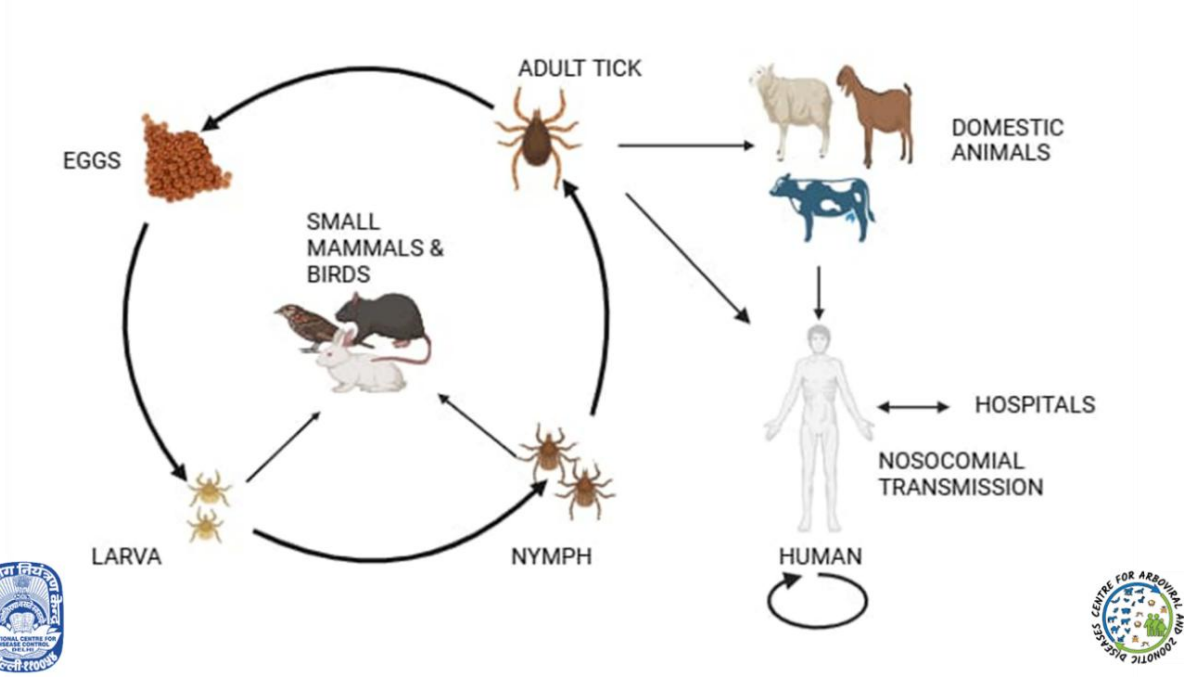


Figure 2: Life cycle of ticks and transmission of CCHF virus.

2.3 Current situation

The geographic range of CCHF virus is the most extensive among the tick-borne viruses that affect human health, and the second most widespread among all medically important arboviruses, after Dengue viruses. The disease is endemic in many countries in Africa, Europe, Middle East and Central Asia, with sporadic outbreaks recorded in Kosovo, Albania, Iran, and Turkey. In endemic countries, majority of cases have occurred in those involved with the livestock industry, such as agricultural workers, slaughterhouse workers and veterinarians. Health care workers attending on suspect/ probable/confirmed CCHF cases and not following contact precautions are at high risk of getting infection. Hospital-acquired infection outbreaks (Nosocomial spread) have been reported in many countries.

In India, the first laboratory confirmed outbreak was reported in January, 2011 in Gujarat where 7 cases and 2 deaths were reported from Ahmedabad. This had nosocomial transmission. Subsequently, outbreaks of CCHF have been reported from different parts of Gujarat (Amreli, Anand, Aravalli, Jamnagar, Kutch, Mehsana, Morbi, Patan, Sabarkantha, Surendranagar, Rajkot, Kheda). Outbreaks have also been reported from UP, Rajasthan & Kerala.

- Rajasthan (2014, 15 & 19)- Outbreaks have been reported from Rajasthan (Chittorgarh, Jodhpur & Sirohi).
- Uttar Pradesh (2015) - An outbreak was also reported from Moradabad.
- Kerala (2018) – A case from Thrissur district (expat from UAE).
- Gujarat (2023)- One case Kutch district and two cases from Amreli district.

3. Clinical Features

The incubation period for the illness depends upon the mode of acquisition of the virus. Following infection via tick bite, the incubation period is usually one to three days, with a maximum of nine days. The incubation period following contact with infected blood or tissues is usually five to six days, with a documented maximum of 13 days. Immunity after infection is probably lifelong.

Recent reports from Gujarat suggest that Chandipura antibodies have been detected from asymptomatic close contacts of confirmed CCHF cases which points towards clinical spectrum ranging from asymptomatic to fatal disease. Onset of symptoms is sudden, with fever, myalgia, dizziness, neck pain, stiffness, backache, headache, sore eyes and photophobia. There may be nausea, vomiting and sore throat early on, which may be accompanied by diarrhoea and generalized abdominal pain. Over the next few days, the patient may experience sharp mood swings, and may become confused and aggressive. After two to four days, the agitation may be replaced by sleepiness, depression and lassitude, and the abdominal pain may localize to the right upper quadrant, with detectable hepatomegaly (liver enlargement). Other clinical features include tachycardia, lymphadenopathy and a petechial rash both on internal mucosal surfaces, such as in the mouth and throat, and on the skin. The petechiae may give way to ecchymoses (like a petechial rash, but covering larger areas) and other haemorrhagic phenomena such as melaena (bleeding from the upper bowel, passed as altered blood in the faeces), haematuria (blood in the urine), epistaxis (nosebleeds) and bleeding from the gums. There is usually evidence of hepatitis. The severely ill may develop hepatorenal and pulmonary failure after the fifth day of illness.

The mortality rate from CCHF is approximately 9-50% with death occurring in the second week of illness. In those patients who recover, improvement generally begins on the

ninth or tenth day after the onset of illness. The convalescence period begins in survivors about 10–20 days after the onset of illness. In the convalescent period, labile pulse, tachycardia, temporary or complete loss of hair, polyneuritis, difficulty in breathing, xerostomia, poor vision, loss of hearing and loss of memory have been reported. Malaria, Leptospirosis, Rickettesial diseases, Meningococcemia, Dengue Haemorrhagic Fever, Haemolytic Uremic Syndrome, and Thrombocytopenic Purpura form the differential diagnosis of CCHF.

4. Laboratory Diagnosis

As CCHF virus is classified as risk group 4 virus and hence the clinical samples should be handled in specially-equipped, high containment biosafety level laboratories based on risk-assessment.

4.1 Collection of Specimen

a) Ante-mortem: Blood sample: Serum/Plasma
b) Postmortem: Tissue sample (liver, spleen, bone marrow, kidney, lung and brain)

In the first few days of illness diagnosis is achieved by virus/genome detection in blood or tissue samples.

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample collection and transport must be in accordance with per laid down guidelines for handling specimens from suspected, probable, or confirmed cases/contacts of high-threat pathogens.

4.3 Laboratory Procedure

a. Virus Isolation: It should always be carried out in maximum bio-containment laboratory i.e. BSL -4. The virus may be isolated from blood or tissue specimens in the first five days of illness, and grown in cell lines.
b. Serology: IgG and IgM antibodies may be detected in serum by enzyme-linked immunoassay from about day six of illness. IgM remains detectable for up to four months, and IgG levels decline but remain detectable for up to five years. Recent or current infection is confirmed by demonstrating seroconversion or a fourfold or greater increase in antibody titre in paired serum samples or detection of IgM antibodies by IgM capture ELISA (MAC ELISA) in a single sample. Viral antigens may often be detected in tissue samples using immune-fluorescence/ELISA
c. Molecular Technique: The Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is the test of choice for laboratory diagnosis of CCHF virus infection for detecting virus specific genome. It is a sensitive and specific method. Specificity and sensitivity can be further enhanced by using automated real time PCR.

5. Treatment

a) Supportive therapy: General supportive therapy is the mainstay of patient management in CCHF. Intensive monitoring to guide volume and blood component replacement is required.

   There is currently no specific antiviral therapy for CCHF. However, ribavirin has been shown to inhibit in-vitro viral replication in Vero cells and reduce the mean time of death in a suckling mouse model of CCHF. Additionally, several case reports have been

published that suggest oral or intravenous ribavirin is effective for treating CCHF infections.

Ribavirin is a member of the nucleoside anti metabolite drugs that interfere with duplication of viral genetic material. This is the only antiviral known to have some affect on the viruses causing VHF.

b) Chemoprophylaxis**:** Prophylactic administration of oral Ribavirin to contacts of CCHF patients is not recommended. Symptomatic contacts can be given a therapeutic dose. Consider full therapeutic dose of Ribavirin for Health Care Workers with severe exposure (Needle stick injury, direct contact with blood /body fluids). For person with mild exposure, observe and closely monitor HCW for any symptoms.

6. Prevention and Control

There is no vaccine available for either people or animals.

Further Reading

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2. Messina, Jane P., et al. "A global compendium of human Crimean-Congo haemorrhagic fever virus occurrence." Scientific data 2 (2015).
3. Shayan, Sara, et al. "Crimean-Congo Hemorrhagic Fever." Laboratory medicine 46.3 (2015): 180-189.
4. Bente, Dennis A., et al. "Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity." Antiviral research 100.1 (2013): 159-189.

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1. Introduction

Dengue fever is the most important mosquito-borne viral disease and a major international public health concern. Dengue fever is a self-limiting disease found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas. Dengue Haemorrhagic Fever (DHF), a potentially lethal complication, was first recognised in the 1950s during the dengue epidemic in the Philippines and Thailand, but today DHF affects most Asian countries and is a leading cause of childhood deaths. In India, the first major outbreak associated with haemorrhagic manifestation occurred in Calcutta in 1963. Since then, there has been a dramatic rise in the incidence of DHF cases.

2. Epidemiology

2.1 Causative Agent

DF/DHF is caused by dengue virus which belongs to genus Flavivirus, family Flaviviridae and includes serotypes 1, 2, 3 and 4 (Den-1, Den-2, Den-3 and Den-4). When a person has had classic dengue (i.e. infection by one serotype), a second infection later by another serotype increases the likelihood of suffering from DHF.

2.2 Mode of Transmission

Aedes aegypti is the main vector of dengue transmission in India. Dengue outbreaks have also been attributed to *Aedes albopictus.* The mosquito is a domestic breeder. Mosquito breeding can occur in any water-catching or water-storage containers, such as desert coolers, over head tanks, discarded buckets, tyres, utensils and large containers used for collecting rain water which are not emptied and cleaned periodically. The mosquitoes rest indoors, in closets and other dark places. Outside, they rest where it is cool and shady. *Aedes* mosquito can fly upto a limited distance of 400 metres but can spread over vast distances mechanically through vehicle means. The outbreaks of DF/DHF are most likely to occur in post-monsoon period when the breeding of the mosquitoes is highest.

For further details on vector, please refer to chapter “Arthropod Vectors of Zoonotic Importance”.

Usually, urban areas, having high population density, poor sanitation and a large number of desert coolers, overhead tanks, discarded buckets, tyres, utensils etc. which promote mosquito breeding, are at high risk. Dengue fever/DHF can also occur in rural areas where the environment is friendly for mosquito breeding like storage water for cattle feeding and drinking, discarded tins, tyre, bottles etc. which are not emptied and changed periodically.

The infection is transmitted by the bite of an infected female mosquito – *Aedes aegypti*. Mosquitoes generally acquire the virus while feeding on the blood of an infected person. After virus incubation for 8 – 10 days, an infected mosquito is capable of transmitting the virus to susceptible individuals for the rest of its life (i.e. 3 weeks).

Humans are the main amplifying host of the virus, although studies have shown that in some parts of the world monkeys may become infected and perhaps serve as a source of virus for the uninfected.

2.3 Current Situation

The global prevalence of dengue has grown significantly in recent decades. The disease is now endemic in more than 100 countries in South-east Asia, Western Pacific, Eastern Mediterranean, Africa, and the Americas. South-east Asia and Western Pacific are most seriously affected. Before 1970 only nine countries had experienced DHF epidemic, a number that had increased more than four-fold by 1995.

About half of the world's population is now at risk of dengue with an estimated 100–400 million infections occurring each year. India is also endemic for Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF). Every year, cases of DF and/or DHF are reported. In 1996, there was a large outbreak of DF and DHF. Cases and deaths were reported from various parts of the country viz. Delhi (10,252 cases and 423 deaths), Haryana (1631 cases and 54 deaths) and Maharashtra (3068 cases and 5 deaths). A total of 16517 cases and 545 deaths were reported from all over the country. Again, in the year 2003, an outbreak of DF and DHF was reported from various parts of the country, especially Delhi, Kerala, Karnataka, Punjab, Tamil Nadu, Uttar Pradesh & Maharashtra.

The state-wise current Dengue situation in India can be accessed from the following link. https://ncvbdc.mohfw.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715

3. Clinical Features

The incubation period of dengue fever is usually 5 – 6 days, but may vary from 3 to 10 days. The virus circulates in the blood of infected humans for two to seven days. Dengue fever is a severe form like illness that affects infants, young children and adults, but seldom causes death. The clinical features of dengue fever vary according to the age of the patient. Infants and young children may have a non-specific febrile illness with rash. Older children and adults may have either a mild febrile illness or classical disease with abrupt onset of high fever, severe headache, muscle and joint pain and rash.

DHF is a potentially deadly complication that is characterized by high fever, accompanied by headache, anorexia, vomiting and abdominal pain. A haemorrhagic diathesis is commonly demonstrated by scattered fine petechiae on the extremities, face, and trunk and in the axillae. A positive tourniquet test is always present. Bleeding from nose, gums and gastrointestinal tract are also seen. The liver is usually enlarged, soft and tender. In moderate DHF cases, all signs and symptoms abate after the fever subsides. In severe cases patient's condition may suddenly deteriorate after a few days of fever, varying degree of circulatory disturbances occur and the patient may rapidly go into a critical state of shock (Dengue Shock Syndrome) and die within 12 – 24 hours, or quickly recover following appropriate volume replacement therapy. Without proper treatment, DHF case fatality rate can exceed 20%. With modern intensive supportive therapy such rate can be reduced to less than 1%. A person with dengue should never take aspirin as it may increase his tendency to bleed. The danger signs in Dengue which require prompt clinical attention are

- Minute spots on the skin suggesting bleeding within the skin
- Nose bleeds and gum bleeds
- Abdominal pains or passage of black and tan like stool
- Refusal to eat and drink
- Abnormal behaviour or drowsiness
- Difficulty in breathing or cold hands and feet, reduced amount of urine being passed

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood/serum (Paired samples)- one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.

CSF-Acute phase (Disease with CNS manifestations)

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

Duration of illness	Recommended Test	Utility
Less than 7 days	Nucleic acid amplification test, NS1 Ag, Virus isolation*	Active infection
More than 7 days	Immunoassay (IgM and IgG antibodies)	Active and Past infection

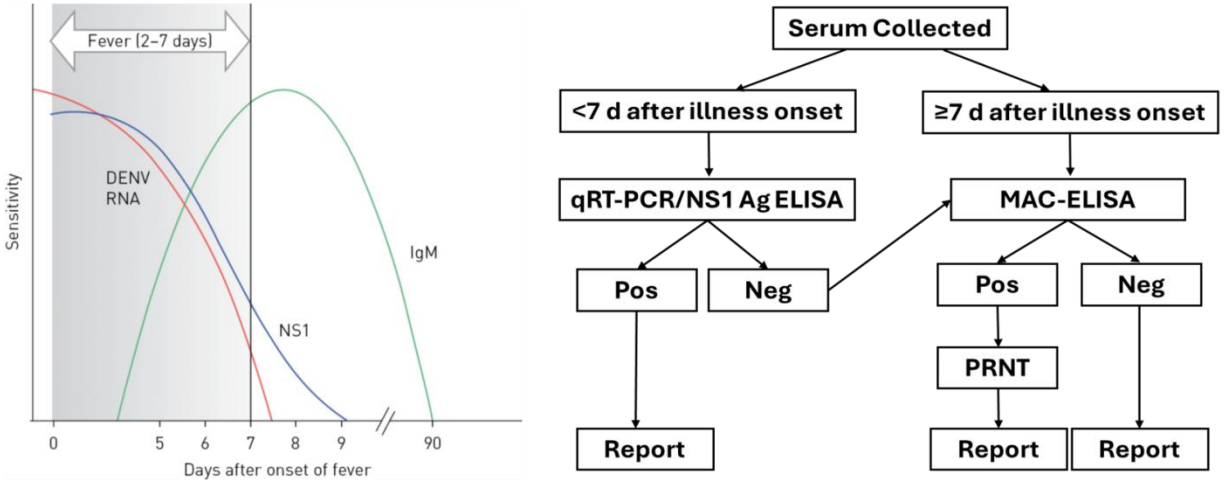


Figure 1: Recommended test based on the immune response post DENV transmission and duration of illness. (*PRNT precisely helps to differentiate cross reacting antibody)

4.3 Laboratory Procedures

a. Hematological test: The platelet count and haematocrit are important investigations for evaluation of Dengue cases. Thrombocytopenia (100,000 cells or less per cumm) and haemoconcentration (>20% rise in average haematrocrit for age and sex) is often reported
b. Isolation of the virus: Virus isolation can be done by inoculation of clinical material in suckling mice, tissue culture or mosquitoes and further detection is performed using fluorescent antibody test or haemagglutination inhibition test.
c. Serological test: Viral antigen (NS1 antigens) can be demonstrated by doing direct fluorescent antibody test using specific monoclonal antibodies for dengue virus/ ELISA. IgM antibodies against dengue virus appear around 5 days after onset of symptoms and are detectable for 1- 3 months after the acute episode. The tests employed are IgM capture ELISA test and Rapid IgM strip test. IgG antibodies appear later than IgM antibodies in primary infection of dengue and persist at high level for 30 – 40 days before declining to levels found in past infection and persist for life. Detection of four-fold or greater increase/fall in IgG titre in paired serum samples taken at an interval of 10 – 14 days confirms the diagnosis of dengue. Tests employed are IgG ELISA for dengue and Haemagglutination Inhibition (HI) Test.
d. Molecular test: Viral RNA or genomic sequence (Cpr-M gene) can also be detected in serum, CSF or culture supernatant by doing polymerase chain reaction and serotype specific PCR/ gene sequencing is done to study circulating serotypes/genotypes.

5. Treatment

Treatment of dengue fever is symptomatic with bed rest, antipyretics and analgesics. ORS solution is recommended for patients with excessive sweating, nausea, vomiting, or diarrhoea to prevent dehydration. Management during febrile phase is with antipyretics and analgesics. Besides this fluid and electrolyte replacement by IV fluids, plasma expanders, if clinically indicated, result in favourable outcomes. In some cases, fresh frozen plasma is indicated and in rare cases like patient with severe shock or massive bleeding, blood transfusion is required. Amount of fluid given should be constantly monitored. Any evidence of swelling, shortness of breath or puffiness may indicate fluid overload. Adoption of appropriate standardized clinical management practices can effectively reduce DHF case fatality rates.

6. Prevention and Control

The only method of controlling or preventing dengue fever and DHF is to combat the vector mosquitoes. In India, *Aedes aegypti* breeds primarily in man made container like water cooler, earthenware jars, concrete cisterns used for domestic water storage, discarded plastic food containers, used automobile tyres and other items that collect rain water. Vector control can be implemented using environmental management and chemical methods. Proper solid waste disposal and improved water storage practices, including covering containers can prevent access by egg-laying female mosquitoes. These methods should be encouraged through community-based programmes. Chemical methods of control include application of appropriate insecticides to larval habitats, particularly those considered useful by householders, e.g., water-storage vessels. These prevent mosquito breeding for several weeks but must be re-applied periodically. During outbreaks, emergency control measures may also include the application of insecticides as space spray to kill adult mosquitoes using portable or truck mounted machine. However, the killing effect is only transient and variable in its effectiveness because the aerosol droplets may not penetrate indoor to microhabitats where adult mosquitoes are sequestered. Regular monitoring of vector's susceptibility to the most widely used insecticides is necessary to ensure the appropriate choice of chemicals. In biological method of control, larvivorous mosquito–eating fish, dragon fly larvae, copepods (mesocyclops), peppermint oil and a fungus lagenidium gigantum have been used with some success. Active monitoring and surveillance of the natural mosquito population should accompany control efforts in order to determine the impact of the dengue control programme.

No effective vaccine is available for dengue. Research into dengue vaccines focuses on the use of live attenuated or inactivated vaccines, infectious clone-derived vaccines, immunogens vector by various recombinant systems, subunit immunogens and nucleic acid vaccines. Among these, intensive and stringent laboratory studies were conducted for live attenuated tetravalent vaccine in Thailand. This vaccine was evaluated in animal models and phase-1 clinical trial of this vaccine was recently completed in Thailand. After two doses, seroconversion to all four serotypes was demonstrated in most vaccinated volunteers and antiviral activity remained quite stable for at least a year. Further, recent clinical trial of a candidate Dengue vaccine have also been started in India.

In order to promote the evaluation of live attenuated vaccines in clinical trials, a group of WHO experts has been developing guidelines for the safety of dengue vaccines.

These guidelines could help public health officials to make decisions about conducting dengue vaccine trials in their countries.

Further Reading

1. Ministry of Health, & Family Welfare National centre for vector borne diseases control Dengue. https://ncvbdc.mohfw.gov.in/index1.php?lang=1&level=1&sublinkid=5776&lid=3690)

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1. Introduction

Japanese Encephalitis (JE) is a zoonotic viral disease caused by a group B arbovirus (flavivirus), involving the Central Nervous System. In nature, the virus is maintained in animals and birds, particularly pigs and ardeid birds (e.g. cattle egrets, pond herons, etc.). Although infection in humans is accidental, the virus can cause serious neurological complications in humans. The majority of infections are not apparent and only 1 in 300 to 1 in 1000 infections result in symptomatic illness. The disease occurs with sudden onset and the common symptoms are headache, high fever, stiff neck, abnormal movements (coarse tremor, convulsions in children), impaired consciousness and coma. Case fatality rate in JE is high, ranging from 20-50%.

JE occurs in a large number of countries/areas of Asia. It is a disease of public health importance because of its epidemic potential and high case fatality rate. In patients who survive, complications may lead to life long sequelae.

2. Epidemiology

2.1 Causative agent

The disease is caused by JE virus, which belongs to genus Flavivirus and family Flaviviridae. The JE virus is further classified based on genetic sequence variations. JEV is categorized into a solitary serotype consisting of five genetically diverse genotypes (I, II, III, IV, and V).

2.2 Mode of Transmission

JE virus has its natural cycle in vertebrates and mosquitoes. The animal hosts include pigs, cattle and horses and amongst birds are the water birds e.g. ponds herons, cattle egrets, poultry birds and ducks play a significant role in the natural history of JE virus. Pigs are the major vertebrate hosts and are considered as amplifying hosts. Currently available evidences does not indicate major role of cattle and horses. Infection in man appears to be correlated with living in close proximity with animal reservoirs, especially pigs.

In India, birds particularly those belonging to family Ardeidae and pigs play important role in maintenance of JE virus in nature. Various studies conducted on detection of the presence of JE antibodies in the sera of birds belonging to different species have indicated that Ardeola grayii (pond heron) and Bubulcus ibis (cattle egret) play a definite role in maintenance of JE virus in nature. In different parts of the country, 12 to 44 percent pig population have been found to be positive for JE antibodies, particularly in JE endemic areas. Besides birds and pigs, bovines and bats have also been found positive for JE antibodies but their role in maintenance of virus in nature is doubtful as the titres found in them are very low.

Mosquitoes belonging to *Culex vishnui* group are most important vector species in India. 11 more species of mosquitoes have been incriminated as vectors of JE. *Culex* mosquitoes generally breed in water bodies with luxurious vegetation like irrigated rice fields, shallow ditches and pools. Mosquitoes are zoophilic, feeding primarily on animals and wild birds. Epidemics usually coincide with monsoons and post-monsoon period when the vector density is high. Female mosquitoes get infected after feeding on a viraemic host and can transmit the virus to other hosts after an extrinsic incubation period of 9 to 12 days.

For further details on vector, please refer to chapter “Arthropod Vectors of Zoonotic Importance”.

In India, JE virus was first isolated from wild caught mosquito species at Vellore in 1956. Since then the virus has been isolated from 12 mosquito species in wild caught

specimen from different parts of the country. Maximum isolations have been made from *Culex vishnui* group consisting of *C. tritaeniorhynchus*, *C. vishnui* and *C. pseudovishnui*.

The infection is transmitted through the bite of an infected culicine mosquito. The transmission cycle is maintained in animals and birds. Infection in man is the dead end of the transmission. Human to human transmission has not been documented.

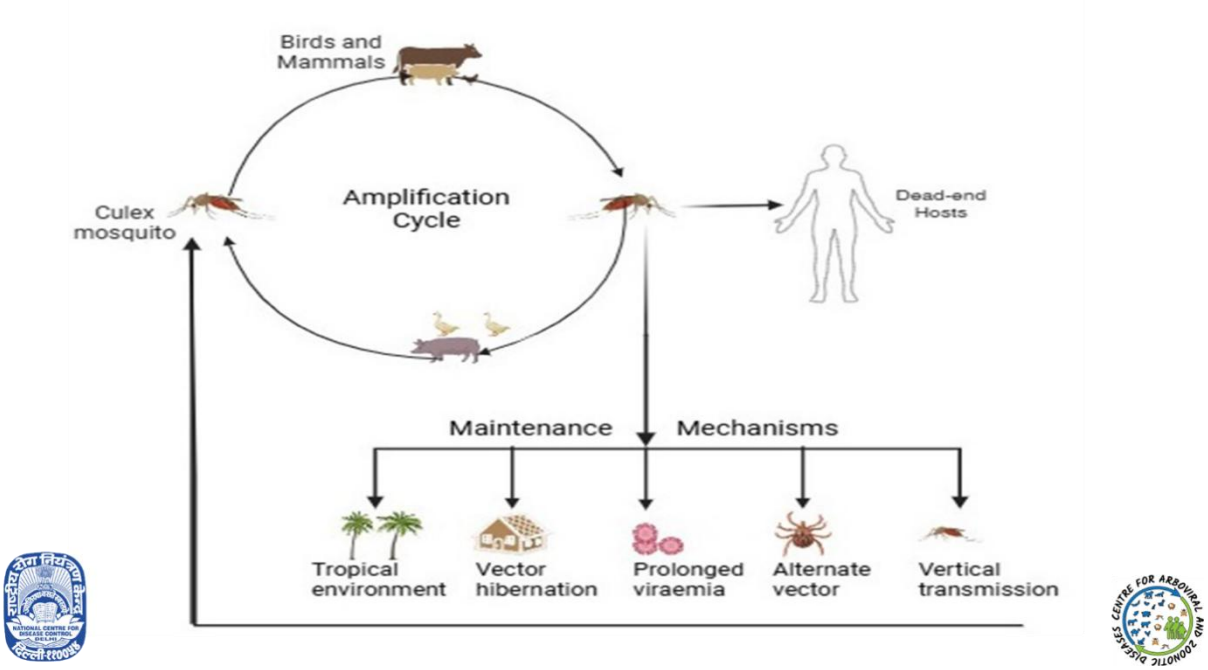


Figure 1: Transmission cycle of virus causing encephalitis.

2.3 Current Situation

JE occurs in a large number of countries of Asia, including Cambodia, China, Indonesia, Japan, Laos, Malaysia, Myanmar, Philippines, Korea, Thailand, Vietnam, South-eastern Russian Federation and the Indian subcontinent. In recent decades, JE has gradually spread to previously non-affected Asian regions. Global studies show that the dominant genotype of the JEV has undergone a progressive shift from GIII to GI due to variations in its adaptability within avian populations.

In India, JE was first recorded in Vellore and Puducherry in mid 1950s. The first major outbreak of JE occurred in 1973 in Bankura & Burdwan districts of West Bengal. In 1976, wide spread outbreaks were reported from Andhra Pradesh, Assam, Karnataka, Tamil Nadu, Uttar Pradesh and West Bengal. In 1978, JE cases were reported from 21 states and Union Territories. The Directorate of NVBDCP is monitoring JE incidence in the country since 1978. The worst ever recorded outbreak in India was reported from Uttar Pradesh during 1988 when 4485 cases with 1413 deaths were recorded from eight districts with case fatality rate of 31.5%. The highly affected states include Andhra Pradesh, Assam, Bihar, Goa, Karnataka, Manipur, Tamil Nadu, Uttar Pradesh and West Bengal. Outbreaks of JE usually coincide with monsoons and post-monsoon period when the vector density is high. However, in endemic areas, sporadic cases may occur throughout the year.

The JE surveillance consists of three major areas: (1) Sero-surveillance to delineate high risk population groups and to monitor JE specific antibodies in sentinel animals or birds as an indication of increased viral activity (2) Vector surveillance in JE prone areas for monitoring vector behaviour and population build up for timely implementation of

intervention methods (3) clinical surveillance through healthcare system for early diagnosis and proper management of AES/JE patients.

The state-wise current JE situation in India can be accessed from the following link: https://ncvbdc.mohfw.gov.in/index1.php?lang=1&level=2&sublinkid=5926&lid=3760

3. Clinical Features

JE cases are mainly reported from age groups below 15 years in areas where disease has become endemic. Various epidemiological studies conducted during investigation of outbreaks, observed that though both sexes are affected, males outnumber females.

The incubation period in human being, following mosquito bite, varies from 5 to 15 days. Japanese Encephalitis virus infection may result in a febrile illness of variable severity associated with neurological symptoms ranging from headache to meningitis or encephalitis. The common symptoms include headache, fever, meningeal signs, stupor, disorientation, coma, tremors, paresis (generalised), hypertonia, and loss of co-ordination. The encephalitis cannot be distinguished clinically from other central nervous system infections.

The patient will give a history of acute onset of fever and change in behaviour or sensorium lasting for more than 24 hours. Focal neurological deficits may or may not be present. In majority of the cases, however, the infection is mild with no overt clinical symptoms or mild fever with headache. Case fatality rate is high i.e. 20 to 40% in severe cases. Patients who recover from the acute episode may have neurological sequelae viz. mental impairment, severe emotional instability, personality changes, paralysis etc.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Humans

Blood/serum (Paired samples)- one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.

CSF-Acute phase (Disease with CNS manifestations). CSF is a preferred sample for laboratory confirmation of JE or identifying other causes of AES and meningitis.

Animals

Blood/serum samples for sero-surveillance and to study circulating genotypes.

Vectors: In an epidemic situation, it is desirable to collect mosquitoes from the affected areas-both indoors and outdoors, so that they may be processed for virus isolation. This may give an indication of the species acting as vectors and also provide some information on the mosquito fauna of the area. Mosquitoes can be collected by standard methods such as aspirator, baited traps, biting collections, and light traps. The mosquitoes should be held alive in 'Barraud Cages' wrapped with moistened lint or cloth.

4.2 Storage and Transport

Humans/Animal specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

For transport of vectors If the collection locality is not far from the laboratory or transportation can be done within a day or two, mosquitoes may be transported alive in Barraud cages. For such transportation, it is necessary to provide raisins soaked in water or a cotton pledget soaked in 10 percent solution of sucrose and glucose inside the Barraud cage. If the collection locality is far from the laboratory and immediate transportation is not

possible, mosquitoes may be identified, pooled species wise and stored in liquid nitrogen, refrigerators or on dry ice for subsequent transportation to the laboratory. If facilities for liquid nitrogen or dry ice storage are not available in the field, transport medium may be used to store the mosquito pools. It is, however, necessary that such pools are constantly kept in the refrigerator or transported on wet ice.

4.3 Laboratory procedures

a. Isolation of the virus: Virus isolation can be done by inoculation of clinical material in suckling mice, tissue culture or mosquitoes and further detection is performed using fluorescent antibody test or haemagglutination inhibition test. Isolation and identification of the JE virus from mosquitoes occasionally from peripheral blood/CSF (within 3 to 4 days after onset of symptoms) or autopsied brain tissue has been successful.
b. Serological test: The detection of antibodies to JE virus can be done routinely by Haemagglutination Inhibition Test (HI) test, IgM Capture ELISA test. Detection of JE virus-specific IgM antibodies by IgM capture ELISA remains the mainstay for diagnosis of JE. JEV IgM antibodies are usually detectable 3 to 8 days after the onset of illness and persist for 30-90 days, however, longer persistence is documented. JE-specific IgM in the CSF or serum reaches > 95% sensitivity 10 days after onset of first symptoms.

   The presence of IgM antibody in a single sample of CSF as detected by an IgM-capture ELISA specifically for JE virus confirms the diagnosis. However, the presence of IgM antibody in a serum sample by an IgM-capture ELISA specifically for JE virus must be reported cautiously in areas, where JE vaccination coverage is high. In addition, a large majority of JE infections are asymptomatic. Therefore, in areas that are highly endemic for JE, it is possible to have AES due to a cause other than JE virus and have JE virus-specific IgM antibody present in serum due to past infection or immunization. In such cases, detection of JEV specific IgM antibodies in CSF confirms the diagnosis

   It is important to differentiate true JE virus infections from false-positive JE results because of cross-reactive epitopes among flaviviruses and low specificity of diagnostic kits. WHO recommends that a validated dengue-specific assay may be carried out on all JE-positive specimens.
c. Molecular test: Viral RNA or genomic sequence can also be detected in clinical specimens/mosquito pools. Gene sequencing is done to study circulating genotypes.

5. Treatment

There is no specific treatment of JE. However, supportive treatment and good nursing care can significantly reduce the case fatality rate. Therefore, encephalitis cases should be referred to a hospital as early as possible so that treatment may start without waiting for serological laboratory results. In the acute phase, clinical management is directed at maintaining fluid and electrolyte balance. Keeping the airway open in a comatose patient, if required oxygen may be given. Appropriate drugs for convulsions may be prescribed.

6. Prevention and Control

Prevention of transmission is possible through vector control. For effective control of vectors, residual insecticidal spraying has been suggested in all animal dwellings with appropriate insecticides before the onset of transmission season.

Safe and effective vaccines are available to prevent JE. Three types of vaccines against JE have been produced and are used worldwide, which are Inactivated mouse brain, Inactivated and Live Attenuated1123421`, Cell culture-derived SA 14-14-2. Live Attenuated, Cell culture-derived SA 14-14-2 is commonly used in Paediatric/adult vaccination.

It has been observed that there is a direct relationship between the time lag in onset of symptoms and initiation of therapy. Immediate management of cases reduces fatality to a considerable extent. Since the disease is predominantly prevalent in rural areas, generating awareness helps in early reporting. Further health education helps in encouraging personnel protection.

Further Reading

1. Ministry of Health & Family Welfare: National Center for Vector-Borne Diseases Control; Japanese Encephalitis https://ncvbdc.mohfw.gov.in/index1.php?lang=1&level=1&sublinkid=5773&lid=3693

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1. Introduction

Kyasanur Forest disease (KFD), also called monkey fever, is a haemorrhagic disease of high mortality and morbidity caused by the tick-borne KFD virus (KFDV). KFD was first identified in a sick black-faced langur monkey in the Kyasanur forest, Shivamogga district, Karnataka, in 1957. In addition to the deaths of the monkeys, an epidemic of human febrile and enteric diseases was witnessed in the Shivamogga district in 1957 and 1958. Serological investigations of these human and monkey cases and virus isolation from Haemaphysalis ticks from the region led to the description of KFDV as a tick-borne virus of the Russian spring-summer virus complex. Since then, KFD has been reported as seasonal outbreak in humans and monkeys from Shivamogga and adjoining endemic areas in Karnataka and has recently spread to other states.

2. Epidemiology

2.1 *Causative agent*

KFDV is an arbovirus classified under the family Flaviviridae and genus Flavivirus. KFDV is a spherical, enveloped virus with a nucleocapsid and a positive sense linear single-stranded RNA genome. The genomic RNA is 10774 base pairs in size, and as established for other Flaviviruses, the genome codes for a single polypeptide that is cleaved into three structural proteins: envelope (E), capsid (C), precursor membrane (prM), and seven non-structural proteins: NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5.

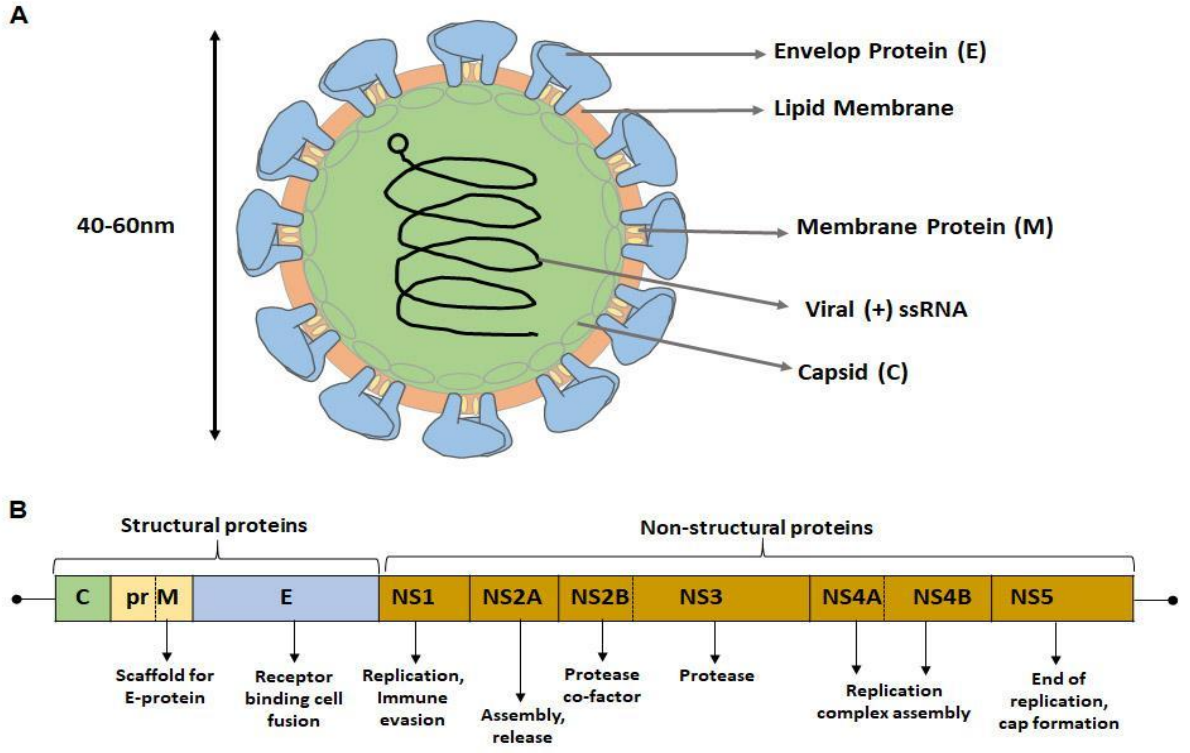


Figure 1: Structure and organization of flavivirus. A) The virion size is 40-60 nm. B) The genome of the virus has a single open reading frame that codes for structural and non-structural proteins.

The virus has a spherical structure composed of the linear positive single stranded (ss) RNA genome, C-capsid, host-derived M-membrane (expressed as prM, the precursor to M), and E-envelope. The genome exhibits 91.6% sequence identity with AHFV, which caused

haemorrhagic fever in Saudi Arabia. The E protein is crucial for viral pathogenesis as it contributes to host-receptor interaction and cellular uptake of the virus. The E-protein comprises of motifs that are potential molecular determinants of haemorrhagic manifestations of these viruses.

Tick-borne encephalitis viruses form a distinct group within the family and include KFDV, Central European encephalitis virus, Louping ill virus, Russian spring-summer encephalitis virus, Omsk hemorrhagic fever virus (OHFV), Alkhurma hemorrhagic fever virus (AHFV), and Powassan virus. Within this group, KFDV, OHFV, and AHFV are associated with hemorrhagic fever and neurological symptoms.

2.2 Mode of transmission

The main vector of KFDV is Haemaphysalis spinigera hard-bodied ticks belonging to the family Ixodidae. KFDV has also been isolated from nine other species of the *Haemaphysalis* genus, including *H. turturis, H. papuana kinneari, H. minuta, H. cuspidate, H. kyasanurensis, H. bispinosa, H. wellingtoni, H. aculeate,* and *H. shimoga*. The transmission of KFDV is through trans-stadial transmission. However, trans-ovarian transmission was reported under laboratory conditions in H. spinigera. KFDV was also isolated in field conditions and in laboratory settings from tick species belonging to Ixodes, Dermacentor, Rhiphicephalus, Amblyomma, and Ornithodoros.

For further details on vector, please refer to chapter “Arthropod Vectors of Zoonotic Importance”.

In KFD, the virus-infected ticks can act as ectoparasites for multiple reservoir hosts. During the feeding process, the tick saliva can enter the host’s body, especially in the blood stream, and infect the host with KFDV. Non-human primates (NHP), particularly Presbytis entullus (black-faced Hanuman langoors) and Macaca radiata (red-faced bonnets), are the amplifying and accidental hosts as they amplify the virus load. Both NHP species are experimentally proven to be highly susceptible to the virus and develop high viremia, resulting in death. Larger animals such as goats, sheep, and cattle also get infected and elicit an antibody response. However, these animals neither amplify the virus nor exhibit predominant symptoms, and hence act as maintenance hosts for the ticks. Immature ticks also feed on dogs, foxes, boars, tigers, deer, rodents (forest rat, shrew, and rabbit), bats, squirrels, porcupines, and ground-dwelling birds that act as hosts in KFD-endemic areas. Humans come into contact with the ticks through exposure to host animals or vegetation and get infected with KFDV from a tick bite. Since no human-to-human transmission of the virus has been reported, humans serve as a dead-end host in the virus transmission cycle.

The life cycle of H. spinigera is approximately 323 (male) and 329 (female) days, which comprises four developmental stages: egg, larva, nymph, and adult. During the post-monsoon period (October–December), the adult ticks lay eggs in sheltered spots such as the soil, wooden surfaces, and under the stones. The eggs hatch into six-legged larvae, which feed on small hosts (rabbits, squirrels, birds, and monkeys) and rest under the vegetation. The ticks get infected with KFDV when the larva feeds on infected animals. After 10–20 days, the larvae are transformed into eight-legged nymphs that attach to hosts such as humans or monkeys for a blood meal, after which the engorged nymph will fall off. The nymph stage is prevalent from January–March. The nymphs will rest for 15–20 days and moult to become adults that rest under the vegetation till the monsoon. During the monsoon (June–September), the adult ticks feed and mate on large animals like cattle, and the fed female tick falls off. After 8–10 days, the female tick lays 4000-5000 eggs in the vegetation and dies.

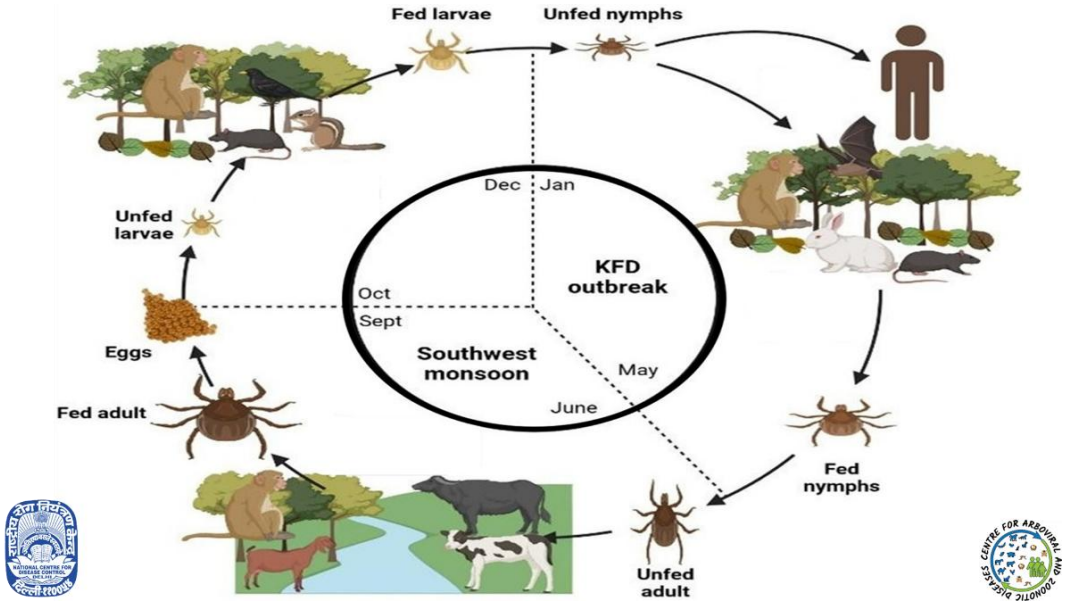


Figure 2: Life cycle of Haemaphysalis spinigera ticks, the primary vector for Kyasanur Forest disease virus (KFDV) and the mode of transmission of the KFDV.

The infected, succumbing animals move towards the outskirts of the forest in search of water, where the animals die and the ticks from their bodies move on to the nearest forest floor, generating hotspots. The ticks attach to humans that come near these hotspots for activities such as farming, firewood collection, hunting, etc.

2.3 Current Situation

The initial description of KFD was done in 1957 by Indian Council of Medical Research (ICMR)- National Institute of Virology, Pune erstwhile Virus Research Centre, in collaboration with the Rockefeller Foundation. Since the virus identification, until 2012, KFD sporadic cases were reported from only Karnataka state among Shivamogga, Uttara Kannada, Chikmagalur, Udupi, Dakshina Kannada, and Chamarajanagara districts. During 2012, KFDV infection was confirmed in the Nilgiris and Wayanad districts of Tamil Nadu and Kerala states, respectively. Again, during 2016-2017, KFDV infections were reported from Maharashtra and Goa states. Till date, KFD-confirmed cases have been reported from Karnataka, Kerala (2013–2015, 2019, 2020), Goa (2015–2020, 2023), and Maharashtra (2016–2023) states during peak seasons. It is evident that KFD infection is confirmed and has progressed from forest ecosystems to cultivated clearings and grasslands along the entire belt of the Western Ghats. Neutralising antibodies were detected among human and cattle samples (1960) from Gujarat and among human samples from West Bengal. It was also interesting that KFD IgG antibodies were detected among human samples (1988-89) from Andaman and Nicobar Island.

Although, the disease manifests as a febrile illness in humans, about 10-20% may progress to haemorrhagic or neurological symptoms. Approximately 400-500 human cases of KFD were reported annually from the endemic regions. However, since 2012, KFD cases have been reported from the neighbouring states of Kerala, Tamil Nadu, Goa, and Maharashtra. Deforestation and other anthropogenic activities altering the tropical forest ecosystem of the Western Ghats are presumably responsible for the geographic expansion of the infection.

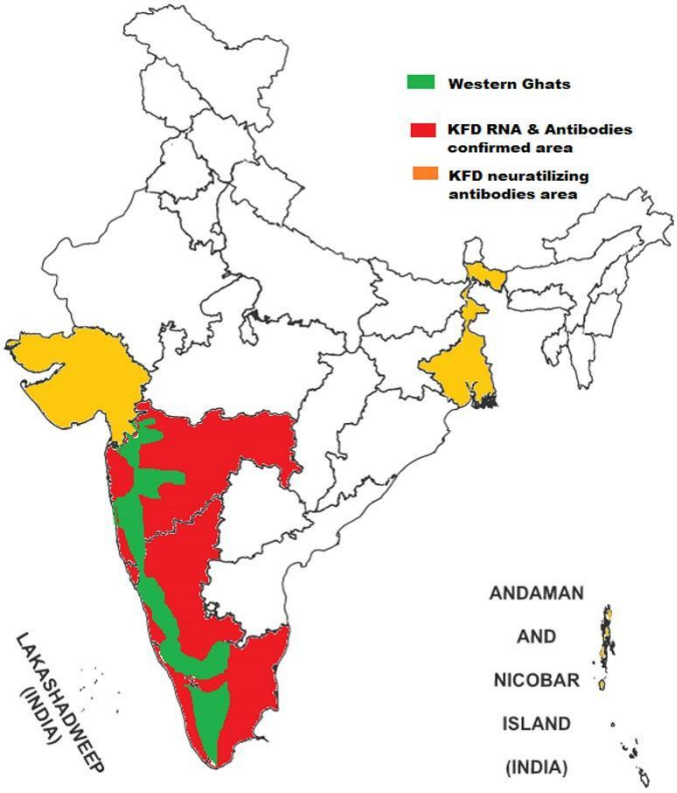


Figure 3: State-wise Kyasanur Forest disease (KFD) positivity in humans.

3. Clinical Features

The preliminary diagnosis of KFD is based on clinical signs along with a history of exposure to forest, vegetation, wildlife, and travel to KFD-endemic areas. Reports of monkey deaths also act as a "sentinel event" to forecast a possible outbreak in an area.

Humans

Clinical symptoms are presented in two phases among KFDV-infected individuals (DHFWS GoK, 2020). After the incubation period of 3-8 days, patients manifest the first phase of illness, characterised by fever (peaking in 3-4 days), eye redness, headache, body pain, sometimes fatigue, joint pain, and lymph node swelling. During the first phase, a few individuals present with gastrointestinal symptoms like vomiting and diarrhoea, hemorrhagic symptoms, and neurological symptoms (CDC, 2013; DHFWS GoK, 2020). During the acute phase, patients present with leucopenia, thrombocytopenia, lower erythrocytes sedimentation rate, albuminuria, urinary sediments, and increased transaminases while fever starts decreasing by 5 to 6 days, and the majority of individuals recover by about 10–15 days. Neurological manifestations have been associated with increased mortality [primarily in first phase]. Pneumonitis, myocarditis, pancreatitis, and acute kidney injury are other complications seen in KFD. Death occurs mostly in the first phase of the illness. The second phase of illness manifests among 10–20% of individuals and is characterised by meningoencephalitis features like fever, headache, neck stiffness, mental disturbance, tremors, visual deficit, convulsions, and coma. The second phase of the illness may be characterised by neurological abnormalities like aseptic meningitis, asthenia, weakness, delirium, convulsions, and focal neurological deficits. Sometimes, the haemorrhagic

manifestations are also reported in the second phase. The disease's convalescent phase is prolonged. The estimated mortality rate is about 3 to 10%.

Animals

An experimental KFD infection of Bonnet Macaques reported that primates presented with fever starting from the 4th day of post-infection present until the 9th day and decreasing from the 10th day onwards. They also presented with ocular discharge, watery dehydration, signs of dehydration, reduced activity and weight, and anorexia.

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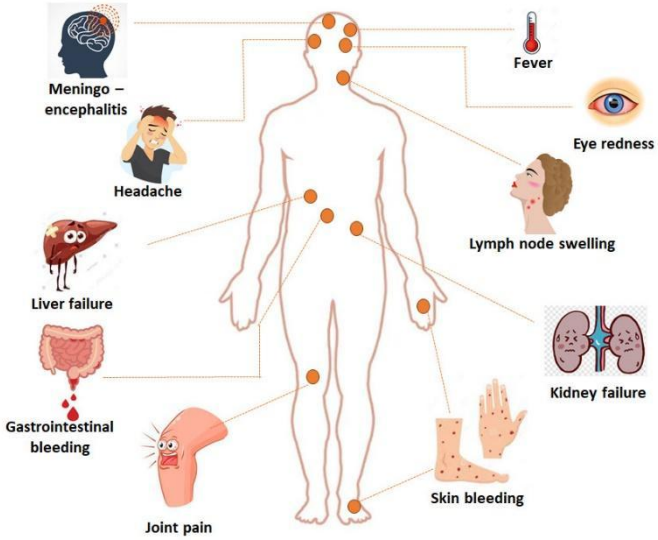


Figure 4: Clinical features of Kyasanur Forest disease.

4. Laboratory Diagnosis

As per the risk group classification of infective microorganisms KFDV is classified as a risk group 3 pathogen. However, for diagnostic activities, a biosafety level (BSL)-2 facility may be considered; however, for isolation from animal and tick, animal and vector based research studies, a high containment facility (BSL-3 laboratory) should be considered.

4.1 Collection of Specimen

Blood/serum (Paired samples)- one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.

CSF-Acute phase (Disease with CNS mainfestations)

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedures

a. Isolation: Isolation of the virus is successfully attempted in animals viz Swiss albino and cell cultures
b. Serological test: Detection of antibodies against KFD has been performed using hemagglutination inhibition, neutralization tests, complement fixation. Recently, point-of-care (POC) tests have been developed and are being evaluated to provide rapid detection in the field, especially in remote territories.
c. Molecular test: Real-time reverse transcription polymerase chain reaction (RT-PCR) is used for KFD diagnosis.

5. Treatment

The treatment is symptomatic and supportive and no specific antiviral is required.

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6. Prevention and Control

The key strategies for prevention and control of KFD are surveillance, vaccination, tick control measures, awareness programmes, and personal protection measures. Since monkey death is closely associated with the onset of clinical cases, it triggers monkey and human surveillance in the surrounding high-risk areas.

Since 1990, a formalin-inactivated chick embryo tissue culture vaccine has been used in KFD-endemic areas of Karnataka. At the moment this vaccine is not available to use and in absence of this vaccine preventive measures become more important.

Tick control strategies include clearing vegetation around human dwellings in the forest area, restricting cattle movement into the forest, and applying tick-repellent to cattle. The tick population is also controlled by the application of acaricides and ecto-parasiticides in hotspots and animal-dwelling areas.

KFD is a zoonotic viral disease with high morbidity and mortality. Considering the rise in the number of cases and transmission of the disease to non-endemic areas, future studies focusing on understanding disease pathogenesis, developing safe and effective vaccines, and developing specific treatment strategies are necessary.

Further Reading

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1. Introduction

West Nile Virus (WNV) is a Culex mosquito-transmitted virus of genus Flavivirus and family Flaviviridae. The genus flavivirus comprises >70 known viruses. Several flaviviruses are significant human pathogens worldwide. WNV infection causes variable clinical manifestations in humans ranging from self-limiting mild, febrile illness to severe neurological complications. Among the flaviviruses, WNV is the most widespread mosquito-borne flavivirus across the globe.

