National Guideline for the Management of Priority Zoonotic Diseases in Bhutan

2025

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Abbreviations

WHO	**World Health Organization**
CDC	**Centres for Disease Control**
RCDC	**Royal Centers for Disease Control**
JDWNRH	**Jigme Dorji Wangchuk National Referral hospital**
CDD	**Communicable Disease Division**
CMO	**Chief Medical Officer**
RDT	**Rapid Diagnostic Test**
PCR	**Polymerase Chain Reaction**
ELISA	**Enzyme Linked Immunosorbent Assay**
MAT	**Microscopic Agglutination Test**
NTS	**Non-typhoidal Salmonellosis**
HEMC	**Health Emergency Management Committee**
HPAI	**Highly Pathogenic Avian Influenza**
ARDS	**Acute Respiratory Distress Syndrome**
IFA	**Indirect Immunofluorescence Assay**
MODS	**Multi-organ dysfunction**
bTB	**Bovine tuberculosis**
LPA	**Line Probe Assay**
CFR	**Case Fatality Rate**
AKI	**Acute Kidney Injury**
EVD	**Ebola virus disease**
RIDTS	**Rapid Influenza Diagnostic Tests**
TAT	**Turnaround Time**

Acknowledgement

The Ministry of Health gratefully acknowledges all the individuals who contributed to the development of this guideline in person and remotely. Special thanks are extended to the World Health Organization (WHO) for its financial support. In particular, the ministry acknowledges the following individuals for their invaluable contributions:

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Foreword

Zoonotic diseases remain a major threat to public health. Around 60% of existing human infectious diseases are zoonotic, and about 75% of new or emerging diseases in humans come from animals. Diseases such as rabies, anthrax, brucellosis, leptospirosis, and avian influenza continue to affect people's health, livelihoods and food security around the world. Increasing human-animal interaction, deforestation, intensive farming, and global travel are contributing to the emergence and spread of these diseases.

Our country faces a high risk of zoonotic diseases due to our way of life and work. More than 60% of our population depends on farming and livestock, leading to frequent contact between humans and animals. Our rich biodiversity and porous, forested borders with neighboring countries also increase the risk of disease spill over and cross-border transmission. In addition, climate change, urbanization, commercial farming, and growing trade and globalization further heighten the risk.

Therefore, it is essential that the country remains well-prepared for zoonotic diseases, especially in areas like surveillance, diagnosis, and case management. Stronger systems will enable early detection, timely treatment, and better protection of public health.

I am pleased that this National Guideline for the Management of Zoonotic Diseases has been developed at an appropriate time. It will be a valuable resource for our health workers in delivering prompt, coordinated and effective care. I sincerely thank all the individuals and institutions who contributed their time, expertise and commitment to developing this important guideline.

(Karma Jamtsho)
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Background

Zoonotic diseases (also known as zoonosis) are infections that spread from animal to human. These diseases can be caused by bacteria, viruses, parasites or other unusual agents. It can spread to humans through direct contact with animals or contaminated food, water, or the environment.

Globally zoonotic diseases are a major public health concern. The World Health Organization estimates more than 60% of new infectious diseases in humans come from animals. Diseases like rabies, anthrax, brucellosis, leptospirosis, and avian influenza continue to affect health, livelihood, and food security worldwide. Factors like increasing contact between people and animals, human-animal, deforestation, intensive farming, and global travel make it easier for these diseases to spread. In the South-East Asia Region, dense populations, farming systems, and rich wildlife make the region a hotspot for zoonotic diseases. Bhutan's close human-animal connections, farming-based economy, and open borders increase its risk. The country often sees outbreaks of rabies, bird flu in poultry, and mosquito-borne diseases along border areas.

Recognizing the rising burden of zoonotic diseases, the Ministry of Health, utilizing a quadripartite tool within a One Health approach, prioritized the following top 10 zoonotic diseases: Rabies, Influenza A H5N1, Leptospirosis, Brucellosis, Enterohemorrhagic Escherichia coli infections, Dengue, Scrub typhus, Bovine tuberculosis, Anthrax, and Salmonellosis.

Situation of zoonoses in the country

Zoonotic diseases, though often underreported, remain a persistent public health challenge in Bhutan. In 2010, an anthrax outbreak in Zhemgang resulted in the deaths of 43 domestic animals and led to 9 human infections, including one fatality. Brucellosis, first detected in humans in 2018, continues to affect high-risk groups such as farmers, farm attendants, and veterinarians. In 2023–24 alone, 38 human brucellosis cases were confirmed out of 282 tested samples.