2. Epidemiology

2.1 Causative Agent

WNV is a small enveloped virus, 50 nm in size with icosahedral symmetry. WNV genome is composed of a single-stranded, positive sense RNA of ~ 11 kb flanked at both the 5' and 3' ends with non-coding regions (NCRs). Viral RNA encodes a single polypeptide that is proteolytically processed to yield three structural proteins [capsid (C), pre-membrane/membrane (prM), envelope (E)] and seven non-structural (NS) proteins (NS1, NS2A/B, NS3, NS4A/B and NS5). Mature C protein assembles with the genomic RNA to form a nucleocapsid, which is wrapped by the lipid bilayer with prM and E proteins to form an infectious virion. M protein is involved in correct folding and maturation of E protein. E glycoprotein is a surface protein forming envelope that plays a role in host cell receptor recognition, attachment, cellular entry, and membrane fusion. The NS proteins are involved in several functions including WNV replication, assembly, virulence, immunomodulation, interferon inhibition, and pathogenicity.

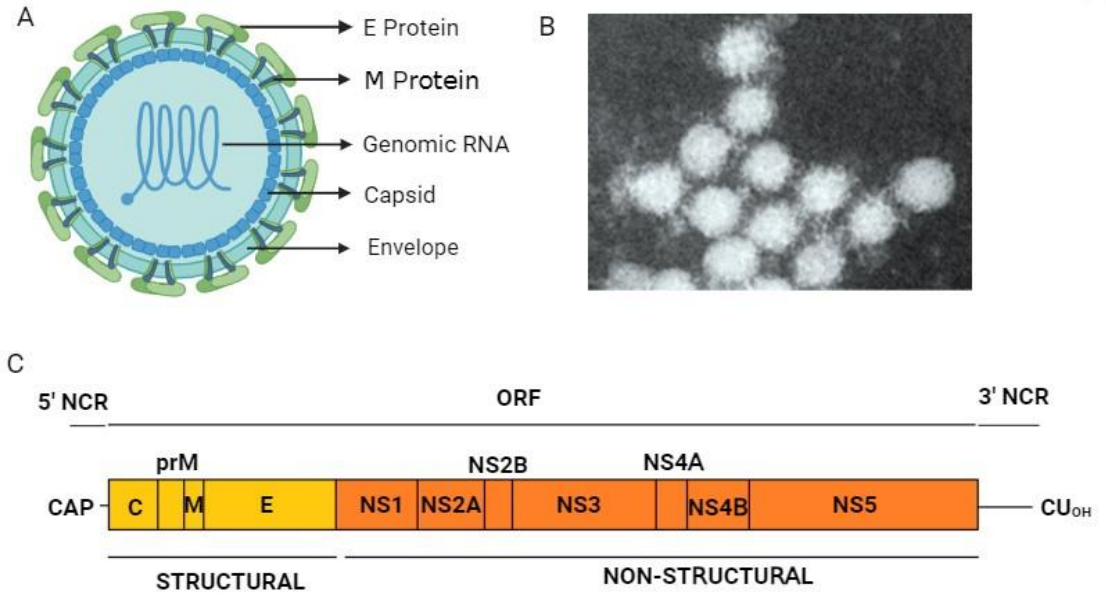


Figure 1: (A) WNV structural organization, (B) Morphology of WNV by TEM, (C) WNV RNA genome.

Based on antigenic similarities, WNV is classified in the Japanese encephalitis sero-complex. Genetically WNV strains show >20% heterogeneity and are classified into nine distinct genetic lineages (L1-L9). L1 strains are the most widespread and forms distinct clades-1a and 1b. L1 clade 1a is comprised of strains from Europe, Africa, Asia, Middle East, and America and are primarily associated with massive outbreaks affecting humans, horses, and birds. The Kunjin virus classified under clade 1b mostly affects horses. Though lineage 2

strains were historically associated with febrile episodes in Africa, they have since been found to cause neurological illness in Europe and South Africa. Lineage 5, after emerging independently in the Indian subcontinent has been associated with occasional neurological illness. Other lineages (L3, 4, 6, 7, 8 & 9) have been isolated only from mosquito or tick vectors and have no documented association with human or vertebrate hosts.

2.2 Mode of Transmission

WNV is maintained in an enzootic ornitophilic-mosquito-bird cycle. Birds including crows, sparrows, and woodpeckers serve as amplifying hosts / natural reservoirs and Culex mosquitoes act as transmission vectors.

For further details on vector, please refer to chapter "Arthropod Vectors of Zoonotic Importance".

Humans and other vertebrates act as incidental/dead end hosts. Migratory birds play an important role in the local and long distance dispersal of WNV.

Culex mosquitoes serves as the primary transmission arthropod vectors worldwide, though WNV has been sporadically isolated from other arthropods like ticks, mites, and hippoboscid flies. In North America, WNV infection has been detected in at least 326 bird species with thousands of bird deaths. In Indian subcontinent, ardeid (heron) birds are likely to serve as reservoirs and sero-positivity has been detected in several wild birds.

Several other animals including domestic and wild mammals, reptiles and amphibians have been described to be susceptible to WNV infection. Other routes, including transfusions, transplants and vertical transmission, have been reported, albeit rarely. Birds faeces, oral and cloacal shedding, and vertical transmission in mosquitoes have been documented as modes of non-vector transmission.

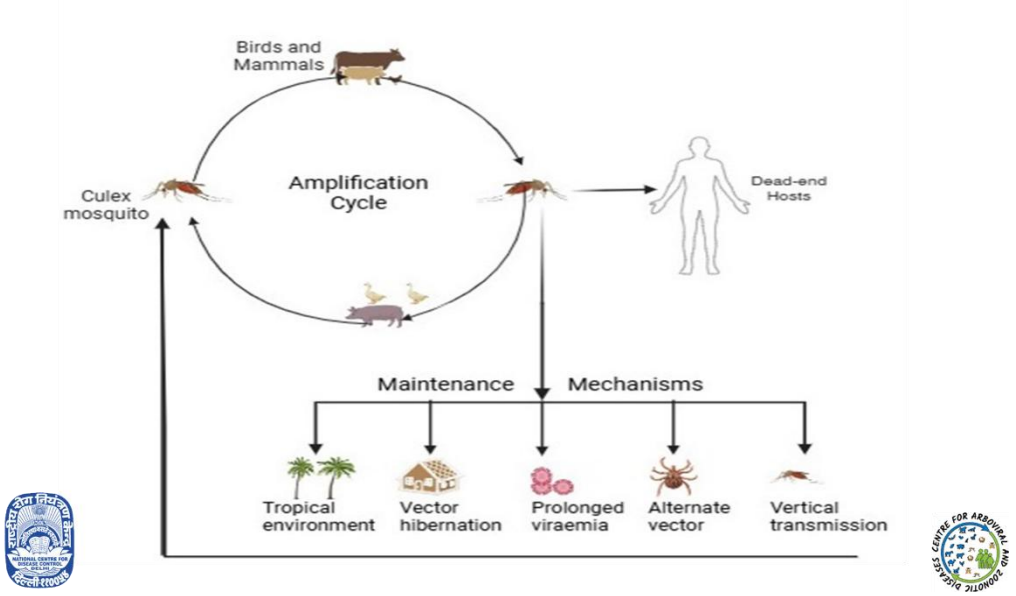


Figure 2: Transmission Cycle of virus causing encephalitis.

2.3 Current Situation

The virus, which was first isolated from West Nile Province of Uganda in 1937, had then been associated with outbreaks of meningoencephalitis in Israel, France, Hungary, Greece, Russia, Austria, Romania, Italy, Asia, and Australia. The entry of the virus to North America was identified following a large scale outbreak of meningoencephalitis in 1999. WNV was detected in India in 1952, with small scale outbreaks being reported from multiple

states including Kerala and Assam. Currently, the virus has wide global distribution and is recognized as a major cause of human viral encephalitis.

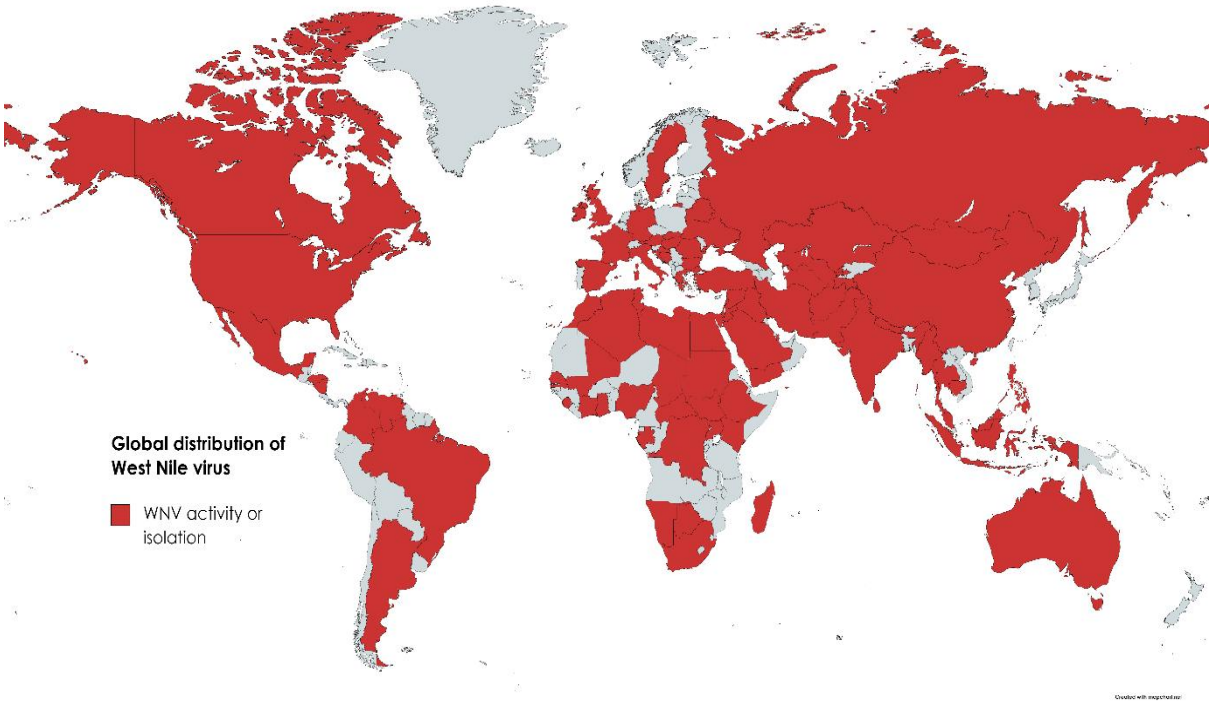


Figure 3: Global distribution of WNV. (Map generated with online Mapchart tool)

In India, the presence of WNV specific neutralizing antibodies in population was documented for the first time in 1952. Further sero-epidemiological investigations confirmed the presence of neutralizing antibodies in 20-30% population of Tamil Nadu (TN), Karnataka, Andhra Pradesh (AP), Maharashtra, Gujarat, Madhya Pradesh (MP), Orissa and Rajasthan. Isolation of WNV has been reported from humans, Culex mosquitoes and certain bat species. During the last decade, Indian sub-continent witnessed the spread of WNV to newer areas of India including the states of Assam, MP and West Bengal (WB) and neighbouring countries including Pakistan and Sri Lanka where in addition to neuroinvasive disease, it caused complications including ocular manifestations and acute flaccid paralysis. The first major WNV acute encephalitis outbreak involving >300 cases and four deaths occurred in Kerala during 2011. Genetic analysis of WNV associated with the recent sporadic cases and outbreaks of high severity suggests close relationship with lineage 1 strains. Over the past two decades, severe outbreaks have occurred globally, attributed to factors such as international travel, urbanization, climate change, vector competence, ecological interactions, and genetic evolution of the pathogen.

3. Clinical Features

Humans

Historically, WNV epidemics of mild febrile illness were localized to Europe, Africa, the Middle East, and Asia. Clinical presentation in humans ranges from asymptomatic, mild febrile, self-limiting illness to neuroinvasive disease leading to death. As estimated, about 80% of human infections remain asymptomatic while about 20% develop systemic febrile illness (WN fever: WNF). Neuroinvasive disease develops in <1% of infected population and

results in aseptic meningitis (WN meningitis: WNM), encephalitis (WN encephalitis: WNE), or an acute poliomyelitis-like syndrome (WNV–associated acute flaccid myelitis: WNV-associated AFM) infections. Clinically WNF is characterized by abrupt onset of fever, headache, fatigue, and myalgia with occasional gastrointestinal symptoms including nausea and vomiting leading to dehydration. Frequently, WNF cases may develop transient, maculopapular, non-pruritic rash over the torso and extremities, excluding palms and soles.
Subset of WNF cases (~5%) develops neurologic disease (WNND) characterized by meningitis, encephalitis, and poliomyelitis-like disease, presenting as acute flaccid paralysis. WNM/WNE is characterized by rapid onset of headache, photophobia, back pain, confusion, and continued fever while WNV associated –AFM is characterized by acute onset of asymmetric weakness and absent reflexes without pain. WNND can result in long-term sequelae lasting from several months to few years with symptoms including cognitive impairments, movement disorders, fatigue, parkinsonism, etc. Ocular manifestations including chorioretinitis, uveitis and vitritis have been increasingly documented during recent outbreaks from Kerala, India.

Animals and Birds

Infections in birds are characterized by non-neurological signs like ruffled feathers, anorexia, dehydration and neurological symptoms like ataxia and tremors. Species like crows act as sentinels since crow deaths in USA precede human outbreaks. As compared to humans, horses develop symptoms only in 10% cases and are characterized by fever, muscle weakness, while in severe neuroinvasive diseases, changes in mentation, locomotor disorders, and/or notable cranial nerve aberrations result to fatal outcome. WNV infection leading to AFM in 40% horses develops monoparesis and paraparesis involving one or all limbs. Mortality has reduced drastically, due to availability of vaccines for horses.

4. Laboratory diagnosis

4.1 Collection of Specimen

Blood/serum (Paired samples)- one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.
CSF-Acute phase (Disease with CNS manifestations)

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample collection and transportation must be in accordance with the International Air Transport Association guidelines.

4.3 Laboratory Procedures

a. Isolation: Isolation of the virus is successfully attempted in cell cultures from clinical specimens/mosquitoes.
b. Serological test: In WNV infection, IgM antibody response mounts the protection during early infection, which appears within 3-4 days of infection in detectable levels. IgG antibody response provoked during the infection may confer long-term immunity against WNV re-infection. IgM antibodies in cerebrospinal fluid using IgM antibody capture (MAC-ELISA) can be treated as confirmatory of infection. Demonstration of IgM antibodies in serum collected during acute phase can be considered to be a presumptive diagnosis, which needs to be confirmed by detection of rise in titre in sera collected during convalescent phase. WNV-IgM antibodies are typically detected in 3-8

days of appearance of clinical symptoms and last for 30 to 90 days. IgG antibody response seems to occur 4–5 days after onset of illness and have a prolonged presence years after infection.

c. Immunohistochemistry (IHC) to detect WNV antigen in patient tissues and the exposure can be detected through demonstration of neutralizing antibodies using plaque-reduction neutralization tests (PRNT).
d. Molecular test: During acute phase of infection, demonstration of WNV RNA in clinical specimens by RT-PCR is used for West Nile virus diagnosis.

5. Treatment

Currently, no approved vaccine or specific therapy exists for humans, and the treatment is usually of supportive nature. Antiviral research is focused on targeting various stages of viral replication, blocking entry, maturation, or multiplication, which are under evaluation. Repurposing drugs like Ribavirin, Mycophenolic acid, and Sodium valproate showed promising outcomes in experimental setups. Therapeutic antibodies targeting WNV particles and the NS1 protein have also emerged as promising alternatives for high-risk populations. Vaccine development has progressed with experimental strategies tested in preclinical models and clinical trials, including live attenuated, inactivated virus-based, and recombinant DNA-based approaches.

6. Prevention and Control

Effective surveillance systems, prompt diagnosis, and vector control measures are crucial for managing and controlling WNV infections. Additionally, public health education and community awareness play a vital role in reducing the risk of exposure to mosquito bites and preventing WNV transmission. Use of insect repellents, wearing long-sleeved shirts and pants, limiting outdoor activities during mosquito breeding season and taking steps to control mosquitoes indoors and outdoors are some of the measures recommended to reduce exposure to the vector.

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1. Introduction

Yellow fever (YF) is an arboviral infection caused by the yellow fever virus (Genus; Flavivirus, Family Flaviviridae). The word "yellow" in the name refers to the jaundice that occurs in some of the affected patients. The disease is highly fatal. Nearly 50 % case fatality rate has been reported in severe cases. During the ancient times, yellow fever was one of the most common causes of feared deaths especially, in the Atlantic trade route. The disease now occurs only in South America, Central America and Africa however, historically, major outbreaks have also been reported from Europe and North America too. The disease is spread by mosquito vector. Several different species of the *Aedes* (in Africa) and Haemagogus (in South America) can transmit the virus. The disease is a major public health concern due to the reason that the mosquitoes capable of transmitting yellow fever exist in regions where the disease does not presently occur.

2. Epidemiology

2.1 Causative Agent

Yellow fever virus (family Flaviviridae and genus Flavivirus) is related to West Nile, Zika, dengue and Japanese encephalitis viruses. The genetic diversity of YFV has been under-investigated mostly due to availability of safe and effective vaccine. There are seven different YFV genotypes proposed: West African genotypes I and II, East African genotype, East/central African genotype, Angolan genotype, and South American genotype I and II. YFV contains a small glycoprotein-containing lipid envelope surrounding a nucleocapsid enclosing single stranded viral RNA. The envelope of virus comprises of two proteins: envelope (E) protein and membrane (M) protein. The E glycoprotein is the major component of the virion surface and is responsible for the receptor-mediated endocytic fusion and subsequent cell entry, as well as direct viral assembly & budding, and immunogenicity. Viral genome has single open reading frame (ORF) encoding three structural proteins (capsid [C], premembrane [PrM], and envelope [E]) and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).

2.2 Mode of Transmission

Yellow fever virus is transmitted to humans primarily through the bite of infected *Aedes* or any other Haemagogus species mosquitoes. Mosquitoes acquire the virus by feeding on infected primates (human or non-human). People infected with yellow fever virus are infectious (viraemic phase) to mosquitoes shortly before onset of fever and up to 5 days after onset of fever. However, the virus has been found in the blood up to 17 days after onset of illness. Yellow fever virus has three transmission cycles: jungle (sylvatic), intermediate (savannah), and urban.

Jungle (sylvatic) cycle: It involves transmission of the virus between non-human primates (e.g., monkeys) and mosquito species found in the forest canopy. The virus is transmitted to humans when they visit or work in the jungles.

Intermediate (savannah) cycle: In Africa, a cycle exists that involves transmission of virus from mosquitoes to humans living or working in jungle border areas. In this cycle, the virus can be transmitted from primates to humans and vice versa.

Urban cycle: It involves transmission of the virus between humans and urban mosquitoes, primarily *Aedes aegypti.* The virus is brought into urban setting by a viremic human infected in jungle or savannah cycles.

The extrinsic incubation period in *Ae. aegpti* is 9-12 days at usual tropical temperatures. Once infected, mosquitoes remain so for life. The disease is not communicated through contact secretions or fomites. Since yellow fever vaccine virus has been documented to be transmitted through breastfeeding and blood transfusions, it is likely that natural yellow fever virus may be transmitted through breastfeeding or exposure to infected blood or organs.

For further details on vector, please refer to chapter "Arthropod Vectors of Zoonotic Importance".

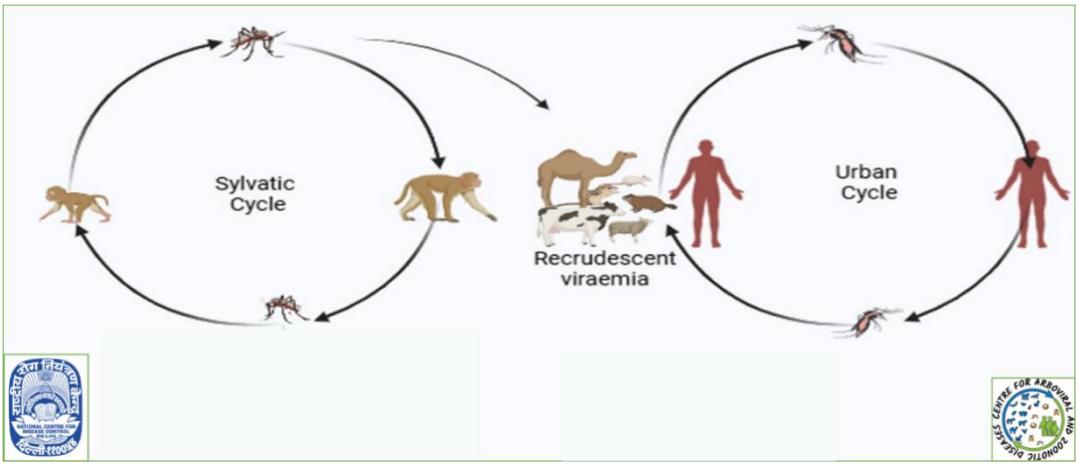


Figure 1: Transmission cyle of virus causing yellow fever.

2.3 Current Situation

Yellow fever virus is found in tropical and subtropical areas of Africa and South America. Urban outbreaks are often reported in Africa, particularly West Africa. In South America, urban outbreaks are uncommon although one occurred in 2008 in Asuncion, Paraguay. According to WHO, yellow fever is endemic in 45 Countries, (32 in Africa and l3 in Central and South America). Most frequently in sub-Saharan West Africa, outbreaks have occurred in Central and East African countries (Central African Republic, Chad, Democratic Republic of Congo, Ethiopia, Sudan Darfur and Uganda. From 2007-2009 human epizootic disease due to yellow fever virus has been seen in many areas of South America that had not seen activity for several decades (Argentina, Southern Brazil, Paraguay, and Trinidad and Tobago). Yellow fever disease and transmission was documented previously in Europe and North America, but no recent cases have been identified. There is no evidence of Yellow fever being present in Asia.

3. Clinical Features

The spectrum of the clinical disease can vary from mild flu like disease to classical triphasic hemorrhagic fever with hepatorenal involvement. An acute phase can last for 4 to 5 days. During the acute phase patients shows the symptoms such as fever, headache, backache, muscle pain, nausea, vomiting and red eyes. Following the acute phase a temporary period of remission occurs in 5% to 20% of cases. After that a period of toxic phase can follow the period of remission and present with symptoms such as jaundice, dark urine, reduced amounts of urine production, bleeding from the gums, nose or in the stool, vomiting blood, hiccups, diarrhea, slow pulse in relation to fever.

A presumptive diagnosis of yellow fever is often based on the patient's clinical

features, places and dates of travel (if the patient is from a non-endemic country or area), activities, and epidemiologic history of the location where the presumed infection occurred.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood/serum (Paired samples) - First acute phase and second convalescent phase after 2-3 weeks of onset of fever.

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedures

a. Isolation: Isolation of virus from blood by inoculation into suckling mice, mosquitoes, or cell cultures has been successful.
b. Serological test: Demonstration of viral antigen in tissues, especially liver, by use of labelled specific antibodies. Serological diagnosis includes demonstrating virus-specific IgM in early sera or a 4-fold or greater rise in titre of virus-specific antibodies in paired acute and convalescent sera. Serological cross-reactions occur with other flaviviruses. In natural infections, antibodies appear in blood within the first week.
c. Molecular test: Demonstration of viral ribonucleic acid (RNA) in blood or tissue by reverse transcription polymerase chain reaction (RTPCR).

5. Treatment

No specific treatment is available for yellow fever. In the toxic phase, supportive treatment includes therapies for treating dehydration and fever. In severe cases, death can occur between the 7th and 10th day after onset of the first symptoms.

6. Prevention and Control

Prevention of yellow fever includes vaccination, as well as avoidance of mosquito bites in areas where yellow fever is endemic. Institutional measures for prevention of yellow fever include vaccination programmes and measures of controlling mosquitoes. Effective vaccine against yellow fever is available and has been proven effective for reducing the occurrences of the disease worldwide. Currently, available vaccines protect against all yellow fever virus strains and are attenuated live virus vaccines derived from a virus originally isolated in 1927. This virus strain was attenuated by passaging in mouse embryo tissue culture and then in chicken embryo tissue culture, resulting in the 17D strain from which all current vaccines are derived. The 17D vaccine is highly effective (approaching 100%). All individuals aged 9 months or older and living in countries or areas at risk should receive yellow fever vaccine.

Further Reading

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1. Introduction

Zika Virus Disease (ZVD) is a mosquito-borne (*Aedes*) viral disease caused by Zika virus (ZIKV). It presents as mild fever, rash (mostly maculopapular), headaches, arthralgia, myalgia, asthenia, and non-purulent conjunctivitis, occurring about two to seven days after the mosquito bite. One out of five people may develop symptoms, but in those who are affected the disease is usually mild and may last between two and seven days. Its clinical manifestation is often similar to dengue which is also transmitted by the same vector. In February 2016, WHO declared Zika-related microcephaly a Public Health Emergency of International Concern (PHEIC).

2. Epidemiology

2.1 Causative Agent

Zika virus belongs to the genus Flavivirus, family Flaviviridae, and is closely related to other flaviviruses of public health relevance including dengue, chikungunya, yellow fever and West Nile viruses. It is a single-strand RNA virus with the genome of 10,794 kb length. Two ZIKV lineages have been described, African and Asian, with the African lineage spitted in East and West African clusters, some authors described three different lineages (West African, East African and Asian). The Asian lineage is expanding, this lineage emerged in the Pacific and in South America and is the currently circulating strain.

2.2 Mode of Transmission

Zika virus is transmitted primarily by *Aedes aegypti* mosquito. *Aedes albopictus* mosquito also might transmit the virus. *Aedes mosquito* is common vector to transmit the viruses that cause dengue, chikungunya, West Nile fever and ZVD. Aedes mosquito can be recognized by white markings on its legs and a marking in the form of a lyre on the upper surface of the thorax. An infective female *Aedes* mosquito acquires the virus while feeding on the blood of an infected person and then viruses are passed on to healthy humans through its bites.

The virus has been isolated from several *Aedes* mosquito species, notably *Aedes aegypti*, which is widespread in the tropics and subtropics, and *Aedes albopictus*, which is established in many parts of Europe, especially in Mediterranean countries. *Aedes polynesiensis* is also suspected to contribute to ZIKV transmission. Other modes of transmission are still under investigation. Cases of nonvector borne ZIKV transmission through sexual intercourse and perinatal transmission have been reported.

For further details on vector, please refer to chapter “Arthropod Vectors of Zoonotic Importance”.

2.3 Current Situation

The virus was first identified in a rhesus monkey in the tropical Zika Forest in Uganda in April 1947 by the scientists of the Yellow Fever Research Institute.

The first human Zika Fever infection was identified in Nigeria in 1954. Until 1981, evidence of human infection with Zika virus was reported from other African countries, such as the Central African Republic, Egypt, Gabon, Sierra Leone, Tanzania, and Uganda, as well as in parts of Asia including India, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam. Despite a global reduction in Zika cases since 2017, the circulation of this mosquito-borne virus has been confirmed in 89 countries around the world. Although incidence levels remain low, sporadic increases have been observed in some countries in recent years.

ZVD has been reported in India as outbreaks in Tamil Nadu (2017), Gujarat (2017),

Madhya Pradesh (2018) and Rajasthan (2018) which were detected during routine laboratory surveillance in patients with no history of travel outside the country. Thus, India seems to be conducive for ZVD transmission because of the availability of non-immune hosts, vectors, and a favourable transmission season. The current situation of Zika virus disease in India can be accessed through https://mohfw.gov.in/?q=pressrelease-32

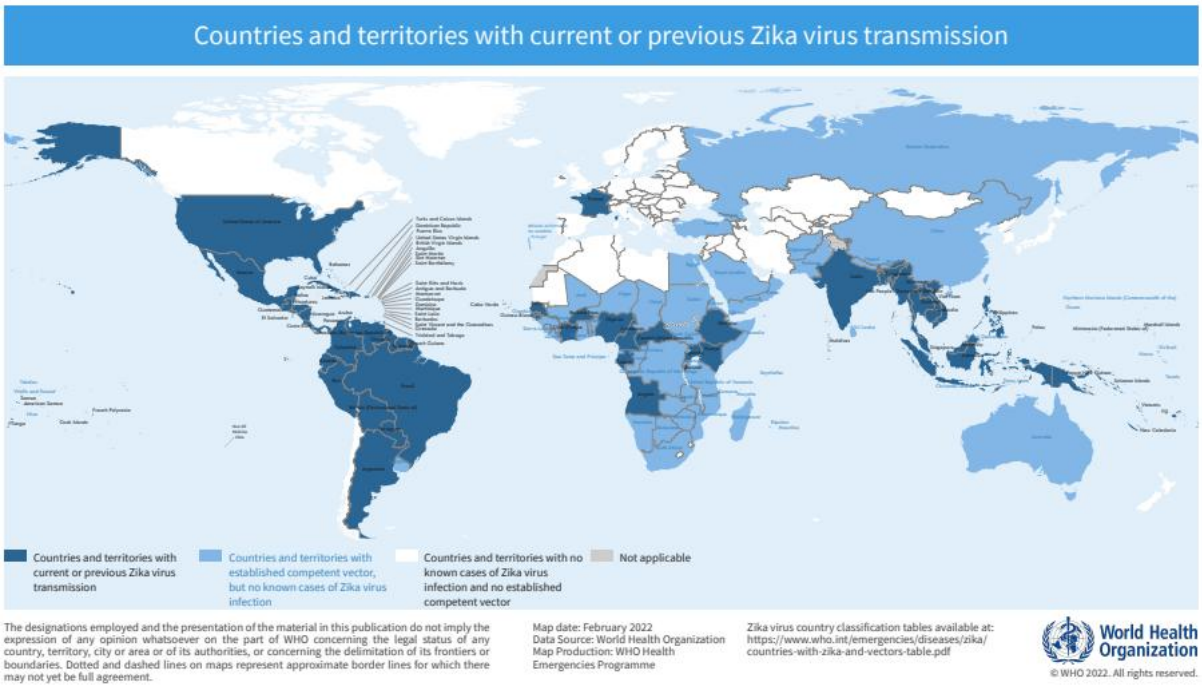


Figure 1: Countries and territories with current and previous ZIKV transmission (WHO,2022).

3. Clinical Features

An estimated 80% of persons who are infected with Zika virus are asymptomatic. In majority of cases, ZVD is a self-limiting disease. The symptoms are similar to other arbovirus infections such as dengue, and include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache. These symptoms are usually mild and last for 2-7 days. Other reported symptoms are dizziness, oedema of the extremities, retro-orbital pain, anorexia, photophobia, gastro-intestinal disorders, sore throat, cough, aphtous ulcers, back pain, sweating and lymphadenopathies. None of these symptoms are specific and ZVD can be misdiagnosed with other bacterial and viral infections, especially with other arboviruses in endemic areas.

Birth defects like microcephaly, neurologic complications like Guillain-Barré syndrome have been associated with Zika virus disease by some studies. There is no evidence that prior Zika virus infection poses a risk for birth defects in future pregnancies.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood/serum (Paired samples)- one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.

CSF-Acute phase (Disease with CNS manifestations)

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2

- 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedures

a. Isolation of the virus: Virus isolation has been successful by inoculation of clinical material in suckling mice, tissue culture or mosquitoes and further detection is performed using fluorescent antibody test or haemagglutination inhibition test.
b. Serological test: Virus-specific IgM and neutralizing antibodies typically develop toward the end of the first week of illness; cross-reaction with related flaviviruses (e.g., dengue and yellow fever viruses) is common and may be difficult to discern. Plaque-reduction neutralization testing can be performed to measure virus-specific neutralizing antibodies and discriminate between cross-reacting antibodies in primary flavivirus infections.
c. Molecular test: Viral RNA or genomic sequence can also be detected in serum, CSF or culture supernatant by doing polymerase chain reaction and gene sequencing is done to study circulating strains.

5. Treatment

Zika Virus Disease is usually relatively mild and requires symptomatic management and no specific antivirals. Acetylsalicylic acid and non-steroidal anti-inflammatory drugs are not recommended due to the increased risk of hemorrhagic syndrome described with other arboviruses as dengue.

6. Prevention and Control

The key to control ZVD is adoption of a comprehensive approach by way of regular vector surveillance and integrated management of the *Aedes* mosquitoes through biological and chemical control that are safe, cost effective; and environmental management, legislations as well as action at household and community levels. There is no approved vaccine against Zika fever in India.

Further Reading

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1. Introduction

The earliest recorded emergence of the avian influenza virus (AIV) in poultry dates back to initial reports from Italy in 1870. The virus manifests in two primary forms: low pathogenic (LP) and highly pathogenic (HP), both of which can inflict substantial economic losses in the poultry industry due to decreased egg and meat yield, elevated mortality rates, and trading constraints. Though waterfowl are common carriers of AIV, they generally do not exhibit symptoms. The first line of defense in preventing poultry infection encompasses biosecurity measures and vaccination. In this chapter, we describe the critical aspects of AIV including its biology, clinical manifestations, and prevention.

2. Epidemiology

2.1 Causative Agent

The avian influenza virus, a subtype of the influenza A virus, predominantly infects bird populations. It belongs to the Orthomyxoviridae family, with a distinguishable envelope and an irregular shape, having a size range of 80 to 120 nm. This virus is classified based on the two surface glycoproteins present on its surface: hemagglutinin (HA) and neuraminidase (NA). There are 16 known HA subtypes (H1 to H16), inclusive of neutralizing epitopes, and nine established NA subtypes (N1 to N9), though the latter does not induce a neutralizing antibody response.

Influenza A may have 144 HA and NA subtype combinations in waterfowl, with no clear link to host range. It is a negative-sense RNA virus, with eight segments encoding proteins for replication. In gallinaceous birds, pathogenicity depends on the hemagglutinin protein's cleavage site, determining systemic or localized infection. The World Organisation for Animal Health classifies avian influenza as highly pathogenic (HPAIV) based on this site, with in vivo tests confirming this through a high fatality rate in chickens. HPAIV typically causes systemic infections, while low pathogenic variants (LPAIV) affect respiratory and digestive systems. Historically, only H5 and H7 subtypes have evolved into HPAIV.

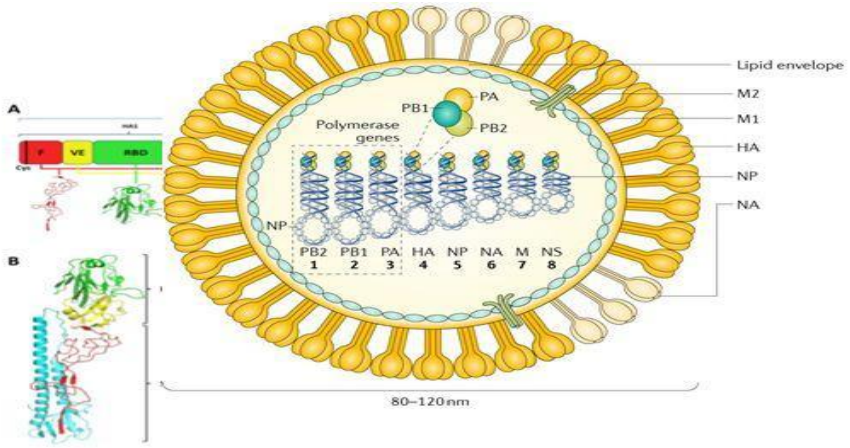


Figure 1: Influenza virus: haemagglutinin and neuraminidase antigens.

2.2 Mode of Transmission

Influenza A viruses mainly reside in aquatic birds, but occasionally infect chickens, turkeys, and mammals, including humans. H5 and H7N9 have crossed species barriers, but most transmissions don't lead to new host adaptations, except for H1N1 in swine and H3N8 in horses. These adaptations occur through changes in receptor binding and polymerase

activity. High pathogenic strains usually evolve from low pathogenic ones adapting to gallinaceous birds. While H5 and H7 viruses often remain low-pathogenic, their shift to chickens or turkeys can increase virulence.

Wild aquatic birds like ducks, geese, and swans are key in spreading avian influenza through their migratory patterns. Their behaviours, such as preening, feeding, and environmental factors like contaminated water facilitate virus transmission. These birds not only transmit but also act as reservoirs for influenza A, harboring and mutating various strains, occasionally leading to highly pathogenic avian influenza (HPAI). Often asymptomatic, they are vital in understanding avian influenza's epidemiology, linking geographic regions, and harboring future strains that threaten poultry and human health. Monitoring and researching these birds is essential for early detection and prevention of outbreaks, crucial for protecting avian and human health.

Wild aquatic birds act as the natural reservoir for diverse avian influenza A viruses, exhibiting silent carriage with minimal to no symptoms. Transmission occurs through direct and indirect contact between birds, facilitated by their migratory habits and ecological interactions. Viral replication within infected birds leads to shedding of new viral particles, perpetuating the cycle. Mutation during replication results in strain diversity. Seasonality, environmental factors, and host susceptibility influence this dynamic cycle. Monitoring wild birds serves as early detection of potential threats to poultry and humans, while understanding the cycle informs vaccine development and control strategies. This intricate life cycle in nature necessitates ongoing surveillance and research to effectively manage avian influenza viruses and protect both birds and humans.

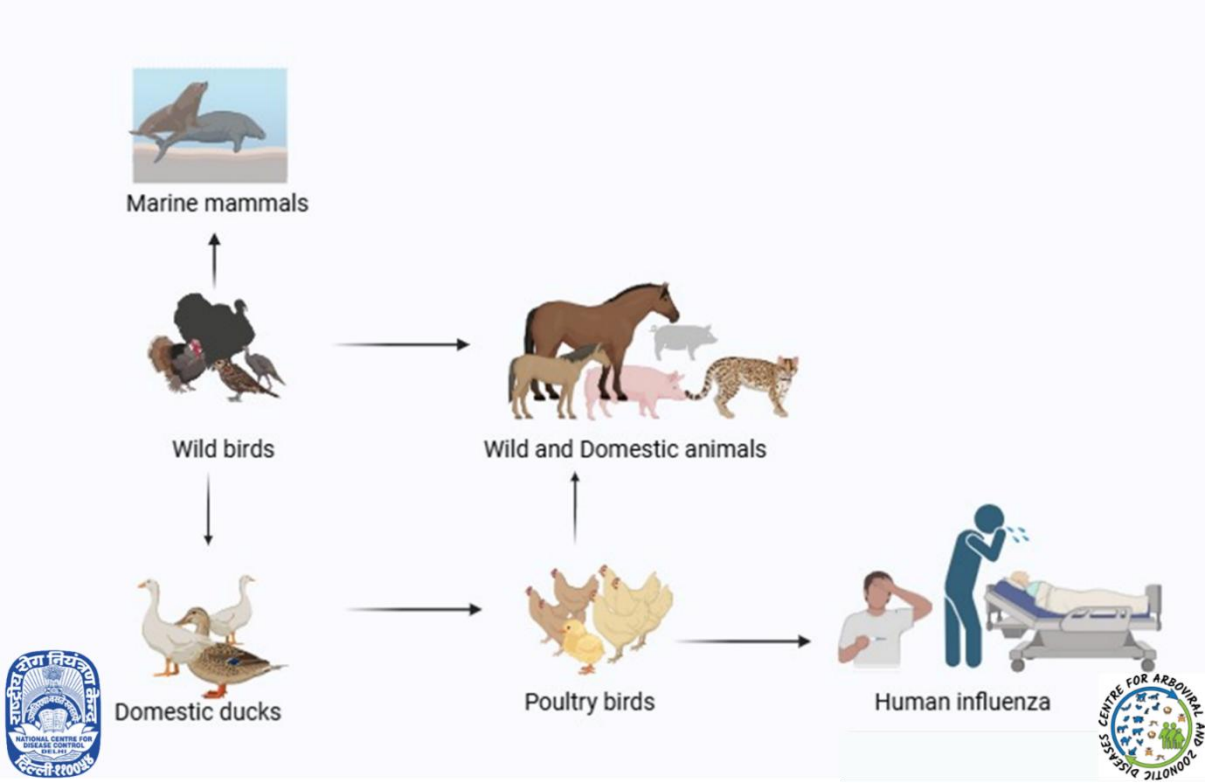


Figure 2: Transmission cycle of Influenza.

2.3 Current Situation

Globally, since 2020, the H5 clade 2.3.4.4b avian influenza, or bird flu, has been rampant, affecting poultry, wild birds, and mammals. By 2022, 67 countries across five continents reported H5N1 outbreaks, resulting in over 131 million poultry losses. In 2023, 14 more countries, mainly in the Americas, faced outbreaks. The virus also impacts diverse mammalian species, including farmed mink, seals, sea lions, cats, and dogs, with human transmission rare but highly lethal.

Swine influenza, caused by type A influenza viruses in pigs, frequently leads to outbreaks. Notable strains in U.S. pig herds include triple reassortant H1N1, H3N2, and H1N2. Pigs, susceptible to avian, human, and swine influenza viruses, can facilitate new viruses through gene reassortment. In humans, infections from variant viruses like H1N1v, H3N2v, and H1N2v, although rare, occur primarily through contact with infected pigs.

The 2012 H3N2 outbreak resulted in 309 human infections, with some severe cases. The 2009 H1N1 influenza virus, initially linked to swine, was found to have a complex gene composition from avian, human, and European and Asian swine viruses. Apart from pigs, other animals like dogs and horses have their own influenza viruses.

India experienced its first highly pathogenic avian influenza H5N1 outbreak in poultry in January 2006 in Maharashtra, attributed to clade 2.2. Since then, annual outbreaks in poultry throughout the country have been caused by newer clades, specifically 2.3.2.1 and 2.3.2.1c. Additionally, surveillance of avian influenza in poultry has identified the presence of low-pathogenicity H9N2 and H4N6 viruses. Notably, on March 15, 2019, a human case of low-pathogenicity avian influenza A(H9N2) was detected in India. To date (Jan 2024), there have been 448 recorded outbreaks of H5N1 and H5N8 avian influenza, affecting domestic or wild birds across 26 of India's 28 states, as reported on the FAO's EMPRES-i disease tracking system. Concurrently, the first outbreaks of highly pathogenic avian influenza H5N8 in Europe were reported in August 2020, with subsequent reports in poultry and wild birds in various countries across Europe, Asia, and Africa, as detailed in the WOAH online report.

Human infections with the A(H5N1) clade 2.3.4.4b virus are rare but severe, with high mortality rates. Most infections result from direct contact with infected birds. Studies show mammalian adaptation markers like 627K or 701N in the *PB2* gene, enhancing replication in mammalian cells. Fortunately, current sequences lack heightened binding to human receptors, and the virus remains susceptible to neuraminidase and endonuclease inhibitors.

Effective management of avian influenza outbreaks involves continuous risk assessment and control strategies, monitoring viral markers, transmission pathways, and potential antiviral resistance.

In April 2024, avian influenza was confirmed in dairy herds across USA with at least one human case presenting with eye inflammation.

3. Clinical Features

Humans

Human symptoms are similar to seasonal influenza, but cases can be severe, especially in individuals with chronic conditions or those under 5, over 65, pregnant, or immunocompromised.

Symptoms of bird flu infections in humans vary widely. They can be as mild as eye irritation or flu-like upper respiratory issues, or as severe as pneumonia requiring hospital care. Common symptoms include fever (100ºF [37.8ºC] or more), cough, sore throat, runny

or stuffy nose, body aches, headaches, tiredness, and breathing difficulties. Less frequent symptoms may include diarrhoea, nausea, vomiting, and seizures. Fever may not be present always.

Poultry

Chickens may exhibit no symptoms upon LPAIV infection in experimental setting, however in field setting infection may presents as a mild to moderate respiratory ailment. Although LPAIV-related mortality is infrequent and minimal, the severity can amplify with concurrent bacterial infections. Notably, the H9N2 subtype of LPAIV, is endemic in Asia and the Middle East, triggers moderate illnesses even without co-infections in chickens, quails, and other small poultry species, some of which have been transmitted to humans. The pathogenic landscape showcases variability in the clinical severity and manifestation of both HPAIV and LPAIV in chickens. HPAIV isolates may lean more towards neurotropism or viscero-tropism, with a range of mean death times. Conversely, LPAIV ordinarily remains asymptomatic in laboratory settings barring co-infections but can escalate to severe conditions in field circumstances due to various external factors.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Humans

Nasopharyngeal and oropharyngeal swab (Dacron or polyester flocked swabs in Viral transport medium)

Bronchoalveolar lavage/Tracheal aspirate/nasopharyngeal aspirate/Sputum in sterile container

Serum (2 samples, acute and convalescent)

Animals

Samples can include swabs of the trachea, oropharynx, and cloaca, as well as blood samples.

4.2 Storage and Transport

Human/Animal specimens should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedure

a. Isolation: Virus isolation and identification, requiring BSL-3 facilities, primarily uses the embryonated chicken egg method, followed by antigenic and genetic characterizations for risk assessment.
b. Serological test: ELISA and other immunoassays are used to detect antibodies indicating past exposure.
c. Molecular Test: Real-time RT-PCR is used to identify active infections. Comprehensive studies, including next-generation sequencing for full genome analysis, to understand avian virus pathogenicity and aid in epidemiological investigations, are done in reference laboratories

5. Treatment

Antiviral drugs are crucial in both preventing and treating influenza virus infections. During a typical influenza season, they are mainly used to treat severely ill patients, especially those with weakened immune systems. In pandemic scenarios, particularly before vaccine availability, these drugs are vital for treating infected patients and preventing

infection in exposed individuals. Two main classes of antiviral drugs approved for influenza include adamantanes and NA inhibitors. Adamantanes, comprising orally administered drugs like amantadine and rimantadine, target the M2 ion channel of influenza A viruses, but due to widespread resistance, their clinical use is no longer recommended globally. NA inhibitors, on the other hand, act on the viral NA protein's enzymatic activity. Oseltamivir, an orally taken prodrug, is metabolized into its active form in the liver, while zanamivir is inhaled as a powder, making it less suitable for those with respiratory issues. Peramivir, administered intravenously, is particularly useful for hospitalized patients. These antiviral options play a significant role in managing influenza infections, offering various administration methods to cater to different patient needs. Oseltamivir is the recommended drug both for prophylaxis and treatment in India.

6. Prevention and Control

Flunov, the only human vaccine authorized in Europe against avian influenza, utilizes an outdated virus clade and is an inactivated, adjuvanted H5N1 vaccine. In response to evolving viral strains, the WHO has proposed a new candidate vaccine virus (CVV) more aligned with the recent H5 viruses in the US, specifically the H5 clade 2.3.4.4b virus CVV (A/American wigeon/South Carolina/22-000345-001/2021).

India's avian influenza prevention strategy in domestic poultry emphasizes biosecurity to minimize contact between domestic and wild or infected birds. Central to this strategy is the "Prevention and Control of Infectious and Contagious Diseases in Animals Act" of 2009. This plan involves zoning during outbreaks, establishing an "Infected Zone" with a 1 km radius around the outbreak center and a "Surveillance Zone" extending up to 10 km, with measures like banning poultry movement and closing markets.

The FAO, WHO, and WOAH advocate a unified approach to avian influenza prevention, balancing public health and ecological factors. This approach includes enhanced biosecurity, timely vaccinations, a robust animal-human health surveillance network adhering to the One Health concept, and guidelines focusing on biosecurity, surveillance, risk assessment, and vaccination. Key components are enhanced surveillance, genetic sequencing, data sharing with international organizations, and rapid outbreak detection and control. They also emphasize joint animal-human health risk assessments, pandemic preparedness, effective risk communication, and specialized training for healthcare workers.

Ground-level interventions target educating high-risk occupational groups, such as veterinarians, farmers, and live animal market workers, on virus prevention and handling of potentially infected animals. Training programs, informed by the WHO resource pack, aim to minimize human exposure at the animal-environment-human interface, thus creating a safer environment through cooperative efforts, informed practices, and advanced surveillance and control technologies.

Further Readings

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1. Introduction

Among several contagions infecting livestock population, zoonosis like brucellosis creates an intensive negative impact economically on the livestock industry and is of enormous public health concern. Disease in humans is popularly known as undulant fever, remitting fever, goat fever, Maltese fever, Gibraltar fever, Crimean fever, Mediterranean fever and in cattle and buffaloes, brucellosis is called as contagious abortion or Bang's disease). As per US CDC reports, 500,000 human brucellosis cases are reported worldwide annually. The disease is disseminated through animal handling, consumption of contaminated meat, unpasteurized milk, dairy products, and unprotected close contact with infected animal secretions. In India, brucellosis infection was first identified in 1942 and from there on, the disease has been extensively reported in several states of India in both livestock and humans. The disease has an added importance to India, where the conditions are conducive for widespread human infection on account of humans cohabitating with animals in unhygienic conditions. Humans are accidental hosts, but brucellosis continues to be a major public health concern worldwide and is the most common zoonotic infection. As a global problem, brucellosis commands all attention because of its significant health and economic implications. In humans, it may take the form of a prolonged, often vague, illness requiring expensive therapy and may produce varying degrees of incapacitation even when the patient is ambulatory. In animals, serious losses occur due to abortions, still births premature birth of weaklings, infertility, loss of meat and milk.

2. Epidemiology

2.1 Causative Agent

Brucellosis is caused by facultative, Gram-negative, aerobic, nonmotile, non-spore-forming bacterium that lacks capsule, forms endospores or native plasmids, and is partially acid-fast. It measures 0.5 to 0.7µm in diameter and 0.6 to 1.5 µm in length. Thirteen species of *Brucella* have been identified so far, of 13 species, six classical and seven novel *Brucella species* have been recognized from a wide spectrum of susceptible hosts. *Brucella species* comprises *B. abortus* (cattle), *B. melitensis* (goats), *B. ovis* (sheep), *B. suis* (swine), *B. canis* (dogs) and other species include *B. inopinata* (marine toads), *B. neotomae* (wood rats), *B. ceti* (whales), *B. microti* (Microtus voles), *B. pinnipedialis* (pinnipeds) and *B. ceti* (marine mammals), *B. papionis* (primates) and *B. vulpis* (foxes). *B. abortus* comprises of mainly eight biovars, which include biovar 1 - 7 and 9, of which 1-3 are predominant in causing infections in humans *B. melitensis* biovars so far recorded comprises of biovars 1, 2 and 3 and all the biovars cause infection in small ruminants. *B. suis* is currently subdivided into five biovars (bv.) with bv. 1, 2 and 3 being responsible for brucellosis in swine, bv. 1 and 3 are pathogenic to humans and bv. 2 appears to be a very rare cause of human infection.

Brucella varies in their ability to infect host species. Cattle are the main hosts for *B. abortus* although camels, deer, dogs, horses, sheep, and humans are also susceptible to infection. *B. melitensis* chiefly infects sheep and goats although cattle and humans may also get infected. This is the most commonly reported species in human brucellosis. *B. suis* species has 5 biovars and except biovar 2, all *B. suis* biovars may be transmitted to humans. *B. canis* infects dogs and may be transmitted to no other species except man. *B. ovis* infects sheep.

2.2 *Mode of Transmission*

A large population of India living in the rural areas have close contact with domestic or wild animal populations owing to their occupation. Brucellosis in cattle seems to be

associated primarily with intensive farming practices in large organized dairy farms. Risk behaviors such as unrestricted trade and movement of animals, use of local cattle yard and fairs for trading, sending dry animals back to villages for maintenance, use of semen from unscreened bulls for artificial insemination and poor farm hygiene probably all contribute to the spread and transmission of the infection. Free grazing and movement with frequent mixing of flocks of sheep and goats also contribute to the high prevalence and wide distribution of brucellosis in these animals in India. Increasing demand for dairy products and protein, changing agricultural methods, and increased trade and movement of animals have caused concerns that the prevalence may increase.

Human brucellosis is primarily caused by *B. melitensis, B. abortus, B. suis* and *B. canis* also cause human brucellosis worldwide. Sheep, goats and their products are major sources of *B. melitensis* infection in human beings. The modes of transmission to man are ingestion, contact, inhalation and accidental inoculation. The transmission of brucella infection to man and its prevalence in different areas of the world depend upon local food habits, methods of processing milk for cream, butter, and cheese, social customs, types of animal husbandry practices, species of brucella prevalent in the region, climatic conditions, and standards of personal and environmental hygiene. The animals that are commonly known to serve as source of human infection are goats, sheep, cattle, buffalo & swine. Infection of reindeer, caribou, camels, and yaks is of epidemiological importance in some parts of the world. Dogs have long been known as carriers of brucella, and the newly recognized species *B. canis* may be transmitted from dogs to man. Farmers, farm labourers, animal attendants, stockmen, shepherds, goatherds, pig keepers, veterinarians, inseminators, slaughtermen, butchers, meat packers, collectors of foetal calf serum, processors of hides, skins and wool, renderers and dairy workers, who work with or are in close contact with animals and animal products, are at high risk of contracting the disease. Laboratory staff involved in culturing Brucella are at particular risk and the staff employed in the maintenance of farm premises, factories or plants used for processing animal products are also at risk of exposure to infection. Brucellosis can spread to humans from animals through the consumption of raw milk or milk products and through direct or indirect contact with aborted materials.

2.3 Current Situation

The presence of brucellosis in India was first established early in the previous century and since then has been reported from almost all states. Epidemiological evidence shows that in India, brucellosis is present in different species of mammalian farm animals including cattle, goats, buffalo, yaks, camels, horses and pigs. Therefore, there is an urgent need for the strict implementation of a control policy not only for cattle but also for small ruminants. Given the potentially huge economic and medical impact, a control policy could be cost-effective. India already has a policy for the control of brucellosis in dairy cattle.

Sporadic cases and occasional outbreaks of human brucellosis have been frequently reported from various parts of India, viz., Andhra Pradesh, Assam, Bihar, Delhi, Gujarat, Haryana, Jammu & Kashmir, Karnataka, Maharashtra. Madhya Pradesh, Odhisa, Pondicherry, Goa, Chandigarh, Punjab, Tamil Nadu, Meghalaya, Uttar Pradesh and West Bengal. Animal brucellosis is reported from practically every State in India except Andaman and Nicobar Islands. The disease among domestic animals is mainly prevalent in buffalo, cattle, goat, sheep, and pigs. Unfortunately, no statistical information is available about the extent of infection in animals and man in various parts of our country and this serious gap in our knowledge is a major handicap in the organization of nationwide programme for brucellosis.

In animals, acute infection of non-phagocytic epithelial cells such as placental trophoblasts, leads to reproductive tract infection, placentitis and abortion. Brucellosis sequestered within monocytes and macrophages of the reticuloendothelial system (RES), such as in lymph nodes, liver, spleen and bone marrow. Localized disease usually involves organs of the RES. Chronic brucellosis may be divided into (1) relapse, (2) chronic localized infection, and (3) delayed convalescence. Chronic localized brucellosis may require surgery (to remove the foci of infection) and antimicrobial therapy. As with most intracellular pathogens, host recovery from brucellosis involves components of cell-mediated immunity (CMI) such as interferon-gamma, cytotoxic CD4(+) and CD8(+) T cells. Once the CMI is activated, the host demonstrates dermal delayed-type hypersensitivity to antigens of Brucella.

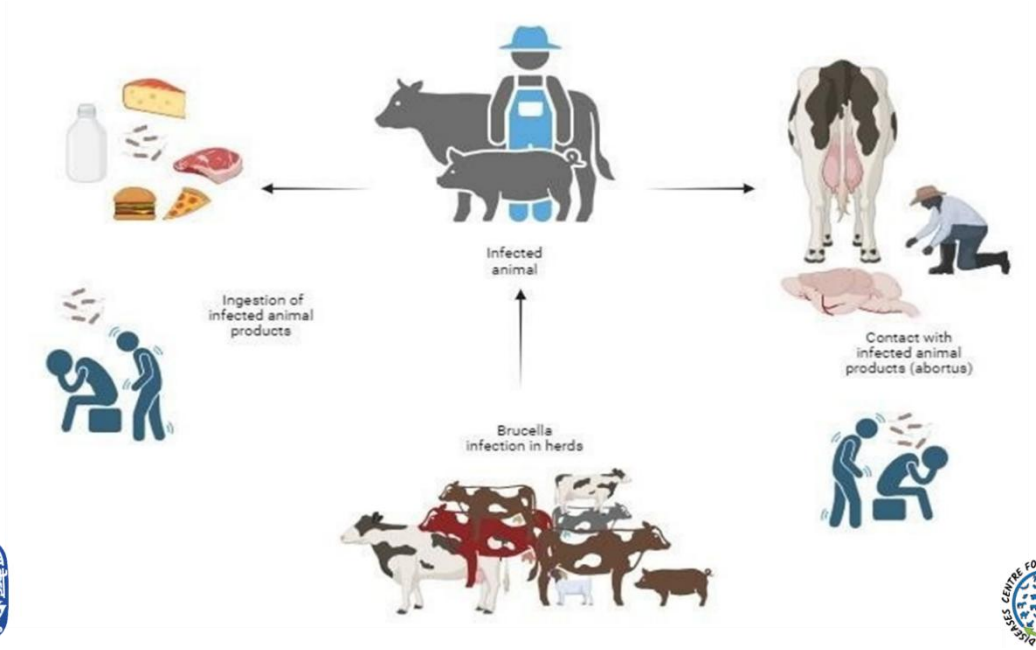


Figure 1: Transmission cycle of Brucellosis.

3. Clinical Features

Humans

Brucella may present as acute infection in humans such as febrile illness in the evening, remitting in the morning. It can also establish chronic infection within phagocytic macrophages to survive and replicate in these immunologic niche cells.

Because human brucellosis can affect any organ and body system, the presenting symptoms of the infection are not pathognomonic, and therefore, the disease may be easily confused with other medical conditions. Undulant fever' (fluctuating, irregular fever), chills, depression, weakness, headache, joint pains, generalized aches and further complications affecting cardiovascular or central nervous systems are multiple manifestations of brucellosis similar to those of other diseases, which often lead to misdiagnosis. The clinical presentation can broadly be characterized as: Acute (Pyrexia): A typical case presenting acutely (3 months with prodromal symptoms such as tiredness, chills, headaches or night sweats) or fever of 38-39°C in the evening and remitting in the morning may be considered as acute presentation.

Complicated Brucellosis: Clinical presentation based on systemic involvement can be classified as Complicated Brucellosis (Skeletal, Neurological, Hepatic, Splenic, cardiovascular, genitourinary, pulmonary, ocular).

Animals

Brucellosis in livestock is characterized by premature abortions in female cattle, typically in the last trimester, resulting in production losses and breeding related complications such as repeat breeding, retained products of conception, metritis, delivery of natimortos, weakness in offspring and reduced milk production in female animals. Most infected animals abort only once in their lifetime, but may remain infected in their entire life. Adult male cattle may develop orchitis, and epididymitis and brucellosis may cause infertility in both sexes. Hygromas can occur in leg joints and are a common manifestation of brucellosis in some tropical countries.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Human

Blood/serum (Paired samples)- one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.

Biopsy material from flat bones, liver or lymph glands.

Animal

The animal sample may vary from milk, blood serum, fluid from hygromas, Vaginal discharges, heart blood, stomach content, lymph nodes, liver, spleen from aborted foetus, placental material (cotyledon, amniotic or chorionic fluid), semen depending upon clincal manifestation.

4.2 Storage and Transport

Specimens should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample collection and transport must be in accordance to as per laid down guidelines for handling specimens from suspected, probable, or confirmed cases/contacts of high-threat pathogens.

4.3 Laboratory Procedure

The clinical presentation of brucellosis in humans is variable and nonspecific and thus, laboratory corroboration of the diagnosis is essential for the patient's proper treatment. The laboratory diagnosis of brucellosis is done to identify the animals that are infected, shedding the organism and spreading the disease. Attention is usually first drawn to the disease in animals due to abortion. Brucellosis can be diagnosed by various direct and indirect methods of diagnosis.

a. Isolation: Diagnosis of brucellosis is quite challenging due to its generic symptoms similar to febrile conditions. Data related to epidemiology, history of infection, clinical symptoms are crucial elements in the prognosis of the disease. Diagnostic methods such as culture on selective Castaneda media and isolation of *Brucella* organism prevail to be gold standard for confirmation for brucellosis prognosis. Modern automated blood culture systems enable detection of acute cases of brucellosis within the routine 5-7 days incubation, however sometimes longer incubation and performance of blind subcultures may be needed for protracted cases. Although bacteriological culture and

isolation are cumbersome /time consuming and pose high level hazard, it is the confirmatory method for the diagnosis of brucellosis.

b. Serological test: Serological tests are important for monitoring, surveillance, control and eradication programs worldwide. Antibodies start to appear in the blood in about a week after infection of Brucella, the IgM appears first followed by the appearance of IgG. The widely used immunoassays for screening of anti-Brucella antibodies generally in livestock species and humans. Milk Ring Test: Milk ring test is a screening test of great value for locating infected bovine herds, especially in areas of low prevalence. This test may be applied to individual animals or to pooled milk samples using a larger volume of milk relative to the pool size. The test is strikingly sensitive, but it is reported to produce false positive results with milk tested from S19 vaccinated animals, in case of colostrum and in milk of animals suffering from mastitis or similar diseases, hence, special attention is needed for milk sample selection.
    i. Rose Bengal Plate test (RBPT): RBPT is an agglutination-based assay, widely used for preliminary screening of brucellosis. It detects IgG1 type than IgM and IgG2. Although the test is sensitive, RBPT may have false positive results in the case of samples from vaccinated animals or by cross-reactivity with antibodies of *Yersinia enterocolitica* or *E. coli* O: 157. Therefore, positive reactions should be investigated using suitable confirmatory strategies. False-negative reactions occur rarely, due to pro-zoning, and retesting by diluting the serum sample /second sample is recommended time. Nevertheless, RBPT appears to be adequate as a screening test for detecting infected herds or to guarantee the absence of infection in brucellosis-free herds.
    ii. Serum Agglutination Test (SAT): SAT is one of the extensively used test modality for screening of human brucellosis Although this test has proved to be successful and proven effective in diagnosing Brucella infection in several brucellosis eradicated countries, this test is no longer advised by WOAH for testing of brucellosis in cattle. SAT is widely employed among humans and titres above 1:80 are considered diagnostic in conjunction with the compatible clinical presentation. A large number of modifications have been made to overcome the problems of specificity/ blocking antibodies associated with SAT such as the acidified antigen, rivanol precipitation and the use of 2-Mercaptoethanol (2-ME) are in common use.
    iii. ELISA-Enzyme Linked Immunosorbent Assays: Indirect ELISA (iELISA), a widely employed antigen based immunoassay which quantifies IgG, IgM and IgA antibodies in serum samples, have proven to be useful tool in diagnosis of Brucella. Several reports highlight the potency of competitive ELISA having recorded a higher specificity and sensitivity
    iv. Brucellin Test: This is a traditional skin test method, based on cell mediated immune assay recorded to be highly specific, however, the sensitivity reported is limited as compared to generic serology-based detection methods for brucellosis.
    v. Complement Fixation Test (CFT): CFT test is antibody specific, reliable in detecting and quantifying anti Brucella IgG1 antibodies, more than IgM antibodies. However, the inference of IgG2 in hampering the complement fixation results in negative test interpretation is reported to be a drawback of CFT. Taking into consideration, the

good test performance of CFT in terms of sensitivity and specificity, which can be due its exceptional ability in identifying IgG1 exclusive for Brucella infection, recommends it as one of the best for the diagnosis of brucellosis.

vi. Fluorescence polarization assay (FPA): FPA is a diagnostic tool which uses *B. abortus* lipopolysaccharide conjugated with FITC tracer. The test is also reported as rapid compared to conventionally available testing methodologies for testing of brucellosis. FPA is considered as confirmatory test for the detection of *B. abortus* S19 vaccinated livestock and its use in human brucellosis diagnosis is yet to be popularised.

vii. Lateral Flow Assay (LFA): LFA for brucellosis detection is an immuno chromatography-based tool for preliminary screening of anti-Brucella antibodies in multiple livestock species and humans. Moreover, LFAs are cost effective, rapid and ready to do assay compared to existing serological or molecular detection methods. A very recent report has shown the potency of LFAs fabricated with anti-Brucella polyclonal antibodies and anti-human IgG and IgM antibodies conjugated with gold coated nanoparticles for brucellosis detection in humans.

c. Molecular Detection: The need for molecular detection arises in initial infection cases. Conventional and multiplex PCR helps to target multiple DNA sequences, in-turn enhancing the test performance in terms of sensitivity, specificity at a faster pace. A number of sequences have been recognized as targets for genus-specific PCR assays for confirmation of *Brucella* species, viz., *omp2, bcsp31,16S rRNA* and *16S-23S* region. The unique repeat sequence (URS)-PCR has been approved for distinctively targeting *B. abortus* and *B. melitensis*. Species level identification of Brucella is critical in order to successfully annihilate brucellosis within a region for identification and categorization of different Brucella isolates. Besides these, AMOS- PCR detects various Brucella biovars including *B. abortus* 1, 2 and 4 and *B. melitensis*.

5. Treatment

Treatment options include doxycycline 100 mg twice a day for 45 days, plus streptomycin 1 g daily for 15 days. The main alternative therapy is doxycycline at 100 mg, twice a day for 45 days, plus rifampicin at 15mg/kg/day (600-900mg) for 45 days. Experience suggests that streptomycin may be substituted with gentamicin 5mg/kg/daily for 7–10 days, but no study directly comparing the two regimes is currently available. The optimal treatment for pregnant women, neonates and children under 8 is not yet determined; for children, options include trimethoprim/sulfamethoxazole (co-trimoxazole) combined with an aminoglycoside (streptomycin, gentamycin) or rifampicin.

6. Prevention and Control

There are two popular vaccines against animal brucellosis. Live attenuated *Brucella abortus* strain 19 (S19 vaccine) is the first effective and most extensively used vaccine for the prevention of brucellosis in cattle.

Successful eradication of brucellosis in animals is expected to prevent the risk of transmission of brucellosis to humans. Brucellosis in livestock sends alarm signals for greater chances of human brucellosis. Hence, proper public awareness of brucellosis within rural populations is one of the inevitable factors in managing the risk of brucellosis in livestock as well as in humans.

National Animal Disease Control Programme (NADCP) is a flagship scheme launched by the Ministry of Fisheries, Animal Husbandry and Dairying in September 2019 for control of Brucellosis by vaccinating 100% bovine female calves of 4-8 months of age.

Brucellosis control systems in developing countries have progressed hierarchically, however, in economically developing countries like India, brucellosis is still challenging. Health awareness training programmes for livestock handlers, veterinarians and farmers highlighting the risk of brucellosis, clinical manifestation in livestock and humans, sources of disease contradiction, preventive strategies, timely screening practices, diagnosis and treatment strategies, and livestock vaccination are crucial elements that would play an important role to curb the disease.

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1. Introduction

Coronaviruses (CoV) have previously been responsible for two significant zoonotic disease outbreaks in the 21st century: severe acute respiratory syndrome (SARS) in 2002 and Middle East Respiratory Syndrome (MERS) in 2012. The COVID-19 pandemic, triggered by the novel coronavirus SARS-CoV-2, marks a pivotal moment in our collective human history, underscoring the profound and devastating impact of zoonotic diseases on a global scale. The aftermath effects of this pandemic have been colossal, leading to massive loss of human lives, disrupting societies, overwhelming healthcare systems, and triggering a worldwide economic slowdown. The propensity of coronaviruses to cause severe respiratory and enteric diseases and their demonstrated ability to cross species barriers highlight their pandemic potential and the need for continual surveillance and research. This catastrophic unfolding of the COVID-19 pandemic beginning in 2019 serves as a potent reminder of the need for robust interdisciplinary collaboration and surveillance systems, bridging virology, biotechnology, ecology, epidemiology, and public health, to ensure early detection and rapid response to emerging zoonotic threats.

2. Epidemiology

2.1 *Causative Agent*

The causative agents of these diseases are viruses from the Coronaviridae family, specifically belonging to the subfamily Coronavirinae, within the order Nidovirales. Coronaviruses are large, enveloped viruses that contain a single strand of positive-sense RNA. The presence of club-shaped spike proteins that protrude from their surface are characteristic of Coronavirus which give the virus a crown-like appearance when viewed under Electron Microscope, which is reminiscent of the solar corona. This distinctive feature is the basis for their name - 'corona' is Latin for 'crown'. Coronaviruses are categorized into four distinct genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. Mammals are primarily infected by Alphacoronaviruses and Betacoronaviruses, whereas Gammacoronaviruses and Deltacoronaviruses are known to mainly affect birds.

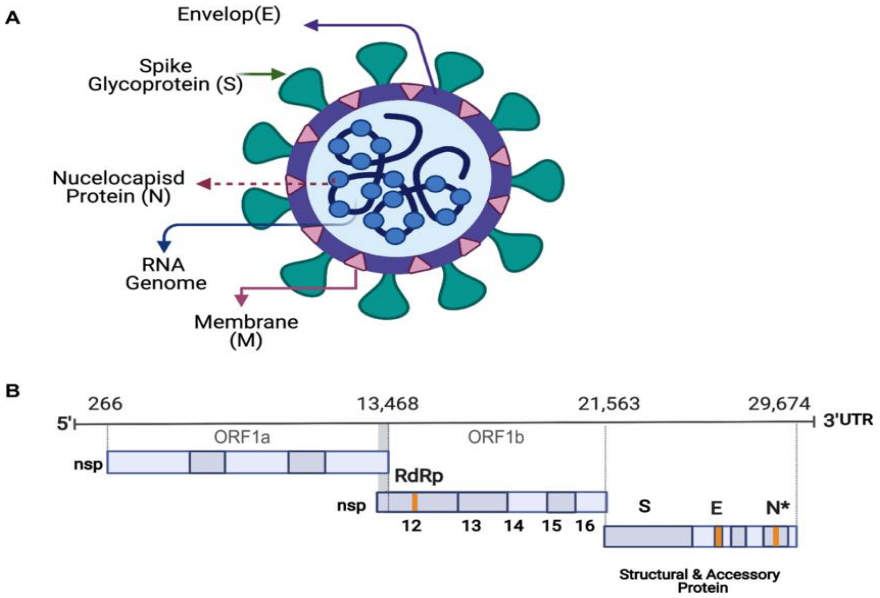


Figure 1: Schematic diagram of SARS-CoV-2: (A) SARS-CoV-2 is a positive-sense, single-stranded enveloped RNA. (Image courtesy Alsobaie, S., 2021)

SARS-CoV-2 entry into host cells is of critical importance and like its predecessors, SARS-CoV and MERS-CoV, SARS-CoV-2 utilizes the Spike (S) protein on its surface to gain entry into host cells. Over time, SARS-CoV-2 has evolved, leading to the emergence of numerous variants, some of which have been classified as Variants of Concern (VOC) due to their potential to impact disease severity, transmissibility and vaccine efficacy. The critical mutations in these VOCs often occur in the S protein, which can affect the virus's transmission dynamics, disease severity, or immune evasion capabilities.

2.2 Mode of Transmission

Coronaviruses have been identified in a wide variety of animals, but their primary natural reservoirs are believed to be bats. Bats are a crucial part of the coronavirus ecology because they host a large number of coronavirus species. Genetic analyses of these viruses suggest a longstanding evolutionary association between bats and coronaviruses. Bats are capable of hosting these viruses without developing symptoms of disease, allowing them to act as persistent reservoirs of the virus. Additionally, bats are the only mammals capable of sustained flight, leading to a wide geographical distribution and increasing the chances of virus spillover to other animals and humans. While bats are considered the original reservoir hosts of SARS-CoV, MERS-CoV, and SARS-CoV-2, these viruses are thought to have been transmitted to humans via intermediate hosts. For SARS-CoV, the intermediate host was the palm civet, while for MERS-CoV, it was dromedary camels. In the case of SARS-CoV-2, pangolins have been suspected as a possible intermediate host. These intermediate hosts are crucial to the spillover process as they enable the virus to adapt to new host species, increasing the virus's ability to infect humans and potentially leading to human-to-human transmission. Intermediate hosts usually come into contact with the virus via shared habitats or overlapping geographical regions with the reservoir host i.e bats. Changes in environmental conditions, human intrusion into animal habitats, and the wildlife trade are factors that increase interactions between bats, intermediate hosts, and humans, leading to an elevated risk of spillover events. However, it is important to note that the identification of the exact intermediate host in the case of SARS-CoV-2 is still a topic of ongoing research. Scientists are actively conducting studies to trace the origins of the virus.

Coronaviruses are primarily transmitted through respiratory droplets from coughs and sneezes, close personal contact, and touching an object or surface with the virus on it, then touching the mouth, nose, or eyes before washing hands. SARS-CoV-2 has also been detected in other types of samples, including stool, blood, and the conjunctival secretions of infected patients, indicating possible additional routes of transmission. However, respiratory droplet transmission is considered the primary route. Direct contact with live wildlife or consumption of wild animals is a significant risk factor for the zoonotic transmission of coronaviruses, as demonstrated in the initial stages of the SARS and COVID-19 outbreaks. The role of asymptomatic and pre-symptomatic individuals in the transmission of SARS-CoV-2 has also been highlighted as a significant factor in the spread of COVID-19. This poses substantial challenges for disease control as infected individuals may transmit the virus before they begin to show symptoms, making it difficult to track and isolate cases. Furthermore, the existence of variants of concern (VOCs) with potentially increased transmissibility, disease severity, or resistance to treatment or vaccination complicates the epidemiology and control of the pandemic. Therefore, understanding the transmission dynamics of SARS-CoV-2 and other coronaviruses, including the roles of different vectors, reservoirs, and hosts, is crucial for developing effective control and prevention strategies.

2.3 Current Situation

Coronaviruses (CoVs) are a large family of viruses that are prevalent worldwide. They are known to infect various species of animals, including camels, cattle, cats, and bats, and can occasionally jump to humans, causing zoonotic diseases. This is especially concerning, as the spillover from animal populations to humans has been the source of major global health emergencies, including severe acute respiratory syndrome (SARS), Middle East Respiratory Syndrome (MERS), and the ongoing pandemic of Coronavirus Disease 2019 (COVID-19). While the majority of known coronaviruses infect animals, seven types are known to cause disease in humans. Four of these - 229E, NL63, OC43, and HKU1 - usually result in mild respiratory disease, similar to the common cold, although they can lead to severe disease in immunocompromised individuals. The other three - SARS-CoV, MERS-CoV, and SARS-CoV-2 - can cause severe respiratory disease in humans and have been responsible for epidemics or pandemics. Beyond household pets, other species like minks and lions have also tested positive for SARS-CoV-2, raising concerns about potential reservoirs. These instances of reverse zoonosis highlight the importance of biosecurity measures and surveillance at the human-animal interface, particularly in settings like mink farms and zoos.

According to data from the Ministry of Health and Family Welfare (MoHFW) as of the end of 2023, India has recorded over 500,000 fatalities attributed to the COVID-19 pandemic. Additionally, there has been a significant burden of morbidity associated with the disease, impacting the health and quality of life of a large portion of the population. Large-scale testing and genomic sequencing are ongoing to monitor any changes in disease trends or viral characteristics. No indigenous cases of MERS and SARS have been reported from India.

Table 1: Coronaviruses of Public health Importance

Corona virus	Outbreak Period/ First Identified	Geographical Focus	Cases/Case Fatality rate (CFR)	Transmission
SARS-CoV	2002-2003	Primarily in East Asia, with spread to multiple countries	Over 8,000 cases confirmed CFR 10%.	Human-to-human transmission primarily via respiratory droplets.
MERS-CoV	2012 in Saudi Arabia	Mainly in the Arabian Peninsula, with cases reported globally.	Over 2,500 confirmed CFR 35%.	Initially zoonotic (from camels to humans) and then human-to-human transmission.
SARS-CoV-2	2019 in Wuhan, China.	Widespread Global pandemic	Over 750 million confirmed CFR 2-3%	Human-to-Human transmission via respiratory droplets and aerosols.

3. Clinical Features

Humans

The clinical features of COVID-19 in humans are varied, ranging from asymptomatic carriers to patients suffering from severe symptoms. The common symptoms associated with this disease include fever, dry cough, fatigue, and in more severe cases, difficulty in

breathing. Other symptoms can include loss of taste and smell, headache, sore throat, and gastrointestinal issues like diarrhea. In more severe cases, COVID-19 can lead to pneumonia, severe acute respiratory syndrome, kidney failure, and even death.

Animals

In animals, the clinical features vary widely depending on the species and the specific coronavirus involved. For instance, Feline Corona viruses (FCoVs) can result in mild intestinal disease, but can also mutate into a variant that causes Feline Infectious Peritonitis (FIP), a fatal systemic disease. Avian Infectious Bronchitis Virus (IBV) leads to respiratory symptoms in birds along with decreased egg production in chickens. The Porcine Epidemic Diarrhea Virus (PEDV) leads to severe gastroenteritis in pigs, especially in suckling piglets. Bovine Coronavirus (BCoV) can cause diarrhea and respiratory infections in cattle and is a significant cause of neonatal calf diarrhea.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Human

Nasopharyngeal and oropharyngeal swab (Dacron or polyester flocked swabs in Viral transport medium)

Broncho alveolar lavage/Tracheal aspirate/nasopharyngeal aspirate/Sputum in sterile container

Serum (2 samples, acute and convalescent)

Animals

Samples include swabs of the trachea, oropharynx, and cloaca, as well as blood samples

4.2 Storage and Transport

Human/Animal specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedure

a. Isolation: Virus isolation and identification, requiring BSL-3 facilities, primarily uses the embryonated chicken egg method, followed by antigenic and genetic characterizations for risk assessment.
b. Serological test: ELISA and other immuneassay are used to detect antibodies indicating past exposure. Rapid antigen tests are also available.
c. Molecular Test: Real-time RT-PCR are used to identify active infections, Comprehensive studies, including next-generation sequencing for full genome analysis, to understand virus pathogenicity and aid in epidemiological investigations is done in reference laboratories.

Recently, more advanced methods such as CRISPR-based diagnostics have been developed.

5. Treatment

The various therapeutic strategies explored, include antiviral drugs, monoclonal antibodies, and immunomodulators. Some, like Remdesivir, have received emergency use authorization for the treatment of COVID-19. Monoclonal antibodies, such as Bamlanivimab and Casirivimab/Imdevimab, have also shown promise, especially when administered early in the disease course. However, the rapidly mutating nature of the virus poses challenges for the long-term efficacy of these therapeutic strategies.

6. Prevention and Control

Non-pharmaceutical interventions (NPIs), such as social distancing, mask wearing, and hand hygiene, have been the cornerstone of public health strategies worldwide to slow the spread of the virus. Additionally, contact tracing, quarantine, and isolation measures have played significant roles. However, compliance with these measures and their impact on mental health, economy, and societal structures present ongoing challenges. Vaccination is a significant public health intervention, with several vaccines available and in use. Early detection through testing, contact tracing, and quarantine measures also form a critical part of disease control strategies.

To control the SARS-CoV-2 pandemic, scientists worldwide embarked on a race to develop vaccines against SARS-CoV-2. In record time, several vaccines have been developed and authorized for use, utilizing different technologies such as mRNA (Pfizer-BioNTech, Moderna), viral vectors (Oxford-AstraZeneca, Johnson & Johnson), and inactivated viruses (Sinovac, Bharat Biotech). These vaccines have demonstrated significant efficacy in clinical trials and real-world studies, significantly reducing the risk of severe disease and hospitalization. However, with the emergence of new VOCs, there is a continuous need to monitor the effectiveness of these vaccines against these new threats.

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1. Introduction

Taeniasis and cysticercosis are diseases caused by the adult and larval stages of the cestode or tapeworm parasites i.e., *Taenia saginata* and *Taenia solium* in their definitive host (humans) and intermediate hosts (cattle, pigs, humans). These intestinal infections, termed taeniasis, normally produce only mild symptoms. Eggs passed in the feces of human carriers can cause further disease if ingested by cattle, pigs, or humans. In these intermediate hosts, the egg develops to the larval (cysticercus) stage, and the disease is termed cysticercosis.

2. Epidemiology

2.1 Causative Agents

The adult stages of *T. saginata* and *T. solium* are flat tapeworms consisting of a scolex (head), and strobila (chain) of proglottids (segments), *T. saginata* can contain 1000 to 2000 proglottids and can vary in length from 4 to 12 m. *T. solium* usually contains fewer than 1000 proglottids and is usually less than 3 m in length. Mature gravid proglottids, containing a uterus filled with as many as 80,000 eggs, detach from the strobila either singly or in multiples and pass out in faeces. The adult worm can lead to an infection within the intestines by adult tapeworms leading to Taeniasis.

The larval stage of *T. saginata* infects cattle, whereas *T. solium* larvae can infect both pigs and humans and can lead to porcine/human cysticercosis. Although larvae invade mainly skeletal muscles, *T. solium* larvae frequently invade the central nervous system of humans, and are, consequently a serious public health problem. The cysticercus is composed of a scolex invaginated within a fluid filled vesicle or bladder. *T. saginata* cysticerci are 7 to 10 x 4 to 6 mm, and *T. solium* cysticerci measure 5 to 20 mm in diameter.

The canine infections are usually caused by *T. crassiceps*, *T. hydatigena, T. multiceps, T. pisiformis, T. serialis.* The feline infections are usually caused by *T. taeniaeformis*.

2.2 Mode of Transmission

Animal to Human Transmission

The cysticerci of *T. saginata* and *T. solium* are transmitted to humans through the ingestion of raw or inadequately cooked beef or pork and contaminated vegetables.

Human to Animal Transmission

Humans harboring tapeworms can contaminate the environment with large number of eggs (250,000 to 500,000 eggs per day per individual). These eggs can survive for long periods and are resistant to moderate desiccation, disinfectants, and low temperature. Tapeworm eggs can be transmitted directly to pigs and cattle by the handling and feeding of these animals by farm workers with egg contaminated hands. Infected farm workers also can cause transmission by contaminating hayfields or hay, silage, and other feeds, as well as irrigation water used on field crops, through indiscriminate defecation. A source of indirect contamination of the environment with eggs is the effluent from sewage treatment plants used for the fertilization of agricultural lands. Taeniid eggs can survive many sewage treatment plants used for the fertilization of agricultural lands. Taeniid eggs can survive many sewage treatment processes. Eggs can also be dispersed by wind and sewage water. Transmission can also occur through birds, earthworms, or beetles that have fed on raw sewage sludge because eggs can survive passage through their digestive tracts and can then contaminate pastures/vegetation where cattles/pigs can get infected while grazing.

Human to Human Transmission

Autoinfection of humans can occur with *T. solium* eggs transmitted directly from anus to mouth, or transmission can occur between individuals through contaminated hands or food. The development of human cysticercosis by internal autoinfection (whereby gravid proglottids enter the stomach via vomiting or reverse peristalsis). However, the frequency of this mode of transmission is never quantified, although there is a possibility that humans most commonly become infected like pigs through the ingestion of eggs contaminating the environment.

Ingestion of the *T. saginata* egg by cattle or the *T. solium* egg by pigs or humans results in the hatching of the larval stage (onchosphere). Activated by host gastric juices, the onchosphere penetrates the host's intestinal mucosa and enters the blood or lymphatic system. The primary site in cattle for development of the *T. saginata* onchosphere to the infective cysticercus stage is the dilated lymphatic spaces in skeletal muscles: some also reach full development in the heart. In pigs and humans, the cysticerci of *T. solium* develop in skeletal muscle, brain, myocardium, and the eye. In humans, localisation in the central nervous system is frequent. The cysticerci in cattle and pigs become infective for humans in 10 to 12 weeks after ingestion of eggs, and the life cycle is completed when these cysticerci are ingested via raw or inadequately cooked beef or pork and contaminated vegetables. The life span of the cysticerci in cattle and pigs is reported to be as short as a few months to as long as 3 years.

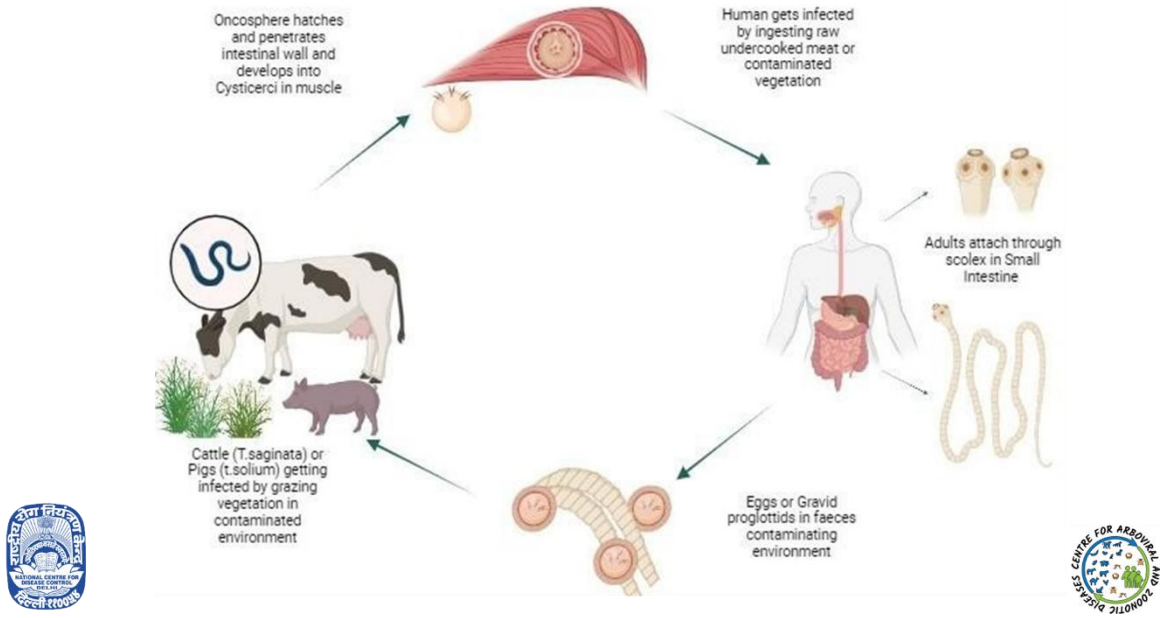


Figure 1: Life cycle of *T. saginata* and *T. solium*.

2.3 Current Situation

Taenia infection affects people worldwide, but is more prevalent in certain areas where people live near pigs, eat undercooked pork, and have poor sanitation. The first report of the incidence of Taenia infection (*T. solium* and *T. saginata*) was studied in certain areas of Tamil Nadu in 1917-1918. Taeniasis and Cysticercosis is prevalent in virtually all states of India. It is generally believed that the disease is more prevalent in north than south India. Neurocysticercosis (NCC) accounts for anywhere between 8-50% of patients presenting with recent onset of seizure. The peculiarity of the disease in India is the high

incidence of patients with the solitary form of the disease, namely solitary cysticercus granuloma (SCG). The incidence of C. bovis in cattle and C. cellulosae in pigs in India is relatively lower as compared to the other neighbouring countries.

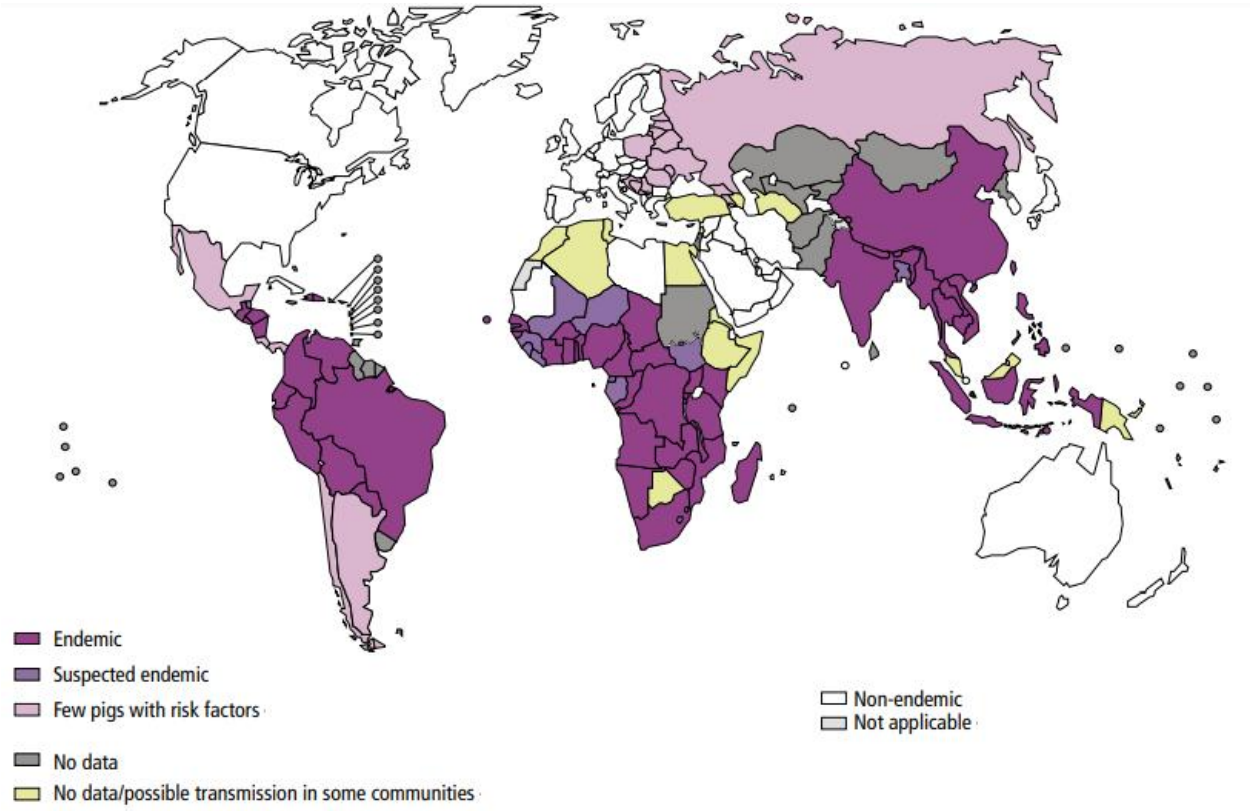


Figure 2: Endemicity of *Taenia Solium*.
(https://iris.who.int/bitstream/handle/10665/353611/WER9717-eng-fre.pdf?sequence=1)

3. Clinical Features

Humans

Infections of adult *T. saginata* and *T. solium* in humans produce only mild symptoms. These might include discharge of proglottid, abdominal pain, nausea, loss of weight, change in appetite, and headache.

Taenia solium cysticerci that develop in muscle tissue present few symptoms, although pain results if muscle cysts encroach on nerve endings. The death and calcification of muscle cysts is a more important cause of discomfort as is eye involvement. The most common locations of cysts in the eye are vitreous humor and subretina, and the inflammatory response to the parasite can lead to retinal detachment or atrophy, iridocyclitis, and chorioiditis. Myocardial cysticercosis frequently occurs in massive infections, yet clinical signs rarely result. Involvement of the central nervous system can cause serious clinical disease resulting from either an inflammatory response (to dead or dying cysticerci) or an obstruction (involving the foramina and ventricular system of the brain). Symptoms include seizures, hydrocephalus, headaches, dizziness, arterial thrombosis, loss of vision and nausea.

Animals

Dogs and cats are infected when they ingest these larval cysts while preying on or scavenging infected vertebrates. However, disease in dogs and cats due to infection with

adult *Taenia species* is rare. Passage of proglottids may be associated with perianal irritation. Occasional intestinal impactions with *Taenia spp.* necessitating surgical removal of a mass of tapeworms from the small intestine have been reported.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood/Serum: Blood sample to be collected for demonstration of antibody against *T. solium* and *T. saginata*

Stool sample: Three sequential stool specimens are necessary for reliable detection of intestinal parasites.

Biopsy Sample: Biopsy may be required in patients with suspected cysticercosis. Biopsy specimens may be taken from subcutaneous nodules or a muscle lesion and occasionally from brain in cases of suspected neurocysticercosis when radiology is not specific.

4.2 Storage and Transport

Collect the specimen in a dry, clean, sterile, leakproof container. Make sure no urine, water, soil or other material gets in the stool container. Fresh stool should be examined, processed, or preserved immediately. All specimens should be sent to laboratory immediately and if delay is anticipated, it must be refrigerated.

4.3 Laboratory Procedures

a. Microscopy: Eggs of *T. saginata* and *T. solium* can be detected in fecal smears or anal swabs. Eggs are not distributed uniformly throughout the stool, and fecal smears are accurate for diagnosis in only about 77% of cases involving a single examination; the anal swab method is about 85%accurate. Tapeworm species can be determined by examination of proglottid morphology (if a proglottid is recovered) but not by eggs alone. The proglottid is pressed between glass slides and the number of uterine branches counted. For *T. saginata* the number of branches is >18; for *T. Solium*, it is <12. Specific diagnosis also can be done by an experienced observer through examination of a scolex, which is usually voided after drug treatment.
b. Histopathology: Calcified intramuscular cysts can be detected by X-Ray analysis. Ocular cysts can be detected by ophthalmoscopy. Computed axial tomography (CAT scan) can detect cysticercosis in the central nervous system, but this procedure is not totally specific. Although the detection of multiple cystic or calcified lesions is considered highly suggestive of cysticercosis, the detection of single lesions or non-specific findings such as hydrocephalus are less diagnostically certain. Also, cysts located in certain regions of the brain (subarachnoid or intraventricular) often are not detectable by this procedure. Thus, definitive diagnosis can be accomplished by surgical removal and biopsy of nodules located on the surface of skin or in subcutaneous tissues and muscles.
c. Serology: Immunodiagnostic tests include complement fixation immunofluorescence, indirect hemagglutination assay, and enzyme linked immunosorbent assay (ELISA). These tests exhibit varying degrees of specificity and sensitivity. Currently, the ELISA shows the highest accuracy which are commercially available. Using either a crude extract of pig cysticerci as antigen or a purified fraction there of (antigen B), antibodies were detected by ELISA in sera or cerebrospinal fluid (CSF) in 70 to 80% of clinically diagnosed cases. False positive reactions were eliminated by combining ELISA with an immunoblotting technique. The complementary use of an ELISA designed for the detection of larval *T. solium* antigens and one designed for the detection of anti T.

solium antibodies in CSF was effective in detecting 69% of patients with a clinical diagnosis of cysticercosis and 100% of patients for whom cysticercosis was confirmed surgically or histologically; no false positive reactions were found. Recently, an ELISA using anyone of three purified proteins isolated from the scolex of *T. solium* metacestodes by monoclonal antibody-immunoaffinity chromatography was able to detect 100% of patients with cysticercosis, with no false positive reactions. These procedures are of greatest value when complemented and correlated with other diagnostic procedures. Enzyme linked immunelectrotransfer blot is a recent technique using *T. solium* metacestode antigen to demonstrate anti-cysticercus antibodies.

d. Molecular methods: DNA probes have been developed for rapid and sensitive diagnostic tests for detection of *T. solium* ova.

5. Treatment

Niclosamide (reported efficacy between 85 and 95%); and praziquantel (95% efficacy with single dose of 10mg per kg body weight) are effective for human taeniasis. Praziquantel has the advantage in that it effectively removes the scolex and causes destruction of the strobila without release of gravid proglottids and it has minimal side effects.

Praziquantel has been shown also to be effective in humans for *T. solium* cysticerci. Previously, the only treatment available was the surgical removal of cysts. Traditional supportive therapy includes anti-seizure and steroid medication and decompression for hydrocephalus. Praziquantel is effective also against cysticerci in cattle and pigs.

6. Prevention and Control

Prevention and Control depends on improved public health measures. Man is the sole definite host; therefore health education is of paramount importance; indiscriminate human defaecation must be strongly discouraged and sewage should be treated to kill T. solium eggs. Husbandry practices must also be improved so that pigs do not wander widely and consume human faeces. The source of infection with adult *T. solium* is infected pork. Cysticerci in meat can be killed by freezing at - 20°C for 12 hours or by cooking at 50°C. Careful inspection of pork is obviously of value. Whenever a *T. solium* infection is diagnosed in man, it should be treated with appropriate antiparasite agent(s); furthermore, family members and close contacts should be investigated for the presence of infection, and treated accordingly.

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1. Introduction

The emergence of Ebola Virus Disease (EVD) in West Africa is unprecedented in many ways. It is certainly one of the largest and deadliest outbreaks in recent times. It began in Guinea in late 2013 and spread to neighboring countries of Liberia and Sierre Leone. The outbreak has mounted exceptional concern, preparedness and response worldwide as it is dreaded as one of the most virulent disease causing high fatality in humans. It has no specific treatment or vaccine despite it being known since 1976 when it first appeared in Democratic Republic of the Congo (DRC) in two simultaneous outbreaks in Nzara, Sudan, and Yambuku, DRC. Formerly known as Ebola hemorrhagic fever, in the current outbreak EVD involved the health care workers and further weakened the already compromised health care system in the affected countries.

2. Epidemiology

2.1 Causative Agent

Ebolavirus is one of 3 members of the Filoviridae family (filovirus), along with genus Marburgvirus and Cuevavirus. There are 5 distinct species of Ebolavirus viz. Bundibugyo ebolavirus (BDBV), Zaire ebolavirus (EBOV), Reston ebolavirus (RESTV), Sudan ebolavirus (SUDV) and Taï Forest ebolavirus (TAFV). While BDBV, EBOV, and SUDV have been associated with large EVD outbreaks in Africa, RESTV and TAFV have not yet been implicated in a human outbreak. The RESTV species, found in Philippines and the People's Republic of China, can infect humans, but no illness or death in humans has been reported.

2.2 Mode of Transmission

The most common mode of human-to-human transmission is direct contact through broken skin or unprotected mucous membranes e.g. the eyes, nose, or mouth, with the blood or body fluids (urine, feces, saliva, semen, and other secretions) of a person who is sick or has died of EVD. However, infection cannot be transmitted before the appearance of symptoms. Transmission may also occur with contaminated needles or infected animals. Ebola does not spread through the air or by water. In Africa, infection has been documented through the handling of infected chimpanzees, gorillas, fruit bats, monkeys, forest antelope and porcupines which were found ill or dead or in the rainforest. People are infectious as long as their blood and secretions contain the virus. Ebola virus has been isolated from semen for more than 5 months after onset of illness. High Risk Group includes those who are in close contact viz. relatives or Health Care Workers (HCWs) of EVD patient, handlers of dead body of an EVD patient.

2.3 Current Situation

The first EVD outbreaks occurred in remote villages in Central Africa, near tropical rainforests. The 2014–2016 outbreak in West Africa was the largest and most complex Ebola outbreak since the virus was first discovered in 1976. It also spread to other countries starting in Guinea then moving across land borders to Sierra Leone and Liberia thereafter.
The natural reservoir of Ebola viruses has not yet been proven conclusively. However, fruit bats Hypsignathusmonstrosus, Epomopsfranqueti and Myonycteristorquata, may be the natural hosts in Africa. Human beings can get infected and initiate human to human transmission by contact with infected animals or their carcasses.

One Imported case has been reported of EVD till date in India. A 26-year-old male, Indian, had travelled from Liberia to India and reached Delhi on 10th November 2014. He underwent the mandatory screening at the Delhi Airport. He carried a certificate of medical clearance from the Ministry of Health and Social Welfare, Government of Liberia mentioning

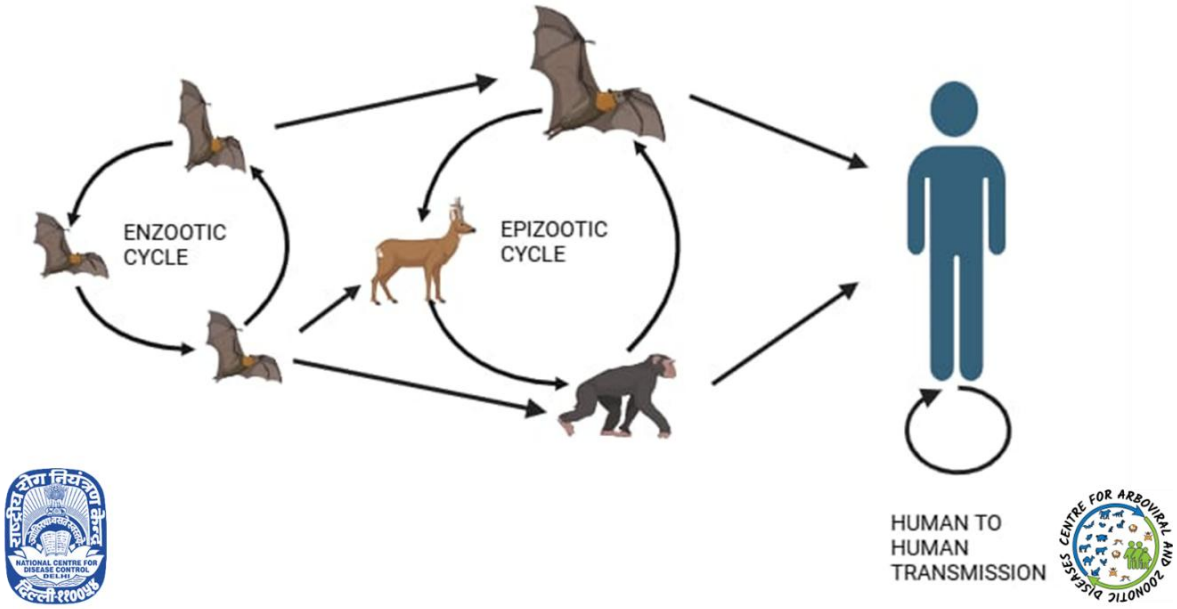


Figure 1: Transmission cycle of Ebola Virus.

that 'he has successfully undergone care and treatment related to Ebola Virus Disease after post-treatment assessment he has been declared free of any clinical signs and symptoms and confirmed negative by laboratory analysis".
As a matter of abundant caution, he was quarantined at the Airport Health Organization's Quarantine Centre at Delhi Airport. Although as per WHO and CDC specifications, he was deemed to be cured. However, as has been reported in the past, the virus may continue to be positive in secretions like urine and semen for a longer time. Before releasing him from quarantine, a decision was taken to test his other body fluids and was released only when clinical specimens turned negative.

3. Clinical Features

After an incubation period of 2-21 days (average 8-10 days) patient develops fever and non-specific symptoms such as severe headache, fatigue, muscle pain, vomiting, diarrhea, abdominal (stomach) pain, or unexplained hemorrhage (bleeding or bruising). Patient may rapidly progress to multi-organ involvement, hemorrhages and shock.

4. Laboratory Diagnosis

As Ebola virus is classified as risk group 4 virus and hence the clinical samples should be handled in specially-equipped, high containment biosafety level laboratories based on risk-assessment.

4.1 Collection of Specimen

Ante-mortem: Blood sample: Serum/Plasma, Urine, Semen, etc.
Postmortem: Tissue sample (liver, spleen, bone marrow, kidney, Lung and brain).
In the first few days of illness diagnosis is achieved by virus/genome detection in blood or tissue samples.

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and

reach reference testing laboratories within 48 hours. Sample collection and transport must be in accordance to the guidelines for handling specimens from suspected, probable, or confirmed cases/contacts of high-threat pathogens.

4.3 Laboratory Procedure

a. Virus Isolation: It should always be carried out in maximum bio-containment laboratory i.e. BSL -4. The virus may be isolated from blood or tissue specimens in the first five days of illness, and grown in cell lines.
b. Serology: Viral antigens may be detected in blood/serum samples using immune-fluorescence/ELISA
c. Molecular Technique: The Real time RT-PCR is the test of choice for laboratory diagnosis of Ebola virus infection for detecting virus specific genome. It is a sensitive and specific method.

5. Treatment

There is no specific anti-viral drug or vaccine available for EVD. Symptomatic treatment is given and complications are managed as they appear. Maintenance of hydration, electrolyte balance and managing infections remains the mainstay of the treatment. ZMapp (Mapp Biopharmaceutical Inc.), cocktail of three different monoclonal antibodies that bind to protein of Ebola virus and some investigational vaccines and drugs are being developed.

6. Prevention and Control

Undoubtedly, EVD with its high mortality and morbidity, rapid and high rate of human-to-human transmission and no approved vaccine or therapeutics, has heightened anxiety across the world. However, the strategies to control EVD are simple and time-tested epidemiological principles of early detection prompt response and prevention of further spread. The importance of communicating clear and accurate information to all stakeholders including the general public is the need of the hour. Central and state governments have demonstrated high-level commitment and preparatory activities are being undertaken.

All the health care workers who come in contact with the suspect/confirmed case of EVD should wear complete PPE which includes double gloves, boot covers that are waterproof and go to at least mid-calf, single-use impermeable gown, respirators, including either N95 respirators or powered air purifying respirator (PAPR), face shield, surgical hoods to ensure complete coverage of the head and neck. The enhanced guidance is centred on the principle of “no skin exposure” when PPE is worn.

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1. Introduction

Echinococcosis is a zoonotic disease, caused by the larval stage of tapeworms (*Echinococcus spp*.). Dogs, Foxes, jackals, etc, are definitive hosts of this parasite. Eggs of these tapeworms are excreted in the feces of infected dogs and other animals. Humans are the accidental hosts of this parasite, who acquire infection through ingestion of contaminated food, water, or soil, or by direct contact with animals.

The two most important and frequently encountered forms of medical and public health importance are cystic Echinococcosis (CE) and alveolar Echinococcosis (AE).

Hydatid disease is prevalent in India and is of great concern due to associated morbidity and huge economic implications. A detailed understanding of the epidemiology of infection in animals is a crucial factor in curbing the transmission to humans.

2. Epidemiology

2.1 Causative Agent

The tapeworm *Echinococcus*, belongs to family Taeniidae. Four species cause infections in humans, *E. granulosus* causes cystic Echinococcosis (CE). *E. multilocularis* which causes alveolar Echinococcosis (AE) is the most common species. Other species like *E. vogeli* and *E. oligarthrus* cause polycystic and unicystic echinococcosis, respectively, known as "Neotropical Echinococcosis" and are rare in humans.

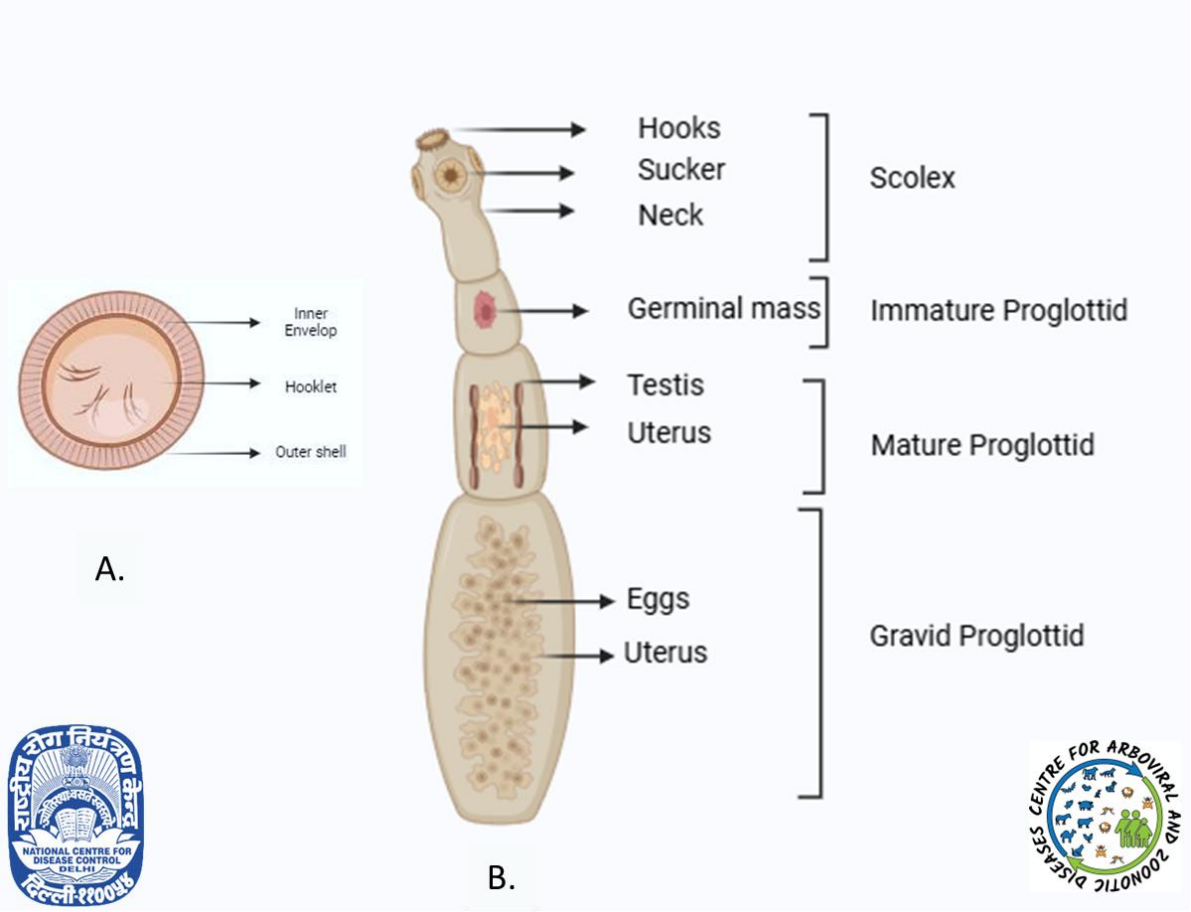


Figure 1: Ova (A.) and Adult form (B.) of *Echinococcus* species.

Developmental stages of Echinococcus are eggs, larvae, and adults.