Bhutan also experienced outbreaks of scrub typhus in 2009 and 2014, impacting 470 individuals mostly farmers, students, and housewives with 88% of cases occurring in rural areas. The highest incidence was seen in subtropical, agriculture-intensive districts along the southern border, with an estimated prevalence of 62 per 100,000 people at risk.

Leptospirosis remains a concern as well, with high infection rates among bovines (36.9%) and dogs (47.6%), and a human seroprevalence of 1.6%.

Since 2010, Bhutan has also recorded 19 outbreaks of H5N1 avian influenza in poultry, particularly in high-density districts. Each outbreak has caused significant economic losses

estimated at Nu. 2.27 million per event. Although no human infections have been reported so far, the threat of zoonotic spillover remains high and could potentially trigger a future pandemic.

Given this growing evidence for the rising burden of zoonoses in the country, it is critical to have strong and coordinated national guidelines to ensure timely detection, response, and management of zoonotic diseases. This guideline brings together Bhutan's priority zoonotic diseases of public health concern under one comprehensive framework.

This national guideline covers the following priority zoonotic diseases and the disease specific management is covered in the following sections.

1. Avian Influenza
2. Leptospirosis
3. Brucellosis
4. Scrub Typhus
5. Bovine Tb
6. Anthrax
7. Rabies
8. Shiga toxin-producing Escherichia Coli (STEC)
9. Non-Typhoidal Salmonellosis
10. Cystic Echinococcosis (CE)
11. Ebola Virus

1.Avian Influenza

1.1. Introduction

Avian influenza is an acute respiratory infection caused by Type A Influenza viruses of animal origin which otherwise primarily infects avian species such as wild birds, waterfowls and domestic birds. The avian Influenza A virus is a single -stranded RNA virus and belongs to Orthomyxoviridae family. The antigenicity of the virus is determined by the surface glycoproteins; haemagglutinin (H) and neuraminidase (N). There are 16 variants of H glycoproteins and 9 variants of N glycoproteins. Although human infections are not common, we can sporadically get infected by certain subtypes of avian Influenza A virus such as A(H5N1), A (H5N6), A (H7N9), A (H9N2). Human infections are primarily acquired through direct contact or close exposure to infected animals or contaminated environments.

The Avian influenza A virus can be classified into Highly Pathogenic Avian Influenza (HPAI) and Low Pathogenic Avian Influenza (LPAI) based on its clinical manifestation in poultry populations. HPAIs are known to cause severe disease and high mortality in poultry whereas LPAIs cause only mild or no illness. However, it should be noted that the LPAIs has the potential to evolve to become highly pathogenic in due course of time. Humans infected with HPAI A (H5N1), HPAI A (H5N6) and HPAI and LPAI A (H7N9) virus subtypes are associated with high mortality.

1.2. Epidemiology

HPAI (H5N1) is one of the several influenza viruses known to cause severe disease in birds with documented evidence of spillover to humans causing illnesses of ranging severity with high fatality rate *(WHO Influenza A: H5N1)*. Since it first emerged in 1996 in Guangdong province, China H5N1 has been reported in 23 different countries with a reported case fatality rate (CFR) of 50% *(WHO Influenza: H5N1 & CDC)*. However, the CFR is reported to be as high as 80% in case of late detection. From 2003 to 2024, the WHO reported 954 H5N1 confirmed cases with 464 deaths (WHO). Fifty-five percent of the total cases and 71.4% of the total deaths were reported from the Asian continent alone *(WHO/GIP data)*. While the South-East Asian countries contributed to the large majority of these cases (around 86%), 13 cases and four deaths were reported from Bangladesh, Pakistan, India and Nepal combined in the last two decades *(WHO Influenza: H5N1 & CDC)*. Other subtypes of avian influenza A reported to cause human infection is **H7N9.**

Bhutan has reported 19 HPAI outbreaks in poultry farms since it was first reported in February 2010 at Rinchending, Chhukha with the most recent outbreak reported at Alley under Sampheling gewog in Chhukha Dzongkhag *(NCAH, MoAL)*. Most outbreaks are attributable to the predominant practice of backyard poultry farming in the country with poor farm biosecurity. No human cases of HPAIs have been reported so far and the disease has remained limited to poultry farms.

1.3. Risk factors

The primary risk factor for human infection by avian influenza appears to be direct or indirect contact with infected or dead poultry, contaminated environment and poultry products.

Box 1. High risk groups

- Children playing with infected poultry, particularly asymptomatic infected ducks
- Poultry handlers in live animal markets/wet markets
- Cullers without proper PPE
- Those handling fighting cocks
- Persons plucking and preparing of diseased birds in wet markets/backyard poultry/kitchens
- Consumption of undercooked poultry products
- Consumption of chicken or duck blood
- Hospital functionaries managing human cases of Avian influenza without proper PPE

Note. From "Interim guidelines for Avian Influenza Case Management'' Copyright 2007 WHO.