Eggs: Small and round (30-43µm in diameter) like other eggs of *Taenia* species, thick-shelled and contain a hexacanth embryo (6-hooked) known as oncosphere. Eggs are the infective form for humans and herbivores like cattle and sheep, etc.

Larvae: The encysted larval (metacestode) stage is known as a bladder-worm or hydatid, and it develops inside the intermediate host (eg. human). Hydatid cysts have three layers, outer pericyst/ectocyst derived from host cells, middle laminated, and inner germinal layer.

Adult worms: Small-sized, about 3-6mm long, have a scolex(head), with three attached segments. The scolex has four lateral suckers and a rostellum along with double crown of 28-50 hooks. The anterior segment is immature while middle segment is mature with functional testes and ovaries. The posterior segment contains gravid uterus.

Genotypes: Parasitic mitochondrial DNA sequence studies have identified 10 different genotypes. These are grouped into 4 different groups: *E. granulosus sensu stricto* (G1-G3), *E. equinus* (G4), *E. ortleppi* (G5), E. canadensis (G6, G7, G8 and G10). *E. felidis* (lion strain) is closely related to *E. granulosus sensu stricto* and is placed within the *E. granulosus complex*. All other strains except G4 have been identified from clinical human cases. Worldwide, G1 genotype is most common in humans.

2.2 Mode of Transmission

CE is usually maintained by the domestic cycle (dog/domestic ungulate) where humans cohabit with dogs fed on raw livestock offal. AE is mainly supported by a sylvatic cycle (fox/rodents), which can be linked with domestic dogs and cats.

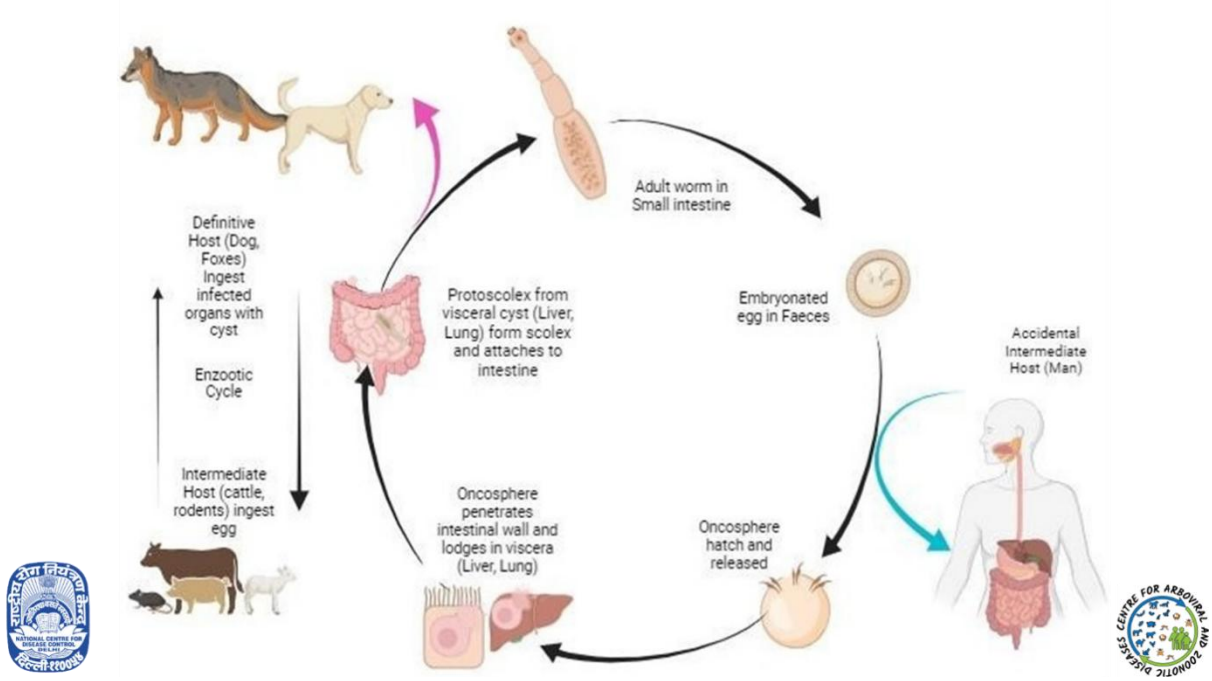


Figure 2: Life cycle of *Echinococcus granulosus*.

The adult *Echinococcus species* reside within the small intestine of the definitive host eg. Dog, Fox, Jackals. Eggs are released by gravid proglottids and excreted in faeces. Eggs are swallowed by a suitable intermediate host usually sheep, goat, pig, cattle or horse during grazing or human (esp. children) due to close contact. The eggs hatch within the small

intestine(duodenum) and discharge hexacanth embryo. The chitinous shells of embryos lyse and penetrate mucosa. These are then carried out to various organs via portal system, pulmonary circulation, and rarely systemic circulation. Wherever it settles, forms a hydatid cyst (liver followed by lung and other visceral organs) (Hydatid: a Greek word means watery vesicles). The intermediate host harbouring cyst is consumed by definitive host where adult form develops within 5-12 weeks, completing the life cycle. As dogs have no access to hydatid cysts developed in viscera of humans, larva cannot develop further and the life cycle of the parasite comes to a dead end.

In case of *E. multilocularis*, major definitive hosts are foxes, dogs, cats, coyotes, wolves, etc. Small rodents act as intermediate hosts.

2.3 Current Situation

The epidemiology of echinococcal disease varies from species to species. *Although E. granulosus is* almost prevalent throughout the world, higher incidence rates are found in South America, the former Soviet Union, the Middle East and the eastern Mediterranean, few sub-Saharan African countries, and western China. In particular, the areas where dogs can consume organs of infected animals, such as the rural and the grazing ones, possess higher infection rates.*E. multilocularis* mostly limited to the Northern Hemisphere in areas such as the northern parts of Europe, Asia, and North America, as well as Central Europe. On the other hand, the remaining two *Echinococcus species, E. vogeli* and *E. oligarthrus*, are only limited to South and Central America.

E. granulosus and *E. multilocularis* are two most common species worldwide including India. The prevalence of echinococcosis increases with age, being more common in elderly and immunocompromised individuals. CE is more common among 30 to 40 years of age group and AE over 50 years of age. Numerous studies have reported a higher prevalence of echinococcosis in old domestic and wild animals. Environmental factors such as seasonal variation, rainfall, high altitude also play an important role in Echinococcosis. Hydatid disease is prevalent in India and is of great importance due to associated morbidity and huge economic burden. Studies on Echinococcosis serosurveillance in India are scanty. The occurrence in humans ranges from 1 to 200 per 100,000 population. Increased incidence level has been documented in Kashmir, Andhra Pradesh, Tamil Nadu, and central India. An increase in seroprevalence from 10.9% in 1984 – 1988 to 28.6% in 2004 to 2015 has been reported, but a decrease to 16.2% was noted from 2016 to 2021. A study on genotypes of Echinococcus from northern India showed that the most common genotype was G3 (53%), followed by G1 (41%).

A study on CE among different animals from Maharashtra showed that prevalence was 3%, 2.05%, 1.28%, 0.09%, and 0.01% among cattle, buffalo, pigs, sheep, and goat, respectively. Another study from southern India reported a prevalence of *E. granulosus* 7.0%, 7.1%, 9.4%, and 11.5% in sheep, cattle, buffalo, and pigs, respectively. Another study carried out in Puducherry showed higher infection rates: in sheep (37.8%) and goats (47.6%). However, in Uttar Pradesh, the prevalence of *E. granulosus* was found to be 2.9%, 1.4% and 0.9% in sheep, goats, and pigs, respectively.

3. Clinical Features

Human

The signs and symptoms vary depending on the site of the cyst occurrence. Common findings in liver Echinococcosis include abdominal pain, decreased appetite, hepatomegaly, a palpable mass, abdominal distention, and in lung Echinococcosis include chronic cough,

chest pain, and shortness of breath. *E. multilocularis* infection is more likely to be symptomatic than *E. granulosus* infection. Extrahepatic primary disease is very rare (<1%).

Animals

In livestock animals, symptoms include reduced growth, decreased production of milk, meat and wool, reduced birth rate and losses due to condemnation of organs at post-mortem examination. The diagnosis of Echinococcosis is typically established by imaging techniques in combination with serology. Ultrasonography is the technique of choice for the diagnosis of both CE and AE in humans, with sensitivity ranging from 90-95%.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood/Serum

Stool sample: Three sequential stool specimens are necessary for reliable detection of intestinal parasites

Fine needle aspiration biopsy performed under ultrasound or CT guidance

4.2 Storage and Transport

Collect the specimen in a dry, clean, sterile, leakproof container. Make sure no urine, water, soil or other material gets in the stool container. Fresh stool should be examined, processed, or preserved immediately. All specimens should be sent to laboratory immediately, and if delay is anticipated, it must be refrigerated at 2-8°C.

4.3 Laboratory Procedure

a. Microscopy: Hydatid fluid is aspirated and examined for presence of Hydatid sand. Hydatid sand comprises of protoscolices (hooklets and scolexes) and brood capsule.

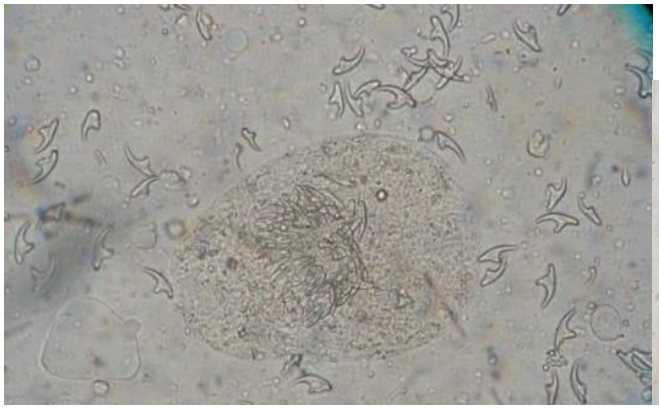


Figure 3: Hooklets of Hydatid.



Figure 4: Hydatid sand.

b. Serology: It is useful for primary diagnosis and follow-up after treatment. Antibody detection is more sensitive than antigen detection for diagnosis *of E. granulosus*.

c. Molecular methods: Molecular methods eg, PCR, end-point PCR assays and qPCR assay targets cytochrome c oxidase I (cox1) and NADH dehydrogenase subunit I (nad1) for diagnosis.

5. Treatment

Humans

The treatment options for Human CE and AE include a) Percutaneous treatment of the hydatid cysts with the PAIR (Puncture, Aspiration, Injection, Re-aspiration) technique; b)

Surgical removal of the cyst. c) Anti-parasitic drug treatment with Albendazole (primary agent) OR Mebendazole (as an alternative).

Animals

The treatment option for animals includes a) Systemic benzimidazole and praziquantel administration b) Ultrasound-guided cyst drainage with ethanol ablation c) Surgical resection.

6. Prevention and Control

Controlling parasitic infection in animals is crucial to reduce the incidence of human disease. Close vigilance and different control measures include:

a) Preventing access of dogs to livestock carcasses or slaughter wastes from farms, households, abattoirs or butchers.
b) Avoiding contact with wild animals such as foxes, coyotes and stray dogs.
c) Control stray dog populations.
d) Treating dogs with an anthelmintic (praziquantel) to kill the adult tapeworm.
e) Detecting cysts at meat inspection, thus targeting infected farms or communities.
f) Vaccinating sheep (or other livestock) to protect against the development of the larval stage of *E. granulosus*.
g) Avoiding consumption of food or water contaminated by fecal matter from dogs.
h) Strict hand hygiene after handling animals and in laboratory.

The cycle of *E. granulosus* in wildlife is not amenable to control, but by discouraging scavenging, and implementing hygiene, the infection of domestic animals and the subsequent spread to humans can be reduced. Control of *E. multilocularis* is much more difficult because of the wildlife cycle between foxes and rodents, but reduction in transmission has been achieved by use of Praziquantel baits for foxes and dosing of owned dogs where spill-over into the dog population occurs.

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1. Introduction

Fungal zoonoses represent a subset of zoonotic diseases where the causative agents are fungi. These pathogens can jump the species barrier, infecting both animals and humans and often require a coordinated effort between veterinary and medical professionals for effective management. One of the defining characteristics of fungal zoonoses is their capacity to emerge or re-emerge unexpectedly. These diseases, which often elude the spotlight of public health scrutiny, have gained prominence in recent years.

2. Epidemiology

Increased incidence of fungal zoonosis is attributable to altered geographic distribution, climate change, environmental factors, host behavior and habitats, globalization and travel, variability in fungal virulence and drug resistance, immunocompromised states, extreme age and occupational activities.

2.1 Altered Geographic Distribution

Blastomycosis, caused by the fungus Blastomyces dermatitidis, and Coccidioidomycosis, caused by *Coccidioides immitis* and *Coccidioides posadasii*, exemplify the evolving geographic distribution of fungal zoonoses in the United States. Traditionally confined to specific regions like the Great Lakes and Mississippi River valleys, though recent reports have documented blastomycosis cases outside these areas. Similarly, Coccidioidomycosis, primarily endemic in the southwestern U.S., has extended its reach due to changing environmental conditions. Cases have emerged in non-traditional endemic regions such as southern Washington and Oregon, indicating a shift in the geographical distribution of these diseases.

2.2 Climate Change and Environmental Factors

Sporotrichosis, typically associated with tropical and subtropical regions, is venturing into new ecological niches. The influence of climate change, which can alter temperature and humidity patterns, may be encouraging the growth and spread of fungi responsible for these infections. The fungus *Sporothrix spp*. responsible for sporotrichosis, can thrive in environments previously considered inhospitable, thereby complicating our understanding of its geographic distribution.

2.3 Changes in Host Behaviour and Habitats

Cryptococcosis wider host range: C*ryptococc*us *neoformans* and *Cryptococcus gattii*, the agents of cryptococcosis, have shown remarkable adaptability to different host species. These fungi, which initially affected primarily immunocompromised individuals, are increasingly found in immunocompetent individuals and a broader range of animal hosts. The ability to infect diverse hosts hints at changes in the behavior and habitats of both fungal pathogens and their hosts, contributing to the emergence of this threat.

2.4 Globalization and Travel

The ease and frequency of international travel and trade play a pivotal role in the spread of fungal zoonotic diseases. Infected individuals or animals can transport fungal spores across borders, introducing these pathogens to new regions. This globalization of fungal zoonoses blurs previously established boundaries of endemicity and further complicates disease control efforts.

2.5 Variability in Fungal Virulence and Drug Resistance

The evolving nature of fungal zoonoses is not solely a result of changing ecological factors but is also influenced by the fungi themselves. Variability in virulence among fungal strains can lead to differing clinical outcomes in infected individuals. Additionally, the

development of drug-resistant strains, as seen in some fungal species, poses significant challenges for treatment and control. For example:

a) *Cryptococcus neoformans* and Virulence Variability: *Cryptococcus neoformans*, a fungus found in soil and bird droppings, can cause severe lung and central nervous system infections, especially in immunocompromised individuals. Variability in virulence factors among different strains of *C. neoformans* influences the severity of the disease. Strains with specific virulence attributes can lead to more aggressive infections and poorer clinical outcomes.

b) *Trichophyton rubrum* and Antifungal Resistance: *Trichophyton rubrum* is a dermatophyte fungus causing common skin infections like athlete's foot and ringworm. There have been reports of antifungal resistance, particularly against azole medications. Resistant strains make topical treatment less effective, leading to persistent or recurrent infections in affected individuals. T. indotineae has become a problematic dermatophyte due to its predominantly in vitro genetic resistance to terbinafine owing to point mutation to the squalene epoxidase gene and itraconazole is effective in such cases of infection.

c) *Blastomyces dermatitidis* and Geographic Variability: *Blastomyces dermatitidis*, causing blastomycosis, exhibits geographic variability in virulence. Strains from different regions can have varying levels of pathogenicity, affecting the clinical presentation and severity of the disease in infected individuals. This variability poses challenges in understanding disease patterns and predicting clinical outcomes accurately.

2.6 Underreporting and Awareness

Underreporting and misdiagnosis are common issues in fungal zoonotic diseases due to their complexity and the need for specialized diagnostic tools and expertise. This lack of awareness can lead to delayed or inadequate treatment, potentially contributing to the emergence of these infections.

2.7 Risk Factors

Fungal zoonotic diseases, which can affect both animals and humans, are influenced by a range of risk factors that determine an individual's or a population's susceptibility to infection. These factors are crucial in understanding how and why certain individuals or communities may be more vulnerable to fungal zoonoses. Here, we delve into the details of these risk factors, shedding light on the complexities of fungal zoonotic disease transmission.

2.8 Immunocompromised States

Individuals with compromised immune systems are at a significantly higher risk of fungal zoonotic infections. This category includes:

a) HIV/AIDS Patients: The human immunodeficiency virus (HIV) weakens the immune system, making affected individuals highly susceptible to fungal infections. Opportunistic fungal pathogens can take advantage of the weakened host defenses. Examples *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis.*

b) Organ Transplant Recipients: Patients who receive organ transplants often take immunosuppressive medications to prevent organ rejection. These medications reduce immune responses, increasing the risk of fungal infections. Example *Sporothrix* species.

c) Patients on Immunosuppressive Therapies: People with autoimmune diseases like rheumatoid arthritis, lupus, or inflammatory bowel disease may require immunosuppressive drugs. These medications can compromise the immune system's ability to fight off fungal pathogens. Examples: *Histoplasma capsulatum, Coccidioides immitis, Sporothrix species*, etc.

2.9 Age and Vulnerability

Certain groups are more susceptible to fungal zoonotic infections:

a) Infants and Elderly: Infants have developing immune systems, while the elderly often have weakened immune defenses. Both age groups may be more vulnerable to fungal infections.
b) Occupational Activities: Occupational or recreational exposure to certain environments or animals can increase the risk of fungal zoonoses:
   Farmers: Farmers are at risk due to their close contact with soil, animals, and organic matter, which can harbor fungal pathogens like *Blastomyces dermatitidis* and *Coccidioides species*.
   Veterinarians: Veterinary professionals working with animals can be exposed to fungal pathogens, especially when handling sick animals or performing surgical procedures.
   Spelunkers (Cave Explorers): Fungi like *Histoplasma capsulatum* are often found in soil enriched with bat or bird droppings, making cave explorers susceptible to histoplasmosis.
c) Geographic and Environmental Factors: Residence or travels can significantly impact the risk of fungal zoonotic infections:
   Endemic Regions: Living or traveling in areas where specific fungi are endemic increases the likelihood of exposure. For instance, living in the southwestern United States elevates the risk of Coccidioidomycosis.
d) Underlying Medical Conditions: Certain medical conditions can predispose individuals to fungal zoonotic infections:
   Diabetes: Poorly controlled diabetes can weaken the immune system and create an environment conducive to fungal growth. Examples *Histoplasma capsulatum, Blastomyces dermatitidis, Sporothrix species.*
   Chronic Lung Disease: Conditions like chronic obstructive pulmonary disease (COPD) can impair lung function, increasing susceptibility to respiratory fungal infections. Example *Histoplasma capsulatum.*
e) Environmental Exposures Environmental factors play a significant role in fungal zoonotic diseases:
   Exposure to Contaminated Soil: Activities involving soil disturbance, such as construction work or gardening, can expose individuals to fungal spores present in the soil. Examples include *Coccidioides immitis* and *Coccidioides posadasii.*
   Animal Contact: Direct contact with infected animals or their excreta can lead to transmission. For instance, handling contaminated bird droppings can result in Cryptococcosis.

3. Diagnosis of Fungal Zoonoses

Fungal zoonotic diseases, are infections caused by fungi capable of infecting both animals and humans, present a wide spectrum of clinical manifestations. Accurate diagnosis is crucial for initiating timely treatment and preventing the progression of fungal zoonotic diseases. The diagnosis typically involves a combination of clinical evaluation, laboratory tests, and, in some cases, specialized procedures are summarized in Table 1

Table 1: Laboratory Diagnosis of Fungal Zoonoses

Fungal Zoonoses Diagnosis	Specimen	Microscopy	Culture	Histopathology	Serological Test	Skin Test	Molecular
Blastomycosis	Sputum, tissue, Fluid samples	Yeast cells with broad-based budding.	Whitish grey on Sabouraud agar	Large, thick-walled yeast cells with single broad-based buds in affected tissues.	Ab detection: Immunodiffusion (ID) and enzyme immunoassays (ELISA).	-	PCR, qPCR, Nested PCR, DNA Sequencing Genotyping: RFLP, MLST
Sporotrichosis	Tissue samples, Pus swabs	Cigar-shaped yeast cells	Cream-colored, but turns brown black once matured on Sabouraud agar	-	-	With Sporothrix antigen to detect delayed hypersensitivity reactions	PCR, qPCR, Nested PCR, DNA Sequencing Genotyping: MLST
Cryptococcosis	CSF, Serum, Blood	India Ink preparation: Cryptococcus neoformans appear as round or oval yeast cells surrounded by a clear zone (halo) due to the India ink repelling the organism.	Creamy to mucoid colonies on Sabouraud agar. India ink stain shows encapsulated yeast cells.	The encapsulated yeast cells are usually 5-20 µm in diameter and have a prominent capsule, which appears as a clear space around the cell.	Cryptococcal antigen test (CrAg)	-	PCR, qPCR, Nested PCR, DNA Sequencing
Histoplasmosis	Tissue, serum,	Oval budding yeast cells within	Mold form with tuberculate	Intracellular yeast cells often within	Ab detection: Immunodiffusion	-	PCR, qPCR, Nested PCR,

Fungal Zoonoses Diagnosis	Specimen	Microscopy	Culture	Histopathology	Serological Test	Skin Test	Molecular
	and Urine	macrophages	macroconidia and microconidia at 25°C, yeast phase at 37°C.	granulomas in affected tissues.	(ID) and enzyme immunoassays (EIAs), Lateral flow assays (LFAs) – strip tests Urine or Serum Antigen Tests		LAMP Assay, DNA Sequencing Genotyping: MLST
Coccidioidomycosis	Serum	Barrel-shaped arthroconidia upon microscopic examination	White to tan colonies on Sabouraud agar.	-	Ab detection: Immunodiffusion (ID), enzyme immunoassays. & Complement Fixation Test (CFT):	-	PCR, qPCR, Nested PCR, DNA Sequencing Genotyping: MLST
Dermatophytes	Skin scraping	KOH (Potassium Hydroxide) Preparation: hyphae and spores can be observed.	White to Cream colonies on Sabouraud Dextrose Agar/ Dermatophyte Test Medium (DTM)	Periodic Acid-Schiff (PAS) Stain**:** Skin samples are stained, highlighting fungal elements under the microscope.	-	-	PCR, qPCR, DNA Sequencing Genotyping: RFLP, RAPD

3.1 Blastomycosis

a) Respiratory Blastomycosis: Patients may present with symptoms resembling acute pulmonary manifestations pneumonia, including fever, cough, chest pain, and difficulty breathing. Severe cases can lead to respiratory failure. Chronic Pulmonary Form may mimic tuberculosis or lung cancer, with prolonged cough, weight loss, and fatigue.

b) Cutaneous Blastomycosis: Localized skin lesions often develop at the site of entry, such as the hands, face, or upper body. These lesions can be ulcerative and may have central crusting.

c) Systemic Dissemination: In some cases, Blastomycosis can disseminate from the initial site of infection to involve other organs, leading to more severe systemic symptoms.

3.2 Coccidioidomycosis

a) Primary Pulmonary Coccidioidomycosis: Patients may experience flu-like symptoms, cough, chest pain, and fever.

b) Disseminated Coccidioidomycosis: The fungus can spread to other organs, leading to more severe symptoms, such as skin lesions, joint pain, and central nervous system involvement.

3.3 Cryptococcosis

a) Pulmonary Cryptococcosis: Respiratory Symptoms: Patients may experience cough, chest pain, and shortness of breath especially with weakened immune systems.

b) Central Nervous System Cryptococcosis: *Cryptococcus neoformans* has a predilection for the central nervous system, and infection can lead to cryptococcal meningitis. Symptoms include headache, neck stiffness, fever, and altered mental status.

c) Cutaneous Cryptococcosis: Rarely, *Cryptococcus* can cause skin lesions, which may resemble cellulitis or other skin infections.

d) Ocular Cryptococcosis: Cryptococcal infection can affect the eyes, causing blurred vision, redness, and eye pain. Severe cases can lead to blindness if not treated promptly.

e) Disseminated Cryptococcosis: In severely immunocompromised patients, the infection can spread to various organs, including the liver, spleen, bones, and joints. This dissemination can cause systemic symptoms such as fever, night sweats, and weight loss.

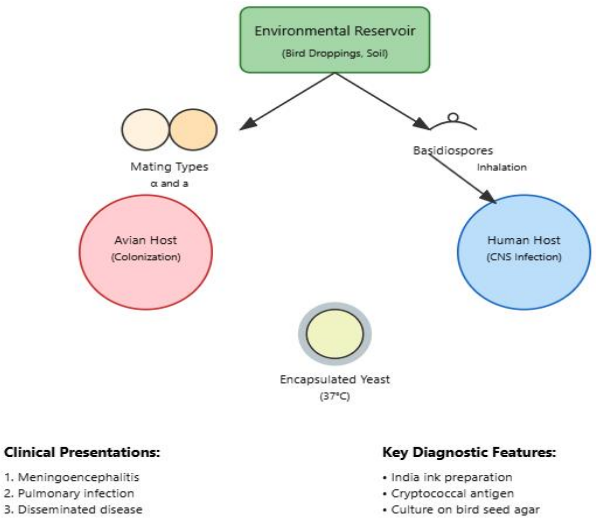


Figure 1: Life Cycle of Cryptococcus.

3.4 Dermatophytosis

Dermatophytes are a group of fungi that cause skin, hair, and nail infections in both animals and humans. These infections, known as dermatophytoses or ringworms, are among the most common fungal zoonoses globally. Crowded living conditions and poor hygiene contribute to its spread. Commonly transmitted between humans and animals, especially cats and dogs. Direct contact with infected skin or contaminated surfaces spreads the infection.

Dermatophyte infections are caused by three main genera and common species leading to human infections are as follows a) *Trichophyton species* (*Trichophyton rubrum, Trichophyton mentagrophytes complex* and *Trichophyton indotinae*): b) *Microsporum species* (*Microsporum canis, Microsporum gypseum*) c) *Epidermophyton species* (*Epidermophyton floccosum*). Dermatophyte infections commonly present as circular, red, and scaly rashes on the skin, leading to the term "ringworm." Dermatophytoses may cause hair loss and crusting of the skin, leading to a condition known as "favus."

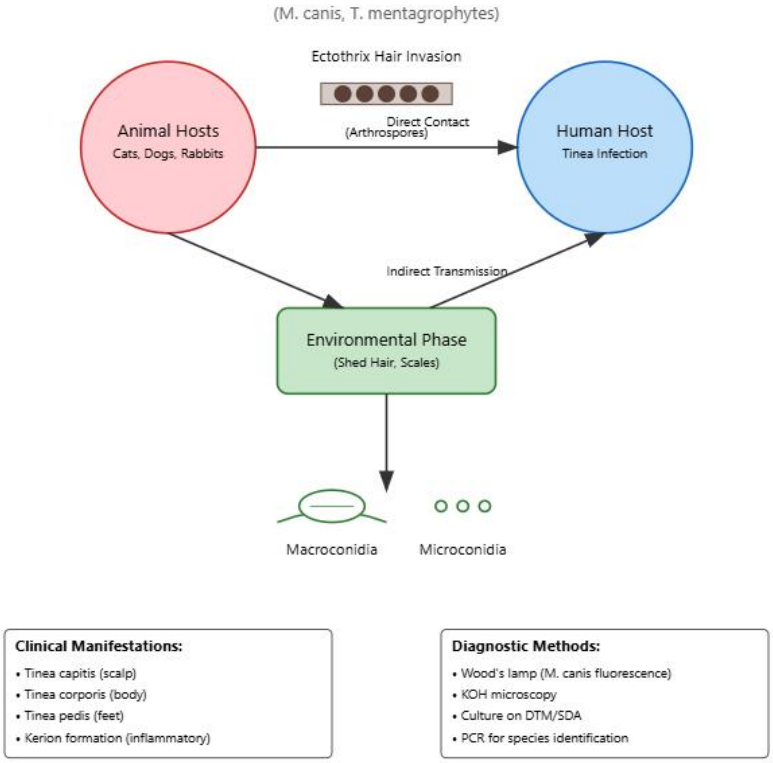


Figure 2: Life cycle of Zoonotic Dermatophytes.

3.5 ***Histoplasmosis***

a) Pulmonary Histoplasmosis: In acute pulmonary form symptoms are similar to those of pneumonia, with fever, cough, chest pain, and fatigue. In chronic pulmonary form lung cavities and symptoms mimicking tuberculosis are seen.

b) Disseminated Histoplasmosis: The fungus can disseminate to various organs, resulting in a range of symptoms, including fever, weight loss, anemia, and hepatosplenomegaly.

3.6 Sporotrichosis

a) Cutaneous Sporotrichosis: Nodular Lymphocutaneous Form: This is the most common presentation, characterized by painless nodules or papules that progress along lymphatic vessels. The lesions may ulcerate and discharge pus. Fixed Cutaneous Form: In this form, skin lesions remain localized without spreading along lymphatic channels.

b) Lymphocutaneous Sporotrichosis: Systemic Dissemination: Rarely, Sporotrichosis can disseminate to other organs, causing more severe systemic symptoms.

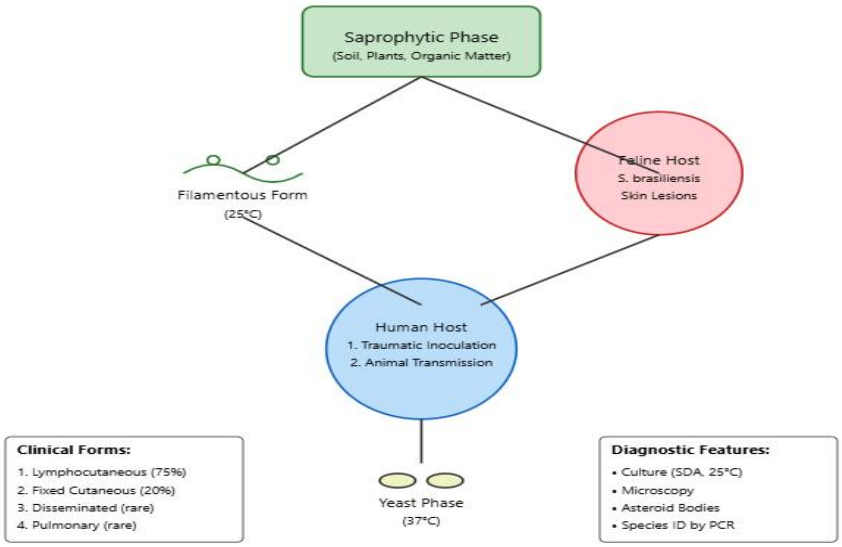


Figure 3: Life cycle of Sporothrix schenckii complex.

4. Treatment

The treatment of fungal zoonotic diseases as per IDSA guidelines typically involves antifungal medications. The choice of medication and duration of treatment depend on the specific fungal pathogen, the severity of the infection, and the patient's overall health. Here are the specific treatments for some common fungal zoonoses.

Table 2: Treatment options for Fungal Zoonoses

Fungal Zoonoses	Treatment
Blastomycosis	● Mild to Moderate Cases: Itraconazole or fluconazole is often prescribed. The duration of treatment is usually 6-12 months, sometimes longer for severe cases or immunocompromised patients. ● Severe or Disseminated Cases: Amphotericin B, a potent antifungal medication, may be used intravenously. This is typically used for severe or life-threatening infections.
Sporotrichosis	● Cutaneous Sporotrichosis: Itraconazole is the primary treatment. Potassium iodide solution can also be used in some cases. ● Lymphocutaneous Sporotrichosis: Itraconazole is the preferred treatment for lymphocutaneous forms as well. Severe cases may require amphotericin B.
Cryptococcosis	● Pulmonary Cryptococcosis: Fluconazole is the first-line treatment. For severe or disseminated cases, amphotericin B followed by fluconazole consolidation therapy is often

Fungal Zoonoses	Treatment
	prescribed. ● Central Nervous System Cryptococcosis: Amphotericin B induction therapy is followed by fluconazole consolidation and maintenance therapy. Intracranial pressure management might be necessary.
Histoplasmosis	● Mild to Moderate Cases: Itraconazole is the drug of choice for non-severe cases. Treatment duration varies from several months to a year or more. ● Severe or Disseminated Cases: Amphotericin B followed by itraconazole consolidation therapy is often used.
Coccidioidomycosis	● Mild to Moderate Cases: Fluconazole is commonly used, especially for those at risk of severe disease. ● Severe or Disseminated Cases: Amphotericin B is used initially for severe infections. Fluconazole is used for long-term suppressive therapy.
Dermatophytosis	Treatment of dermatophyte infections typically involves antifungal medications. ● Topical treatments like creams and ointments (Clotrimazole, Miconazole, Terbinafine Ketoconazole Creams for skin infection, and Ketoconazole or selenium sulfide-based shampoos for scalp infection) are effective for mild cases. ● Oral antifungals, such as terbinafine and itraconazole, are prescribed for moderate to severe infections. Infected animals might require antifungal baths or dips. Timely and appropriate treatment is essential to prevent the spread of infection. ● *T. indotineae* has become a problematic dermatophyte due to its predominantly in vitro genetic resistance to terbinafine owing to point mutation to the squalene epoxidase gene. It also displays in vivo resistance to terbinafine. The most efficacious drug currently for this terbinafine-resistant dermatophytosis, based on sound evidence is itraconazole.

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1. Introduction

Hantaviruses are rodent-borne and have been isolated from various rodent species. Notably, each hantavirus infects only a specific rodent species, which serves as the reservoir for that particular virus. Human infection with hantaviruses occurs through exposure to aerosolized rodent excreta, such as urine, droppings, or saliva, which can become airborne and be inhaled. Thottapalayam (1966) was the first indigenous Indian hantavirus species isolated from the spleen of the shrew (insectivore), Suncus murinus, captured in Vellore, South India, during field studies of Japanese encephalitis. In a CMC Vellore study in 2005, 23 (14.7%) out of 152 serum were positive for IgM Ab to Hantavirus in febrile patients while 5.7% healthy donors were also positive. Seoul like virus 12% & Puumala 5% was found in Indians presenting with Leptospira like clinical picture from Cochin & Chennai in 2006. The seroepidemiological study from CMC Vellore in 2008 indicated 4% prevalence of Hantavirus in India. 28 cases were confirmed among patients with chronic renal disease, warehouse workers & Irula tribe (snake & rat catchers) in Vellore District, Tamil Nadu, in 2008. Another study found 38/661 samples seropositive by ELISA/IFA in high-risk group.

2. Epidemiology

2.1 Causative Agent

Hantavirus is a single-stranded negative-sense RNA. The virus particle is spherical or oval-shaped with diameter of 80-120 nm consists of a unique grid-like surface pattern which is transmembrane glycoproteins. Hantaviruses survive for 12 hours at 4°C with high salt concentration and non-physiological pH and 1-3 days after drying. The virus contains a granular, filamentous interior consists of genome and protein structure which is approximately 13 kb. Hanta virus consists of three segments designated as small (S), medium (M) and large (L).

Hantavirus Pulmonary Syndrome (HPS) caused by rodents in the US belongs to the Muridae family, Sigmodontinae subfamily. Some of the rodent hosts in the United States are Deer mouse (*Peromyscus maniculatus*) which is a carrier of Sin Nombre strain, primary agent of HPS in the US. There were 250-300 cases since discovery with more than 50% mortality rate. White-footed Mouse (*Peromyscus leucopus*) is a carrier of New York strain. Cotton Rat (Sigmodon hispidus) is a carrier of Black Creek Canal strain and Rice Rat (Oryzomys palustris) being a carrier of Bayou strain with 40% mortality rate. There are 23 well described Hantaviruses in the World.

2.2 Mode of Transmission

- Contact with rodent excretions or via rodent bite
- Inhalation of aerosols or dust carrying infective material
- Human-to-human transmission is rare but has been demonstrated for Andes virus
- Rodent-to-rodent transmission occurs via biting or scratching; vertical transmission is not believed to occur due to maternal antibody protection

Wild-caught rodents have often been used in laboratory research, sometimes transmitting diseases to researchers, especially during winter when mice seek shelter and are more likely to come into contact with humans. Rodent populations tend to be densest at this time.

2.3 Current Situation

Old World hantaviruses are associated with distinct geographic and environmental patterns. Rural diseases are mainly caused by Hantaan, Dobrava-Belgrade, and Puumala

viruses, with Hantaan found in China, Russia, and the Korean Peninsula, Dobrava-Belgrade in the Balkans and Greece, and Puumala in northern Europe, especially Scandinavia. Urban diseases are often linked to Seoul virus, which is widely distributed globally due to the popularity of pet rats, but is most common in Japan, the Korean Peninsula, China, and the Americas.

New World hantaviruses gained attention in 1993 following outbreaks of Sin Nombre virus in the southwestern United States. These outbreaks were linked to two unusually wet winters, which led to an abundance of grain and vegetation, supporting a surge in mouse populations. Consequently, human cases increased, with infections typically occurring in the spring and summer, aligning with the peak of rodent activity.

In 2022, the United States reported a total of 864 laboratory-confirmed cases of hantavirus disease since surveillance began in 1993. This total includes both Hantavirus Pulmonary Syndrome (HPS) and non-pulmonary Hantavirus infections. Few Sporadic cases of hantavirus infection have been reported among wild small mammals from South India.

3. Clinical Features

Human to human transmission of hanta viruses has not been documented. Clinically Hantavirus disease can present in two different ways:

A) Stages of Hemorrhagic Fever with Renal Syndrome (HFRS) - After an incubation period of 1 or 2 weeks (4-40 days), HFRS exhibits 5 phases-1) Febrile Phase 2) Hypotensive Phase 3) Oliguric Phase 4) Diuretic Phase and 5) Convalescent Phase

B) Stages of Hantavirus Pulmonary Syndrome (HPS) -After asymptomatic incubation of 4-30 days, HPS has four phases-1) Febrile Phase 2) Cardiopulmonary Phase 3) Diuretic Phase and 4) Convalescent Phase

4. Laboratory Diagnosis

4.1 Collection of Specimen

Whole blood, Kidney, Lung, Blood, saliva, urine, or feces of infected rodent, serum

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.2 Laboratory Procedure

a) Identification of the agent can be done by Viral isolation is consistently difficult and rarely performed outside of a laboratory setting, Reverse-transcriptase polymerase chain reaction (RT-PCR) and Immunohistochemistry (IHC).

b) Serological tests includes Antibody capture enzyme-linked immunosorbent assays (ELISAs) for assessing hantavirus exposure in rodents due to the risk of working with infectious samples. Spot cards are often used by veterinarians to assess the serologic status of pet rats, otherwise 100µL of blood collected in EDTA can be used for serologic testing, Immunofluorescent assay (IFA), Enzyme immunoassay (EIA).

5. Treatment

There is no effective treatment of all hantavirus infections. However, aggressive supportive therapy may be administered to control the infection by fluid management, hemodynamic

monitoring, ventilatory support, peritoneal dialysis, pressor agents to support blood pressure, and inotropic agents which increases cardiac muscle contractility.
For future strategies for treatment, the hantavirus/β-3 integrin interaction may be targeted and Immunologic approaches may be applied to target host inflammatory responses and to find out inhibitors of TNF-a.

6. Prevention and Control:

- Rodent control is the primary mechanism by which human infections can be prevented. If the rodent density is particularly high, baiting and trapping may be necessary
- Researchers and laboratory staff should avoid utilizing wild rodents trapped in endemic areas whenever possible
- Vaccines: Formalin-inactivated rodent brain-derived vaccines for HFRS derived from SEOV and HTNV infections is commercially produced in South Korea as Hantavax. This vaccine has a reported seroconversion rate of 97% one month after the second dose and is considered safe, with only minor side effects. In China, Cell culture-derived vaccines are manufactured showing an average prevention rates of more than 90% for four years after primary vaccination in a trial with more than 100,000 participants.

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1. Introduction

Leishmaniasis is a vector-borne disease caused by different species of protozoan parasites of the genus Leishmania. Leishmaniasis is a major health problem yet neglected tropical diseases. Human leishmaniasis occurs in different forms, consisting of a broad range of manifestations extending from Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL), Diffuse Cutaneous Leishmaniasis (DCL), Visceral Leishmaniasis (VL) and Post-Kala-azar Dermal Leishmaniasis (PKDL). Among these different forms, VL or Kala-azar is more severe and prevalent. Clinical symptoms include irregular bouts of fever, considerable weight loss, fatigue, anaemia and substantial swelling of the liver and spleen.

2. Epidemiology

2.1 Causative Agent

Leishmania, classified under the kingdom Protista is cauasative agent of VL, CL and PKDL. It is categorized under the family of the Trypanosomatidae. Genus Leishmania comprises many species, which are classified as *L. donovani complex* (*L. donovani, L. infantum* and *L. chagasi*), *L. mexicana complex* (*L. mexicana*, *L. amazonensis* and *L. venezuelensis*) and other species such as *L. tropica, L. major* and *L. aethiopica*.

Leishmania parasites have two main morphologies, or forms, during their life cycle:

Amastigotes: These spherical or ovoid cells are found in the mammalian host and are 1–5 µm long by 1–2 µm wide. They have a large nucleus, a prominent kinetoplast, and a short axoneme. Amastigotes reside in macrophages of the host.

Promastigotes: These thin, elongated cells are found in the sand fly and are 5–14 µm long by 1.5–3.5 µm wide. They have an anterior kinetoplast and a free flagellum. Promastigotes propagates inside sand fly gut.

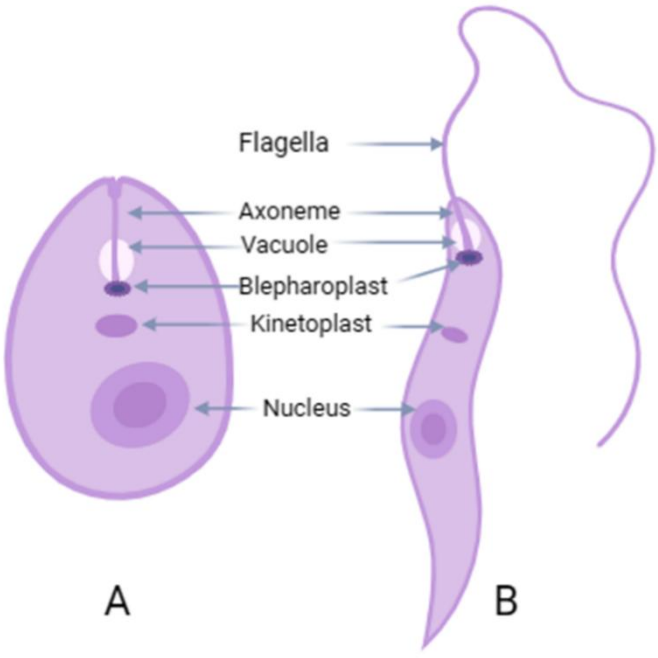


Figure 1: Leishmania: A) Amastigote form, B) Promastigote form.

2.2 Mode of Transmission

About 21 Leishmania species have been identified to be pathogenic to the humans and of the 500 species of phlebotomine species, about 90 species of female Phlebotomous belong to 6 genera are suspected or proven vectors transmitting parasites from animal-to-animal, animal-to-man, and/or man-to-man.

Like any other vector borne zoonotic disease, Leishmania can get transmitted from animal reservoirs to human in various manners, even if it requires an insect vector. The most common modes and risk factors include domestic animals like, goat, sheep, cattle or dogs, in the vicinity of human population. Farmers and rural population are at highest risk. This, by-and-large a poor population, provides conducive environment for the vector to hop on and hop off between animals and humans and transmit the parasites through bites to both- the animal and humans. In some tribes forest animals including the farm rats and foxes are hunted for food. During the hunting process, these tribes are exposed to sand-flies hiding in the burrows and nests of these animals. Yet another mode is during deforestation. The workers when they visit and stay in the forests for the purpose, they expose to the vector and they can get the infection. Last but not the least important mode is mixing of the domestic animals with forest animals which are well known reservoirs of the parasites. In this way the domestic animals can bring the infection into the residential areas and the transmission can occur in these foci.

Primary reservoir hosts of *Leishmania* are sylvatic mammals, such as forest rodents and wild canids. Because of the increasing process of domiciliation of the zoonotic cycle of transmission of leishmaniasis, synanthropic and domestic animals have assumed a significant role as a reservoir of infection. Dogs are the main domestic reservoirs whereas wolves and foxes in the case of wild animal. Another important feature for the parasite life cycle and its reservoirs is the behaviour of vector species and their host feeding preference. In VL endemic area, the vector*, P. argentipes*, feeds on both bovids and humans, but with a preference for cattle. These animals, in endemic settings, must be frequently bitten by sand-flies infected with L. donovani, but nothing is known about their susceptibility to infection in these animals. The presence of leishmanial antibodies in animals suggests that these animals are bitten by sandfly as confirmed by molecular method.

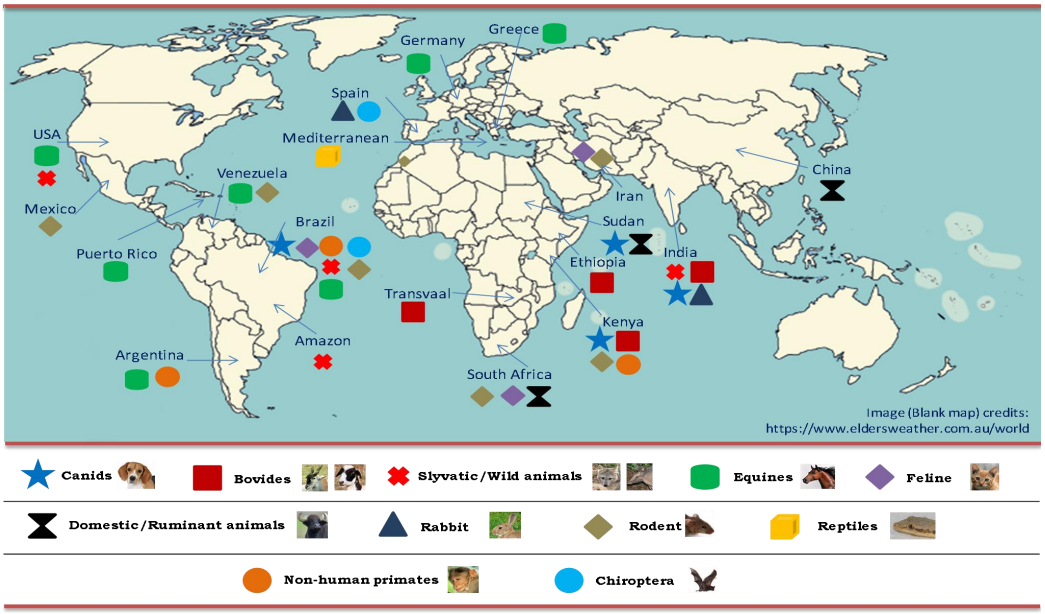


Figure 1: Global distribution of animal Leishmaniasis (adopted from WHO).

There are many Leishmania reservoirs all over the world. Animal reservoirs are important for maintaining the life cycle of many Leishmania species and hence are important for transmission of zoonotic and rural/sylvatic infections. In the Americas the most common reservoirs are sloths, opossums, small forest rodents such as the hyrax and peri-domestic dogs. In India, humans are considered a reservoir host for leishmaniasis.

2.3 Current Situation

VL is considered as one of the highest parasitic killer diseases with an estimation of 20,000–30,000 deaths per year globally. More than 90% of global VL cases occur in five countries: India, Sudan, South Sudan, Ethiopia and Brazil.

An estimated 50 000 to 90 000 new cases of Human VL occur worldwide annually. Most cases occur in Brazil, east Africa and India. CL cases occur in the Americas, the Mediterranean basin, the Middle East and central Asia. Majority of mucocutaneous leishmaniasis cases occur in Bolivia (the Plurinational State of), Brazil, Ethiopia and Peru.

The current situation of VL, HIV-VL coinfection and PKDL infection in India can be accessed
https://ncvbdc.mohfw.gov.in/index1.php?lang=1&level=2&sublinkid=5945&lid=3750

Natural vertebrate hosts of Leishmania parasites are mammals of the orders: Edentata (e.g., Armadillos, Sloths), Carnivora (e.g., Dogs, Cats), Hyracoidea (e.g., Hyraxes), Rodentia (e.g., Rats, Gerbils), Primates (e.g., Humans, Monkeys), Marsupialia (e.g., Opossums), and Perissodactyla (e.g., Horses).

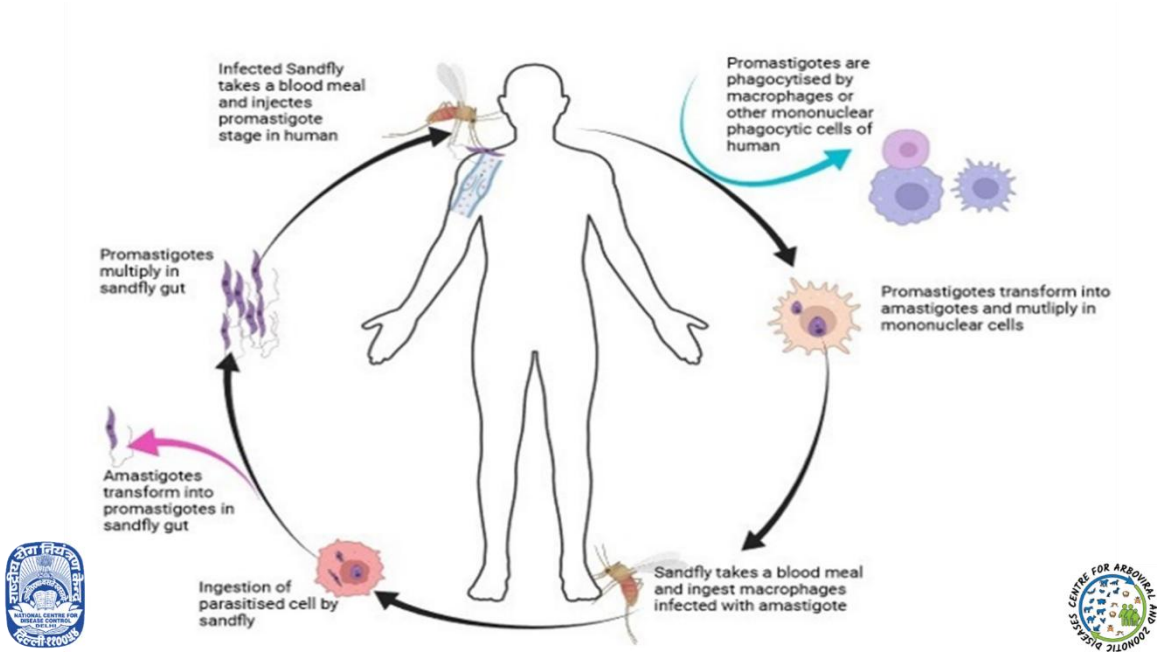


Figure 2: Transmission cycle of Leishmaniasis.

3. Clinical Features

Clinical manifestations of leishmaniasis depend on complex interactions between the virulence characteristics of infecting Leishmania species and the immune responses of its host. Visceral Leishmaniasis: The clinical manifestations of VL are more or less the same in all age groups. After inoculation of the parasites in the host skin by the bite of a sand fly,

man may remain completely asymptomatic or oligosymptomatic in the beginning, and the diseases may develop gradually into the active VL over several months. Asymptomatic infection is transiently seropositive but may not show clinical sign of the disease. The illness may present with malaise, intermittent diarrhea, lethargy, and intermittent fever. However, on examination, most will have high fever, marked hepatomegaly, splenomegaly, pancytopenia, weight loss, and hypergammaglobulinemia. Other common symptoms include vomiting, cough, fatigue, weight loss, leukopenia, anemia, thrombocytopenia and lymphadenopathy may also be present. Prolonged fever with anorexia and severe cachexia are the major presenting features of VL. The disease may often get complicated with secondary bacterial or superinfections, such as pneumonia, septicemia, otitis media, urinary tract infections with VL patients, leading to death in children. The clinical signs and symptoms in VL are nonpathognomonic, and these can mimic several other conditions. Whenever there is a clinical suspicion of leishmaniasis, the diagnosis should be confirmed by laboratory tests.

Cutaneous Leishmaniasis: Cutaneous leishmaniasis is the most common form and causes skin lesions, mainly ulcers, on exposed parts of the body. These can leave life-long scars and cause serious disability or stigma.

PKDL: Post Kala-azar Dermal Leishmaniasis (PKDL) is a condition when Leishmania donovani invades skin cells, resides and develops there and manifests as dermal lesions. Some of the kala-azar cases manifest PKDL after a few years of treatment. Recently it is believed that PKDL may appear without passing through visceral stage. However, adequate data is yet to be generated on course of PKDL manifestation.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Bone marrow aspirate, splenic aspirate, lymph node aspirate, buffy coat of peripheral blood.

4.2 Storage and Transport

Air dried smear on a glass slide from bone marrow/splenic aspirate/lymph node aspirate/skin biopsy/skin scraping to be sent in triple layer package with wrapping the slides individually inside a polythene bag. While for serology (Serum) and molecular testing (Whole blood in K3-EDTA vial), samples are to be sent in cold chain maintained at 2-8°C for transportation times less than 48 hours.

4.3 Laboratory Procedure

a. Microscopy: The demonstration of the parasite is confirmatory diagnosis after staining the slide smears with Romanowsky stains. In cases of VL, as mentioned above, the microscopic demonstration of Leishmania donovani (LD) bodies is specific and considered gold standard, but inexperienced microscopist may confuse Histoplasma capsulatum, a dimorphic fungus, with LD bodies.
b. Isolation: For culture isolation of the causative agent, various culture media, such as Novy-MacNeal-Nicolle (NNN) medium, Schneider's drosophila medium, RPMI 1640, brain–heart infusion, or Evan's-modified Tobie's medium can be used. The culture isolation of the parasite has several other uses, such as, genotyping, animal inoculation for pathogenicity testing, and for the screening of the therapeutic agents.
c. Serology: The immunological diagnosis is based on the presence of specific humoral response and considered a major diagnostics test for VL. Several antigens or antibody detection tests have been developed. The nonspecific (total immunoglobulin or cytokine detection) tests include Shia's test, Napier's test, Water test, tumor necrosis

factor-α, and others. Direct (antibody detection) tests include indirect haemagglutination test, countercurrent immunoelectrophoresis, and immunodiffusion test which have been used in the past, but are very rarely used these days due to cumbersome procedure, lack of sensitivity, and poor specificity. The indirect fluorescent antibody (IFA) test, direct agglutination test (DAT), enzyme-linked immunosorbent assay (ELISA), and immunochromatographic test (ICT) are currently in use and carry very high accuracy. The ICTs are easy, point-of-care tests and are economically affordable. In Kala-azar elimination programme, Government of India, rolled out these tests free of cost to screen all suspected cases in the endemic areas.

Antigen detection is considered to be more specific than antibody-based immunodiagnostic tests. KAtex is highly useful for the detection of VL in the urine sample, especially in infants. KAtex detects a low molecular weight (5–20 kDa), heat stable carbohydrate antigen, which is present in both promastigote and amastigote forms of LD in the urine specimen.

d. Molecular test: The molecular tools have been used effectively for the detection of asymptomatic, follow-up cases of VL. These tests are also able to track relapses and reinfections cases effectively. The conventional polymerase chain reaction (PCR) and its variations have been serving as important diagnostic tools for VL diagnosis. The PCR-based tools utilized specific primers set which targets specific multicopy genes, for example, rRNA, kDNA minicircles, mini exon derived RNA (med RNA), genomic DNA, splice leader mini-exon (SLME), telomeric repeats and internal transcribed spacer (ITS) regions for the detection of parasite DNA directly from clinical samples in the diagnosis of pediatrics VL. The sensitivity and specificity of conventional PCR among clinical samples i.e. blood sample, bone marrow or lymph node samples were observed. The seminested-PCR and qRT-PCR have potential to overcome these limitations and are also able to quantify the parasite burden possible with excellent diagnostic accuracy with tiny samples. Recently, oral fluid (saliva) based qRT-PCR was developed for VL diagnosis, which is highly useful in infant VL cases. The assay showed 94.6% sensitivity and 90% specificity.

5. Treatment

Chemotherapy has remained the mainstay for the control of leishmaniasis due to lack of effective vaccines. Currently, a wide variety of the drugs is available as an option for the treatment of leishmaniasis. There are approximately 25 compounds and formulations showing antileishmanial effects, but only a few have been proven worthy, such as the pentavalent antimonials, that is, sodium stibogluconate (Pentostam) and meglumine antimonite (glucantime); amphotericin B and its lipid formulations; pentamidine, miltefosine and paromomycin. The drug regimens with sufficient efficacy against leishmaniasis include amphotericin B, pentavalent antimonial drugs, paromomycin, and miltefosine. However, antimony compounds are rarely used in some South American countries and not anymore in the old world leishmaniasis.

Combined chemotherapy has various prospective advantages over the single therapy such as shortening duration of treatment and reducing the overall dose of medicines, thereby decreasing drug toxicity, cost, and development of drug resistance. Various drug combinations, such as liposomal amphotericin (L-AMB) with miltefosine, L-AMB plus paromomycin, and miltefosine plus paromomycin sulfate were tested in clinical trials. All combinations were noninferior to the standard treatment with amphotericin B.

6. Prevention and Control

Vector control is the most crucial step in the control of vector-borne diseases including Leishmaniasis. Sand fly control is accomplished by using light traps, sticky papers, and insecticides. Perhaps fly bite can also be reduced or prevented by use of insect repellents, parathyroid impregnated bed nets and curtains, staying away from endemic areas, and stopping outdoor activities especially at the insect active time. Control of reservoirs is largely limited to culling of infected animal reservoirs and treatment of infected humans to stop the further spread of the infection. Poor protein, energy, iron, vitamin A, and zinc nutritional status increase the risk that an infection will progress to clinically manifest. Population movement: Epidemics of VL, in both the old and the new world, are often associated with migration and the introduction of non-immune people into areas with existing endemic or enzootic transmission cycles. Climate changes: Leishmaniasis is a climate-sensitive disease, occupying a characteristic climate space that is strongly affected by changes in rainfall, atmospheric temperature, and humidity, which can have strong effects on the ecology of vectors and reservoir hosts by altering their distribution and influencing their survival and population sizes.

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1. Introduction

Leptospirosis is one of the most widespread direct zoonoses, many mammalian species, act as reservoir hosts. The clinical spectrum of disease ranges from subclinical infection to a severe, life-threatening syndrome or combination of syndromes associated with multiorgan involvement with a high case fatality rate of up to about 24% or more. One of the reasons for the emergence in the recent past in Southeast Asia is global warming that leads to extreme weather events such as cyclones and floods and thus exposure of a large number of people to a contaminated environment. Disease caused by pathogenic spirochete of the genus Leptospira. Leptospira comprises of 66 species, more than 300 serovars under about 30 serogroups or more were reported across the globe. Leptospirosis is transmitted by direct or indirect contact with the infective agent or exposure to a contaminated environment with infected animal urine. A wide range of wild and domestic animals are involved in transmission dynamics. In resource-poor settings, leptospirosis is underrated due to the lack of proper diagnosis which leads to high morbidity and mortality. The non-specific clinical symptoms overlapping other febrile illnesses and lack of proper early diagnosis and improper disease monitoring system because leptospirosis is underreported or overlooked.

2. Epidemiology

2.1 Causative Agent

Leptospirosis is a zoonotic bacterial disease. Bacteriologists, and among them Robert Koch, after discovery of Weils disease tried to isolate etiologic agent with no success. Etiologic agent was first isolated in 1915 by Inada and Ido.

The microorganisms responsible for leptospirosis belong to genus Leptospira of the family Leptospiraceae that traditionally consists of two species; *L. interrogans* and *L. biflexa*. The former includes all pathogenic serovars and the latter the saprophytic strains. Leptospira strains have been divided into 30 serogroups of which two belong to saprophytic leptospires. Each serogroup consists of several strains designated as serovar that is basically taxon.

Leptospires are highly motile, obligate aerobic spirochetes with a length of about 6-20µm and 0.1µm diameter. The genus Leptospira is phenotypically classified into two species L. interorgans (Pathogenic) and *L. biflexa* (Saprophytic). The species *L. interorgans* comprises about 250 serovars and is arranged into about 25 serogroups. The species *L. biflexa* comprises about 45 serovars and is arranged into 38 serogroups. The genus Leptospira has been genetically classified into 20 species and 300 serovars under 25 serogroups. Leptospira belongs to the family Leptospiraceae and the order Spirochaetales. Dark-field or phase contrast microscopy of wet preparation is required for direct observation of leptospires since the bacteria are too thin and transparent and poorly stained. The Leptospira genome possesses two circular chromosomes. The largest replicon, with lengths ranging from 3.6 to 4.3 Mb, has traits unique to bacterial chromosomes. It carries the majority of the bacterium's vital housekeeping genes as well as the replication proteins *dnaA, dnaN, gyrA*, and *gyrB*. The smaller chromosome, 277-350Kb in size, has a plasmid-like replication origin. It contains vital genes, that are involved in amino acid synthesis.

2.2 Mode of Transmission

Animals are the hosts and can be divided into maintenance hosts and incidental hosts. Though the primary reservoir hosts of leptospirosis are rodents, especially rats. Leptospires live in the renal tubules of infected mammals and shed in the urine. Rodents are asymptomatic carriers and are responsible for the contamination of the environment and transmission of disease. Leptospira forms environmental biofilms and is resistant to physical stress such as temperature and pH. Commonly observed in surface waters of paddy fields, walls of sewage canals, and thus continuous environmental sources.

The complex life cycle of pathogenic leptospires involves shedding in the urine of infected mammals, long-term survival in the ambient environment, obtaining a new host through the exposure of the host to a contaminated environment, and achieving systemic infection, especially dissemination to the kidneys through hematogenous route and survival in renal tubules due to its immune privilege. The pathogenic leptospires can survive for several months in water and water-logged soil.

2.3 Current Situation

Leptospirosis is found worldwide and affects both humans and animals. Every year, approx. 1.03 million leptospirosis cases and 58,900 deaths are estimated worldwide. The disease is prevalent in tropical and subtropical regions, whereas few cases are reported in temperate regions. Leptospira infects a different wide range of both wild and domestic animals such as cattle, dogs, horses, swine, goats, and sheep. Environmental source.

Leptospirosis is found endemic in different states across India and has been reported viz. Andaman and Nicobar Islands, Andhra Pradesh, Assam, Chattisgarh, Delhi, Gujarat, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Puducherry, Tamil Nadu, Telangana, and Uttar Pradesh.

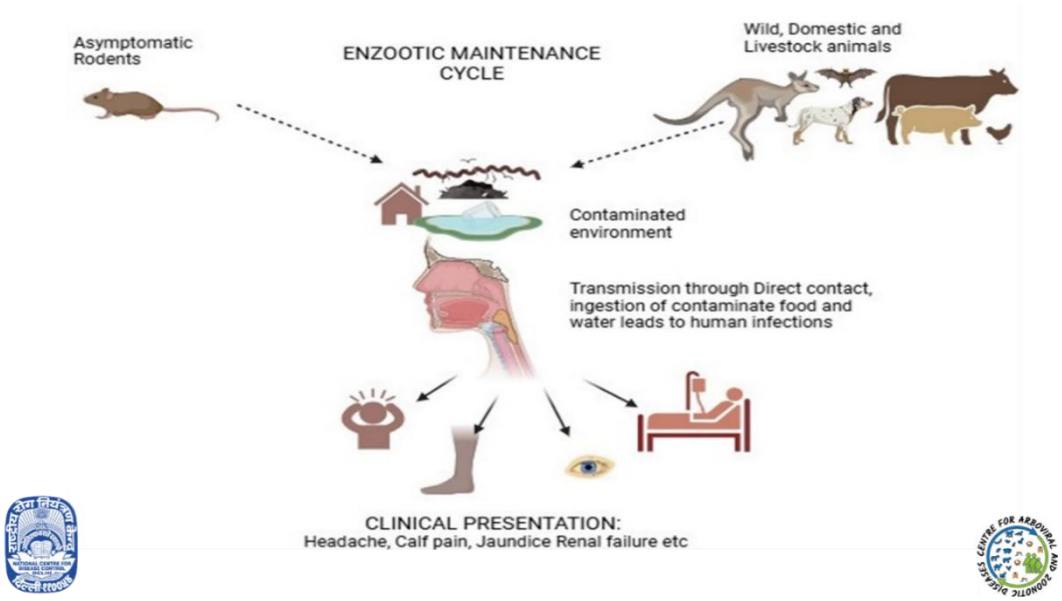


Figure 1: Transmission cycle of Leptospirosis.

3. Clinical Features

Leptospirosis is a systemic disease that affects both humans and animals with quite variable clinical manifestations ranging from mild, self-limiting febrile illness to severe

fulminant leptospirosis with multiple organ dysfunction. The majority of leptospiral infections present subclinical manifestations and patients do not seek medical attention. About 10% of leptospirosis cases progress to severe forms of the disease. The signs and symptoms are non-specific and simulate other common febrile illnesses; thereby, it is not sufficiently characteristic for proper diagnosis. The incubation period ranges from 7 to 12 days. The biphasic forms of leptospirosis are the acute leptospiremic (septicaemic) phase and the immune leptospiruric phase with immune responses.

Acute leptospiremic phase: The primary phase is characterized by non-specific acute febrile illnesses, including sudden onset of fever, myalgia, severe headache, chills, abdominal pain, conjunctival suffusion, nausea, diarrhoea, rash, jaundice, and this phase lasts about a week.

Immune leptospiruric phase: The septicaemic phase is followed by the immune phase, which is evidenced by IgM antibody production and shedding of leptospires in urine and the phase occurs during the second week of the illness. In this phase, a severe form of clinical presentation occurs based on the virulent type of pathogen and the extent of various organ involvement.

Syndromes or combination Syndromes: Clinical syndromes/manifestations with variable severity include Weil's syndrome (hepato-renal injury), hemorrhagic pneumonitis, acute respiratory distress syndrome (ARDS), Weil's syndrome associated with either gastrointestinal involvement or lung involvement or both and anicteric leptospirosis/acute febrile illness without organ injury (Mild case).

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood and CSF should be collected within the first 10 days of illness. After the first week of illness, urine may be a better source of Leptospira.

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Leptospira transport medium (LTM) is a culture medium that can be used to transport bovine urine samples for leptospira isolation.

4.3 Laboratory Procedure

a. Microscopy: Leptospires in clinical samples can be visualized during the acute stage by Dark-field microscopy because they arethin rods that are actively motile when compared with borrelia and treponema and move with rapid spinning. Alternative rotation, flexion, and extension. The threshold of microscopic detection of leptospires are nearly 104 leptospires per mL of blood samples, which allows at least one leptospire per field to be visible. Warthin-Starry silver staining has been widely used in the past, but outdated and rarely used now.
b. Isolation: Isolation and identification are the gold-strandard methods to identify causative and circulating serovars. Blood, cerebrospinal fluid, dialysate fluid, and urine are commonly used specimens and provide a definite diagnosis it is rarely recommended due to their limitations, i.e. expensive, time-consuming, and technically demanding. The recommended culture medium is Ellinghausen, McCullo, ugh's medium

modified by Johnson & Harris 1967 (EMJH Medium). Note: All the above techniques are recommended for the first few days of the onset of the disease.

c. Serological test: Serological methods can detect either antibodies or antigens present in the test sample. The antigen detection techniques include Radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), and fluoroimmunoassay, which can detect leptospires from 10^2 to 10^5 cells per ml. The methods are recommended for the first few days of the onset of the disease.

Antibody detection techniques are mainly classified into genus-specific and serogroup-specific methods. MAT is the gold standard test for serological characterization and sero-epidemiological studies. ELISA (IgM) is an alternative or additional diagnostic tool for leptospirosis diagnosis and is extensively used. Antibody-based tests can diagnose leptospirosis after the 5th to 7th day of disease onset. Seroconversion in paired serology helps in diagnosis.

The sensitivity of the serological test is observed high when the test is performed on paired sera (collected during acute and convalescent stages). Reference or representative strains of circulating serogroups or endemic serovars are used as panel antigens in MAT. The size of the MAT panel is based on circulating or endemic serovars of serogroups. One culture for each serovar of serogroup needs to be added. If reference strains are not available any characterized isolates of each serogroup need to be added to the MAT panel. If the information on circulating or endemic serovars of serogroups is not available, a total of 100 serum or plasma samples collected from patients with febrile illness and diagnosed as fever of unknown origin needs to be sent to reference laboratories for generation of information.

Criteria for serological diagnosis of leptospirosis include Four-fold or greater rise in the titer between acute and convalescent serum specimens collected from suspected cases/ Sero-conversion between acute and convalescent-stage samples (Based on endemicity in the region).

High Seropositvity in a single serum sample (However, the significant titre in the case of single serum samples may vary from one geographical area to another).

No single serological test can be considered as the diagnostic tire of current clinical infection because confirmed patients may have seropositivity even several years of past infection (clinical or sub-clinical). Hence single MAT titre needs to be correlated with clinical condition.

d. Moleculer Test: Many molecular diagnostic methods have been developed for the detection of leptospirosis. These include conventional PCR or its modifications. they are useful in detection in acute leptospiremic phase and has been primarily used for the diagnosis of leptospirosis, which tends to overcome the limitations of serological methods due to its high sensitivity and ability to provide an early diagnosis.

Generally, PCR techniques have been designed to target the housekeeping genes (*16Srrs, secY, gyrB*) of all leptospiral species or the genes (*lipL32, lfb1*) which are specific for pathogenic species. The threshold for detecting leptospirosis is usually 10-100 leptospires/mL of blood or urine. The leptospiral load in clinical samples is measured by using the panel of standard DNA. Over the last two decades, nested PCR targeting the *16S rRNA* gene has been widely used for the detection of leptospiral DNA from different clinical samples, including blood, urine, or cerebrospinal fluid, and gives rapid

and accurate results. The *secY* gene amplification phylogenetically discriminates the pathogenic Leptospira species and has been widely used.

Identification of biomarkers and genotyping of host gene polymorphisms can achieve early detection, monitor disease progression, and follow-up prognosis of leptospirosis even before initiating host antibody production in response to leptospiral infection.

5. Treatment

Once the diagnosis is established, immediate treatment should be started based on severity of diseases. Amoxycillin/ Ampicillin in divided doses for 7 days is recommended for mild to moderate cases. Crystalline penicillin should be given for 7 days as per recommended dose. Ceftriaxone/ Cefotaxime/ Erythromycin for individuals who are sensitive to penicillin group of drugs for 7 days is recommended.

In disease progression multiple organs such as kidney, liver, lungs, CVS and CNS may be involved. All suspected leptospirosis cases whether positive or negative with rapid immunodiagnostic test having feature of organ dysfunction as follows should be immediately shifted to higher centre and systemically managed as per organ involvement.

The unfavorable indices are age (>50 years) and development of Jaundice. The more severe cases however are associated with a high mortality. So, prompt diagnosis and treatment help in terminating most of the fatal effect.

6. Prevention and Control

a) Personal protection: Hygienic methods such as avoidance of direct and indirect human contact with animal urine are recommended as preventive measures. Workers in flooded fields should be cautioned against direct contact with contaminated water or mud and should be advised to use rubber shoes and gloves. In case of any cuts or abrasion on the lower extremities of the body, the worker should apply an antiseptic ointment e.g. betadine, before entering the field and after exit. Those at risk should cover all cuts with waterproof plasters and wear protective clothing or other materials. Immersion in natural waters such as rivers, fakes and canals should be avoided, and individuals should wear footwear and wash or shower after canoeing, windsurfing or swimming.

   Low lying or swampy areas should be drained or fenced, and access to stagnant water or runoff from corrals should be prevented. Contact between domestic animals and wildlife should be controlled, if possible. Inside buildings, pens should be cleaned and disinfected after occupancy by infected animals. In hog barns, the pen partitions should be solid for at least part of their height to prevent urine splashing from one pen to the next. Pits rather than common manure alleys should be used wherever possible to prevent contamination from pen to pen. And finally, a minimized contact with fresh water, mud, and vegetation that might be contaminated with the urine of infected animals, especially rodents is sensible.

b) Health education: The main preventive measure for leptospirosis is to create awareness about the disease and its prevention. This has to be conceptualized through intensive educational campaign, IEC templates/software for audio visual, print, press, outdoor outreach modes, new and emerging electronic media.

c) Chemoprophylaxis: Antimicrobial prevention is only indicated for those people who are under risk of exposure_except on epidemics - to leptospires (doxycylcine 200mg/week) and for those ones with known exposure (doxycycline 100mg for 7 days) such as

agricultural workers (eg. paddy field workers, canal cleaning workers in endemic areas) from where clustering of cases has been reported. The chemoprophylaxis should be for six weeks and never to be extended for more than eight weeks.

d) Rodent control: It is established beyond doubt that rodents are the major reservoirs of bacterium *L. interrogans*. Four species of rodents *Rattus rattus* (House rat), *Rattus norvegicus* (Norway rat), *Bandicota bengalensis* (Lesser bandicoot) and *Bandicota indica* (Larger bandicoot) are so far found to be reservoirs for this bacterium in India. Hence controlling these reservoir species with proper strategy planning and management planning will reduce the incidence of the disease in the affected areas.

   The strategic planning should include identifying the reservoir species of affected area, delineating areas for anti-rodent activities, completion of activities in pre monsoon months, adopting appropriate technology for anti-rodent operations.

e) Mapping of water bodies for establishing a proper drainage system: The mapping of water bodies and human activities in water logged areas should be carried out. This will help to identify the high risk population. Farmers may be educated to drain out the urine from the cattle shed into a pit, instead of letting it flow and mix with water bodies (rivers, ponds etc.).

f) Health impact assessment: Health impact assessment should be made mandatory for all developmental projects along with environmental assessment.

g) Vaccination of animals: Leptospiral vaccines confer a limited duration of immunity. Boosters are needed every one to two years. Vaccination should however be very selective and used only in endemic situations having high incidence of leptospirosis. The vaccine must contain the dominant local serovars. While this prevents illness, it does not necessarily protect from infection and renal shedding.

   Vaccines consist of inactivated bacteria containing the leptospires Leptospira canicola and Leptospira icterohaemorrhagiae. Vaccines will neither prevent urinary shedding in infected animals nor eliminate the organism from these animals. Therefore, the vaccines do not prevent infection, but rather are effective in reducing the occurrence and severity of disease. Further, duration of protection following vaccination is relatively short, usually no more than 6 months and the immunity imparted is specific to the individual leptospires contained in the vaccine. Immunisation of cattle will reduce the excretion of leptospires in their urine. The rat population needs to be reduced, with destruction of their habitats and rat proofing buildings.

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1. Introduction

Listeriosis, caused by *Listeria monocytogenes*, has been implicated in increasing foodborne outbreaks worldwide. The disease in humans is mainly transmitted through the consumption of contaminated food products and is rarely occupational. In humans, especially immunocompromised individuals, the disease is manifested in various forms ranging from severe sepsis, febrile gastroenteritis, stillbirth, abortions, and meningoencephalitis, while, in animals, it leads to reproductive disorders, abortions, stillbirths, mastitis, and encephalitis. Though listeriosis is a rare disease, it has a high case fatality rate (20–30%).

2. Epidemiology

2.1 Causative Agent

With the recent discovery of several new species, the genus *Listeria* comprises 30 species. *L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri, L. grayi, L. marthii, L. costaricensis, L. rocourtiae, L. fleischmannii, L. newyorkensis, L. weihenstephanensis, L. floridensis, L. aquatica, L. thailandensis, L. cornellensis, L. riparia, L. booriae, L. goaensis, L. grandensis, L. valentina, L. farberi, L. portnoyi, L. cossartiae, L. rustica, L. immobilis*, *L. ilorinensis, L. tempestatis sp. nov.* and *L. rocourtiae subsp. hofi.* Among all the species, *L. monocytogenes* is the most frequently reported pathogen in humans and animals, while *L. ivanovii* causes disease in animals.

L. monocytogenes is a highly heterogeneous species that currently classified into four evolutionary lineages, fourteen serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7, 4h), and four PCR serogroups. The pathogen, *L. monocytogenes* is ubiquitous with a wide host range including 40 mammals, 20 birds, crustaceans, ticks, and fishes.

2.2 Mode of Transmission

Human listeriosis is mainly transmitted through the consumption of contaminated food products, although zoonotic and vertical transmission from mother to fetus is another possibility. Nosocomial transmission as seen in cutaneous lesions among veterinarians is uncommon. *L. monocytogenes* has been reported from diverse sources, including water, soil, plants, and animals. Foods and their processing environments are commonly contaminated with this pathogen.

Significantly, there might be a direct association between human infections and listeric infections in farm animals and their environments. Moreover, *Listeria* spp. have been isolated from 1% to 7% of the intestinal content of healthy animals and from 5% to 10% of the stools of healthy human adults.

Foods of animal origin consumed without adequate processing, as well as plant products contaminated by manure from infected or shedding animals, may aid in the transmission of listeriosis. Also, there are reports of *Listeria* isolation from invertebrates, house flies, caterpillars and invading slugs. Even isolation of *L. monocytogenes* from invertebrates (fly and tick) collected from goat farm environments has been documented. It is stated that these vectors may serve as vehicles for the transmission of *Listeria* in food-processing facilities and also can serve as a threat to animal feed safety. In addition, rodents have been reported to disseminate *Listeria* into the natural environment and possibly transmit the pathogen between wild animals and humans.

L. monocytogenes has been isolated from the faeces of clinically affected animals including healthy animals suggesting them as latent carriers. It has been hypothesized that

in the ecological system, livestock farms may function as a natural reservoir for *L. monocytogenes*. Isolation of *L. monocytogenes* has also been reported from bone marrow of humans.

L. monocytogenes have a diverse assortment of virulence factors. The life cycle of the pathogen reflects its notable adaptation to intracellular survival and multiplication in professional phagocytic and non-phagocytic cells of vertebrates and invertebrates. The pathogenic *Listeria* species are capable to breach the intestinal, blood-brain, and fetoplacental barriers, and also invade and replicate in phagocytic and nonphagocytic cells and has a tropism for all tissues.

Internalins, A and B mediate the entry of the pathogen, while LLO and PI-PLC help to lyse the primary single-membraned vacuoles and subsequent escape of *L. monocytogenes* into the cytoplasm of the infected cell. The intracellular mobility and cell-to-cell spread are facilitated by bacterial surface protein, ActA forming the polarized actin tails enabling the bacterium to infect the second cell.

Several virulence-associated proteins, such as PIPLC, LLO, Mpl, ActA, and PC-PLC are encoded in a 9.6 kb virulence gene cluster, regulated by the pleiotropic virulence regulator, PrfA. Several other genes, such as *iap, bsh, vip, inlJ, auto, ami, bilA* are also involved in *L. monocytogenes* virulence and pathogenicity. A hybrid sub-lineage of the major lineage II (HSL-II) and serotype 4h of *L. monocytogenes* were discovered while characterizing the isolates from severe ovine listeriosis outbreaks.

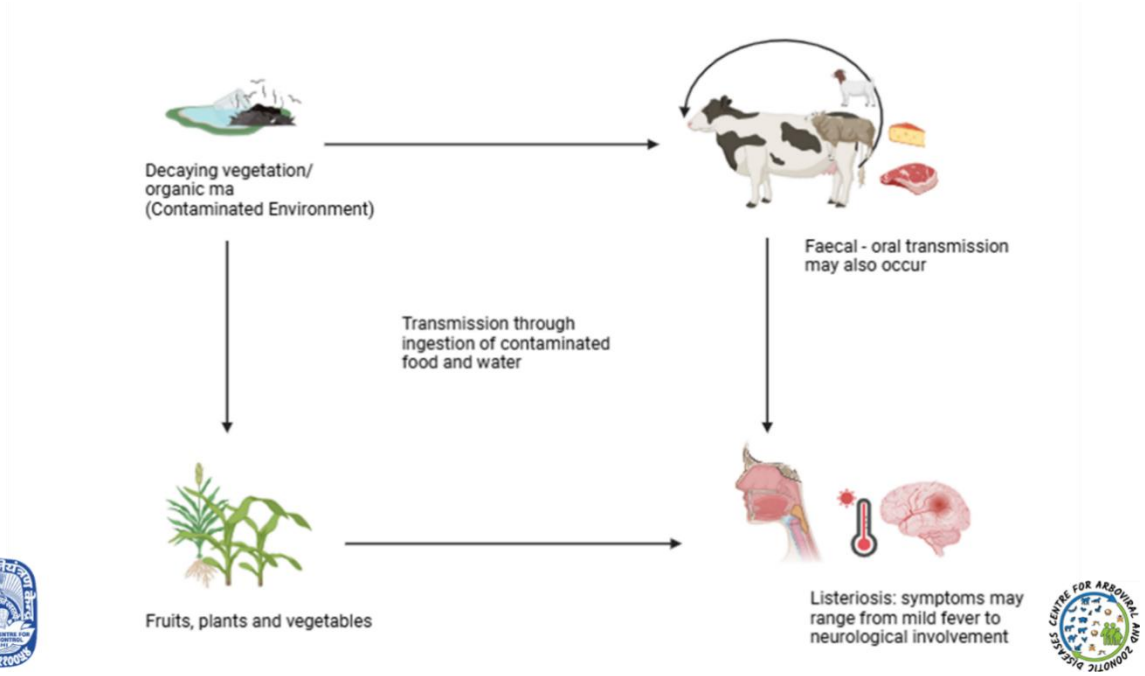


Figure 1: Transmission cycle of Listeriosis.

2.2 Current Situation

The annual global burden of listeriosis in 2010 was estimated to be more than 23,000 illnesses and almost 5500 deaths and 172,823 disability-adjusted life-years. Recently, International surveillance systems could easily identify sources of contamination and strains through genetic data sharing. As an important model for intracellular infections and host-pathogen interactions, *L. monocytogenes* is a widely studied pathogen.

Considerable economic losses have been reported due to outbreaks and sporadic cases of human listeriosis infections. During 2017 – 2018, South Africa encountered the world's largest listeriosis foodborne outbreak. It was characterised by a progressive increase with over 1060 laboratory-confirmed cases and 216 deaths. There is a paucity of data on the occurrence of *L. monocytogenes* from most AFRO, EMRO, and SEARO WHO regions which are known to have a significant immunocompromised population.

In India, data on the detection of *L. monocytogenes* and its molecular epidemiology studies are emerging recently. The disease has been reported in humans and animals, and the pathogen has been isolated from varied food sources. Also, extensive reviews on the occurrence of *Listeria* infections in the Indian subcontinent are available.

In India, *L. monocytogenes* has been identified as one of the etiological agents resulting in abortions and premature births among humans. Also, genital listeriosis has been reported as the most common clinical form in humans. Abortion, stillbirth, neonatal sepsis, and meningitis are the common sequelae of perinatal human listerial infection.

The occurrence of *Listeria* in different environmental niches, including foods, soil, vegetables, mangrove swamps, seafood, freshwater fishes, clinical cases, and also insects has been recorded. The organism has also been isolated from animal clinical cases and wildlife samples. A novel species of *Listeria*, *L. goaensis,* has been identified and characterized from India. Also, biofilm-forming abilities of *L. monocytogenes* strains isolated from clinical, environmental, and foods have been demonstrated. Interestingly, *L. monocytogenes* serogroup 4b isolates from India were genetically similar, however, isolates of serotypes 1/2a and 1/2b were relatively diverse and unique. Also, whole-genome-based studies have revealed diverse *L. monocytogenes* isolates of serotype 1/2a and 1/2b, respectively. Besides, serotype 4b ST328 clone, a predominant and unique serotype of *L. monocytogenes* has been reported in India. This clone was repeatedly isolated from different sources, varied temporally and spatially.

3. Clinical Features

Humans

The predisposing factors for the occurrence of listeriosis in humans include elderly patients (age ≥ 60 years) having implications such as primary bacteremia, involvement of the central nervous system, non-hematological malignancies, organ transplantation, patients with AIDS, diabetics, alcoholism, chronic kidney disease, cardiovascular disease, and pulmonary disease.

Human listeriosis can occur in different forms either as severe or mild invasive or non-invasive febrile gastroenteritis. It depends on various factors such as the age of the infected person, its immune status, the amount of bacterial cells consumed, and the virulence of the infective strain. Neuroinfection is unusually threatening due to the high case fatality rate. Listeriosis can cause severe invasive infections in high-risk groups such as young and old individuals, pregnant women, and immunocompromised persons. Clinically, listeriosis is most often presented as bacteremia, meningitis, or meningoencephalitis, and also as miscarriage or neonatal sepsis in pregnancy-associated infections.

Animals

L. monocytogenes causes invasive and often fatal diseases including CNS infection in numerous animal species including farm ruminants, horses, dogs, pigs, deer, South American camelids, and cats. Sporadic as well as outbreak forms of listeriosis have been reported from the animal population. The clinical manifestations varied with the species

affected; however, spontaneous abortions, sub-clinical mastitis, meningoencephalitis, and endometritis were the most common forms reported. In sheep, the disease is referred to as a circling disease. In cattle, listeriosis is manifested as a cause of mastitis, abortion, repeat breeding, infertility, encephalitis, and septicaemia.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Human

Blood, cerebrospinal fluid

Animal samples

Blood, cerebrospinal fluid, meconium of newborns or fetus in abortion cases, faeces, vomitus, vaginal secretions of infected animals, primary production

Food, Feed and Environmental samples: food and feed products, milk and dairy products, red meat, poultry products (raw or cooked ready-to-eat), eggs, egg products

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours.

4.3 Laboratory Procedure

Human/Animal samples

a. Isolation: The confirmatory diagnosis can be made by isolation of the pathogen from clinical specimens such as blood, cerebrospinal fluid, and meconium of newborns or fetus in abortion cases, and faeces, vomitus and vaginal secretions of infected individuals or animals. *L. monocytogenes* is a Gram-positive, rod-shaped, non-spore-forming, non-capsulated, facultative intracellular anaerobic bacterium and exhibits characteristic tumbling motility at or around 25°C.

   L. monocytogenes is morphologically indistinguishable from other *Listeria* species, and therefore, to differentiate *L. monocytogenes* from other *Listeria* species, Christie–Atkins–Munch-Petersen (CAMP) reaction with *Rhodococcus equi* and *Staphylococcus aureus*, lecithinase reaction, haemolysis on blood agar, phosphatidylinositol specific phospholipase C activity (PI-PLC) on chromogenic media (Agar *Listeria* Ottavani Agosti – ALOA medium) are oftenly used to discriminate pathogenic and non-pathogenic *Listeria*. Further, to determine the virulence potential of *L. monocytogenes* isolates, in vitro, hemolytic titer assay, tissue culture plaque assay, and in vivo, mouse pathogenicity, embryonated egg inoculation method and *Galleria mellonella* larvae model are often used by researchers.

b. Serological test: Lateral flow assays, biosensors, ELISA and immunofluorescence tests have been developed for diagnosis of listeriosis.

c. Molecular test: Several molecular methods including PCR, Real time PCR, RT-PCR have been devised for detection of this pathogen. Additionally, phenotype-based subtyping methods are commonly used for *L. monocytogenes*, which include serotyping, phage typing, and multilocus enzyme electrophoresis, while, the genetic subtyping approaches include PCR-based approaches (e.g., RAPD), PCR-restriction fragment length polymorphism, ribotyping, pulsed-field gel electrophoresis and DNA sequencing-based subtyping techniques e.g. multilocus sequence typing, etc

Environmental Samples

In foods, a zero-tolerance level (absence in 25 g of food) has been implemented for this pathogen and to detect *Listeria* in various foods, either the FDA bacteriological and analytical method (BAM) and/or the International Organization of Standards (ISO) 11290 method are widely used. In FDA, BAM method, the food sample is enriched in *Listeria* Enrichment broth (LEB) having selective agents such as acriflavine, and nalidixic acid, followed by plating onto selective agar (PALCAM). In the ISO-11290 method, the first enrichment (pre-enrichment) is performed in Half-Fraser broth, followed by enrichment in Full-Strength Fraser broth, and then selective plating either on Oxford or PALCAM agar. Besides this USDA-FSIS and Association of Analytical Chemists (AOAC/IDF) method contains a modification of the University of Vermont media (UVM) containing acriflavine and nalidixic acid for primary enrichment followed by secondary enrichment in Fraser broth and plating onto Modified Oxford (MOX) agar containing the selective agents, moxalactam and colistin sulphate. In general, the ISO-11290 method is mainly employed for the detection of *Listeria* in food and feed products, primary production, and environmental samples, FDA/AOAC method for milk and dairy products, whereas, the USDA-FSIS method is often used for red meat and poultry (raw or cooked ready-to-eat), eggs, egg products and environmental samples.

5. Treatment

Ampicillin or penicillin are generally used in combination with gentamicin to treat listeric infections. Alternatively**,** co-trimoxazole is also recommended for CNS infection as it has excellent penetration into the cerebrospinal fluid. Besides, vancomycin may be a choice for primary bacteremia, while erythromycin can be used to treat listeriosis in cases of pregnancy. To treat bacteremia cases 1 to 2 weeks of treatment is generally recommended, while for CNS infection, the treatment duration may be longer i.e. approximately 6 to 8 weeks.

6. Prevention and Control

L. monocytogenes is capable of persisting at a wide range of temperatures and also propagates in food having a low moisture content, and a high salt concentration. The pathogen, being psychrophilic in nature makes it most difficult to control in the food stored at refrigeration temperature. Besides, *L. monocytogenes* has a unique ability to form biofilms on a variety of surfaces present in food processing environments, thereby allowing them to persist for a longer duration. This persistence of *L. monocytogenes* depends on its tolerance to sanitizers, disinfectants, growth at low temperatures, resistance to heavy metals, or ability to develop a biofilm.

Various biocontrol strategies with the potential to act against listerial biofilms such as bacteriophages, their endolysins, competitive bacterial species, and their antimicrobial products such as bacteriocins, and plant-derived products have been addressed by researchers to control this pathogen in the food industry. Besides, the use of a chitosan coating and Mentha aquatica *L. essence* and *Lactobacillus rhamnosus* had been found to inhibit *L. monocytogenes* in cheese. An antimicrobial peptide (AMP), sonorensin, derived from *Bacillus sonorensis* was found to control the growth of undesirable bacteria including *L. monocytogenes*.

Additionally, surveillance systems need to be strengthened in the food chain to assist in preventing and facilitating early detection of sporadic cases and outbreaks of

infections. Addressing the challenges of emerging and resurging pathogens requires intersectoral collaboration, coordination, and communication through the One Health approach. Presently, there is no vaccine available against listeriosis and hence, proper hygienic measures at food processing units and health education of the personnel remain important in the prevention of this pathogen.

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1. Introduction

Lyme disease is a bacterial disease commonly seen in temperate regions of the Northern hemisphere. It is caused by the Spirochaetes of the *Borrelia burgdorferi sensulato complex*. Lyme disease is transmitted by the bite of the infected black legged hard ticks of genus *Ixodes*. It is manifested by a wide spectrum of clinical symptoms affecting primarily skin, nervous system, heart and joints.

2. Epidemiology

2.1 Causative Agent

Lyme disease is a bacterial infection caused by spirochetes of the *Borrelia burgdorferi sensulato complex* (also more recently grouped under a new genus taxon named Borreliella). It is a vigorously motile, corkscrew-shaped bacteria. The cell envelope of *B. burgdorferi is* exceptional and differs significantly from the typical Gram-negative bacteria. Unlike other gram-negative bacteria, *B. burgdorferi* lacks lipopolysaccharides, instead they have immunoreactive glycolipids. Also, flagella are located in the periplasmic space, while other bacteria commonly have them outside the cell.

The *Borrelia burgdorferi sensulato genospecies complex* (Bbsl) includes the three most frequent agents of Lyme borreliosis worldwide—*Borrelia burgdorferi* (*sensustricto*), *Borrelia afzelii*, and *Borrelia garinii*. In Europe, five human-pathogenic genospecies from the *Borrelia burgdorferi sensulato* complex have so far been isolated: *B. afzelii* is the most frequent, followed by *B. garinii, B. bavariensis, B. burgdorferi sensustricto* and *B. spielmanii*.

2.2 Mode of transmission

Agents of Lyme disease are transmitted to birds, mammals and humans by the bite of hard-bodied black-legged ticks of the *Ixodes ricinusor Ixodes persulcatus spp*. complex during the blood meal. Ticks are obligate hematophagous at all stages of their life cycle, which makes them competent for transmitting pathogens at various stages. In Europe, the transmission is primarily from *I. ricinus*, in Asia from *I. persulcatus* and in the USA predominantly from *I. scapularis*. Before they lay their eggs and throughout their whole life cycle from larva to adult tick, ticks feed on blood. It is at this time that they can acquire and/or transmit *Borrelia*.

Ixodid (hard) ticks are obligate blood-sucking ectoparasites with two body parts and eight legs. They have a characteristic hard cuticle, a terminal capitulum which can be seen in dorsal view and a large shield-shaped plate (scutum). Ticks undergo incomplete metamorphosis whereby eggs hatch larvae which moult to nymphs and then adults. This tick is primarily found in deciduous forests, tall grasslands, and shrubs bordering forest edges. Ticks along with their immature stages parasitize small to medium-sized mammals and birds and the adult females parasitizes large mammals such as deer, cattle, sheep and hares.

The principal reservoir host of *B. burgdorferi* is the white-footed mouse (*Peromyscus leucopus*), but chipmunks (*Tamias striatus*), short-tailed and masked shrews (*Blarina brevicauda* and *Sorex cinereus*), and eastern gray squirrels (*Sciurus carolinensis*) also serve as infectious hosts.

The disease is transmitted by the bite of ticks which have a 2-to-3-year life cycle. During this time, they go through four life stages: egg, larva, nymph, and adult. Adult ticks

feed and mate on large animals, especially deer, in the fall and early spring. Female ticks then drop off these animals to lay eggs on the ground. After the egg hatches, the larva and nymph each must take a blood meal to develop to the next life stage, and the female needs blood to produce eggs. *B. burgdorferi* infection is acquired by feeding on an infected reservoir animal (usually rodents), and the bacterium is retained during the subsequent stages (that is, trans-stadially) after each blood meal and moult. The bacteria are passed along to the next life stage. In the course of their routine feeding habits, infected nymphs and adult ticks bite other small rodents, animals, and people, passing on the bacteria that causes Lyme disease.

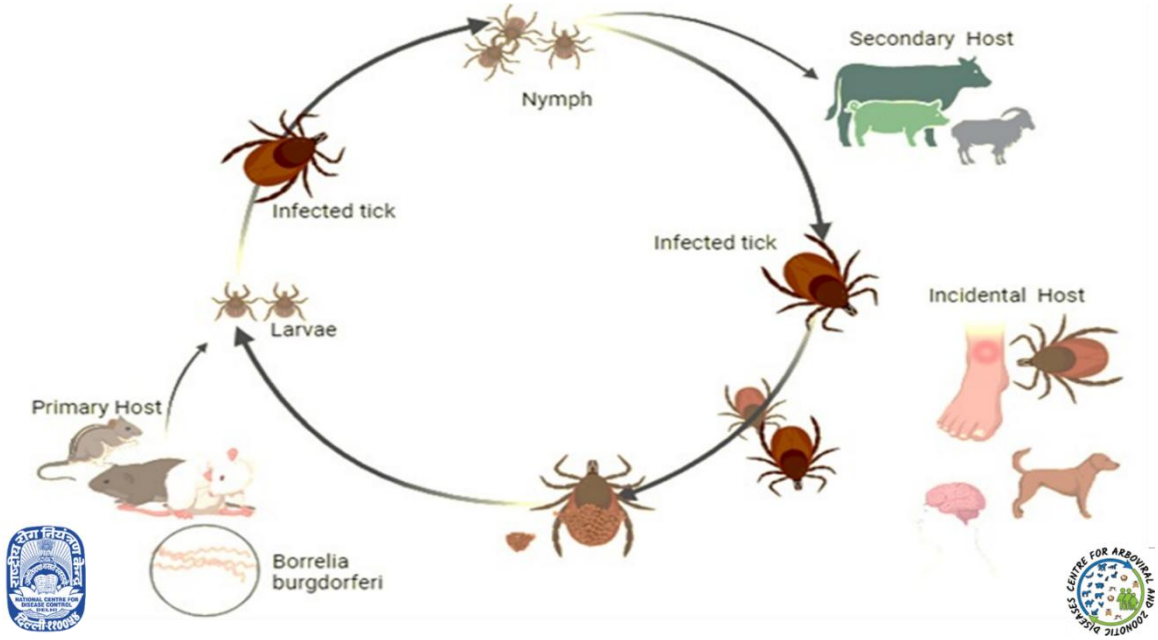


Figure 1: Life cycle of tick and Transmission cycle of Lyme Disease.

2.2 Current Situation

Overall, in the United States and Europe, Lyme disease is the most frequently reported tick-borne illness. The disease was first recognized as "Lyme arthritis" during studies of a cluster of children in Connecticut who were thought to have juvenile rheumatoid arthritis in 1977. The disease was made a nationally reportable condition in the US in 1991, and ever since then, both the incidence and geographic distribution of cases in the country have significantly increased. There may also be an increase in occurrence in various parts of Europe.

Prevalence of Lyme disease in India is still unknown. There are some isolated case reports from different parts of the country. In India, first case of Lyme disease was reported from Shimla, Himachal Pradesh in 1990 with a clinical picture of meningitis, carditis and arthritis. In the subsequent years, new cases of Lyme disease are increasingly being reported. A study among military personnel revealed 13 % prevalence of Lyme disease in North East India. Few reports are also available from southern part of India. During 1994, serologically positive cases of Lyme disease were reported from Coonoor in Nilgiri hills, Tamil Nadu state. In 2010, a case of Lyme disease was reported with neuroretinitis from Nagarhole forest in Western Ghats of Karnataka. Recently, death and four positive cases

were reported from Wayanad district, Kerala. It indicates that the disease is active in Western Ghats.

3. Clinical Features

Approximately 1.6%–7% of infected individuals may have asymptomatic infection. Erythema migrans (EM) at the site of the tick bite is the most common clinical symptom, and it eventually goes away even without antibiotic therapy. EM is traditionally described as an expanding, annular, erythematous skin lesion with central clearing, the so-called classic "bull's eye rash". However, the infecting pathogen has potential to spread to other tissues and organs, leading to severe manifestations involving skin, nervous system, joints, or heart. Most cases of Lyme disease present as one of three stages, which may occur sequentially if an earlier stage was untreated: early localized disease (usually < 30 days from exposure), early disseminated disease (< 3 months after exposure) and late disseminated disease (> 3 months after exposure). Most of the cases are being diagnosed based on clinical findings and ELISA due to lack of confirmatory assays.

Table 1: Clinical manifestations of Lyme disease

Stage	System	Manifestation
Early localized disease (usually < 30 days from exposure)	Skin	Erythema migrans (note: must be > 5 cm in diameter, painless and slowly expanding
	Systemic	Fever, Athralgias, Headache
Early disseminated disease (< 3 months after exposure)	Skin	Multiple erythema migrans
	Systemic	Fever, Athralgias, Headache, lymphadenopathy
	Heart	Atrioventricular block Tachyarrhythmias Myopericarditis Myocardial dysfunction
	CNS	Aseptic meningitis Cranial neuropathy (especially facial nerve palsy) Motor or sensory radiculopathy
	Ocular	Conjunctivitis (rare)
Late disseminated disease (> 3 months after exposure)	CNS	Encephalopathy Axonal polyradiculoneuropathy Chronic encephalomyelitis
	Musculoskeletal.	Oligoarticular arthritis
	Ocular	Retinitis (rare)

Animals

Animals, both wild and domestic, may become infected with Lyme disease bacteria and some of these (dogs, for instance) may develop arthritis. These animals can carry infected ticks into areas where humans live.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood, cerebrospinal fluid, Urine, Skin specimen

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours.

4.3 Laboratory Procedure

a. Microscopy: direct visualization of Lyme-related Borreliae in blood or other infected tissues is very challenging and easily subject to misinterpretation. The direct visual detection is not sensitive or practical as a first-line diagnostic approach, or even an adjunctive test as the in vivo bacterial burden is usually very low in primary tissue samples. The spirochetes can be visualized using Romanowsky stains (e.g., Giemsa) or with silver impregnation or immunohistochemical staining techniques using light microscopy. The spirochetes can be visualized using fluorescence microscopy, for example with the acridine orange stain or with direct fluorescent antibody staining. Dark-field or phase-contrast microscopy can be used to see live, cultivated organisms.
b. Culture: Lyme-related Borreliae can be cultivated in vivo using laboratory animals (e.g., mice, hamsters, or rabbits) or in vitro using artificial media. Because of their specific nutritional requirements, they don't grow in routine bacteriologic culture media. The most successful media have been modifications of Kelly's medium, such as BSK-II medium, BSK-H medium, and Kelly-Pettenkofer (MKP) medium. The spirochetes are microaerophilic in nature and their growth can be detected within 1 or 2 weeks, though 8 to 12 weeks of incubation should be allowed before termination. Detection of growth in the culture may be performed using microscopy or PCR methods.
c. Serological test: Most diagnostic test development has focused on indirect detection of Lyme disease by assessing the antibody response because spirochetes only transiently enter the bloodstream of infected persons in small numbers therefore direct detection of *B. burgdorferi* by PCR or culture has been challenging. Two-tiered serology is the corner stone for the laboratory diagnosis of Lyme disease. The serology involves detection of IgM using a sensitive enzyme immunoassay (EIA) or immunofluorescent assay (IFA), and IgG antibodies. It is recommended that a standardized Western immunoblot be performed on all specimens that are positive or equivocal by a sensitive EIA or IFA. Specimens that test negative using a sensitive EIA or IFA do not require additional testing.

   Both IgM and IgG procedures should be carried out when Western immunoblot is employed within the first 4 weeks after disease onset (early Lyme disease). A positive IgM test result alone is not advised for use in identifying active disease in individuals with illnesses lasting more than 1 month since there is a substantial risk of a false-positive test result for a current infection in these individuals. If a patient with suspected early Lyme disease has a negative serology, serologic evidence of infection is best obtained by testing of paired acute- and convalescent-phase serum samples. Serum samples from persons with disseminated or late-stage Lyme disease almost always have a strong IgG response to Borrelia burgdorferi antigens.

   Two of the following three bands—24 kDa (OspC), 39 kDa (BmpA), and 41 kDa (Fla)—should be present for an IgM immunoblot to be deemed positive. Further, IgG immunoblot should be considered positive if five of the following 10 bands are present: 18 kDa, 21 kDa (OspC), 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa (Fla), 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa.

d. Nucleic acid amplification tests -Several PCR assays targeting different *B. burgdorferi* genes, including chromosomal targets (e.g., *flaB, recA, 16S rRNA gene, oppA1*) and plasmid targets have been developed and evaluated. However, except for analysis of joint fluid prior to antimicrobial therapy in Lyme arthritis, PCR has been a low-yield procedure in Lyme borreliosis after the weeks of infection.

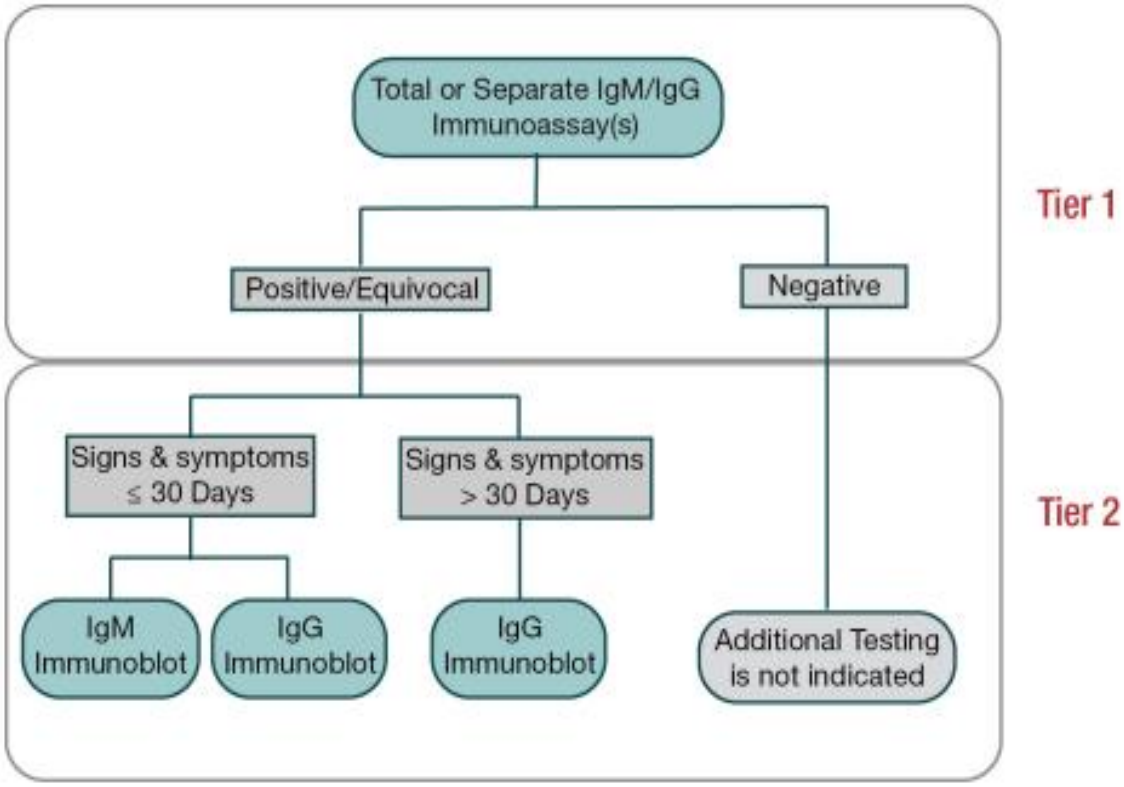


Figure 2: Two-Tiered Testing for Lyme Disease.

5. Treatment

The treatment of Lyme disease depends on the stage and organ system involved. Most cases can be treated with oral antibiotics, with the exception of neurological problems and Lyme arthritis, for which intravenous medication is more effective. Anyone over the age of 8 years with an early localised or disseminated disease is advised to take doxycycline for 14 to 21 days, with the exception of pregnant women. Amoxicillin is the second choice alternative to be used in children or pregnant women. Cefuroxime axetil is a third-choice alternative in case of any allergy. Erythromycin or its cogeners, a fourth-choice alternative, should only be taken by people who cannot take doxycycline, amoxicillin, or cefuroxime axetil.

6. Prevention and Control

Removal of the tick from the site of attachment within 24 hours of attachment is usually sufficient to prevent Lyme disease as 24 to 72 hours of tick attachment is necessary before transmission of the spirochete occurs. However, if an engorged nymphal *I. scapularis* tick is found, treating the patient with a single dosage of 200 mg of doxycycline within 72 hours of the tick bite usually prevents Lyme disease.

Interventions that target the vector or the reservoir have the potential to permanently change the trajectory of the illness, both in terms of incidence and geographic distribution.

a) Vector control: Reduction of local tick populations through the application of acaricides. Use of biopesticides (different essential oils, garlic, and nootkatone) to control the vector. Alternative methods for killing ticks include using compounds that can disrupt mating, such as sex pheromones, or that draw pests into traps. Reducing and managing deer populations in areas where Lyme disease is prevalent may also help reduce tick abundance.
b) Reservoirs based targets: One of the most highly efficient methods for lowering the carriage of a tick-borne infection is the use of reservoir-targeted antibiotics (doxycycline), and work is also being done to create a reservoir-targeted vaccine.

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1. Introduction

Melioidosis, [me"-le-do'sis] caused by Gram-negative environmental saprophyte, Burkholderia pseudomallei has earned its nomenclature from the combination of Greek words "melis", "eidos" and "osis" meaning "distemper of asses", "resemblance" and "abnormal disease" respectively. Historically, described as "Whitmore's disease" to honour British pathologist Alfred Whitmore who described mysterious glanders like illness, some of them progressing to sepsis in morphia addicts. The ability of *B. pseudomallei* to persist in abiotic environments like soil and water and to infect a wide range of hosts including humans and various animals yet with rare, questionable evidences of animal to human transmission fits melioidosis into a sapronosis or at best sapro-zoonosis category rather than a true zoonosis. The clinical spectrum of melioidosis in humans is broad, non-specific and encompasses dormant seroconversion, acute, fulminant pneumonia/septicaemia, internal organ abscesses, chronic/ persistent localized suppurative infections and is determined by a complex interplay of host factors, route of acquiring infection, dose of inoculum as well as bacterial virulence factors.