1.4. Mode of transmission

Humans mainly acquire avian influenza infection by:

- Directly touching infected birds (live or dead), feces and secretions of infected birds, contaminated environment.
- Ingestion of undercooked or uncooked poultry products including blood.
- Close contact (within 1 meter) with a person infected with the virus, although less common.

Route of transmission: Mucous membrane of eyes, nose and mouth, ingestion, inhalation and through broken skin.

1.5. Incubation period

The time period from exposure to onset of illness ranges from **2-8 days** with the median incubation of 4 days.

1.6. Infectious period

One day prior to the onset of symptoms until 7 days after the resolution of fever in adults and up to 21 days in children (because of prolonged viral shedding).

1.7. Clinical Features

H5N1 infection can result in mild symptomatic illness to life threatening disease. The clinical presentation may depend on several factors including the nature of the exposure, age, immunocompromised state and comorbidities of the patients. The most consistent clinical features include fever of > 38°C, cough and shortness of breath. The initial presentation may be similar to seasonal influenza, however in H5N1 there is longer incubation period, earlier onset of pneumonia and rapid progression to respiratory distress with high case fatality rate.

BOX 2. Clinical features

- Fever > 38°C
- Cough
- Shortness of breath
- Diarrhea

Infrequent features

- Vomiting
- Abdominal pain
- Chest pain
- Abnormal bleeding from nose and /or gums
- Encephalopathy (rare)

Note: Adapted from Interim guidelines for Avian Influenza management, 2007, WHO.

1.7.1. Complications

- Severe pneumonia
- Acute respiratory distress syndrome (ARDs)
- Respiratory failure
- Sepsis with multiorgan dysfunction (MODs)
- Acute kidney injury (AKI)
- Myocarditis
- Meningoencephalitis

1.7.2. Differential Diagnosis

- Seasonal flu
- SARS-CoV-2 infection
- Respiratory syncytial virus infection
- Common cold caused by other viruses – rhinovirus, parainfluenza and coronavirus
- Pneumonia caused by bacteria, fungi, and protozoa.

1.8. Laboratory Diagnostic Approach for Avian Influenza

1.8.1. Collection of specimens

Sample should be collected by trained personnel considering all biosafety measures including the use of personal protective equipment appropriate for respiratory viruses. Recommended samples are the same type of samples used for influenza routine surveillance. Nasopharyngeal swab and oropharyngeal swab are the optimal specimen collection method for influenza testing. However, combined nasal and throat swab specimens or aspirate specimens can be collected.

Sample collection is recommended within 4 days of symptoms onset for highest influenza virus yield and better detection.

In human, the commonly specimen are collected as follow

- Nasopharyngeal and oropharyngeal swab (Dracon or polyester flocked swabs in the viral transport medium)
- Patient with severe respiratory disease with suspected infection should also collect lower respiratory tracts specimens such as bronchoalveolar lavage/endotracheal aspirate / nasopharyngeal aspirate/ induced sputum in sterile container
- If the patient has conjunctivitis (with or without respiratory symptoms) conjunctival swabs can also be collected in combination with nasopharyngeal and oropharyngeal swabs.

Note: For more detailed information with collection procedure, refer National Respiratory virus sentinel surveillance guidelines.

1.8.2. Sample storage and transport

Accuracy of the diagnosis will depend on the quality of sample collected, how the specimens were stored and made shipment to higher testing laboratories.
Specimens should be shipped immediately and if delay is anticipated, it must be stored at -20/-80*C. The samples should be transported in triple-layer packaging in cold chain (2-8*C) and