2. Epidemiology

2.*1 Causative Agent*

Burkholderia pseudomallei is a non-fermenting Gram-negative bacillus which is oxidase positive and motile. Earlier, taxonomically placed in rRNA homology group II of the genus Pseudomonas, it is now included under genus *Burkholderia sensu stricto* and *Burkholderia pseudomallei complex* (Bpc) species group along with *B. mallei, B. thailandensis, B. oklahomensis, B. humptydooensis, B. singularis*, and two recently described novel Species: *Burkholderia mayonis sp. nov.* and *Burkholderia savannae sp.,* resulting in a total number of eight species. The individual members of the (Bpc) species group have unique ecological niche. *B. mallei*, a host adapted obligate pathogen of true zoonotic potential without any saprophytic reservoir, has been demonstrated to be a clone of *B. pseudomallei* based on genomic studies [99% nucleotide sequence identity], however the species status has been retained by virtue of strong clinical as well as epidemiological dissimilarities between glanders and melioidosis. *B. pseudomallei* possesses unique bipartite genome structure, with one large chromosome, referred to as chromosome 1, responsible for core functions, and the small chromosome, chromosome 2 assigned for various accessory functions. *B. pseudomallei* is an opportunistic pathogen, and the role of various virulence factors, e.g., capsular polysaccharide, adhesins, type III and type VI secretion systems, actin based motility systems in the disease process continues to remain a topic of research.

2.2 Modes of Transmission

Percutaneous inoculation through pre-existing skin lesions (from contaminated soil and water) is the principal mode of infection acquisition, followed by inhalation and ingestion. Inhalation mode is important with high wind speed and mostly results in acute pneumonic/septicaemic form of the disease manifestation. Ingestion of unchlorinated water is also regarded as an important mode of transmission. Other less important modes of transmission include laboratory acquired infection, occasional evidences of mother to child transmission and sexual transmission. There has been a handful of reports of possible zoonotic transmission from aquarium fish, outbreak in Paris Zoo resulting in two cases of human fatalities etc. Global trade of exotic animals, livestock as well as animal products has allowed the introduction of *B. pseudomallei* into previous non-endemic regions. As the

causative agent is present abundantly in the abiotic environment, possibility of both animals and human beings acquiring infection from common environmental sources cannot be ruled out in the cases of possible zoonotic transmission.

Traditional Risk Factors	Putative Risk Factors	Environmental and Occupational Risk Factors
• Poor glycaemic control in diabetes mellitus, (12-fold increased risk) • Heavy regular alcohol consumption, • Chronic renal disease • Chronic pulmonary diseases	• Cancer chemotherapy, • Immunosuppressive medications/Steroid therapy • Cardiac diseases like rheumatic heart disease, congestive heart failure, • Chronic granulomatous disease, • Iron overload conditions like thalassemia and pulmonary hemosiderosis, • history of kava consumption • Tuberculosis	• Farmers, Construction workers, Gardeners, Fishermen, swimming in muddy water, Military Personnel • Rainfall, humidity, wind speed, cloud cover, flood, cyclonic storm

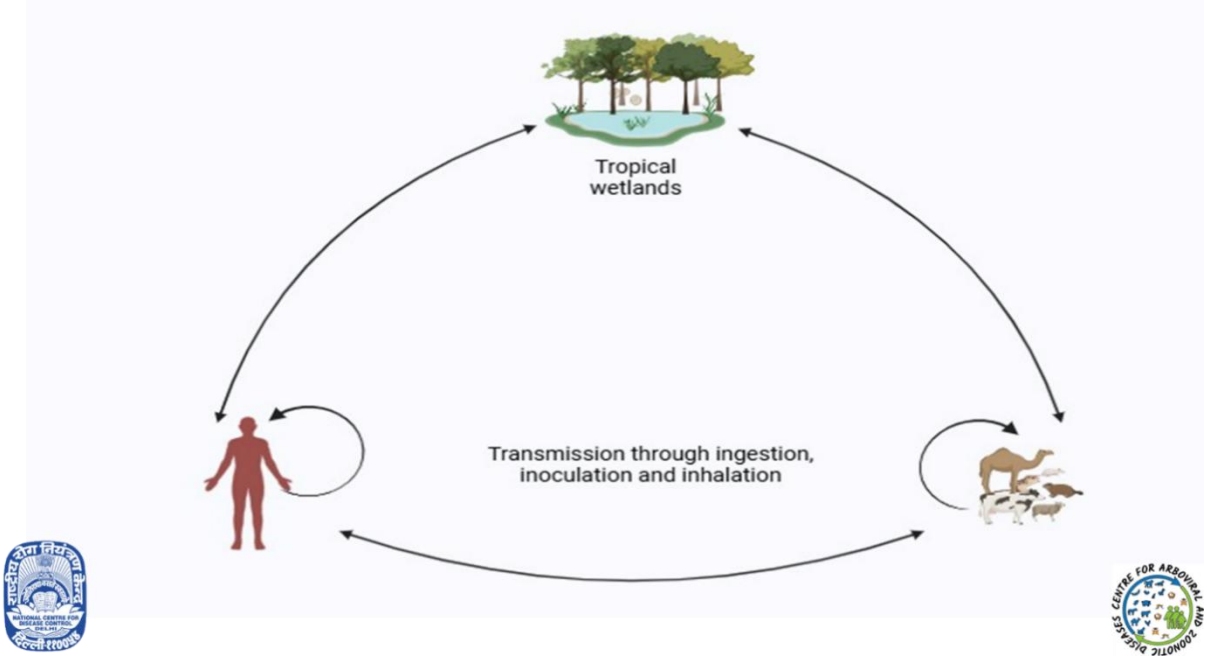


Figure 1: Transmission Cycle of Melioidosis.

2.3 Current Situation

Till 1991, it was known that melioidosis was highly endemic to Southeast Asian countries (North eastern Thailand, Malaysia, Singapore) and Northern territories of Australia. In 2016, a landmark modelling study, listed 45 countries as melioidosis endemic based on reported cases of human/animal infections and/or demonstration of presence of *B. pseudomallei* in the environment and 34 countries were labelled as probably endemic because of conducive climatic factors. South and South East Asian countries and Australia

bear the highest disease burden, and cases are now being recognized Southern United States, previously considered as non-endemic. Though melioidosis is not an officially notifiable disease in many of the countries, the number of culture -confirmed cases across various countries can be accessed from https://www.melioidosis.info/map.aspx.

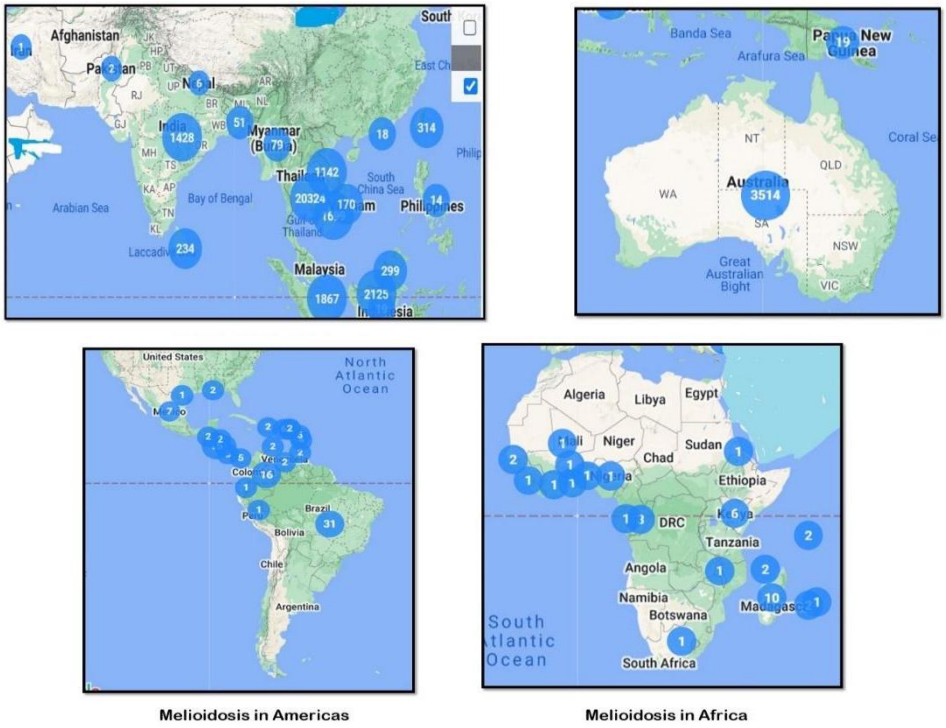


Figure 2: Culture confirmed cases of Melioidosis. (https://www.melioidosis.info/map.aspx)

Indian subcontinent is predicted to have annual incidence of ≈52,000 melioidosis cases. Melioidosis is not included as a notifiable disease in India, hence the available information about the diseases is from published case series/case reports. In the last decade, more than 1000 cases have been reported from various parts of India, mainly from Karnataka, Tamil Nādu, Odisha, and Kerala. Barring few northern states, melioidosis cases are being recognised from all over India, due to increased awareness and microbiology laboratory capacity building.

3. Clinical Features

Humans

Melioidosis has earned the name Great mimicker because of a wide range of non-specific signs and symptoms, thus confounding the clinical diagnosis. The disease can have acute (88%), chronic ≈2 months (9%) presentations as well as reactivation of latent infection (3%). The disease can involve virtually every organ system of the body and can be either localized or disseminated. Melioidosis should be included in the differentials in the following conditions, in endemic places, including India.

a) Prolonged fever (>3 weeks) is not responding to conventional antimicrobials without an immediately apparent etiology, especially during the rainy season.

b) Acute onset community acquired sepsis, defined as "presence of ≥ two of the following, i.e., temperature < 36°C or > 38°C, tachycardia (Heart rate> 90 beats/minute), tachypnoea (respiratory rate >20/minute, total white cell count>12,000 cells/mm3 or band forms > 10%.
c) Acute onset community-acquired pneumonia presenting with cough purulent expectoration, shortness of breath and/or pleuritic chest pain with variable radiological features, e.g., minimal infiltrates/cavitation/diffuse parenchymal disease) and not responding to conventional antimicrobials (e.g., standard dosing regimens of β lactams, macrolides, or fluoroquinolones).
d) Parenchymal visceral abscesses (e.g., spleen, liver, kidneys) with characteristic ultrasonographic features (Swiss cheese appearance of tiny, dispersed abscesses). Intracerebral abscesses, prostatic abscesses, and parotid abscesses (especially in children)
e) Chronic ulcerative/pustular cutaneous lesions, unresponsive to conventional antimicrobials.
f) Fever, weight loss, and productive cough with predominantly upper lobe infiltrates on chest radiography mimicking pulmonary tuberculosis.
g) Lymphadenitis, particularly involving the cervical lymph nodes.
h) Neuromelioidosis with various combinations of Cranial nerve palsies, Cerebellar signs, and peripheral weakness.
i) Bone and Joint Infections e.g., chronic osteomyelitis, and septic arthritis.
j) Other rarer presentations of pyogenic lesions, including thyroid and scrotal abscesses, breast abscess, infected pancreatic pseudocyst

Animals

The wide range of susceptible animals are sheep, goats, and pigs; (Most commonly affected) Cattle, Buffalo, Horses, Mules, Deer Camels, Alpacas, Dogs, cats, Dolphins, Wallabies, Koalas, Human and non-human primates Birds, Tropical fish, and Reptiles including crocodiles are also susceptible. The clinical features can be of wide range and vary widely between species, e.g., respiratory involvement in sheep, mastitis in goats, fever and nervous system involvement in cattle, camelids pigs etc.

There are reports of melioidosis outbreaks in Zoo animals from Thailand, captive animals in Australia, and recent reports of fatal outbreak affecting monkeys in a zoo in Hongkong. A recent human case of possible zoonotic transmission of *B. pseudomallei* from tropical fish aquarium in the United States, suggest that the risk of human cases from animal sources should be more rigorously investigated by genetic analysis studies.

4. Laboratory Diagnosis

4.1 Collection of Specimen

For optimal isolation of *B. pseudomallei* in culture, a combination of blood, throat swab/sputum, and centrifuged urine deposit are recommended specimens in all cases of suspected melioidosis irrespective of presenting symptoms. Specimens such as pus/exudate, USG-guided aspirates from deep- seated organ abscesses and lymph node aspirates have a high bacterial load. In few cases, invasive sampling methods like endobronchial ultrasonography (EBUS) may be useful in rarer presentations like isolated mediastinal lymphadenopathy.

4.2 Storage and Transport

The samples should be transported in Triple layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Specimen should be shipped immediately and if delay is anticipated, it must be stored in cold chain.

4.3 Laboratory Procedures

Simple bench side tests such as Gram stain finding of bipolar or irregular staining, oxidase positivity and triple disc screening (resistant to polymyxin B 300 Units/Colistin 10µg, Gentamicin 10 µg, and susceptible to Amox/clav 20/10µg disc) provide presumptive species-level identification in resource constrained laboratories. A recent meta-analysis of non-culture diagnostic tests for melioidosis have concluded that direct PCR based assays, LFI and immunofluorescence-based assays cannot substitute culture due to issues in sensitivity. Confirmation is to be done by isolation

a) Isolation: Handling of samples for suspected melioidosis should ideally be performed inside a class II biological safety cabinet with gloves and mask. Culture positivity is essential for definitive diagnosis, though culture has the limitation of suboptimal sensitivity (≈60%). Blood cultures provide highest yield, and needs to be repeated if the initial cultures are negative and there is high index of clinical suspicion. Routine bacteriological culture media (e.g., 5% sheep blood agar, MacConkey agar) support the growth of *B. pseudomallei*, and a prolonged incubation up to five days is necessary to compensate for slow growth of *B. pseudomallei*. In clinical specimens heavily colonized with commensal flora, e.g., sputum, throat swab etc., initial enrichment culture in CVC-50 broth containing colistin, followed by plating of surface pellicle onto selective media such as Ashdown agar, has been shown to increase the case detection of melioidosis. Urine sample requires centrifugation followed by culturing of centrifuged deposit on Ashdown agar for optimal recovery of *B. pseudomallei.* Ashdown medium and CVC 50 broth are not commercially available and have to be in-house prepared. Colony morphologies on various culture media at various stages of incubation provide initial valuable clues to identification.

Seven distinct *B. pseudomallei* colony morphotypes have been described, with type I being the most common morphotype. Most morphotypes produce rough colonies, except type III and VI morphotypes which produce smooth colonies.

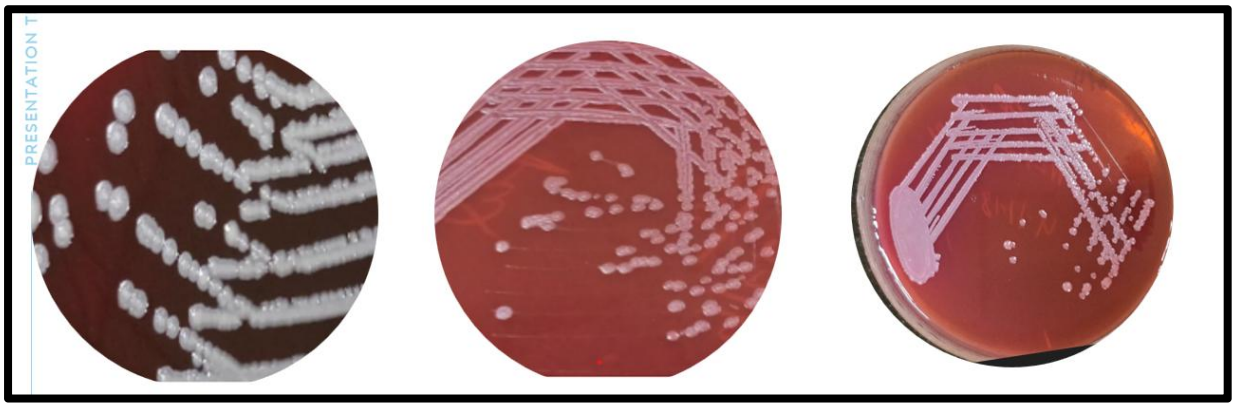


Figure 3: Characteristic colony morphologies of *B. pseudomallei* **A)** On sheep blood agar, *B. pseudomallei* colonies are cream-colored with slight metallic sheen **B)** On MacConkey agar, colonies are initially lactose non-fermenting and opaque with a metallic sheen and after 48-72 hours of incubation, characteristic rugose appearance with central umbonation is evident. **C)** On Ashdown's agar, colonies are small at overnight incubation and gradually evolve to purple, flat, dry, wrinkled appearance after 48-72 hours.

Misidentification as *Pseudomonas aeruginosa* by diagnostic microbiology laboratories is very common and it is often stated as a golden rule that in cases of oxidase positive Gram – negative bacilli, resistance to either Polymyxin B 300 Units or Colistin 10µg

on disc diffusion, the possibility of *B. pseudomallei* should be strongly considered. Battery of biochemical tests can be put to distinguish between closely related members of Burkholderia sensu stricto.

Commercial identification platforms such as Vitek 2 (Biomerieux, USA) has geographically variable levels of accuracy in correct identification ranging from 19-98% and 53-88% in isolates from Australia and Malaysia respectively. Incorrect identification is also reported with other commercial platforms like API 20 E and BD phoenix. *B. pseudomallei* is currently not included in the in vitro diagnostic use (IVD) database and is part of research use only (RUO) databases of two commercially available mass spectrometry (MS) systems (MALDI Biotyper, Bruker Daltonik GmbH, and the Vitek MS BioMérieux).

Antimicrobial susceptibility testing (AST)- Even though there are scarce reports of resistance, routine AST is recommended for all culture-confirmed cases of melioidosis for the drugs recommended for intensive as well as eradication phases. Breakpoints for minimum inhibitory concentrations (MIC) as well as zone diameters may be used for AST. However, disk diffusion method may overestimate Cotrimoxazole resistance, hence confirmation by an alternate MIC method is indicated, as co-trimoxazole remains the most valuable drug in the eradication phase.

b) Serological test: Latex agglutination (Mahidol University, Thailand) and Lateral flow immunoassay (LFI, In Bios) detecting specific 200 kDa exopolysccharide and capsular polysaccharides remain valuable, quick tool for final identification from colonies as well as from positive blood culture broth, however commercial availability remains an issue. Given the limitations of culture, there is a need of antigen detection tests which can be deployed directly from various samples. A commercially available test detects capsular polysaccharide antigen in a lateral flow immunoassay format (InBios Active Melioidosis Detect lateral flow test, research only use) have been validated in few studies, including In India. The test can be performed on pus/exudate/aspirate, urine, sputum/BAL as well from serum specimens. Sputum and pus samples have shown to have better sensitivity (85% and 83% respectively) followed by urine (63%) and serum samples have the least sensitivity (27%). Testing multiple specimens from suspected cases increases the sensitivity to 91% in culture confirmed cases. Serodiagnosis methods like Indirect haemagglutination assay (IHA) have limited role in clinical diagnosis in endemic countries like India.

c) Molecular test: A validated PCR (type III secretion system gene) performed by quality assured laboratory can provide reliable results. Molecular confirmation remains the reference method for final species level identification and the type III secretion system gene cluster1 (TTS1) remains the most plausible target for discriminating between closely related members.

5. Treatment

Melioidosis treatment is prolonged and is divided into two phases, i.e., intensive phase with either I.V ceftazidime or a carbapenem (preferably Meropenem) for at least two weeks and eradication phase with oral antibiotics (preferably co-trimoxazole, and alternatives being amox-clav/doxycycline in patients experiencing adverse drug reactions to co-trimoxazole). Though ceftazidime (2gm IV 6-8 hourly) is the drug of choice, Meropenem (1-2gm IV 8hourly) is the preferred drug in intensive phase in patients with septic shock, neurological manifestations, and patients with clinical nonresponse to ceftazidime. A repeat blood culture is recommended in bacteremic cases towards the end of first week, and a positive blood culture warrants prolongation of the intensive phase. Intensive phase is

continued beyond two weeks in complicated pneumonia, deep seated abscesses and neurological melioidosis to 4-8 weeks respectively. Melioidosis cases without pneumonia benefitted with the addition of co-trimoxazole (up to 320/1600 mg 12hrly) to the intensive phase therapy. Adherence to therapy in the eradication phase (12-20 weeks) is crucial to prevent recrudescence and relapse. Prolonged eradication phase (up to 6months) is recommended in osteomyelitis, neurological and mycotic aneurysm cases. The adverse drug reactions to co-trimoxazole remains a cause of concern and non-compliance in the eradication phase is the most common cause for relapse. In addition to antimicrobial therapy, surgical drainage of abscesses particularly in prostatic, bone and joint infections and intensive care management of sepsis/septic shock is also of paramount importance. The role of G-CSF in melioidosis sepsis cases remains unclear.

6. Prevention and Control

Human beings

a) Personal Protective wear should be worn particularly by farmers, construction site workers, and gardeners to prevent percutaneous transmission e.g., knee length gum boots, trousers while working outdoors with soil contact. Health care workers should wear PPE during aerosol generating propagative processes. Covering of open wounds in extremities, avoidance of topical herbal formulations, hand washing with clean water after soil contact.

b) Advisory for staying indoors during windy, dusty weather, Kalbaisakhi, topical cyclonic storm. Properly treated potable water consumption should be done in endemic areas. Proper washing of raw vegetables and fruits with clean water, pasteurization of goat milk.

c) Vaccine is the most promising approach, however, no licensed vaccine is available so far. There is requirement of multi-component vaccine strategy in high-risk individuals. The combination of Capsular polysaccharide (CPS)-CRM197 (non-toxigenic diphtheria toxin mutant) & Recombinant hcp1(hemolysin-coregulated protein 1) may be the candidate vaccine to enter human trial.

d) Post exposure prophylaxis by Oral co-trimoxazole for 21 days is drug of choice. Risk-benefit judgement required in cases of definite high-risk exposure e.g., laboratory exposure in high-risk individuals.

Animals

In Endemic areas to prevent outbreak in herds the rearing of livestock animals on artificial hard, concrete surfaces for avoiding access to soil. Isolation of infected animals, body surface antisepsis with potassium hypochlorite and cresol, with particular attention to lower limbs, frequent disposal of infected excreta, proper disposal of infected carcasses should be mandated.

Increasing awareness, capacity building of microbiology laboratories is of paramount importance to reduce the mortality, morbidity of melioidosis in India. At the same time core research is warranted for development of a suitable point of care test to reduce the turn - around time for diagnosis as well as development of a suitable vaccine.

Further Reading

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1. Introduction

Mpox, earlier referred to as Monkeypox, is a viral disease of zoonotic origin with Smallpox like symptoms, but with less clinical severity. WHO declared it a Public Health Emergency of International Concern (PHEIC) first in July 2022 and recently again in August 2024. Mpox was first discovered in 1958 in colonies of monkeys kept for research, hence the name 'Mpox' was derived. It was first identified in humans in 1970 in the Democratic Republic of the Congo (DRC) in a 9-year-old boy in a region where smallpox had been eliminated in 1968. Since 1970, cases of Mpox have been reported in 15 African countries. In 2003, the first Mpox outbreak outside Africa was reported in USA and was linked to contact with infected pet prairie dogs. Spillover of Mpox virus is likely linked to specific latitude and ecological conditions in Africa.

2. Epidemiology

2.1 Causative Agent

Mpox disease is caused by the Mpox virus (MPXV). It is an enveloped double-stranded DNA virus of the Orthopoxvirus genus in the Poxviridae family, which includes variola, cowpox, vaccinia, and other viruses. There are two distinct genetic clades of the Mpox virus – Central African (Congo Basin) clade and West African clade. WHO renamed the Congo Basin Clade (more virulent and transmissible) as Clade I (with subclades Ia and Ib) and the West African Clade as Clade II (with subclades IIa and IIb).

2.2 Mode of Transmission

The natural reservoir is yet unknown. However, certain rodents (including rope squirrels, tree squirrels, Gambian pouched rats, dormice) and non-human primates are known to be naturally susceptible to Mpox virus infection. The incubation period (interval from infection to onset of symptoms) of Mpox is usually from 6 to 13 days but can range from 5 to 21 days. A person is not contagious during this period. Physicians are currently recommended to monitor patients for up to 21 days. Period of communicability: 1-2 days before the rash to until all the scabs fall off/get subsided.

As per reports, clade Ib is spreading primarily through household contacts and frequently infects children. Clade IIb, which had prompted the previous WHO global warning in 2022, spread mainly through sexual contact. While clade Ib causes a similar illness to clade IIb, it is considered capable of spreading faster and killing more people.

Human-to-human transmission

- Close contact with infected person: Through large respiratory droplets. Being face-to-face (talking or breathing close enough for droplets to carry) with an infected person is a risk factor.
- Direct contact with the body secretions of infected persons: It can also be transmitted through direct contact with body fluids or lesion material, and indirect contact with lesion material, such as through contaminated clothing or linens of an infected person.
- Sexual transmission: Transmission between sexual partners, having skin-to-skin contact, including sex and mouth-to-mouth or mouth-to-skin contact are among the risk factors.
- Vertical transmission: Pregnant women with Mpox can pass the virus to the foetus during pregnancy or to the new-born during and after birth.

Animal-to-human transmission

- Animal-to-human Mpox virus transmission may occur by bite or scratch of infected animals like small mammals including rodents (rats, squirrels) and non-human primates (monkeys, apes) or through bush meat preparation.

Risk Factors: Skinning infected animals or eating their meat if not cooked thoroughly also exposes one to the disease. People with low or compromised immunity are at a higher risk. People are less likely to get Mpox from a pet, but it's possible. Close contact with a pet that is infected, including petting, cuddling, hugging, kissing, licking, and sharing sleeping spaces or food, can spread Mpox to a person.

2.3 Current Situation

Earlier regarded as a disease largely confined to Africa, in a series of outbreaks reported from Europe in 2022, it was the first time that chains of transmission were without known epidemiological links to West or Central Africa. Since 1st January 2022, Mpox cases have been reported to WHO from 121 Member States across all 6 WHO regions. As of 31st July 2024, a total of 1,02,997 laboratory-confirmed cases and 186 probable cases, including 223 deaths, have been reported to WHO. As of July 2024, the number of monthly reported new cases has increased by 8.8%, compared to the previous month. Majority of cases reported in the past month were mainly notified from African Region (54.9%) and the Region of the Americas (24.2%). The 10 most affected countries globally since 1 Jan 2022 are: USA (n = 33,556), Brazil (n = 11,841), Spain (n = 8,104), DRC (n = 4,385), France (n = 4,283), Colombia (n = 4,256), Mexico (n = 4,132), UK (n = 4,018), Peru (n = 3,939), and Germany (n = 3,886). Together, these account for 80% of cases reported globally.



Figure 1: Global distribution of MPXV clades (WHO).

Till recently, all the sequences in present global outbreak were associated with Clade IIb. However, since early 2024, there has been an upsurge in the number of cases and deaths due to Mpox (Clade Ib) in African region. CDC has vaccination recommendations for people traveling to countries with clade I outbreaks. As of December 11, 2024, these countries

include Burundi, Central African Republic, Democratic Republic of the Congo, Republic of the Congo, Rwanda, and Uganda.

In India, the first case of Mpox was reported in Kollam, Kerala on 14th July 2022. As per latest data, there are no active mpox cases across the country. However, since 2022, total 33 laboratory-confirmed mpox cases (17 cases from Kerala and 16 cases from Delhi) have been reported by the States and UTs in the country. Self reporting to the health care provider has been the modality for case detection in India.

3. Clinical Features

Mpox is usually a self-limiting disease with symptoms lasting from 2-4 weeks. Severe cases occur more commonly among children and are related to extent of virus exposure, patient health status and nature of complications. The extent to which asymptomatic infection occurs is unknown. Mpox disease is characterized by an incubation period, prodrome, and rash.

- Prodrome (0-5 days) - A person may be contagious during this period. Instruct the patients to isolate if they develop symptoms like:
  - Fever
  - Lymphadenopathy: Typically occurs with fever onset ➢ Peri-auricular, axillary, cervical or inguinal ➢ Unilateral or bilateral
  - Headache, muscle aches, exhaustion
  - Chills and/or sweats
  - Sore throat and cough
- Skin involvement (rash) - Lesions typically develop simultaneously and evolve together on any given part of the body. The evolution of lesions progresses through four stages —macular, papular, vesicular, to pustular —before scabbing over and desquamation. A person is contagious until after all scabs on the skin have fallen off and a fresh layer of intact skin has formed underneath.
  - Usually begins within 1-3 days of fever onset, lasting for around 2-4 weeks
  - Deep-seated, well-circumscribed and often develop umbilication Lesions are often painful until healing phase when they become itchy (in crust stage)
  - Stages of rash (slow evolution)
    - Enanthem- first lesions on tongue and mouth
    - Macules starting from face spreading to arms, legs, palms, and soles (centrifugal distribution), within 24 hours
    - The rash goes through a macular, papular, vesicular and pustular phase. Classic lesion is vesico-pustular.
  - Involvement by area: face (98%), palms and soles (95%), oral mucous membranes (70%), genitalia (28%), conjunctiva (20%). Generally skin rashes are more apparent on the limbs and face than on the trunk. During the 2022 outbreak in the European Region, most of the cases were picked up in sexual health clinics, with patients presenting with lesions on their genitals and anus.

    The lesion heals with hyper-pigmented atrophic scars, hypo-pigmented atrophic scars, patchy alopecia, hypertrophic skin scarring and contracture/deformity of facial muscles following healing of ulcerated facial

lesions. A notable predilection for palm and soles is characteristic of Mpox. At any one point in time, pleomorphic forms can also be seen in a patient.

- The skin manifestation depends on vaccination status, age, nutritional status, associated HIV status. Mpox chiefly occurs in communities where there is often a high background prevalence of malnutrition, parasitic infections, and other significant heath-compromising conditions, any of which could impact the prognosis of a patient with MPX. g. The total lesion burden at the apex of rash can be quite high (>500 lesions) or relatively slight (<25)
- Complications of Mpox in children can be severe and may include secondary bacterial infections, severe skin infections, dehydration, pneumonia, encephalitis, ocular complications and even longer sequelae such as scarring of skin lesions.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Rash phase:

- Skin lesion material from multiple sites, including swabs of lesion surface and/or exudate, roofs from more than one lesion, or lesion crusts.
- Nasopharyngeal/Oropharyngeal swab is recommended for diagnosis, in addition to skin lesion material.
- Blood and Urine sample

Recovery phase: Blood and Urine sample

PPE to be donned before collecting the specimens.

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedures

For the confirmation of Mpox on the suspected clinical specimens:

a) Mpox-specific conventional PCR or real time PCR for Mpox DNA is recommended on clinically suspected cases.

b) Additionally, virus isolation and the Next Generation Sequencing of clinical samples (Miniseq and Nextseq) used for the characterization of the positive clinical specimens.

5. Treatment

Treatment of Mpox is primarily supportive. Principles of Management include:

- Patient isolation and protection of compromised skin and mucous membranes
  - Isolation of the patient in a separate room at hospital/ at home.
  - Patient to wear a triple layer mask.
  - Skin lesions should be covered to the best extent possible (e.g. long sleeves, long pants) to minimize risk of contact with others.
  - Isolation to be continued until all lesions have resolved and scabs have completely fallen off and a fresh layer of intact skin transformed.
- Rehydration therapy and nutritional support.
- Symptom alleviation.

- Antibiotics to treat secondary bacterial infections if they develop.
- Monitoring and treatment of complications.

6. Prevention and Control

Raising awareness of risk factors and educating people about the measures to reduce exposure to the virus is the main prevention strategy for Mpox. Several measures can be taken to prevent infection with Mpox virus:

- Avoid contact with any materials, such as bedding, clothing etc. that has been in contact with a sick person.
- Isolate infected patients from others: Affected individuals should avoid close contact with immune-compromised persons and pregnant women until all crusts are gone. Isolation precautions should be continued until all lesions have resolved and a fresh layer of skin has formed.
- Practice good hand hygiene after contact with infected animals or humans. For example, washing hands with soap and water or using an alcohol-based hand sanitizer.
- Use appropriate personal protective equipment (PPE) when caring for patients.
- Correct containment and disposal of contaminated waste (e.g., dressings) in accordance with Biomedical Waste Management guidelines for infectious waste.

Three vaccines are currently licensed for mpox:

i. Modified vaccinia Ankara-BN (e.g., MVA-BN or JYNNEOS, Imvamune or Imvanex), a 2-dose 3rd generation smallpox vaccine that is a highly-attenuated replication-deficient vaccinia virus vaccine, approved in the USA, Canada and Europe;
ii. LC16-KMB (licensed in Japan) and
iii. OrthopoxVac (licensed in the Russian Federation).

- WHO advises vaccination for individuals only at high risk of exposure, such as those with certain occupations or circumstances, and travellers who may be at risk, as determined by a healthcare provider.
- Based on currently assessed risks and benefits, mass vaccination is NOT recommended by WHO for Mpox at present. India has also not issued any advisory pertaining to Mpox vaccination at present.

Further Reading

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2. World Health Organization. Fact Sheet. Mpox. https://www.who.int/news-room/fact-sheets/detail/mpox
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1. Introduction

Nipah virus disease (NVD) is an emerging zoonotic infectious disease caused by Nipah virus (NiV) belonging to the genus Henipavirus within the family Paramyxoviridae. The name "Nipah" refers to the place, Sungai Nipah (literally '*nipah* river') in Port Dickson, Negeri Sembilan, the source of the human case from which Nipah virus was first isolated. Its first recognition was held during a large outbreak of 276 reported cases in Malaysia and Singapore from September 1998 to May 1999. The Nipah virus has been classified as a Risk Group 4 pathogen by WHO, and Category C agent by the Center for Disease Control and Prevention. The case fatality rate is estimated at 40% to 75%.

2. Epidemiology

2.1 Causative Agent

Nipah is a zoonotic virus and a member of the family Paramyxoviridae and genus Henipavirus. Nipah virus genome is a negative-sense, single-stranded RNA (non-segmented) of over 18 kbp, substantially longer than other paramyxoviruses. The enveloped virus particles are variable in shape, and can be filamentous or spherical; they contain a helical nucleocapsid. The NiV genome codes for six open-reading frames (structural proteins). The gene order from 3' to 5' is N (nucleocapsid), P (phosphoprotein), M (matrix), F (fusion), G (glycoprotein), and L (RNA polymerase). The *P* open reading frame also encodes three non-structural proteins, C, V, and W. Based on sequence differences of either whole or partial nucleocapsid (N) gene, NiV strains are divided into two clades (Clade I: NiV-B in Bangladesh and India, and Clade II: NiV-M in Malaysia) which suggests two separate NiV introductions in these countries.

2.2 Mode of Transmission

Large fruit bats or flying foxes (bats in the *Pteropus* genus) are the natural reservoir of NiV. These are distributed at the coastal regions and in several islands in the Indian ocean, India, south-east Asia and Oceania. NiV has a broad host range and includes humans, bats, pigs, sheep, goats, dogs, cats, and horses.

The incubation period of NiV generally ranges between 4–21 days; however, longer incubation periods have also been observed, notably during the outbreak in Malaysia when the incubation period was up to two months. It is presumed that infected persons remain infectious till 21 days after the onset of symptoms. NiV can be transmitted to people via:

- Direct contact with infected animals, such as bats or pigs, or their excretions and secretions (such as blood, urine or saliva).
- Consumption of unprocessed meat from infected animals, or raw food products contaminated by body fluids of infected animals (such as palm sap or fruit contaminated by an infected bat).
- Close contact with a person infected with NiV or their body fluids (including nasal or respiratory droplets, urine, or blood).

2.3 Current Situation

Nipah virus (NiV) was first discovered in 1999 following an outbreak of disease in pigs and people in Malaysia and Singapore. This outbreak resulted in nearly 300 human cases and more than 100 deaths, and caused substantial economic impact as more than 1 million pigs were killed to help control the outbreak. While there have been no other known

outbreaks of NiV in Malaysia and Singapore since 1999, outbreaks have been recorded almost annually in some parts of Asia since then—primarily in Bangladesh and India. The virus has been shown to spread from person to-person in these outbreaks, raising concerns about the potential for NiV to cause a global pandemic.

Since its discovery, there have been almost 726 cases of NVD and 427 deaths reported globally with cases mainly from the Southeast Asia region. Of these, 46% of cases have been reported from Bangladesh, Malaysia (36.5%), India (13.5%), Philippines (2.5%), and Singapore (1.5%). In Bangladesh, Nipah virus infection outbreaks are seasonal, corresponding with the harvesting season of date palm sap from November to March.

In India, initial NiV outbreaks in humans were reported from West Bengal in 2001 and 2007. Later after almost a decade, in 2018, Nipah virus outbreak was reported in Kozhikode and Malappuram, Kerala (23 cases, CFR: 89.4 %). In 2019 and 2021, single case was reported from Ernakulum and Kozhikode respectively. In 2023, a total of 6 cases have been reported from Kozhikode District, Karela. Out of these reported cases, two succumbed to the disease. Recently in 2024, India reported two fatal cases of Nipah virus from Mallapuram, Kerala.

3. Clinical Features

Human

Human infections range from asymptomatic to acute respiratory infection (mild, severe), and fatal encephalitis. Infected people initially develop symptoms including fever, headaches, myalgia (muscle pain), vomiting and sore throat. This can be followed by dizziness, drowsiness, altered consciousness, and neurological signs that indicate acute encephalitis. Some people can also experience atypical pneumonia and severe respiratory problems, including acute respiratory distress. Encephalitis and seizures occur in severe cases, progressing to coma within 24 to 48 hours.

The incubation period (interval from infection to the onset of symptoms) is believed to range from 4 to 14 days. However, an incubation period as long as 45 days has been reported.

Most people who survive acute encephalitis make a full recovery, but long-term neurologic conditions have been reported in survivors. Approximately 20% of patients are left with residual neurological consequences such as seizure disorder and personality changes. A small number of people who recover subsequently relapse or develop delayed onset encephalitis.

Animal

NiV can infect and cause subclinical or clinical diseases in domestic animals such as dogs, cats, pigs, horses, goats and sheep. The broad species tropism is also demonstrated by experimental studies showing NiV infection of hamster, guinea pigs, and ferrets. The illness is similar to the human disease i.e. neurological or respiratory. It seems to be mostly acute and self-limiting, but can also be fatal. NiV infection remains asymptomatic in fruit bats. NiV infection presents as mild to severe disease in intermediate hosts (swine and equine). In pigs, most of the infections are asymptomatic and vary by the age of the host. The incubation period in experimental studies was observed to be 5-7 days post infection. The older pigs appear to be affected more than young pigs. The virus shows neural and

respiratory tropism in pigs. The symptoms may include neurological manifestations such as twitching, muscle spasm, and ataxic gait. In horses, NiV infection is presented as a severe respiratory disease with fever and tachycardia leading to a rapid deterioration of health. The incubation period is between 5-16 days. The fatality rate in horses is >75%.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Swab (Nasal and oropharyngeal)
Blood/Serum. Blood/serum (Paired samples)- one acute phase or immediately on reporting of case and second after 2-3 weeks of onset of fever.
Urine sample
CSF (Disease with CNS manifestations)
Tissue sample-brain, kidney, spleen etc.

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedures

a) Molecular test: In acute phase, NiV infection can be confirmed by detecting NiV-specific RNA in the clinical samples using RT-PCR.
b) Serological test: In later phase of illness (10-14 days post the symptom onset), the antibody against NiV infection can be demonstrated using enzyme-linked immunosorbent assay (ELISA)

5. Treatment

Currently there is no known treatment or vaccine available for either people or animals. Treatment is limited to intensive supportive care, including rest, hydration, and treatment of symptoms as they occur. Ribavirin, an antiviral may have a role in reducing mortality among patients with encephalitis caused by Nipah virus disease, but its efficacy is unclear. Immunotherapeutic treatments (monoclonal antibody therapies) are currently under development and evaluation for treatment of NiV infections.

6. Prevention and Control

- **Reducing the risk of bat-to-human transmission:** Decreasing bat access to date palm sap and other fresh food products with protective coverings (such as bamboo sap skirts) may be helpful. Consumption of raw date palm juice should be avoided. Freshly collected date palm juice should be boiled, fruits should be thoroughly washed and peeled before consumption.
- **Reducing the risk of animal-to-human transmission:** Need to avoid contact with fruit bats and other domestic animals, especially pigs, dogs, cattle etc. and with their secretions and body fluids. Gloves and other protective clothing should be worn while handling sick animals or their tissues, and during slaughtering and culling procedures.
- **Reducing the risk of human-to-human transmission:** Close unprotected physical contact with Nipah virus-infected people should be avoided.
    - Regular hand washing.

- Travelers to the areas affected should be aware of the possible risk of infection and follow any local guidance issued to minimize this risk.

- **Risk assessment in field investigations and control of contacts and the immediate environment**
  a. Review the situation of the area, investigation site, and any local assistance prior to commencement of any examination of live or dead animals.
  b. Communicate any concerns or advise precautions to assistants through IEC regarding possible risk factors for the disease.
  c. Establishment of a dedicated isolation facility for confirmed /suspected cases.
  d. Appropriate PPE should be worn by caregivers, treating physicians, healthcare professionals, and laboratory personnel while handling the patients or samples suspected of NiV infection.
  e. Wash hands and equipment(s) after examinations or specimen collection. Disinfect protective clothing, refuse and biological waste and dispose it off safely.
  f. Handling of dead bodies should be done in accordance with the govt. advisory
- **Other Considerations for Prevention and Control**
  a. Since the original source of transmission by various species of fruit bats, it may be possible to reduce the transmission of Nipah to pigs by removing the fruit source from a farm, practicing import/export caution, and enhanced biosecurity planning.
  b. Concurrent disinfection: Slaughter of infected pigs, horses or swine with burial or incineration of carcass under the supervision of health authority.
  c. Quarantine: Restrict movement of pigs and horses from infected farms to other areas.
  a. Enhanced hygiene and updated protocols on pig operations are essential.

Further Reading

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PLAGUE
CHAPTER 26

1. Introduction

Plague is an acute communicable zoonotic disease caused by the bacterium *Yersinia pestis*, which is usually found in small mammals and their fleas. It is transmitted between animals through fleas. Humans can get the infection by the bite of infected vector fleas, by unprotected contact with infectious body fluids or contaminated materials, or by inhaling respiratory droplets/small particles from a patient with pneumonic plague. Plague is a very severe disease in humans, particularly in its septicaemic (systemic infection caused by circulating bacteria in the bloodstream) and pneumonic forms, with a case-fatality ratio of 30% to 100% if left untreated. The pneumonic form is invariably fatal unless treated early. It is especially contagious and can trigger severe epidemics through person-to-person contact via droplets in the air. It was the cause of some of the most devastating epidemics in history.

It has been responsible for three human pandemics: the Justinian plague (sixth to eighth centuries), the Black Death (fourteenth to nineteenth centuries) when as much as one-third of Europe's population died and the modern plague (nineteenth century to the present day) Huge pandemics also arose in Asia eventually spreading around the world and causing millions of deaths. The recent identification of strains resistant to multiple drugs and the potential use of *Y. pestis* as an agent of biological warfare mean that plague still poses a threat to human health. Plague has been classified as a re-emerging disease by the World Health Organization due to the worldwide increasing incidence of human plague.

2. Epidemiology

2.1 Causative agent

Yersinia pestis formerly *Pasteurella pestis* is a causative agent of Plague. It is a Gram-negative, coccobacilli, about (1.5-2 X 0.5-0.7) microns in size, arranged singly in short chains or in small groups. When stained with Giemsa or methylene blue, it shows bipolar staining with two ends densely stained and the central area clear. Polymorphism is very common in old cultures, involution forms are seen as coccoid, club shaped, filamentous and giant forms. This involution in culture can be enhanced in media containing 3 % NaCl. The bacterium is non-motile, non-spore forming and non-acid fast. Capsules are present but may be seen in cultures grown at 37°C rather than at the optimum temperature of 28°C. *Y. pestis* can be divided into three biovars, i.e., antiqua, mediaevalis, and orientalis, according to their ability to reduce nitrate and utilize glycerol.

2.2 Mode of transmission

The epidemiology of plague is extremely complex. Infection depends upon the maintenance of a great variety of rodents and vectors, which differ from country to country and also over time. Ecological studies point to a multiplicity of factors concerned with the fluctuating balance that exists between rodents of a greater and lesser degree of susceptibility to the plague bacillus, and also in the degree of risk to which humans are exposed.

a) Vector:

Many species of fleas can act as vectors for *Y. Pestis*. There are 3000 species of fleas of which at least 31 are proven vector of plague, and there are around 220 rodents which can carry plague. The rat flea is the most important vector for the plague bacterium. Rat fleas (domestic plague) and wild rodent fleas (natural foci) are important for plague transmission. The fleas are piercing, sucking, wingless insects. Both sexes can transmit the disease. Xenopsylla cheopis is the most common vector of plague in rats, typically found in rat burrows. However, the rat flea can also adapt to different environments, particularly in

port cities. When rats are absent, the flea can bite human beings. *X. brasiliensis* is also an effective vector, but it prefers rural areas and typically inhabit roofs rather than burrows, distinguishing it from its urban counterpart.

There is some evidence that when a man is infected, *Pulex irritans* may spread the infection to other members of the household without the intervention of rat or rat flea. Fortunately, *Pulex irritans* has not been reported from India.

In India, fleas considered important for Plague transmission are *Xenopsylla cheopis, X. astia, X. brasiliensis.* Out of these *X. cheopis* is the principal vector.



Figure 1: *Xenopsylla cheopis.*

b) Reservoir host:

Plague is primarily a disease of rodents. The infection is maintained in the natural foci of the disease in wild rodent colonies through transmission between them by their flea ectoparasites. Wild plague exists in its natural foci independent of human populations and their activity. Domestic plague is intimately associated with rodents living with humans and can produce epidemics in both human and animal populations. Wild and peri-domestic rodents are susceptible to the infection but resistant to the disease, domestic rodents are susceptible both to the infection and the disease—hence the phenomenon of "rat falls"(Rat fall is defined as more than one dead rat in a house, or more than one house with dead rats, where it has been ascertained that the deaths among the rats have not been due to poisoning).

Among the wild and peri-domestic rodents, the role of Indian gerbil (Tatera indica) and bandicoot rat (Bandicota bengalensis) as a natural reservoir of plague is well established. Among the commensal rodents, Rattus rattus and Mus musculus are hosts for *Y. pestis*. The marmot is a primary carrier of the plague bacillus in many Asian countries. Marmots are the only creatures besides humans who can pass pneumonic plague from one to another under normal (not laboratory) circumstances.

c) Life cycle:

- Flea life cycle: Fleas lay their eggs in the moist soil of rodent burrows; larvae feed on rodent feces, and develop to form pupae and later fleas. Adult fleas parasitise rodents and can become infected with *Y. pestis.*
- Enzootic cycle: Rodents act as hosts and reservoirs for *Y. pestis*, vectored by the fleas *X. cheopis, X. brasiliensis,* and *S. fonquerniei*.
- Epizootic cycles occur when fleas infect mammals which are not the natural hosts of *Y. pestis*.
- These newly infected mammals may spread plague to new areas and lacking resistance, may die quickly by *Y. pestis*.

- Humans may become infected via the bite of a flea originating from an infected rodent or other mammal.

Bubonic plague may develop into pneumonic plague by affecting the lungs to induce cough which generates respiratory droplets containing *Y. pestis* which are inhaled by other individuals, resulting in direct transmission between people.

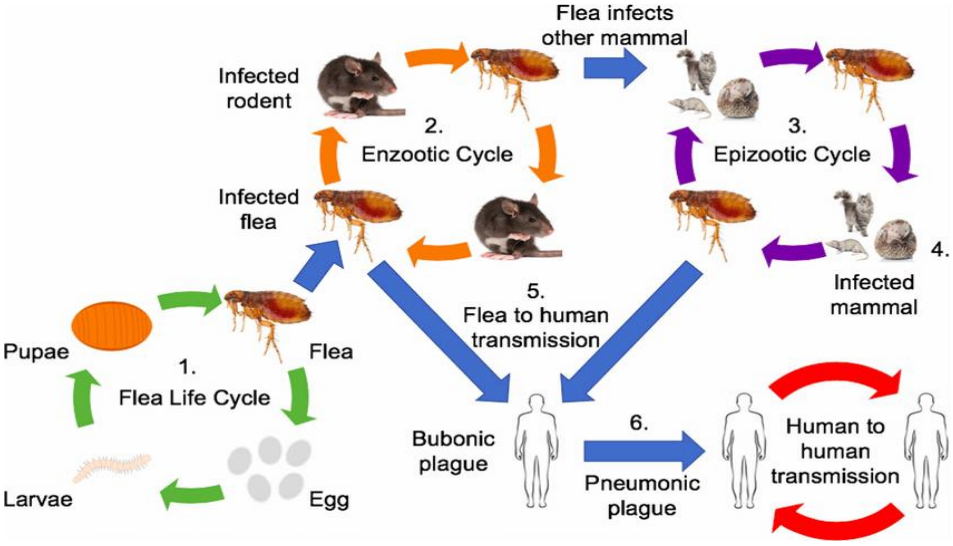


Figure 2: Life cycle of flea and transmission cycle of Plague.

2.3 Current situation

Plague is found in all continents, except Oceania. There is a risk of human plague wherever the presence of plague natural foci (the bacteria, an animal reservoir and a vector) and human population co-exist. Plague epidemics have occurred in Africa, Asia, and South America; but since the 1990s, most human cases have occurred in Africa. The three most endemic countries are the Democratic Republic of Congo, Madagascar, and Peru. In Madagascar cases of bubonic plague are reported nearly every year, during the epidemic season (between September and April). Almost all of the cases reported in the last 20 years have occurred among people living in small towns and villages or agricultural areas rather than in larger towns and cities.

Plague is a disease of great antiquity in India and finds mention in Bhagwat Purana. The recorded evidence of disease is available only from the last pandemic in the year 1898. According to an estimate the disease took over 12.5 million lives in the current pandemic. Nevertheless, the disease trend has shown a decline. It almost disappeared from the entire country in the early 1960s.

Human Plague again stuck the country after a lapse of 28 years after 1966 with 46 cases and 2 deaths, when in August 1994 cases of bubonic Plague were reported from Mamla Village of Beed district in Maharashtra and of pneumonic plague from Surat in Gujrat state in September 1994. Subsequently, suspected cases of pneumonic plague were reported from Delhi, Varanasi, Karnataka and other states. A total of 876 cases and 54 deaths were reported. After a gap of 8 years an outbreak of pneumonic plague occurred in Himachal Pradesh in the year 2002 with 16 cases and 4 deaths. A localized outbreak of bubonic Plague was reported from Uttarakhand in 2004 with 8 cases and 3 deaths. National Centre for Disease Control is monitoring the surveillance of plague in endemic states and all

international seaports to ensure that there is zero migration of any rodent through sea route along with ships.

Table 1 Details of Plague Surveillance sites- State Plague Control units and International Seaport

State	State Plague Control units	International Seaports
Andhra Pradesh	Chittoor district,	Vishakapatnam
Tamil Nadu	Niligiris and Krishnagiri districts,	Tuticorin
Karnataka	Kolar and Bengaluru rural district	Mangalore
Maharashtra	Beed district	Mumbai
Gujarat	Surat, Urban and Rural district	Kandla
Uttarakhand	Barkot, Uttarkashi district	-
Himachal Pradesh.	Rohru, Shimla district,	-
Kerala	-	Cochin
Goa	-	Goa
Orissa	-	Paradip
West Bengal	-	Kolkata

3. Clinical Features

Yersinia pestis infection in humans occurs in one of three main clinical forms: bubonic, septicaemic and pneumonic. Bubonic plague is characterized by regional lymphadenopathy resulting from cutaneous or mucous membrane exposure. The plague bacterium penetrates the human through skin lesions or through the mucous membranes of the mouth, nose or eyes.

Plague should be considered in any patient with clinical signs of plague and a recent history of residence in or travel to the plague endemic areas. Brief about all three types of Plague is given in table 2 below

Table 2: Clinical forms of Plague

Bubonic	Pneumonic	Septicaemic
● Most common form ● ~80% of cases ● Incubation 2 to 6 days ● Clinical signs Fever, malaise, chills, headache ● Bubo: swollen, painful lymph node ● Mortality (untreated): 50-60%	● Incubation: 1 to 6 days ● Primary—Y. pestis inhaled ● Secondary—septicemic form spreads ● Clinical signs: Fever, chills, headache, septicemia, Respiratory distress, hemoptysis ● Person-to-person possible ● Potential use as bioweapon	● Primary or secondary ● Rapid onset ● Clinical signs Signs of sepsis ± bubo ● Necrosis of extremities ● Microthrombi block capillaries ● “Black Death” ● Mortality (untreated): 100%



Figure 3: patient with bubonic plague (inguinal bubo).



Figure 4: patient with bubonic plague (axillary bubo).



Figure 5: Patient with septicemic plague with peripheral necrosis.

Although the majority of patients with bubonic plague present with a bubo, some may have nonspecific symptoms. Primary pneumonic plague results from inhalation of aerosolized droplets containing *Y. pestis.* Septicaemic plague can present with prominent gastrointestinal symptoms such as nausea, vomiting, diarrhoea, and abdominal pain. Additional rare forms of plague include pharyngeal and meningeal.

4. Laboratory Diagnosis

The laboratory plays a vital role in surveillance and diagnosis of plague. Plague surveillance requires laboratory support for evidence of plague activity in rodents, carnivores, human and fleas.

Serological investigations are carried out for the detection of antibodies in rodents, carnivores and human sera. Rodents must be identified as their importance in public health can differ according to the species. Rodent organs are processed for the isolation of *Y. pestis.*

Fleas are collected, identified and flea index is calculated (average number of fleas per rat). During times of high suspicion fleas are also processed for direct detection of plague bacilli.

Clinical specimens should be collected urgently when plague is suspected and specific antimicrobial treatment is begun without waiting for the laboratory report.

A brief summary of the specimens required is shown in Table 3.

Table 3: Laboratory specimens required for Plague diagnosis

Clinical Presentation	Specimen
Bubonic	Bubo fluid/aspirate, Serum
Pneumonic	Bronchial/tracheal washing, Sputum, Serum, Blood
Septicaemic	Blood
Post-mortem	Biopsy from lymph nodes, lungs, bone marrow, spleen, liver

4.1 Specimen Collection and Transportation

Specimens from suspected Human case:

- Bubonic: Aspirated fluid from affected bubo is the preferred sample for microscopic examination and isolation.
- Pneumonic: Sputum and throat swab smear need to be examined with a Direct fluorescent Antibody (DFA) test. A bronchial/tracheal washing should be taken for culture.
- Septicaemic: Blood culture should be taken whenever it is possible
- Post-mortem specimens: The lymphoid tissues, lung, and bone marrow samples may yield evidence of plague infection by DFA or by detection of *Y.pestis* by PCR.

Cary-Blair transport media is usually adequate for transporting all clinical and autopsy material except for blood collected for bacteriological and serological studies which should be placed in blood culture bottle. Specimens in transport medium, Blood culture bottles and serum vials should have triple layer packaging and sent directly to the reference laboratory in cold chain (2-8°C), and if delay is anticipated and molecular detection is required, samples must be transported at -20°C/dry ice.

a) Rodent and Flea specimens:

- Dead rodents: Rodent carcasses or tissues should have triple layer packaging and can be transported on wet ice, dry ice, freezer packs or in special containers filled with liquid nitrogen. If these are not available, samples (such as liver or spleen) can be taken from carcasses and sent in Cary-Blair transport media in cold chain.
- Trapping of live rodents: Multiple catch live traps are preferable to snap or deadfall traps because fleas tend to leave a dead host's body as it cools. Live traps are useful for capturing hosts for flea collection, and tissue and blood samples. Traps must be set at specific sites where there are burrows, nests, runways or other evidence of rodent activity.
- Rodent sera: Blood can be collected from rodents by a cardiac puncture in serum vial which should have triple layer packaging and can be transported directly in sterile, sealed tubes in cold chain.
- Vectors: If hosts are captured live, they should be anesthetized and placed in a white enamel pan and brushed vigorously from the tail end forwards with a toothbrush or comb. This will dislodge fleas from the hosts. Fleas will fall to the bottom of the pan and can be collected by flea-sucking tubes and placed in vials of 70% ethanol which should have triple layer packaging. These may then be transported to the nearest laboratory capable of identification and further processing. Fleas can also be collected from rodent burrows by burrow swabbing.
- Carnivore sera—Blood from dogs can be obtained from large veins in the forelegs or hind legs after properly restraining and muzzling them. Samples collected can be transported directly in sterile, sealed tubes following transportation guidelines.

As these specimens may harbor high-threat pathogens hence the safe transport of infectious substances and diagnostic specimens (WHO/EMC/97.3) for shipping dangerous goods should apply when the specimens are to be shipped via air transport either domestically or internationally.

4.2 Laboratory procedure

Handling of *Y. pestis* or material suspected to harbour this organism requires biosafety level II (BSL-II) environment and precautions. However, when large volumes of cultures are handled, BSL-III facilities are required. Only trained personnel, working in a

restricted area, should undertake plague diagnostic work. The infectious material must be handled only in biosafety cabinets (with vertical laminar airflow under negative pressure and vented to the outside). The waste disposal must also conform to all other the National/Institutional guidelines.

Microscopy: A preliminary diagnosis by the examination of smears of clinical material show the presence of gram negative coccobacilli in gram staining and characteristic bipolar / safetypin appearance in wayson staining (Wayson's stain consists of basic fuchsin, methylene blue, absolute methanol and phenol.)

Direct Fluorescent antibody (DFA) staining is helpful in the rapid presumptive diagnosis of plague. DFA test is based on the presence of bacterial envelope fraction I antigen, which is produced most readily at 37°C but not at temperatures below 28°C. Consequently, DFA test is done best on smears of animal tissue, aspirates of buboes, or isolates incubated at 37°C. This test uses antiserum to fraction I antigen which has been conjugated with a fluorescent dye such as FITC.

Culture and Identification: Various bacteriological media that can be used are described below:

- Blood agar: Small, grey-white, transluscent colonies, 1-2 mm in diameter in 48 hours, Non hemolytic colonies are seen at 28°C or 37°C
- Brain heart infusion broth: Stalactite growth with flocculation on the sides of tube is seen.
- MacConkey agar: Non-lactose fermenting colonies are seen at 28°C or 37°C
- CIN Agar: Yersinia grows as pink to red centered colonies surrounded by transparent edge giving the appearance of a “bulls- eye”

Specific bacteriophage lysis test: *Y.pestis* cells are sensitive to lysis by a temperature-specific bacteriophage, thus this feature is used to confirm its identification. Plague bacteria are susceptible to bacteriophage lysis at 25°C and at 37°C grown at 6 percent Sheep Blood Agar. *Y. pseudotuberculosis* may be differentiated from *Y. pestis* by the loss of lysis at incubation temperatures of less than 28°C. The cultures of *Y. pestis* All22 (positive) and *Y. pseudotuberculosis* (negative) are used for quality control.

Biochemical tests: *Y. pestis* is relatively inactive in routine biochemical tests, therefore biochemical testing is not critical for identification of plague. However some of the tests are useful in differentiating *Y. pestis* from *Y. pseudotuberculosis* e.g Urease (negative in *Y. pestis*)

Although animal pathogenicity testing is rare nowadays, however animals like Guinea pigs & White mice can be used to characterize plague bacilli.

Serological tests: Various serological tests such as Passive Haemagglutination and Passive haemagglutination Inhibition Tests are available to detect antibodies against fraction 1 antigen of Yersinia pestis in the serum of the patient but none are commercially available. The presence of a single high titer of plague antibody in individual who has not been vaccinated earlier or a fourfold rise in convalescent sample is significant. The development of a rapid diagnostic test (RDT), based on immunochromatographic detection of the F1 antigen, which is specific to plague bacilli, is a major step forward in case management and surveillance of plague. This test is able to reliably confirm a suspect case in 15 minutes, and is sufficiently simple and robust for use in the field at peripheral level

Molecular techniques: Molecular techniques are powerful tools that can be used to provide confirmation about the etiological agent that cannot be obtained by traditional diagnostic methods. The presence of *CaF1, pla* and *inv* gene is significant for confirmation of *Y.pestis*.

5. Treatment

Gentamicin and fluoroquinolones are first-line treatments. Duration of treatment is 10 to 14 days, but treatment can be extended for patients with ongoing fever or other concerning signs. Patients can be treated with intravenous or oral antimicrobials, depending on severity of illness and other clinical factors. The regimens may need to be adjusted depending on a patient's age, medical history, underlying health conditions, or allergies.

6. Prevention and control

Standard precautions like Handwashing, using personal protective equipment, cleaning and disinfection of environmental surfaces, beds, bedrails, bedside equipment and other frequently touched surfaces, Occupational health and personal safety measures, droplet precautions must be taken care. Prevention and control of plague is primarily based on the following activities:

6.1 Surveillance

A natural decline in human plague incidence would not justify the conclusion that plague has disappeared from an area. Plague is not static but shifts from place to place through the contiguity of colony infection among wild rodents, which eventually transfers the infection to the commensal rodents on their path. The natural foci of plague are known to be maintained in wild rodents in a cyclic pattern. Sudden ecological changes might create a spill-over of sylvatic plague into domestic environments. Therefore, continuous surveillance and vigilance should be maintained as a part of epidemic preparedness and early warning systems. Surveillance needs to be done with the following objectives,

- To detect early warning signals of an outbreak.
- To institute timely and appropriate control measures.
- To assess the impact of intervention measures.
- To ensure early containment of the outbreak.
- To identify local ecological factors or human activities that may result in increased plague exposure risks for humans
- To detect trends in the epidemiology and epizootiology of plague in a given region (e.g., by mapping out the various species of fleas vis-à-vis their sustaining host)

Surveillance components are 1. Rodent surveillance. 2. Vector (flea) surveillance. 3. Carnivore (dog) surveillance. 4. Human surveillance.

Criteria for confirmation of plague

Suspected case: Compatible clinical presentation; and consistent epidemiological features, such as exposure to infected animals or humans and/ or evidence of flea bites and/ or residence in or travel to a known endemic focus within the previous 10 days.

Presumptive case: Meets the definition for the suspect case plus, at least 2 of the 4 following tests must be positive:

- Microscopy: material from bubo, blood or sputum contains Gram-negative coccobacilli, bipolar after Wayson or Giemsa staining;
- F1 antigen detection in bubo aspirate, blood or sputum;
- a single anti-F1 serology without evidence of previous *Yersinia pestis* infection or vaccination;
- PCR detection of *Y. pestis* in bubo aspirate, blood or sputum.

Confirmed case: Meets the definition for suspected case plus, an isolate from a clinical sample identified as *Y. pestis* (colonial morphology and two of the four following tests must be positive:

- phage lysis of cultures at 20–25°C and 37°C
- F1 antigen detection
- PCR
- *Y. pestis* biochemical profile or a fourfold difference in anti-F1 antibody titre in paired serum samples; or Direct Validated PCR on clinical samples;
  or (in endemic areas when no other confirmatory test can be performed) a positive rapid diagnostic test using immunochromatography to detect F1 antigen.

6.2 Rodent Control

Prevention is based on controlling rodent populations in rural and urban settings as much as possible. Rodent control activities should be undertaken during interepidemic periods only. Environment sanitation, Physical/Chemical/Biological methods of rodent control may be employed. Environment sanitation involves proper garbage disposal, proper drainage systems, rat-proof food storage and the rat-proofing of building structures. Physical method includes Trap barrier system (TBS) by putting fences around crops. Chemical method involves Chronic or multiple-dose poisons like Warfarin, Diphacinone, Pindone and Acute or single-dose poison like Zinc phosphide, Bromadiolone, Coumatetralyl, aluminium phosphide. Biological control involves Pathogenic bacteria, viruses, protozoans as microgen and helminths, nematodes and arthropods as micro-parasites have biocontrol potential.

6.3 Vector Control

Vector control is done when rat fall is reported in a locality/ whenever there is an increase in the population of fleas/ /Specific Flea Index found to be more than 1.0 through active surveillance in areas of known foci of natural plague/ On receipt of specific information from state or central government authorities about a flea nuisance. It is done by Personal prophylactic measures, Insufflation and Residual insecticide spray.

- Personal prophylactic measures: Repellents like benzylbenzoate, diethyltolumide (DEET), dimethylphtholate (DMP) on the body or on clothing to avoid flea bites, cots at least 0.5 meters from the ground level need to be used for sleeping, high-necked shoes or socks up to the knees need to be used.
- Insufflation: The method of treatment of rodent burrows and rats runs with 10% DDT or 5% malathion dust powder.
- Residual insecticide: spray Insecticides used for residual insecticide spray are malathion, deltamethrin, cyfluthrin and lambdacyhalothrin.

6.4 Prophylaxis:

The preferred antimicrobials for preventive therapy are tetracyclines, chloramphenicol, or one of the effective sulfonamides. The duration of prophylactic therapy is seven days which is required for lab professionals.

Plague vaccine is no longer commercially available. The vaccine is indicated for persons whose work routinely brings them into close contact with *Y. pestis*, such as laboratory technicians working in plague laboratories and persons studying infected rodent colonies or engaged in field operations in areas with enzootic plague.

Further Reading

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1. Introduction

Rabies is an ancient viral, zoonotic disease, transmitted to humans mostly through bites of infected dogs. This acute progressive fatal encephalomyelitis, caused by viruses from the Lyssavirus genus, kills tens of thousands of people world-wide every year, mainly in Asia and Africa.

2. Epidemiology

2.1 Causative Agent

Rabies is caused by viruses of the Lyssavirus genus belonging to the Rhabdoviridae family of order Mononegavirales. The rabies virus (RABV), the prototype virus of the Lyssavirus genus is the most common causative agent of rabies. The lyssavirus structure is bullet-shaped, measuring around 180nm (130-250nm) in length and 75nm (60-110nm) in diameter. The two distinct parts include the nucleocapsid core and lipoprotein envelope. The core is made up of tightly coiled ribonucleoprotein (RNP), containing the nucleic acid required for transcription and replication. The lipid envelope covering the core has several spiky surface glycoprotein protrusions that help in attaching virus to receptors on the host cells. The 12 kb negative sense single-stranded RNA genome encodes five viral proteins- Nucleoprotein (N), Phosphoprotein (P), Matrix protein (M), Glycoprotein (G), Large protein (L) or RNA-dependent RNA polymerase.

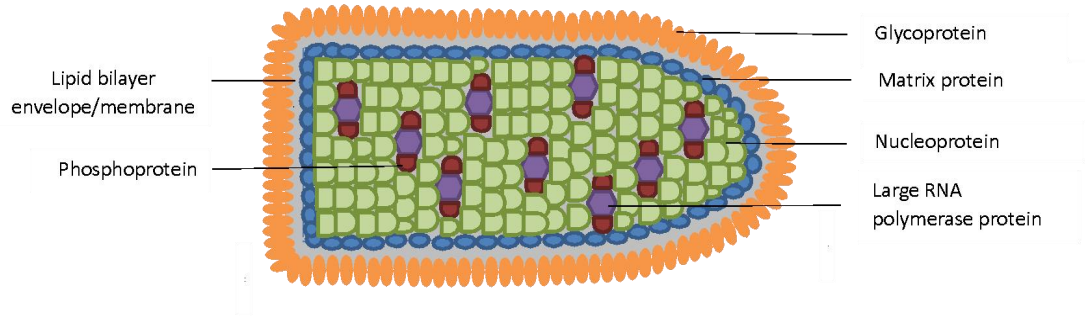


Figure 1: Structure of Rabies Virus.

Sixteen lyssavirus species, sub-divided into three phylogroups are currently recognised by the International Committee on the Taxonomy of Viruses. These are Phylogroup 1: Rabies virus (RABV), Aravan virus, Australian bat lyssavirus, Bokeloh bat lyssavirus, Duvenhage virus, European bat lyssavirus 1 and 2, Gannoruwa bat lyssavirus, Irkut virus and Khujand virus; Phylogroup 2: Lagos bat virus, Mokola virus, Shimoni bat virus; Phylogroup 3: Ikoma lyssavirus, Lleida bat lyssavirus and West Caucasian bat lyssavirus.

2.2 Mode of transmission:

All mammals, domestic or wild, can be potentially infected with rabies. Domestic dogs are responsible for transmission of rabies in 99% of human cases; other vectors which can transmit the disease include domestic cats, foxes, jackals, wolves, raccoons, mongooses, bears, badgers, monkeys, bats etc.

Bats are the reservoir hosts for most lyssavirus species. However, RABV is maintained mostly in mesocarnivores such as domestic dogs, foxes, coyotes, raccoon dogs, raccoons, mongooses, and skunks, except in the Americas where multiple bat species harbour RABV. The lyssaviruses are maintained in ecosystem through enzootic cycles (intraspecies), cross species transmission (spill over) and host shift events (new reservoirs).

Most often, RABV is transmitted via the saliva of an infected animal through bites or scratches, or licks on mucous membrane or abraded skin. The less common modes of transmission reported include exposure of mucous membrane and/or inhalation of aerosols in bat-infested caves, in people handling infected carcasses, or live virus in laboratories, and through tissue or organ transplants from infected donors.

RABV is a neurotropic virus, which replicates at the site of the inoculation, binds to post-synaptic nicotinic acetylcholine receptors and spreads along neuronal pathways to reach the brain and cause an acute encephalitis. No viraemia is seen in rabies. RABV replicates in the neurons and then spreads centrifugally along the peripheral nerves to various organs. This phase corresponds to symptom onset in the host. Despite the fatal outcome in rabies, inflammatory changes observed in the brain are minimal. Hence neuronal dysfunction has been suggested to be possibly contributing to rabies pathogenesis.

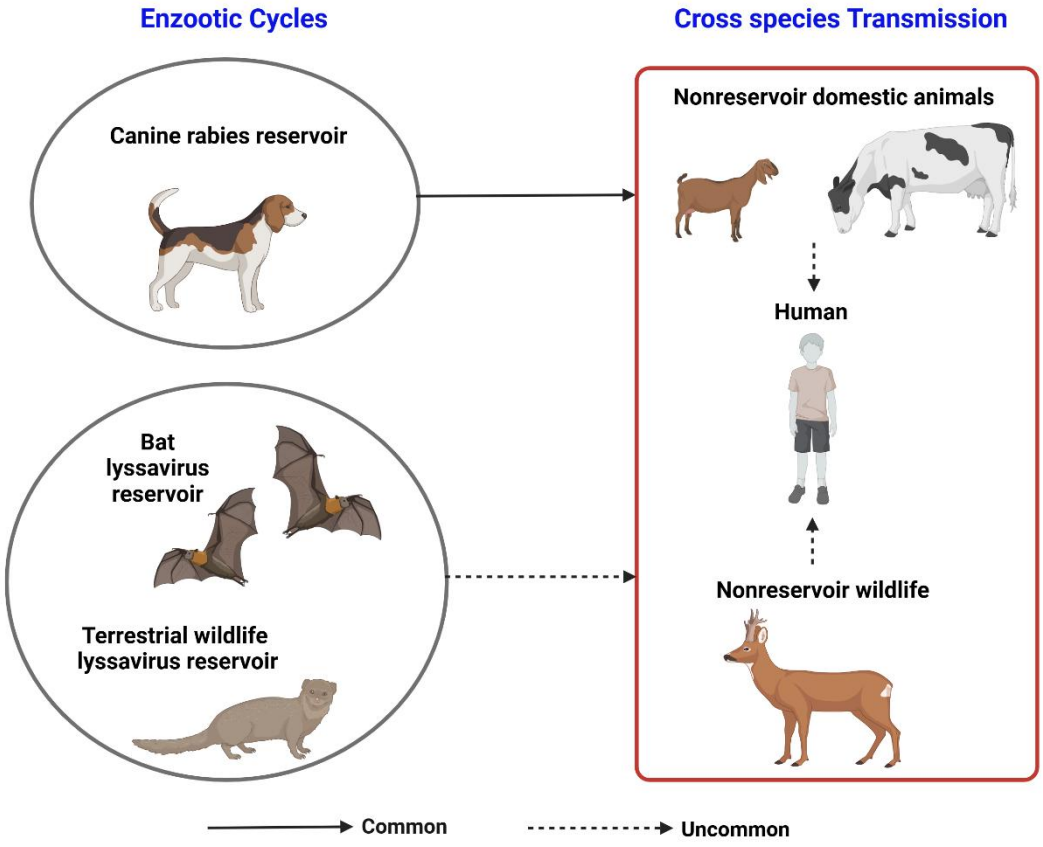


Figure 2: Lyssavirus Ecosystem.

2.3 Current Situation

Rabies is seen in all continents of the world except Antarctica. Dog-mediated rabies has not been seen in Australia and several pacific-island nations, and it has been eliminated in North America, western Europe, Japan and parts of Asia and South America. However, it is endemic in more than 150 countries and territories globally. Every year around 60,000 human deaths occur due to rabies worldwide, mainly in Asia and Africa; about 40% of these victims are children. Rabies largely affects poor and rural populations. The global financial burden due to dog-mediated rabies is estimated to be US$ 8.6 billion (95% confidence

interval, 2.9–21.5 billion), with an associated loss of 3.7 million disability-adjusted life-years (DALYs).

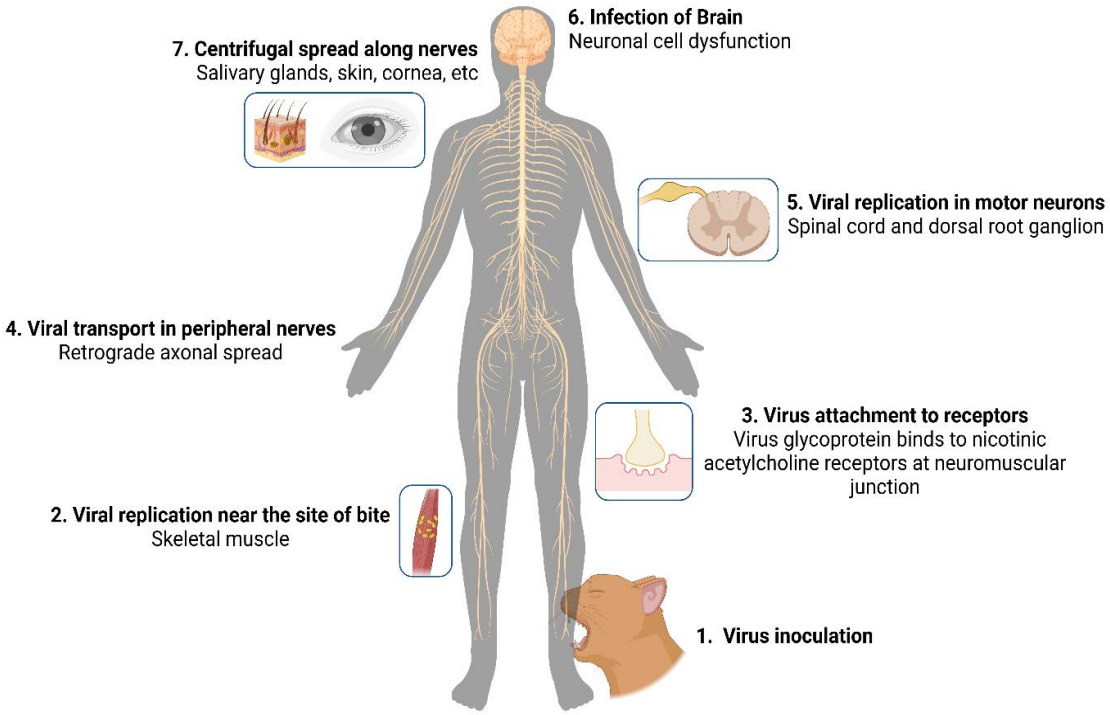


Figure 3: Pathogenesis of Rabies.

Rabies is prevalent in all states and union territories of India except the Andaman, Nicobar, and Lakshadweep islands, which have been historically free of rabies. India has the highest global burden of rabies with an estimated 20,000 deaths annually, and contributes to one-third of the global and two-thirds of the burden in Asia. This estimate may not be accurate as rabies is often underreported in India. The average number of animal bites reported on IDSP portal during 2012-2023 are 3-4 million per year (Range 1.7- 7.5 million per year) and more than 2 million people seek PEP every year. More than 95% of human rabies cases in India can be attributed to transmission from infected domestic dogs; other animals which transmit the disease include cats, foxes, wolves, jackals, mongooses and monkeys. There is no adequate evidence to suggest transmission of rabies to humans from bats in India. Not much data on rabies in wild-life is available due to lack of adequate surveillance. Though rabies in livestock leading to huge economic losses is reported from several parts of India, the exact burden is not known.

3. Clinical Features

Humans

The incubation period is usually about 20 to 90 days following an exposure, though it can range from about a week to more than a year. The clinical course of the illness includes the prodrome, acute neurological phase, coma and death. Two clinical forms of rabies are

well recognised-encephalitic (furious) and paralytic (dumb), which can be differentiated only in the acute neurological phase.

The prodromal features in both the clinical forms of rabies are non-specific and include fever, chills, malaise, sore throat, headache, fatigue, loss of appetite, lack of sleep, anxiety and irritability. However, pain, paraesthesia or itching near the site of exposure, may be seen in 50-80% of cases and is highly suggestive of rabies.

The acute neurological phase in encephalitic rabies is characterised by hyperactivity, agitation, hypersalivation, hallucinations, and hydrophobia and aerophobia, which are the hallmarks of encephalitic rabies. The paralytic form presents with muscle weakness in the bitten limb, progressing to ascending flaccid paralysis involving all the limbs. Without intensive care, both forms of rabies eventually lead to death due to respiratory or cardiac failure within two weeks.

Animals

The incubation period has been studied in various animal species under experimental conditions, since it is difficult to track them in a natural state. The average incubation period in dogs is about 2-8 weeks (range 10 days to 6 months).

The clinical signs of rabies in animals vary, however, usually hypersalivation, paralysis, lethargy, unprovoked abnormal aggression, abnormal vocalization, diurnal activity of nocturnal species, loss of inhibition etc is seen in rabid animals. Hydrophobia is not observed in animals.

Two clinical forms of rabies are described in dogs-furious and dumb (paralytic) form. Dogs with furious rabies exhibit behavioural changes, restlessness, irritability, aggression (attacking inanimate objects, animals, people), disorientation and seizures. Paralytic form is characterised by paralysis of throat and jaw muscles, foaming at mouth, laboured breathing, choking, weakness and respiratory failure. Death usually occurs within 5-7 days of disease onset.

4. Laboratory Diagnosis

Since the diagnosis of rabies based solely on clinical features, is difficult and often unreliable, laboratory confirmation must be done wherever feasible. Laboratory confirmation in suspected human rabies cases can be done antemortem or post-mortem. In animals only post-mortem testing is recommended.

The gold-standard for confirmation of rabies in humans and animals is antigen detection in brain tissue obtained post-mortem using the Direct Fluorescent Antibody test (dFAT). Other tests which can be used on brain tissue include the Direct Rapid Immunohistochemical Test (dRIT), polymerase chain reaction (PCR) for viral RNA detection and histopathological techniques for detection of Negri bodies, which are characteristic inclusions seen in rabies. Rapid Immunochromatographic Diagnostic Tests (RIDT) can be used as screening tests, especially on the field for diagnosing rabies in animals.

Tests on human antemortem samples include viral RNA detection using polymerase chain reaction (PCR) based assays on pooled saliva, nuchal skin biopsy and cerebrospinal fluid (CSF). Detection of neutralizing antibodies using the Rapid Fluorescent Focus Inhibition Test (RFFIT) or Fluorescent Antibody Virus Neutralization (FAVN) or antibodies against the viral glycoprotein using ELISA in CSF and serum samples can help in diagnosis of rabies in previously unvaccinated individuals. However, antemortem tests have low sensitivity and multiple samples have to be tested to confirm the diagnosis. Also, negative antemortem tests cannot rule out a diagnosis of rabies.

Table 1 Laboratory Diagnostics tests for Rabies.

Target	Antigen detection		Inclusions (Negri bodies)		Viral RNA		Virus Isolation		Antibody detection	
	Sample	**Method**	**Sample**	**Method**	**Sample**	**Method**	**Sample**	**Method**	**Sample**	**Method**
Human (Ante-mortem)*	Nuchal skin	dFAT	-	-	Saliva, Nuchal skin, CSF	RT-PCR	CSF, Saliva	RTCIT	CSF, Serum	RFFIT/FAVN/ ELISA
Human (Post-mortem)	Brain	dFAT	Brain	Seller's technique / H & E staining	Brain	RT-PCR	Brain	RTCIT	-	-
Animal (Post-mortem)	Brain	dFAT/ dRIT/ RIDT	Brain	Seller's technique / H & E staining	Brain	RT-PCR	Brain	RTCIT	-	-

Abbreviations: dFAT - Direct fluorescent antibody technique; dRIT - Direct rapid immunohistochemical test; RIDT - Rapid immunochromatographic diagnostic test; H & E - Haematoxylin & eosin; RT-PCR - Reverse transcriptase polymerase chain reaction; RTCIT - Rabies tissue culture infection test; RFFIT - Rapid fluorescent focus inhibition test; FAVN - Fluorescent antibody virus neutralization test; ELISA - Enzyme-linked immunosorbent assay; CSF - Cerebrospinal fluid

*Positive results in ante-mortem samples are diagnostic, but negative results do not rule out rabies.

5. Treatment

Presently, there is no effective therapy recognised for rabies. The clinical course is rapid with an invariably fatal outcome. The patient should preferably be managed in a quiet, dark, draft-free room to avoid any stimulation. Treatment is mainly supportive and may include the use of sedative, anti-pyretic, analgesic and anti-convulsant drugs to relieve symptoms.

6. Prevention and Control

The World Health Organization (WHO), World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) have set a global target to achieve zero human deaths due to dog-mediated rabies by 2030 (ZBT). The Ministry of Health and Family Welfare, Government of India is implementing the National Rabies Control programme since the 12th Five Year plan. The National Action Plan for the Elimination of dog-mediated Rabies (NAPRE) in India was launched in 2021 to provide a broad framework for combating rabies. Since rabies is a zoonotic disease, a 'One Health' approach with multisectoral collaboration involving the human, animal, environmental and other sectors is imperative to achieve this goal. As demonstrated in several countries world-wide, elimination of dog-mediated human rabies can be achieved through mass dog vaccination, rabies health-education, access to post-exposure prophylaxis and enhanced human and animal rabies surveillance.

Despite being a vaccine-preventable disease, thousands of people continue to die of rabies, mainly because of lack of awareness. Educating health care workers, and public engagement to increase awareness about the disease in the community, and the need to seek Post exposure prophylaxis (PEP) following potential exposures is essential to prevent deaths due to rabies.

6.1 Immunoprophylaxis

Rabies in humans is completely preventable with appropriate immunization offered after a potentially rabid exposure (post-exposure prophylaxis) or in individuals with a high risk of exposure (pre-exposure prophylaxis). Post-exposure prophylaxis (PEP) consists of wound management, active immunization with anti-rabies vaccines and passive immunization with rabies immunoglobulin (RIG) or monoclonal antibodies (RmAbs) in and around the wounds, as indicated, depending on the categorization of exposures. Pre-exposure prophylaxis (PrEP) is recommended in individuals with a continual/high risk of exposures related to occupation or other activities such as veterinary professionals, animal handlers, rabies researchers, children in rabies-endemic regions, and travellers visiting rabies-endemic areas. Currently, only cell-culture based rabies vaccines, which are safe and efficacious are approved for use in India. The vaccination regimens for PEP and PrEP recommended in India are summarized in Table-3.

6.2 Dog Population Management (DPM) and vaccination

DPM: DPM aims to reduce the birth of unvaccinated puppies and improving the welfare and life expectancy of vaccinated dogs, DPM helps maintain herd immunity within the dog population, contributing to more effective rabies control.

DPM supports sustainable rabies control by focusing on reducing population turnover. DPM can help to reduce breeding behaviours, which helps lower the risk of rabies transmission by decreasing contact between dogs. Additionally, promoting responsible dog ownership encourages timely rabies vaccination, ensuring owned dogs remain protected and strengthening overall immunity in the community.

Table-2: WHO Category of exposure and recommended post-exposure prophylaxis (PEP)

Category of Exposure	Exposure details	Recommended PEP (In unimmunized)
I	Touching or feeding animals, licks on intact skin	Thorough washing of exposed skin surfaces
II	Nibbling of uncovered skin, Minor scratches or abrasions without bleeding	Thorough wound washing and active immunization with rabies vaccines
III	Single or multiple transdermal bites or scratches, contamination of mucous membrane or broken skin with saliva from animal licks, exposures due to direct contact with bats	Thorough wound washing, active immunization with rabies vaccines and passive immunization with rabies immunoglobulin (RIG) or rabies monoclonal antibodies (RmAbs)

Table-3: Rabies vaccination regimens in India

Type of Prophylaxis	**Vaccination route**	**Days of vaccination**	**Dose/per visit**	**Passive immunization**
Post-exposure prophylaxis (Primary)	IM	0,3,7,14, 28 (Five visits)	Entire vial (0.5/1 mL)	To be given as indicated
	ID	0,3,7, 28 (Four visits)	0.1 mL at 2 sites (0.2 mL)	To be given as indicated
Post-exposure prophylaxis in previously immunized (re-exposures)	IM	0,3 (Two visits)	Entire vial (0.5/1 mL)	Not required
	ID	0,3 (Two visits)	0.1 mL	Not required
Pre-exposure prophylaxis	IM	0,7, 21/28 (Three visits)	Entire vial (0.5/1 mL)	Not required
	ID	0,7, 21/28 (Three visits)	0.1 mL	Not required

IM-Intramuscular; ID-Intradermal
Day 0 (zero) is the day when first dose of vaccine is given

Vaccination: Mass canine vaccination is one of the most effective ways to control and even eliminate rabies cases. It is estimated that by vaccinating 70% of the dogs where infection is still rife, rabies could be eradicated in dogs and the number of human cases would rapidly drop to almost zero.

Types of vaccine: Types of Vaccines available are-

a. Modified Live Virus (MLV) Vaccines: In addition to their use in pet dogs and cats, these have been used widely for oral immunization of wildlife (e.g. foxes in Canada and Europe, raccoon dogs in Finland). All are all safe derivatives of the SAD (Street Alabama Dufferin) virus strain.
b. Vectored Recombinant Rabies Vaccines: Recombinant vaccine viruses are particularly safe because they contain only the rabies virus glycoprotein G gene that is relevant for protection. Poxvirus (vaccinia and canary pox) and adenovirus vectors expressing the rabies virus glycoprotein are used routinely in North America for the

control of rabies in wildlife (vaccinia and adenovirus vectors) by the oral route, and in cats (canary pox vector) by the parenteral route. These vaccines are avirulent in all avian and mammalian species tested.

c. Inactivated (Killed) Vaccines: The use of killed vaccines is the rule for individual dog and cat protection and mass canine vaccination programmes. The killed vaccines are easier to manage than live preparations because of their stability at ambient temperatures, and accidents of self-inoculation do not represent a risk, as would be the case for MLV vaccines.

There is no pet dogs (non- shelter) vaccine policy /local vaccine policy in the country. However, there is evidence of rabies found in puppies of age less than a month. The policy of dog vaccination in non-endemic areas are given in the table below. In rabies-endemic areas, dogs and cats should be vaccinated for protection in both pets and humans, even if the legislation does not require this.

Table: Rabies vaccination for pet dogs-non shelter (adopted by WSAVA guidelines for the vaccination of dogs and cats, 2014)

Vaccine	Puppies ≤16 weeks	Dogs≥16 weeks	Revaccination	Comments and recommendation
Rabies (inactivated)	Follow any local laws or regulations as a priority. Follow the product leaflets of locally manufactured vaccines. In some countries, the first dose is generally not given before 12weeks of age	Follow any local laws or regulations as a priority. Follow the product leaflets of locally manufactured vaccines	Follow any local laws or regulations as a priority. Follow the product leaflets of locally manufactured vaccines. Revaccination at 1year of age (or in some countries 1year after the primary vaccination) is required. Canine rabies vaccines with either a 1- or 3-year DOI are available. Timing of boosters is determined by the licensed DOI, but in some areas may	Core for dogs wherever the disease is endemic or wherever local laws or regulations require

Oral Vaccination: All vaccines currently used for oral vaccination programmes are either modified live-virus vaccines or live recombinant vaccines. At present, two oral vaccines are recommended by WHO: a recombinant vaccine – VRG vaccine, and a highly attenuated vaccine – SAG2.

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1. Introduction

Rickettsial diseases (RD) are zoonosis caused by a diverse collection of obligate intracellular, coccobacillary gram-negative bacteria from the order Rickettsiales. These are well known as pathogens of vertebrate for which the vectors are hematophagous arthropods: ticks, mites, fleas, and lice. Many of them are highly contagious and even a low number of pathogens can result in infection.

The family Rickettsiaceae includes two genera namely *Rickettsiae* and *Orientia*. *Rickettsia* (0.3-0.5µm x 0.8-1µm) is smaller than *Orientia* (0.5-0.8 µm x 1.2-3.0 µm). The *Orientia* differs from other *Rickettsia* but is similar to *Ehrlichia* and *Anaplasma* in that it lacks LPS and peptidoglycan. The cell wall of *rickettsia* is surrounded by the slime-layer (S-layer) which consists of "surface cell antigens" (Sca).

These organisms can be stained by Giemsa or Gimenez stain. Cultivation and isolation cannot be done on routine culture media; it requires cell cultures, embryonic egg, or animal experiments at laboratories that meet high biosafety standards (BSL- 3). Due to reductive evolution, rickettsial organisms have smaller genomes (1.1–2.1 Mb) and consist of a single circular chromosome. Table 1 summarizes the important rickettsial pathogens, vectors, distribution, and associated clinical features.

2. Epidemiology

2.1 Causative Agent

The bacterial order Rickettsiales belongs to class Alphaproteobacteria, and phylum Proteobacteria. The order consists of two families Rickettsiaceae and Anaplasmataceae. *Rickettsia* and *Orientia* are members of Rickettsiaceae, while *Ehrlichia, Anaplasma, Neorickettsia, Neoehrlichia*, and *Wolbachia* belong to Anaplasmataceae.
This chapter predominantly focuses on *Rickettsia* and *Orientia*, responsible for most human rickettsial infections in India.

Rickettsia are currently categorized into four main groups based on genetic, biological, and antigenic characteristics: 1) ancestral group (AG), 2) spotted fever group (SFG), 3) typhus group (TG), and 4) transitional group (TRG). The AG includes R. canadensis and R. bellii, associated with ticks. The SFG contains vast number of species like *R. reckettsii*, *R. africae*, and *R. conorii*, primarily associated with ticks. The TG consists of *R. prowazekii* and *R. typhi*, transmitted by with lice and fleas, respectively. The TRG includes *R. akari, R. australis*, and *R. felis*, transmitted by mites and flies.

The genus Orientia comprises of *O. tsutsugamushi* and newly discovered *O. chuto* and *O. chiloensis* makes up the scrub typhus group, transmitted via mite bites. *O. tsutsugamushi* is broken into several prototypes including the three original serotypes; Kato, Karp, and Gilliam.

2.2 Mode of Transmission

Rickettsial organisms rely on vector for transmission, with the mode of infection varying based on the vector type and specific rickettsial species involved. Rick factor for rickettsial infections includes exposure to vectors such as ticks and mite, as well as contact with rodents (mice, rats). Travelling to tropics; living and working close to forests, scrub, natural vegetation, and bush; exposure to cat or dogs. Additionally, practices like infrequent bathing, changing clothes, and raising domestic animals contribute significantly to the risk.

SFGR are mostly harbored by ticks and are transmitted to host through bites during feeding. These zoonotic infections can be maintained in nature by transovarial or

transstadial transmission in ticks.

TGR are carried by body lice (*Pediculus humanus corporis*) and fleas (*Xenopsylla cheopis* or *Ctenocephalides felis*). They are transmitted when the vector's feces contaminate bite wounds during feeding. The act of scratching or rubbing the bite wound introduces the bacteria into the human host, initiating infection. Remarkably, these pathogens can remain viable in dried vector feces for several days, potentially becoming aerosolized and transmitted through inhalation. Individuals with recrudescent typhus (Brill-Zinsser disease) can serve as reservoirs capable of infecting lice.

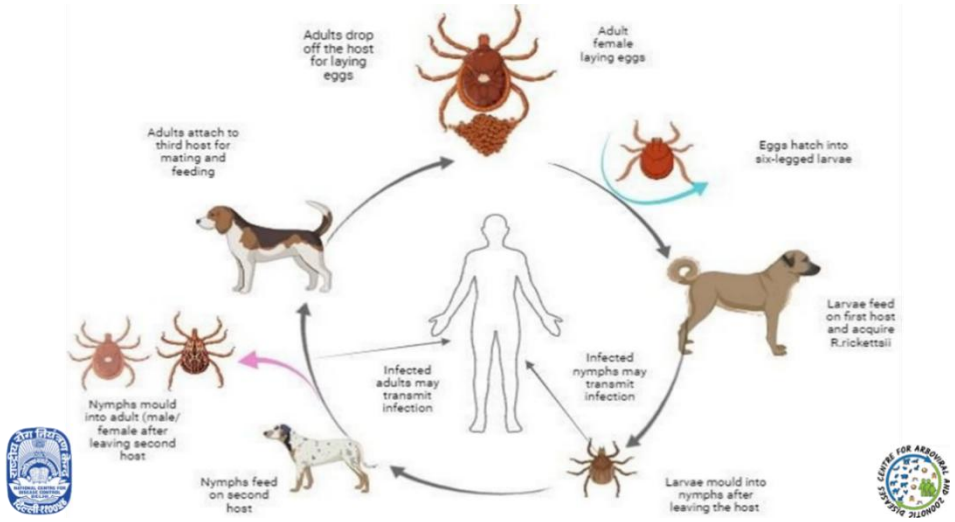


Figure 1: Transmission cycle of spotted fever rickettsiosis.

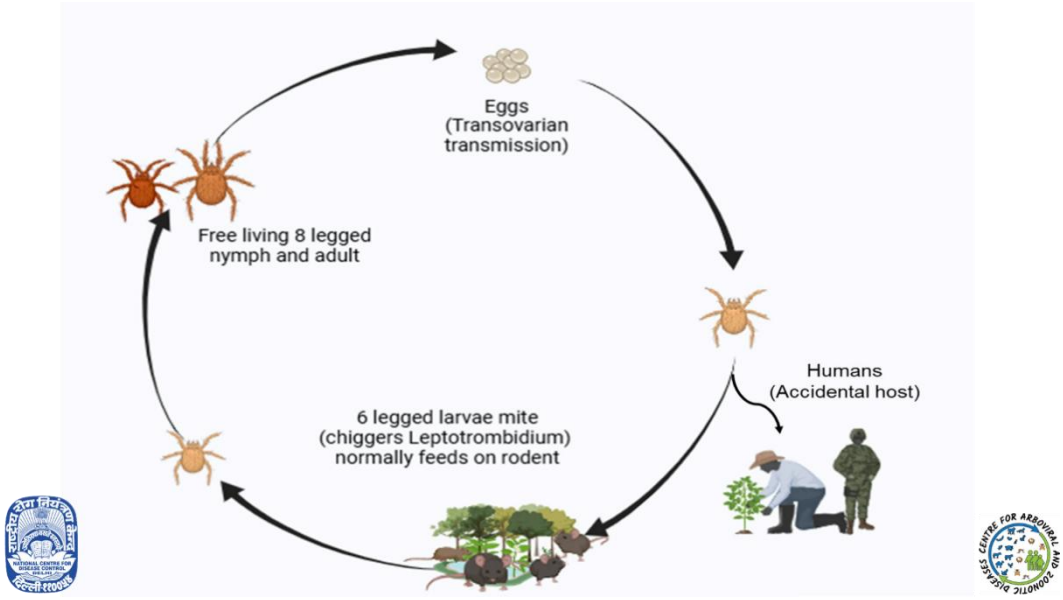


Figure 2: Transmission Cycle: Scrub Typhus.

ST is transmitted through the bite of larval mites, or "chiggers," belonging to the Trombiculidae family, specifically the genus, and subgenus Leptotrombidium. Only the larval stage of these mites feeds on blood, and their natural hosts are small rodents, particularly wild rats of the Rattus subgenus. In Indian settings, Leptotrobium deliense is

the most common mite species. Once these mites feed on the body fluids of small mammals, they become infected and can maintain the infection throughout their various life stages. As adults, they pass the infection to their eggs and other developmental stages through transovarial and transtadial transmission. This continuous cycle allows chigger mite populations to autonomously sustain their infectivity over extended periods. The mite larvae, responsible for transmitting the disease to humans and other vertebrates, insert their mouthparts into hair follicles or pores rather than directly biting or piercing the skin. Their salivary glands contain a substantial amount of *O. tsutsugamushi*, which is injected into the host during feeding. This process often leaves a characteristic black eschar at the bite site, aiding in the diagnosis.

2.3 Current Situation

Rickettsial organisms have a worldwide distribution, determined by vector ecology, such as altitude or temperature. *Orientia tsutsugamushi* causes scrub typhus, once considered endemic only to the "tsutsugamushi triangle" from northern Japan to northern Australia, and Pakistan. Recent reports from South America, Africa, the Middle East, and Europe suggests wider geographic range. Globally, about one billion people are at risk for scrub typhus and an estimated one million cases occur annually. The name "scrub typhus" originates from exposure in areas with secondary vegetation, but recent findings indicate its presence in mountain deserts, sandy beaches, and equatorial rainforests.

Rickettsial diseases have been documented in India since the 1930s with reports of scrub typhus from Kumaon region, in soldiers during the Second World War in Assam, scrub and murine typhus from Jabalpur area in Madhya Pradesh and of murine typhus from Kashmir. Surveillance in animals and humans in different parts of India has documented significant levels of exposure to infections. With the increasing awareness, availability of diagnostics and improved surveillance, scrub typhus and other Rickettsiosis is being widely detected from various parts of the country including north eastern states (Assam, Arunachal Pradesh, Manipur, Mizoram, Meghalaya, Nagaland, Sikkim, Tripura), Jammu and Kashmir, Uttar Pradesh, Delhi, Himachal Pradesh, Uttarakhand, Bihar, West Bengal, Rajasthan, Gujarat, Maharashtra, Karnataka, Tamil Nadu, Puducherry and Kerala. In some regions, scrub typhus accounts for up to 50 per cent of undifferentiated fever presenting to hospital.

3. Clinical Features

Clinical presentations vary widely depending on the causative agent and can range from mild to severe, affecting individuals of all age groups. Typically, the disease manifests after an incubation period of 1-2 weeks with symptoms including fever, severe headache, rash, myalgia, cough, and gastrointestinal issues. These symptoms, while common in acute rickettsial infections, are nonspecific and can resemble other infections, necessitating further tests for accurate diagnosis. In most of tick/mite-transmitted rickettsioses, a distinctive maculopapular, vesicular, or petechial rash or an eschar (tache noir) at the site of the tick/mite bite often occurs, resulting from vascular endothelium damage and perivascular mononuclear cell infiltration causing vasodilation. The rash's distribution varies by the specific rickettsial disease. For example, in murine typhus, it primarily affects the trunk but can extend to the extremities and occasionally the palms and soles. In epidemic typhus, the rash starts in the axillary folds and upper trunk, spreading outward to the limbs. Rocky Mountain spotted fever presents with a centripetal rash, typically beginning around the wrists and ankles before appearing on the trunk. Furthermore, an

eschar or "techinoir" (skin necrosis with a dark scab) is a common feature of several rickettsial diseases, including boutonneuse fever, Japanese spotted fever, African tick bite fever, Flinders Island spotted fever, North Asian tick typhus, rickettsialpox, Queensland tick typhus, and tick-borne lymphadenopathy. Scrub typhus frequently manifests an eschar at the site bitten by larval mite (Chiggers).

While many RD lead to mild or moderate illness, some, such as Mediterranean spotted fever, scrub typhus, epidemic typhus, and Rocky Mountain spotted fever, can be severe and fatal in 20–60% of untreated cases.

Associated complications include encephalitis, coma, seizures, regional lymphadenopathy, thrombosis, intravascular complications, acute renal failure (due to hypovolemia and hypotensive shock), severe pneumonitis/peritonitis, acute respiratory distress syndrome (ARDS), myocarditis, and multiorgan failure. However, prompt treatment significantly reduce mortality rates, with treated patients experiencing lower fatality rates.

4. Laboratory Diagnosis

4.1 Collection of Specimens

Serum, EDTA blood, Eschar material, etc.

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedure

Serology: Serological assay remains mainstay in the diagnosis of rickettsial infection due to their ease of use and test availability. It aims to detect pathogen specific antibodies, generally after 5-10 days of infection. Available serological tests include nonspecific Weil-Felix test (WF) and the specific tests utilizing rickettsial antigens, such as complement fixation (CF), indirect haemagglutination (HA), latex agglutination (LA), indirect Immunoperoxidase assay (IPA), indirect immunofluorescence assay (IFA), Enzyme-linked Immunosorbent Assay (ELISA). Among these most predominantly used formats are ELISA and IFA. Commercial rapid flow assays are also available for scrub typhus diagnosis, exhibiting good specificity.

Culture: Despite its high specificity, culture remains limited in sensitivity due to the intracellular localization of rickettsial pathogens. Various cell lines, such as HeLa cells, L929 cells, Vero cells, and BHK-21 cells, have been used for cultivation. However, it is a time-consuming and resource-intensive method, typically taking four weeks for identification and requires BSL III facility.

Molecular testing: Nucleic acid amplification techniques (NAATs), like PCR, offer high specificity and are among the most specific tests for RD. PCR can detect rickettsial DNA in various samples, with eschar samples showing high sensitivity and specificity. SFGR and TGR have multiple gene targets for their detection, such as gltA, 16S rRNA, 17-kDa, ompA, ompB, SCA4, and 23S- 5S, have been used for diagnosis.

Gene targets for detection and diagnosis of Orientia include 56 kDa, 47 kDa, 16 S rRNA, and groEL genes, with high specificity. Real-time PCR assays provide timely results

and have advantages like cost-effectiveness and reduced risk of contamination. Recently, Loop-mediated isothermal amplification (LAMP) assays targeting groEl and 47 kDa genes have been described, offering simpler implementation but with lower sensitivity.

Whole-genome sequences and next-generation sequencing methods have been used to elucidate phylogeny, study virulence, and identify new Rickettsia species directly from clinical samples, providing clinically actionable results.

5. Treatment

Timely antibiotic therapy is crucial for reducing the course, morbidity, and mortality of rickettsial infections. Physicians need to be aware of clinical features, available diagnostic tests, and therapy options. Specific antibiotic therapy i.e., Doxycycline, Azithromycin is the treatment of choice. The details may be referred from DHR-ICMR Guidelines for Diagnosis & Management of Rickettsial Disease in India (2015).

6. Prevention and Control

An effective vaccine for humans has not been developed till now, mainly due to serotypic heterogeneity of the organism. Preventive measures for general public.

- People who cannot avoid infested terrain should wear protective clothing (Full pant, full sleeves, and shoes), impregnate their clothing and bedding with a miticide.
- People should wash themselves and their clothes after every potential exposure.
- Insect repellents containing dimethyl phthalate (DMP), benzyl benzoate and diethyl toluamide (DEET) can be applied to the skin and clothing to prevent chigger bites.
- It is advisable to not sit or lie on bare ground or grass; use a suitable ground sheet or other ground cover.
- Clearing of vegetation and chemical treatment of the vegetation/ soil may help to break up the cycle of transmission from chiggers to humans.

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Table 1: Important rickettsial pathogens, vectors, distribution, and associated clinical features

Disease	Organism	Vector	Animal reservoir (s)	Life cycle	Geographical distribution	Eschar	Rash	Regional lymph-adenopathy	Mortality rate
Tick-transmitted spotted fevers									
Rocky Mountain spotted fever	Rickettsia rickettsii	Dermacentor variabilis, Dermacentor andersoni, Rhipicephalus sanguineus, Amblyomma cajennense and Amblyomma aureolatum	Rodents	Transovarian in ticks and rodent ticks	Western hemisphere	Rare	Yes	No	High
Mediterranean spotted fever or Boutonneuse fever	Rickettsia conorii	R. sanguineus and Rhipicephalus pumilio	Dogs, rodents	Transovarian in ticks	Southern Europe, southern and western Asia, Africa, India	Frequent	Maculop apular	No	Mild to moderate
African tick bite fever	Rickettsia africae	Amblyomma hebraeum and Amblyomma variegatum	Ruminants	Transovarian in ticks	Africa and the West Indies	Frequent and often multiple	Papular or vesicular; often sparse or absent	Yes	None reported
Maculatum disease	Rickettsia parkeri	Amblyomma maculatum and Amblyomma triste	Rodents	Ticks	Western hemisphere	Yes	Often	Yes	None reported
Far Eastern spotted fever	R. heilong-jiangensis	Haemaphysalis concinna Dysmicoccus sylvarum	Rodents	Tick	Far East of Russia, Northern China, eastern Asia	Yes	Yes	Yes	None reported
Aneruptive fever	R. helvetica	Ixodes ricinus Dermacentor reticulatus		Tick	Central and northern Europe, Asia	No	Yes	No	

Mediterranean spotted fever-like disease	R. massiliae	Rhipicephalus sp.	Unknown, possibly dogs	Tick	France, Greece, Spain, Portugal, Switzerland, Sicily, central Africa, Mali, United States	Yes	Maculopapular	No	
Mediterranean spotted fever-like illness	R. monacensis	Ixodes ricinus	Lizards, possibly Birds	Tick	Europe, North Africa	Yes	Yes	Yes	
Tickborne lymphadenopathy (TIBOLA), Dermcentor - borne necrosis & lymphadenopathy (DEBONEL)	R. raoultii	Dermacentor spp.	Unknown	Transovarian in ticks	Europe, Asia	Yes	Yes	Yes	None reported
TIBOLA, DEBONEL	R. slovaca	Dermacentor marginatus Dermacentor reticulatus	Lagomorphs, rodents, European boar	Tick	Southern and eastern Europe, Asia; recently in US tick colony (unknown origin)	Yes	Yes	Yes	
North Asian tick typhus, Siberian tick typhus	R. sibirica	Hyalomma asiaticum H. truncatum Dermacentor marginatus D. nuttalli	Rodents	Tick	Russia, China, Mongolia	Yes	Yes	Yes	Mild
Flea-transmitted diseases									
Flea-borne spotted fever		Ctenocephalides felis	Fleas	Transovarian in the cat flea	Worldwide	Sometimes	Sometimes	No	None reported
Murine typhus	Rickettsia typhi	Xenopsylla cheopis and Ctenocephalides felis	Rodents	Rat-flea for *X. cheopis* and Opossum flea for *C. felis*	Worldwide	No	Yes	No	Low
Louse-transmitted disease									

Epidemic typhus	Rickettsia prowazekii	Pediculus humanus humanus	Humans	Human louse	Worldwide	No	Yes	No	High
Epimic typhus	R. prowazekii	Fleas and lice of flying squirrels and Glaucomys volans volans	Flying squirrels	Flying squirrel flea and louse ectoparasite	United States	No	Yes	No	Low
Mite-transmitted diseases									
Rickettsialpox	Rickettsia akari	Liponyssoides sanguinus	Rodents	Transovarian in mites	Worldwide	Yes	Yes	Yes	Non reported
Scrub typhus	Orientia tsutsugamushi Orientia chuto Orientia chiloensis	Leptotrombidium spp.	Rodents	Transovarian in mite (chigger)	Asia-Pacific region from maritime Russia and China to Indonesia and North Australia to Afghanistan UAE Chile	Yes	Yes	Yes	Moderate

1. Introduction

Toxoplasmosis in third world countries is a major health hazard but the most neglected one. The presence of food-borne pathogens in a country's food supply not only affects the health of the local population, but also represents a potential for spread of pathogens to visitors to the country and to consumers in countries which import food products. In the United States of America, food-borne toxoplasmosis is considered to be the third leading cause of death amongst the food-borne diseases.

The consequences of food-borne illness can be particularly devastating during pregnancy because both the woman and her fetus are at risk. The pregnancy-induced decrease in cell-mediated immune functions leads to increased susceptibility of the pregnant woman to contain infections. *Toxoplasma gondii* is one such intracellular pathogens that have a predilection for the maternal-fetal unit and may induce serious disease in the mother and/or fetus. The pregnant woman, in order to protect herself and her fetus from the consequences of food-borne illness, must practice a high standard of food hygiene and eating habits.

2. Epidemiology

2.1 *Causative Agent*

The parasite was first described in 1908 by Nicholle and Manceaux in the blood, spleen, and liver of North African rodent- gundi (Ctenodactylus gondii) which was captured in Tunisia. Cats including wild Felidae were found to act as the definite host and all other warm-blooded animals including humans are known to serve as intermediate hosts. The oocysts are formed as a result of a sexual cycle in the intestine of the cats. The trophozoites (tachyzoites and bradyzoites) are also conoid in shape and measure 2-4 x 4-7 µm. The encysted stage (tissue cyst) contains relatively slowly multiplying organisms (called bradyzoites, brady = slow). Tissue cysts persist in the body as long as the host lives. The tissue cyst is a dead-end phase of the parasite in the intermediate host waiting to be eaten by the definitive host, cat. In feline intestinal epithelial cells, bradyzoites first undergo a special form of asexual development and merozoites liberated from schizonts produce male (micro) and female (macro) gamonts. After fertilization, a wall is formed around the zygote and an oocyst is produced, excreted in the faeces of infected cats and in the environment, they sporulate. The sporulated *T. gondii* oocysts are infectious to humans and all other warm-blooded hosts.

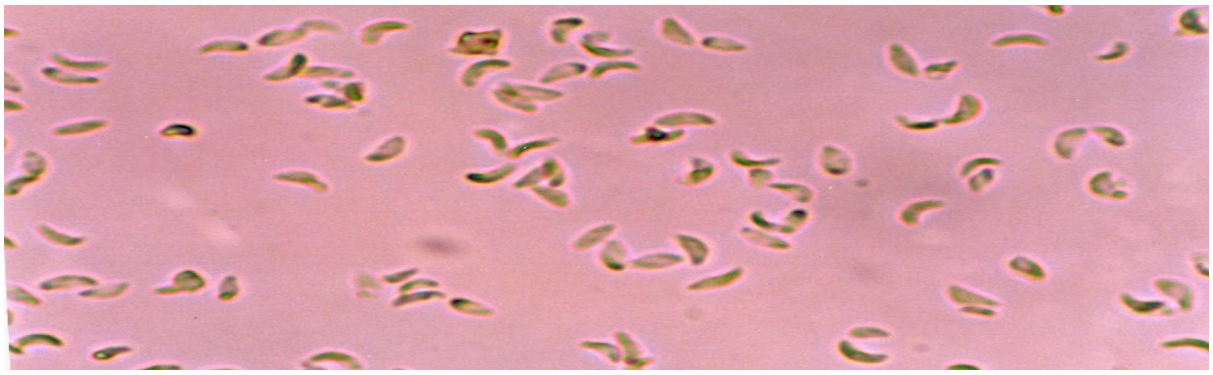


Figure 1: Tachyzoites of *Toxoplasma gondii* isolated from the aspirated mouse ascitic fluid after 96 hours of intraperitoneal inoculation. Note numerous sickles shaped sluggishly motile tachyzoites.

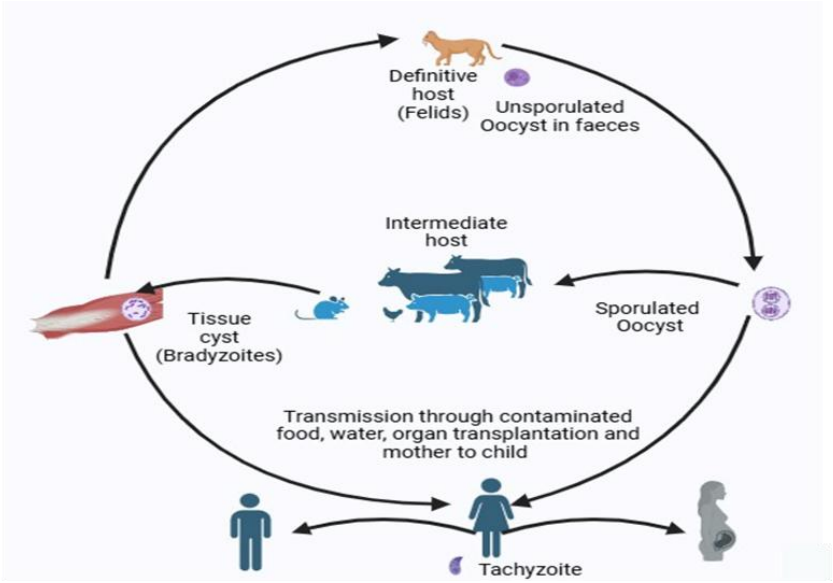


Figure 2: Transmission cycle of toxoplasmosis.

2.2 Mode of transmission

Toxoplasma is known to encyst in tissues of both humans and animals. Each cyst contains many viable trophozoites of the parasite and these are highly infectious and capable of invading the intestinal wall of the carnivore /omnivores. Several serological studies also confirmed that several animals including meat giving animals are infected with *Toxoplasma gondii* and they transmit this infection to those who consume their meat. Outbreaks of toxoplasmosis associated with raw meat consumption have been reported.

Bradyzoites are transmitted through ingestion of the tissue cyst. Tissue cysts can remain viable for upto 3 hours incubation in digestive fluid. This brief period can allow the bradyzoite to enter the host via the stomach or intestinal epithelial lining. The tissue cyst's ability to remain viable in host for the lifetime maximizes the time available for transmission.

There are 4 established routes of transmission of *Toxoplasma gondii* (a) by ingesting uncooked meat containing tissue cysts, (b) by ingesting the food and water contaminated with oocysts from infected cat faeces, (c) congenitally from mother-to-fetus where transplacental infection occurs when an uninfected mother acquires infection during pregnancy (d) through organ transplantation including the whole blood transfusion from a latently infection donor to seronegative recipient. There are circumstantial evidences that Toxoplasmosis can also be acquired from males to female through sexual route. There are other rare modes of transmission such as through blood transfusion, and laboratory acquired. Overall, less than 1% of the general human population becomes infected congenitally

Sheep are commonly infected with *Toxoplasma gondii*. Seroprevalence appears to increase with age. Approximately, 42-48% of the sheep or lamb are positive for antibody to Toxoplasma. The seroprevalence of toxoplasmosis in pigs is reported to vary from less than 1% to as high as 100%. Outbreaks of human toxoplasmosis after consuming uncooked pork have been reported from Asian countries too.

Though in India and other countries in the continent mutton is the commonest

meat consumed, the chances of toxoplasmosis through this route are rare, because the meat is cooked adequately. In several other countries the natives hunt and consume raw meat of wild goats.

Thus, wild animal meat can serve as a source of infection for hunters and their families. Those who are involved in skinning and handling meat scraps, are also at risk of getting infected. There are anecdotal reports of toxoplasmosis associated with consumption of raw milk and hen eggs. But parasite has not yet been isolated from the eggs commonly consumed by man. If at all the infection can occur through contaminated egg shell if such eggs are laid by the hen on the soil containing Toxoplasma oocysts. The survival of Toxoplasma has been reported for up to 10 days in milk and unprocessed homemade cheese.

There are epidemiological evidences that suggest that Toxoplasmosis could be transmitted through unprotected sexual mode, from males to females. However, more vertical studies are required to establish this mode of transmission.

2.3 Current Situation

Studies have indicated variable prevalence rates in poultry, varying from as low as 6.2% in Mexico, to as high as 65.5% in Argentina.Very little information is available about Toxoplasma infection in other animals such as horses, camels, kangaroos and elephants whose meat is often consumed in some countries. The parasites were also isolated from the tissues of horses. Tissue cysts in these animals also can persist for a very long time as in humans but at least for a year. *Toxoplasma gondii* has been demonstrated in the muscles of naturally infected deer, bear, moose, pronghorn and elk.

In India, serologic studies reveal that a large section of the population has had the disease at one time or the other. A wide variation in the prevalence and incidence rates of this infection has been reported. Seroprevalence and incidence of toxoplasmosis in Indian women of child bearing age has remained a contentious issue. Different laboratories have used different patient recruitment criteria, methods and variable results, making these data unreliable. In an estimated prevalence of 22.4% (328); the highest prevalence being in South India (37.3%) followed by East India (21.2%) and North India (19.7%). West Indian women had the lowest seroprevalence (8.8%). There are several risk-factors of infection. These included lower socioeconomic status, residing in mud plastered houses, consumption of raw salad, drinking untreated water, owning pets and advanced age. Overall, the incidence rate of toxoplasmosis is approximately 1.43%. Extrapolating the data, we estimate that between 56,737 to 176,882 children per year may be born in India with a possible risk of congenital toxoplasmosis, which can manifest itself in-utero or several years after birth.

3. Clinical Features

The severity and likelihood of infection depend on the trimester of pregnancy the mother becomes infected with *T. gondii*. Toxoplasmosis is more severe in infants whose mothers become infected during the first trimester than those during the third trimester. Transmission of *T. gondii* from preconception seropositive mothers to their babies is rare, but occasional reports of seropositive mothers transmitting *Toxoplasma* to their child are on record. Although most congenitally infected children are asymptomatic at birth, they may develop some symptoms later in life. Loss of vision is the most common (up to 95%) sequel in congenitally infected children. Hydrocephalus, retinochoroiditis, chorioretinitis, intracerebral calcification, mental retardation, loss of hearing, and very rarely death may also occur.

Toxoplasmosis in AIDS patients and other immunocompromised patients can be life-threatening and in most of these individuals, the disease is caused by reactivation of the parasite from the latent tissue cysts. Heart and other organ transplantation recipients are at risk for developing toxoplasmosis because of lowering of host resistance by immunosuppressive medication. Similarly, cancer patients are also at risk of developing clinical toxoplasmosis. In most of these immunocompromised patients, toxoplasmosis results from reactivation of a latent infection, especially due to the rupture of tissue cysts which lead to the renewed multiplication of the parasite's tissue forms. Although any organ may be involved, encephalitis is the predominant presentation of toxoplasmosis in AIDS patients.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Bonem marrow, Splenic puncture, CSF, Tissue biopsy and serum

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedure

Microscopy: Trophozoites in smear of bone marrow, splenic aspirate, CSF & tissue biopsy

Serology: Detection of serum IgG and IgM antibodies against *Toxoplasma gondii* measure the response to infection. The commonly used tests are IgM, IgG Enzyme linked Immunosorbent assay (ELISA), and IgG avidity test which helps to differentiate recent and recent/past and past infections.

Molecular: PCR is often performed on the amniotic fluid at 18 gestation weeks to determine if the infant is infected. The most frequent PCR target for amplification is B1 repititive gene.

5. Treatment

Pyrimethamine, is considered the most effective drug against toxoplasmosis, and must be given with folinic acid (leucovorin). The second drug of choice, such as sulfadiazine or clindamycin (if the patient has a hypersensitivity reaction to sulfa drugs), should also be included. The fixed combination of trimethoprim with sulfamethoxazole has been used as an alternative. Occasionally, other drugs such as atovaquone and pyrimethamine plus azithromycin, are also prescribed. If visceral disease is clinically evident or symptoms are severe or persistent, treatment may be indicated for 2 to 4 weeks. Treatment for ocular diseases should be based on a complete ophthalmologic evaluation.

Management of maternal and fetal infection varies. In general, spiramycin is recommended for women whose infections were acquired and diagnosed before 18 weeks gestation and infection of the fetus is not documented or suspected. Spiramycin acts to reduce transmission (as it gets concentrated in the placenta) to the fetus and is most effective if initiated within 8 weeks of seroconversion. Pyrimethamine, sulfadiazine and leucovorin are recommended for infections acquired at or after 18 weeks gestation or infection in the fetus is documented or suspected.

6. Prevention

Man is infected by Toxoplasma from tissue cysts in meat, the oocysts in soil and by tachyzoites transplacentally. In view of the prevalence of Toxoplasma infection in domestic animals proper cooking of meat is essential. Heating meat to 66°C kills the organisms. Freezing causes reduction of organisms but is not adequate. The hazards inherent in handling raw meat as in slaughterhouses and in the home are well recognized and measures such as hand washing or wearing of rubber gloves should be adopted.

Freshly passed oocysts in cat's faeces are non-sporulated and therefore non-infective. Proper disposal at this stage by incineration or flushing down the toilet obviates the risk of infection. However, sporulated occysts can remain infective for about a year in moist soil. As cats are usually infected by ingestion of raw meat, wild birds and rodents, effective prevention can be achieved by control of the diet of pet cats and elimination of stray. Sand and soiled represent a durable source of infection; therefore, children's play areas should be made inaccessble to cats. Pet cats shedding oocysts can be temporarily isolated and treated with sulfadiazine and pyrimethamine. Other drugs used for cats are 2 suiphameyl 1 -4 diaminodiphenyl sulfone (SDDS) and clindamycin but these do not completely stop the shedding of oocysts. Immunoprophylaxis has been attempted in animals using phenol killed toxoplasma and irradiated toxoplasma with some success.

As the foetus and neonate represent the most vulnerable section avoidance of infection by pregnant women is very important. Routine serological check - up in antenatal clinics and treatment of infected mothers would be a valuable step in protecting the foetus.

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1. Introduction

Trypanosomes, which belong to the Trypanosomatidae family, are parasitic protozoa that can be found worldwide. They infect humans, domestic animals, and wild animals and are primarily transmitted by blood-sucking insects. The emergence of a-HT, particularly in the Indian subcontinent, is evident. However, detecting the true prevalence of a-HT is challenging due to the lack of sensitive and specific diagnostic tools. It is postulated that the available records of a-HT may have been underestimated due to a poor health alert system, low public awareness, and limited access to diagnostic healthcare services.

2. Epidemiology

2.1 Causative Agent

The classification of trypanosomes is based on their development method in the invertebrate host, resulting in two broad categories known as salivarian and stercorarian trypanosomes. Salivarian trypanosomes develop in the anterior part of the digestive tract of their vector and can cause pathogenic infections in humans, domestic animals, and wild animals. The main species in this group include *T. brucei ssp, T. evansi, T. congolense* and *T. vivax*. On the other hand, stercorarian trypanosomes develop in the posterior part of the digestive tract of their vectors. Except *Trypanosoma cruzi* (sub-genus Schizotrypanum), stercorarian trypanosomes are generally considered non-pathogenic and belong to subgenus Megatrypanum (e.g., *T. theileri* in bovines and antelopes and *T. melophagium* in sheep) or subgenus Herpetosoma (e.g., *T. lewisi, T. musculi* and various *T. lewisi*-like species widely distributed in rodents). However, in recent past, *T. lewisi* has emerged as neglected zoonotic pathogen, many cases have been reported from human beings. Out of the 125 *Trypanosoma* species found in mammals, approximately 10% are considered pathogenic to humans and/or other mammals.

2.2 Mode of Transmission

Trypanosoma evansi belongs to the Salivarian group. The vectors responsible for the transmission of *Trypanosoma evansi* are typically biting flies, particularly tabanid flies (commonly known as horseflies) and stable flies (*Stomoxys calcitrans*). By feeding on the blood of diseased animals, usually domestic and wild mammals, including camels, horses, cattle, and other livestock, these flies become infected with *T. evansi*. Through their bites during repeated feeding, infected flies can spread the pathogen to additional susceptible animals. *T. evansi* has evolved to infect a variety of hosts and does not require a particular vector, in contrast to other *Trypanosoma species* that depend on tsetse flies as vectors. Its extensive distribution in numerous areas of the world is a result of its tolerance to varied host species and vector types

Trypanosoma lewisi belongs to the Stercorarian group. The parasite commonly infects rats and is found worldwide. It is not known to cause disease in its host and is limited to specific host species. Transmission occurs through the Northern rat flea (*Ceratophyllus fasciatus*) and the oriental rat flea (*Xenopsylla cheopis*) in a cyclic manner within the posterior station of the rat flea. In humans, *T. lewisi* acts as an opportunistic hemoparasite, with possible transmission through contamination of an open wound with flea feces or via a flea bite. In sporadic cases, sting marks have been reported in other infected children, such as in the leg and axilla. Infections are mostly reported in people of low socioeconomic status and in areas populated with rats. These factors may contribute to the coexistence of infected individuals in environments with a higher flea population in rats, potentially leading to the spread of this uncommon zoonotic trypomastigote infection.

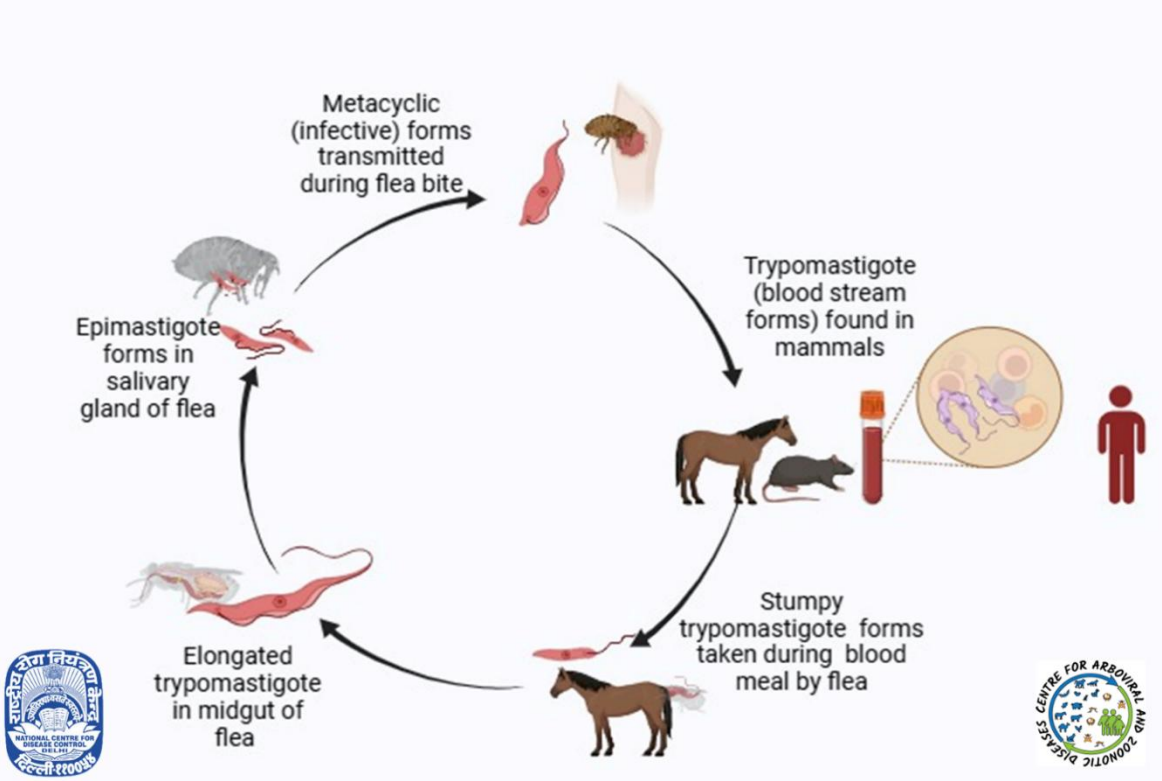


Figure 1: Transmission cycle of Trypanosomiasis.

2.3 Current Situation:

Human trypanosomiasis is prevalent in Africa and Latin America. In Africa, it is commonly referred to as sleeping sickness and is caused by *T. brucei rhodesiense* and *T. brucei gambiense*. This infection is limited to the hot and humid regions of Africa, where tsetse flies (*Glossina* spp.) thrive and play a crucial role in transmitting the disease. In Latin America, the corresponding condition Chagas disease, which is caused by *T. cruzi*. These infections are not known to occur in the Indian subcontinent.

The majority of trypanosomes were traditionally believed only to infect animals. Examples include *T. b. brucei, T. congolense*, and *T. vivax*, which are responsible for the complex animal trypanosomiasis known as "nagana" in Africa. *T. evansi* is the causative agent of a widely distributed disease called "surra," affecting domestic and wild animals in Asia, Africa, South America, and even Europe. *T. lewisi*, a trypanosome of rats transmitted by fleas, is found worldwide.

However, in recent years, *T. evansi* and *T. lewisi* have emerged as potentially pathogenic for humans, causing atypical human trypanosomiasis (a-HT). Based on reported human cases of trypanosomiasis, the majority were caused by *T. lewisi*, followed by *T. evansi, T. brucei, T. vivax* and *T. congolense*. The hemoflagellates are identified based on its morphological features, which was further confirmed using a polymerase chain reaction (PCR) assay for species confirmation. A total of 10 cases had been reported from India, including 8 cases of *T. lewisi* and 2 cases of *T. evansi* presented respectively from four States viz., Gujarat, Madhya Pradesh, Maharashtra, Uttar Pradesh. While the typical human trypanosomiasis (sleeping sickness) and Chagas disease, are prevalent in Africa and Latin America, respectively, the Asian continent is experiencing the highest incidence of a-HT caused by *T. lewisi* and *T. evansi*.

A cross-sectional investigation of uncommon Human Trypanosomiasis caused by *Trypanosoma evansi* in Kolkata and Canning Areas of West Bengal, India was reported in 2014. The results indicated a 5.2% (9/173) prevalence of *T. evansi* infection based on the CATT serological test. Additionally, PCR targeting VSG gene sequences suggested an active *T. evansi* infection in 2.89% (5/173) of the samples.

3. Clinical Features

Human

The clinical manifestations of trypanosomiasis in human beings may exhibit non-specific symptoms in early stage of infection including headache, malaise, arthralgia, weight loss, fatigue, and intermittent fever with rigors. There may be involvement of the nervous system in late stages. It is important to note that the clinical presentation of trypanosomiasis can vary depending on the stage of infection and the species of Trypanosoma involved. The fever sometimes not a notable clinical sign; however, patients suffer with insomnia, tachycardia, lymphadenomegaly, hepatosplenomegaly, tachycardia, painful thumbs, lack of memory and persistence in pain in right hand.

Animal

Affected animals with *T. evansi* infection commonly experience anemia, weight loss, reduced productivity, and often mortality. Animals with acute *T. evansi* infection usually have a short clinical course of disease and die within weeks to months of infection. In contrast, some chronic infections of *T. evansi* may persist for years. In horses, donkeys, and mules, the incubation period of disease ranges from 5 to 60 days. Presenting signs may include weight loss, lethargy, anemia, progressive paresis, loss of draught capability, enlarged lymph nodes, petechiae of serous membranes, oedema of dependent parts, urticaria, and alopecia, exudation, or necrosis of the coronary bands. Recurrent episodes of pyrexia are associated with transient parasitaemia. Abortions associated with trypanosomosis have been reported in buffaloes, mares, ewes, cattle, pigs and camels. The severity of illness depends upon the Trypanosoma strain, concurrent infections, stress, and environmental factors.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Blood, Bone marrow, Lymphnode aspirate, CSF etc.,

4.2 Storage and Transport

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory Procedure

The diagnosis of *Trypanosoma lewisi* infection involved two primary methods. Initially, blood smears are examined using microscopy to detect the presence of Trypanosoma parasites, confirming the infection. Subsequently, the Polymerase Chain Reaction (PCR) technique was employed to amplify a specific 623 bp fragment of ITS1, which is characteristic of *T. lewisi*. This combined approach provided reliable and accurate evidence for the presence of *T. lewisi* in the samples under investigation. Diagnostic techniques for animal trypanosomiasis can be categorized into three types: parasite detection, antibody detection, and DNA detection.

Microscopy: Demonstration of trypanosomes in unstained and Leishman stained films. The simplest technique involves the microscopic examination of fresh wet blood films, which provides a quick and inexpensive method but has lower sensitivity. A more sensitive method is the Giemsa-stained thin blood smear (GSBS), which allows identification of Trypanosoma parasites based on their morphology

Another approach is the hematocrit concentration technique (HCT) or Woo method, where a capillary tube is used to collect a blood sample that is then centrifuged to concentrate the parasites. The sediment is examined microscopically for the presence of Trypanosoma parasites. The buffy coat method (BCM or Murray method) is also employed, which involves collecting and examining the layer between the red blood cells and plasma.

Serology: Serology plays a crucial role in surveillance and the identification of infected animals, particularly before their transfer to trypanosome-free areas. Initially, ELISAs based on monoclonal antibodies for detection of antigens were developed; however, subsequent field evaluations revealed significant limitations in terms of sensitivity and specificity, leading to their abandonment. On the other hand, antibody detection methods have demonstrated high sensitivity in providing evidence of host-parasite contact. The World Organization for Aniaml Health (WOAH) has validated four main antibody detection techniques for routine use in trypanosomiasis diagnosis. These include indirect fluorescent antibody test (IFAT), whole-cell lysate soluble antigens (WCLSA) antibody-detection ELISA, and Card Agglutination Test for *Trypanosome*s (CATT/*T. evansi*) used for Surra.

Molecular Tests: DNA detection techniques are valuable tools for the detection of *trypanosomes* in both hosts and vectors. Polymerase chain reaction (PCR) amplification of trypanosome DNA using specific primer pairs has been developed. These primer sets target highly repetitive satellite DNA sequences, which can have 10,000-20,000 tandem repeats per genome. The WOAH provides gold standard primer sets for various trypanosome subgenera, species, and types. DNA detection methods offer higher sensitivity and specificity compared to parasitological methods. However, they have certain limitations in terms of laboratory infrastructural requirements.

5. Treatment

The treatment of *Trypanosoma evansi* infection in both humans and animals typically involves the use of trypanocidal drugs. In human cases of *T. evansi* infection, successful treatment has been reported, with a weekly dose of 1 g of suramin for 5 weeks without any complications.

T. lewisi infection is typically self-limiting in both rodents and humans. Most of *T. lewisi* cases resolve spontaneously without specific treatment. Some literature suggests using trypanocide-based therapies, while others indicate successful recovery without any specific intervention.

Presently, three drugs have been used to treat animals suffering with *T. evansi* infection. These drugs include quinapyramine methyl sulphate/chloride alone or combination salts, isometamedium hydrochloride and diminazene aceturate. It is essential to provide an adequate water supply to avoid a toxic effect on the kidneys, which can be fatal.

6. Prevention and Control

Trypanosoma evansi and *Trypanosoma lewisi* infections in humans can be prevented by taking a number of precautions to reduce exposure to the parasites and their vectors. It

is essential to employ a variety of prophylactic techniques to reduce the risk of infection because these trypanosomes can be spread through contact with diseased animals or contaminated materials as well as by biting insects like flies.

a) Vector control: It is crucial to limit interaction with the parasite-transmission vectors. Insecticide-treated bed nets can be used to prevent fly bites, together with the use of insect repellents, protective clothing, and other measures. Controlling the vector fly population can also greatly lower the risk of illness, especially in regions with high transmission rates.
b) Avoiding contact with infected animals: Transmission can be prevented by limiting contact with infected animals, notably rodents for *T. lewisi* and livestock for *T. evansi*. Direct contact with animal blood, tissues, and body fluids must be avoided, especially when the animal is sick or dead.
c) Proper hygiene practices: Infection can be avoided by maintaining proper hygiene habits. After touching animals or animal products, thoroughly washing your hands with soap and water can help lower the chance of transmission.
d) Screening and diagnosis: Trypanosome infections must be diagnosed and treated as soon as possible. Regular health examinations and quick diagnostic tests for suspected instances can speed up treatment and stop the spread of disease.
e) Quarantine measures: To prevent the spread of the parasites to others, isolating and quarantining individuals with confirmed or suspected trypanosome infections could be helpful.
f) Public awareness and education: Early diagnosis and control of outbreaks depend on educating local people, tourists, and healthcare professionals about the hazards of trypanosome infections and the preventive actions.
g) Research and surveillance: It is essential to carry research on the epidemiology and transmission of *T. evansi* and *T. lewisi* to know the patterns of infection and conduct effective prevention strategies. Surveillance systems can help monitor disease prevalence and identify high-risk areas.

Currently, there are no vaccines available for *T. evansi* and *T. lewisi* infections. Therefore, implementing above mentioned preventive measures to reduce the risk of exposure to the infection

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1. Introduction

Zoonotic tuberculosis (zTB) is a chronic bacterial disease primarily caused by *Mycobacterium bovis*, which belongs to the *Mycobacterium tuberculosis* complex (MTBC). The recent evidence of *M. orygis* as a predominant cause of zoonotic tuberculosis in India is quite relevant as is endemic to bovine tuberculosis. This disease has an enormous impact on animal and human health, especially the communities that rely on livestock for their livelihood, due to high livestock morbidity, reduced milk and meat productivity. The restrictions on the international trade of animals and their products from TB-infected countries cause a huge economic burden in developing countries. Hence, bovine tuberculosis (bTB) is one of the most economically significant zoonotic diseases in the world accounting for an estimated loss of three billion US dollars per year. Despite a well-recognized risk associated with tuberculosis, zTB is poorly monitored and continues to be an unaddressed global problem.

2. Epidemiology

2.1 *Causative Agent*

The primary and major pathogens of MTBC causing tuberculosis in domestic and wild animals are *M. bovis, M. caprae, M. microti, M. africanum, M. orygis* and *M. pinnipedii.* The World Health Organization (WHO) has reported zTB to be predominantly caused by M. bovis. However, infection of humans with any member of the MTBC (other than BCG vaccine strain) may result in zoonotic tuberculosis.

2.2 Mode of Transmission

The most common routes of transmission of the agent from animal to humans is consumption of contaminated raw milk, improperly cooked contaminated meat, and unpasteurized dairy products. However, airborne transmission due to inhalation of infectious droplet nuclei by human living in close contact or proximity to infected animals poses an occupational risk to people who work with infected animals or animal products, such as farmers, animal handlers and transporters, veterinarians, para-veterinarians, butchers, zookeepers and abattoir workers. People involved in these occupational activities may develop pulmonary and extrapulmonary tuberculosis, putting other humans and susceptible animals in contact at risk.

In recent decades, several nations have documented animal tuberculosis reservoirs, and members of the MTBC are frequently recovered from free-living and captive wildlife hosts. Spillover from suspected maintenance wildlife reservoir hosts has been reported in certain regions of Canada (elk), the Iberian Peninsula (wild boar and several cervid species), Ireland and the United Kingdom (European badger), New Zealand (brush-tailed possum), South Africa (African buffalo), and certain regions of the United States (white-tailed deer). In regions where MTBC circulates in wildlife, multiple spillover hosts ranging from rodents and small mammals to carnivores and ungulates may exist. Humans may represent a maintenance host in areas with a high prevalence of human TB and close contact with animals, with spillover from humans to animals reported for *M. tuberculosis, M. bovis* and *M. orygis*.

Although *M. bovis* can be transmitted between animals, from animals to humans or vice versa, but rarely between humans. With regard to the transmissibility of other major MTBCs of animal origin like *M. orygis* and *M. caprae,* enough data are not available. Transmission of MTBC between animals occurs mainly through aerosols, although transmission through other routes like oral, cutaneous, congenital and genital routes have

also been reported. Even calves can be infected by ingesting colostrum or milk from infected cows. Female animals are reported to be at a greater risk of bTB due to lactation, gestation, and parturition. Apart from age and gender, cross-species transmission, immune suppression, sharing of drinking and grazing locations, environment or weather, and physiological and pathological variations are some of the risk factors that play an essential role in the spread of disease among animals.

2.3 *Current Situation*

M. orygis, a new member of MTBC has recently been reported to be associated with zTB, most notably in South Asia. However, accurate estimates of its occurrence in humans and animals are still lacking. Another species of MTBC, *M. caprae* is also recognized as an important zoonotic MTBC and a hidden cause of tuberculosis in Central and Southern European countries and Turkey. This species has also been reported in animals from Japan, China and Peru. Evidence of such new zoonotic Mycobacterial species necessitates an immediate assessment of prevalence, potential drivers and risk in order to develop appropriate interventions. Furthermore, *M. tuberculosis* and *M. africanum* although are primarily human pathogens, yet reverse zoonosis is reported in animals.

WHO estimated 1,40,000 new zTB human cases globally in 2019 due to *M. bovis*. However, the estimates of global burden of zTB is grossly underestimated because of limited laboratory capacities for species-level confirmation of MTBC, weak or inexistence of surveillance and lack of data. Reporting of zTB cases in human are uncommon in countries where bovine TB is controlled, and food safety standards are followed strictly. But there is a lack of data on the occurrence of zoonotic TB in south-east Asia, which includes major cattle producing middle- and low-income countries like India, Bangladesh, Pakistan, Myanmar, and Indonesia.

India with 303.76 million bovines is one of the largest producers of milk and meat worldwide. India has an estimated 21.8 million bTB infected cattle which makes it a country with one of the largest infected herds in the world. In 2020 alone, India accounted for 26% and 34% of world's TB prevalence and death in humans, respectively. In India, the cases of infection caused by MTBC members other than *M. tuberculosis* have been reported from time to time. The consumption of raw milk and contacts with TB patients were found to be important determinants of zoonotic TB in human. The recent reports on isolation of *M. orygis* from several human patients, primarily with extrapulmonary or disseminated forms of the disease, is probably the largest reported series of *M. orygis* cases in humans from South Asia. Earlier and even in recent past either *M. bovis* in human or *M. tuberculosis* in animals alone or mixed infection along with *M. tuberculosis* has been reported by several researchers that demonstrate the zoonosis as well as the reverse zoonosis of TB in Indian settings.

3. Clinical Features

Humans

In humans, *M. bovis* infection usually has a prolonged course, with symptoms appearing months or years later. The bacteria can sometimes remain dormant in the host without causing disease. The most common clinical manifestation of *M. bovis* infection in humans is associated with the extra-pulmonary form of the disease; however, approximately half of zoonotic TB cases involve the lung and may be responsible for human-

to-human transmission. Extrapulmonary tuberculosis is mainly caused by hematogenous dissemination, although infection may spread directly from an adjacent organ. The common symptoms include loss of appetite, fluctuating fever, malaise, intermittent hacking cough and weight loss, and may vary depending on involvement of the organ with enlarged prominent associated lymph nodes. Pulmonary tuberculosis in human by MTBC of animal origin shows similar clinical manifestations as also those caused by *M. tuberculosis* in terms of clinical, radiological, and anatomo-pathological features or even severity.

Due to consumption of contaminated milk, meat and their products, the predominant site of infection of *M. bovis* is reported to be the intestine that may heal or may progress in the intestines or disseminate to other organs. Recently during 2018–2019, M. orygis infection are confirmed in 8 human patients with extrapulmonary TB from a tertiary care hospital in South India with two having concomitant pulmonary involvement. The most prevalent non-pulmonary manifestations in humans include cervical lymphadenitis, meningitis, chronic skin tuberculosis, intestinal infections, and involvement of bones.

Animals

Tuberculosis in animal has no pathognomic or distinctive clinical features. Clinical signs in animals may appear after several months to years after infection and sometimes infected animals may remain dormant for an extended duration and reactivate after several years. The most frequently observed clinical signs in cattle include a progressive weight loss, weakness, low-grade elevated or fluctuating body temperature, coughing and lymphadenopathy. Involvement of lung may be manifested by a cough, which can be induced by changes in temperature or manual pressure on the trachea. Sometimes, dyspnoea and other signs of low-grade pneumonia are also involved. Extreme emaciation and acute respiratory distress may occur during the terminal stages of tuberculosis. Lymph nodes of the head and neck may become visibly affected and sometimes rupture and drain. In advanced cases, lymph nodes are often greatly enlarged and may obstruct air passages. Involvement of the digestive tract is manifested by intermittent diarrhoea and constipation in some instances. Lesions involving the female genitalia may occur, however, male genitalia are seldom involved.

4. Laboratory Diagnosis

4.1 Collection of Specimen

Antemortem and postmortem samples, and serum. For the diagnosis of zoonotic tuberculosis, careful collection of appropriate samples from both humans and animals is crucial. In suspected human pulmonary infections, deep-cough sputum samples are typically collected in the early morning on three consecutive days, ensuring at least eight hours between samples within any 24-hour period. In cases of extrapulmonary TB, the type of specimen collected depends on the site of infection and may include tissue biopsies from affected organs, body fluids (cerebrospinal, pericardial, synovial, ascitic, bone-marrow), urine, or fine needle aspirates from lymph nodes. From animals, suitable samples depend on the stage of infection and type of diagnostic tests available, commonly involving nasal swab, milk, lymph node aspirates or serum samples. Proper handling and timely processing of these specimens are essential to ensure accurate diagnosis and effective disease management.

4.2 Storage and Transport:

Specimen should be shipped immediately and if delay is anticipated, it must be stored at -70°C. The samples should be transported in triple-layer packaging in cold chain (2 - 8°C) and reach reference testing laboratories within 48 hours. Sample transport must be in accordance to the International Air Transport Association guidelines.

4.3 Laboratory procedures

A combination of clinical examination, radiography, microscopy, bacterial culture, serological method, and tuberculin skin test are the most used screening and identification methods for zoonotic TB. In case of humans, rapid diagnosis of tuberculosis is required for early treatment and to prevent the emergence of drug-resistant strains. TB in animals is diagnosed by tuberculin tests, interferon gamma release assay (IGRA), isolation, histology, and molecular methods.

Microscopy Examination: In the peripheral laboratories, sputum smear microscopy, and LED-Fluorescence microscopy (LED-FM) are still the favoured procedures. However, most of these commonly used tests cannot differentiate mycobacterial species among the MTBC. Therefore, there is an urgent need of diagnostics for species-level confirmation of MTBC towards confirmation of zTB rather than those established for diagnosis of *M. tuberculosis* complex.

Culture and Identification: Currently, zTB is diagnosed by culture isolation of mycobacteria supplemented by MTBC identification by lateral immune-chromatography assay (MPT 64 antigen detection), subspecies identification by polymerase chain reaction (PCR), or whole genome sequencing of culture isolates. As these techniques are expensive and complex, they are not routinely used in most of the low and middle-income nations, where the potential risk of zTB is higher.

Antemortem tests in animals: Tuberculosis in live animal species like cattle, goats, and deer is often diagnosed using tests for cellular immunity (tuberculin or gamma interferon test). In wildlife, enzyme-linked immunosorbent assays (ELISA) and lateral flow tests are used for detection of serum antibodies.

Molecular Tests: Numerous molecular approaches are also being developed, including loop-mediated isothermal amplification (LAMP), line probe assay (LPA), whole genome sequencing (WGS), and other non-invasive lateral flow urine lipoarabinomannan assay (LF-LAM) and eNose assays. WHO has also endorsed a range of new and rapid diagnostic tests such as the Xpert MTB/RIF Ultra and Truenat assay. The advanced molecular techniques used in human are also being explored for animals.

5. Treatment

As zTB in humans is predominantly extrapulmonary in nature, it is often misdiagnosed resulting in delay in initial treatment. Further, most healthcare providers initiate treatment without drug susceptibility testing, therefore, patients with zoonotic TB may receive inadequate treatment. Nonetheless, the basic regimen recommended for the treatment of TB caused by *M. tuberculosis*, consists of a first phase with isoniazid, rifampicin, pyrazinamide, and ethambutol, followed by a maintenance phase with isoniazid and rifampicin. But zTB poses difficulties in patient treatment and recovery, because *M. bovis*, the most common MTBC of animal origin, is naturally resistant to pyrazinamide, one of the four first-line drugs used in tuberculosis treatment. Some authors suggest that resistance to pyrazinamide might compromise the effectiveness of treatment with fatal consequences, including an increase in the selective pressure to develop resistance to other

antituberculosis drugs, prolonging the treatment. However, in confirmed cases of zTB, some recommend the interruption of pyrazinamide use and the administration of isoniazid and rifampicin for an extended period of time. On the other hand, wild-type *M. bovis* is susceptible to most other antibiotics used to treat TB: streptomycin, ofloxacin, and ethionamide. However, it is more commonly associated with resistance to streptomycin than is *M. tuberculosis*, possibly because of the use of antibiotics in cattle.

6. Prevention and Control

Although significant progress has been made towards lowering human tuberculosis, animal TB still persists, and hence, zTB remains a concern with an unknown incidence. Despite advances in research, much of our understanding with regard to transmission mechanisms, diagnostics, control, and multi-host infection systems of zTB is still unclear. Initiatives for proper surveillance of tuberculosis both in human and animal population should be a priority, especially in endemic settings. Strong multi-disciplinary action is required to estimate the scale of animal tuberculosis burden and its transmission at human-animal interface. As the usage of dairy products rises over the world, the incidence of zTB is expected to rise. Therefore, implementing various food safety efforts, including pasteurization of milk, are the most effective way to prevent zTB.

Further Reading

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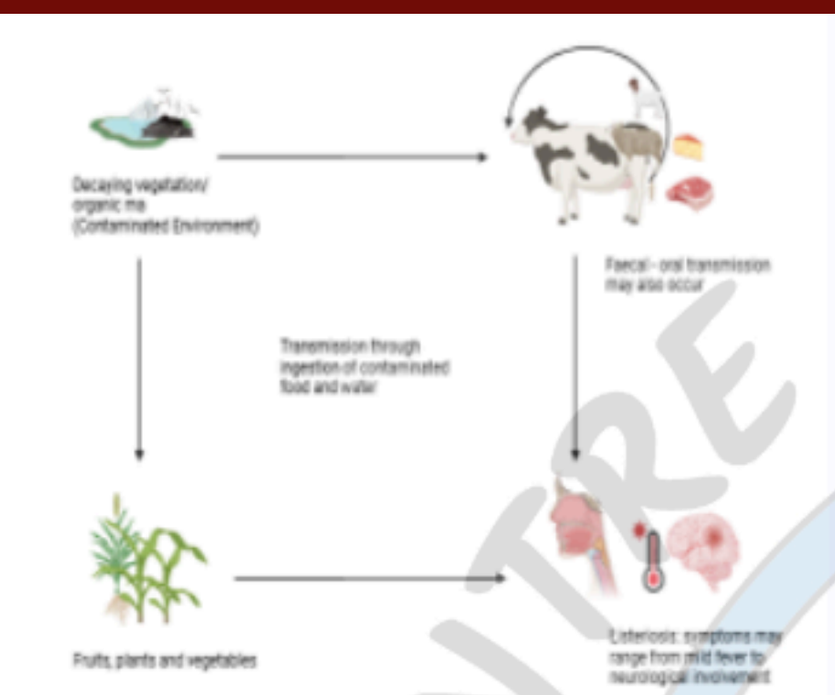
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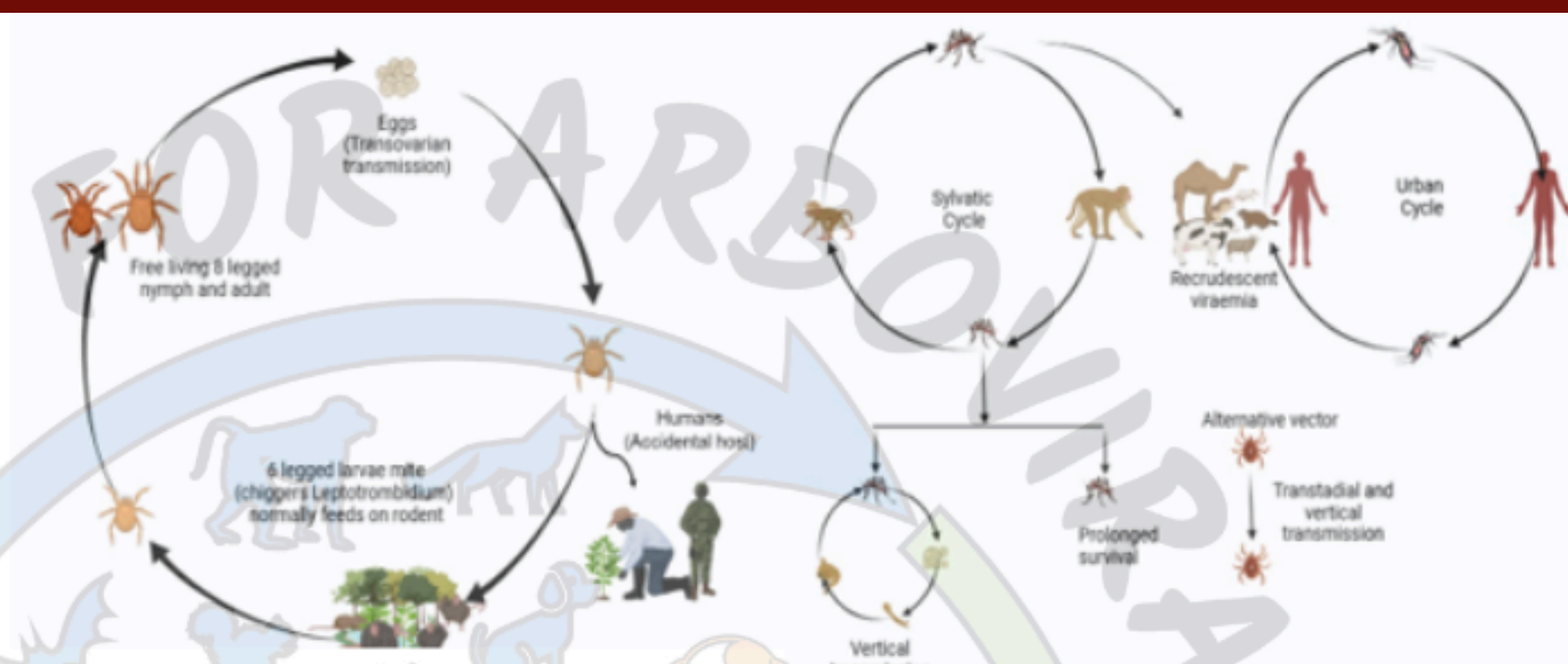
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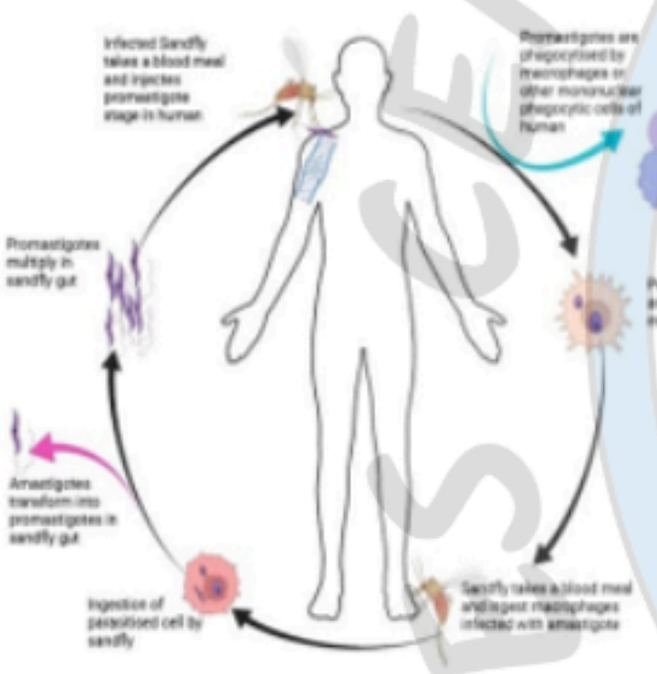
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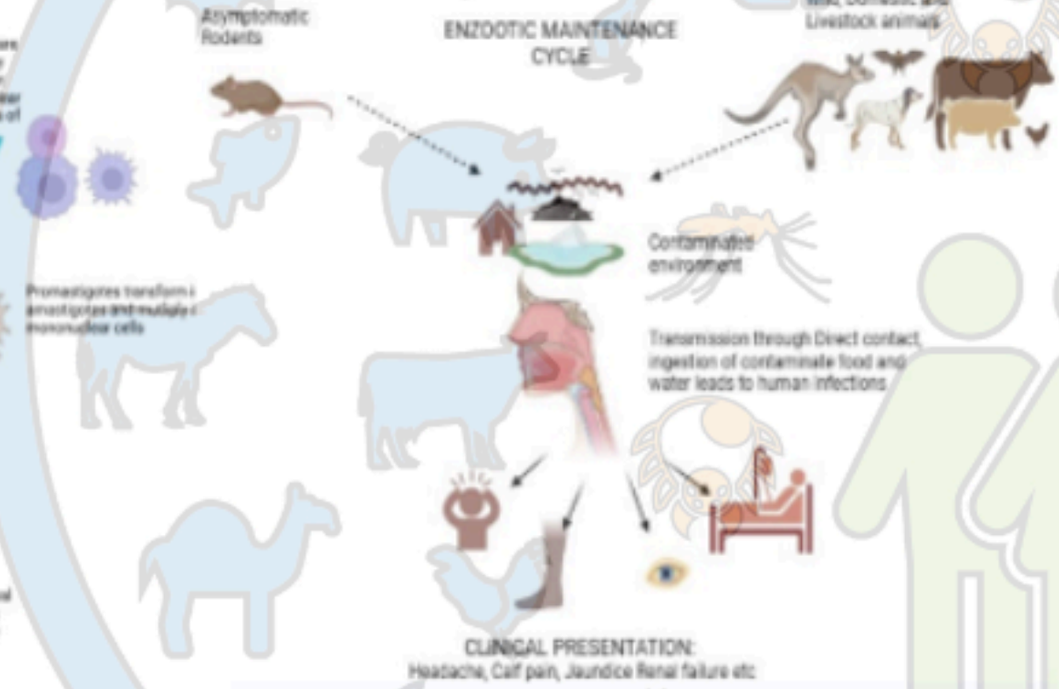
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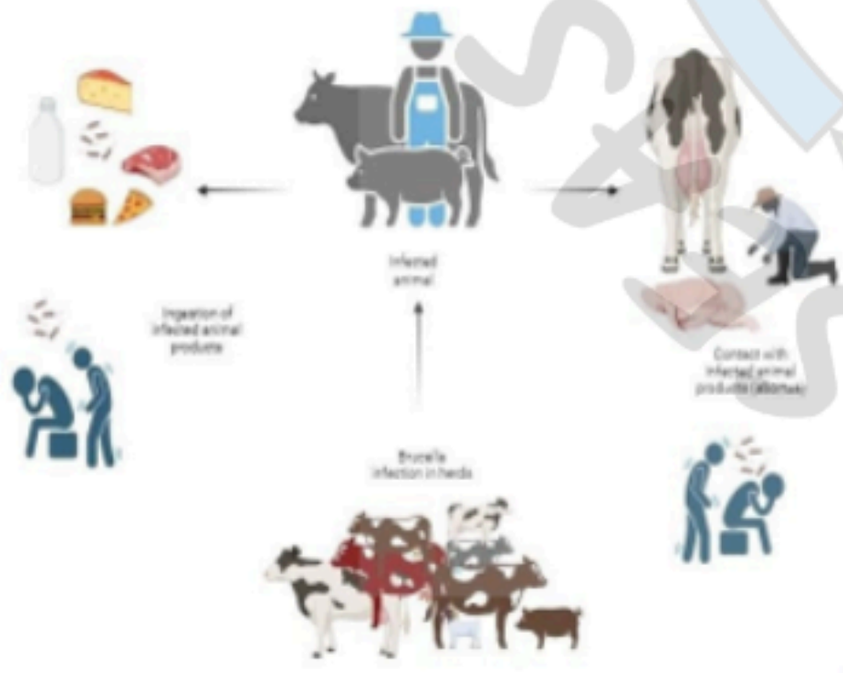


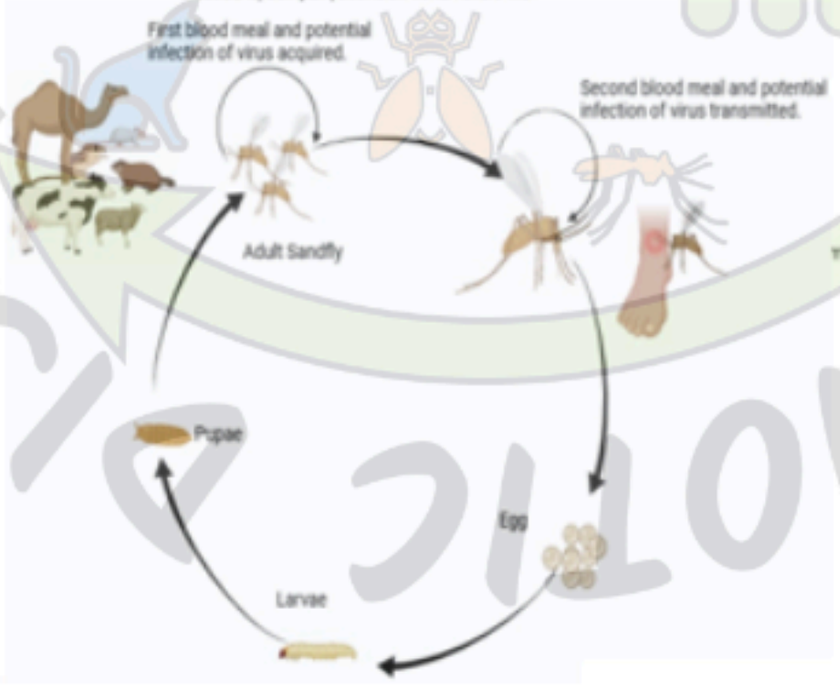


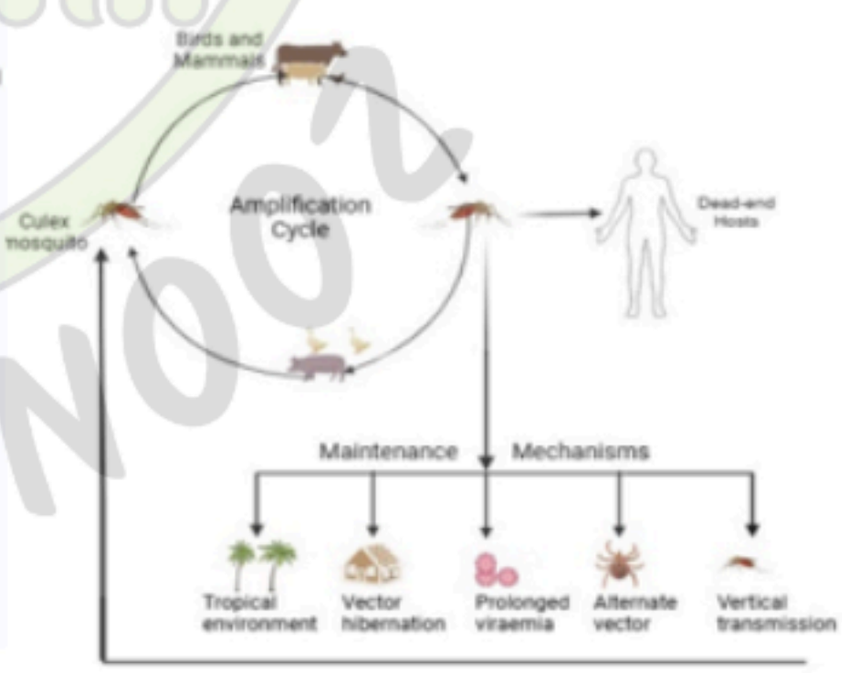












Manual of Zoonotic Diseases of Public Health Importance is a resource material focusing on endemic, emerging and reemerging zoonotic diseases in India, such as Arboviruses, Rickettsia, Plague, Anthrax, Rabies, and leptospirosis. It also gives insight into various newer emerging diseases like Monkeypox, Nipah virus disease, Zika virus disease, Crimean Congo Hemorrhagic fever and Ebola virus disease. This manual will provide an overview to the public health experts, laboratory professionals, State directorates, veterinary experts, airport health officials, and medical and paramedical professionals about various epidemiological and laboratory aspects and measures for prevention and control of zoonotic diseases as "One Health" approach.