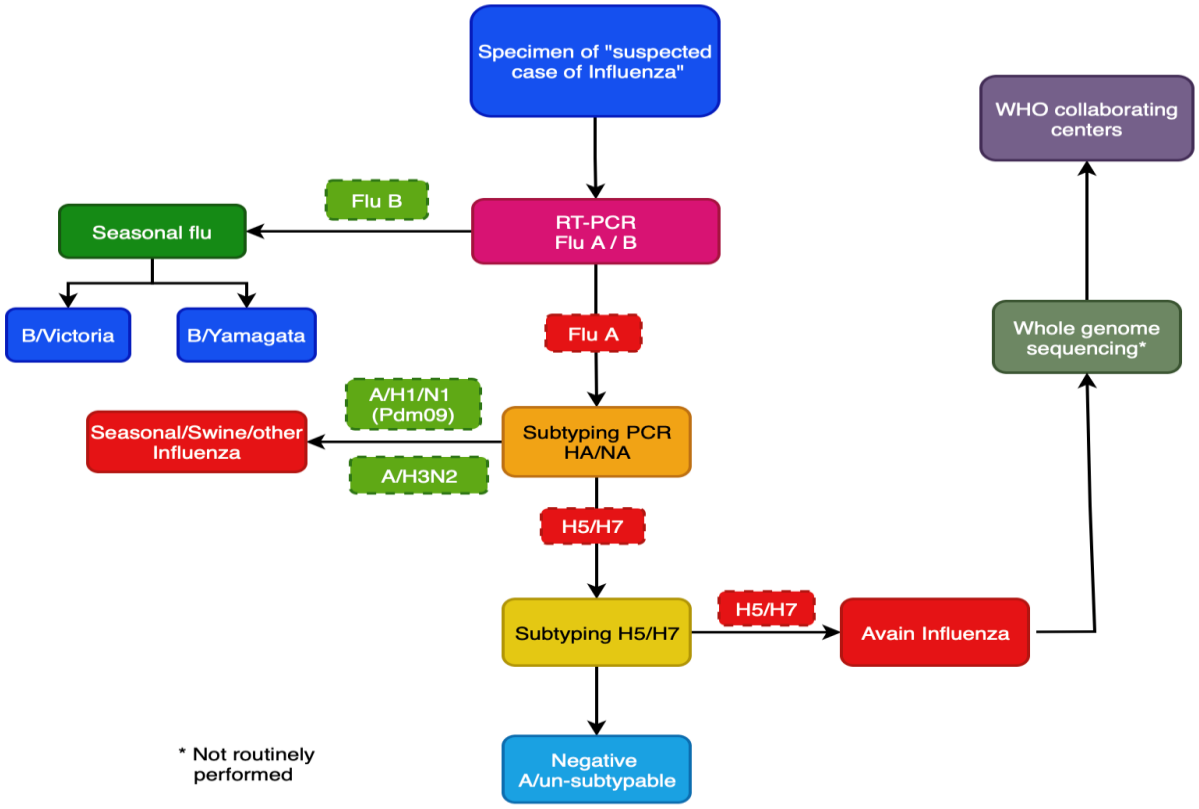
ship to testing laboratories. Sample transport must be in accordance with the international Air Transport Association guidelines.
The laboratory should be notified in advance by phone that the specimens will be sent to ensure prioritization by laboratory personnel and for the safety of laboratory personnel while handling.

For more detailed information with sample packaging and shipment procedure, refer to National guidelines for sample collection, packaging, transport of clinical specimens.

Note: Each sample should be properly labeled mentioning name, date of collection, and accompanied with a duly filled case investigation form (CIF) with relevant clinical details should be included.

1.8.3. Sample flow and laboratory testing algorithm

In Bhutan, National Influenza Centers (NIC) and district ILI/SARI testing laboratories equipped with RT-PCR facilities, use molecular diagnostics to test for the general influenza virus from the clinical specimens.
Sentinel sites/ decentralized laboratories should refer influenza A suspected cases samples to the NIC for H5 subtyping is not possible.



Laboratory testing algorithm for Influenza

1.8.4. Laboratory testing for influenza

Testing for influenza can be performed either using molecular assay and the rapid diagnostic test kit. Clinicians should always consider diagnostic testing for other respiratory pathogens that can cause acute febrile respiratory illness depending on the local epidemiology of circulating respiratory viruses (e.g., SARS-CoV-2) since novel influenza A virus infections of humans are very rare, even in exposed persons.

1.8.4.1. Rapid influenza Diagnostic test (RIDTs)

It is an immunoassay that can identify the presence of influenza A and B viral nucleoprotein antigens in respiratory specimens and display the result in a qualitative way (positive vs negative). RIDTs can distinguish between influenza A or B viruses but do not provide information on influenza A virus subtype. In addition, RIDTs cannot distinguish between seasonal influenza A virus infection and novel influenza A virus infection (due to infection with avian or variant influenza A viruses).
However, RIDTs have limited sensitivity to detect influenza viruses in respiratory specimens compared to RT-PCR or viral culture and negative RIDT test results should be interpreted with caution given the potential for false negative results, especially during peak influenza activity in a community.

Advantages of RIDTs;

- Rapid diagnosis which aids in early case identification, initiation of specific antiviral therapy and implementation of infection control measures for patients suffering from influenza.
- Can be used as point of care test to be performed under field setting.
- Simple to perform, non- laboratory health personnel can perform the test with adequate PPEs.
- Provide results within 15 - 30 minutes.

Disadvantages of RIDTs;

- Distinguish between influenza A or B viruses but do not provide information on influenza A virus subtype.
- Issue with sensitivity and specificity of the test kit.

1.8.4.2. Molecular assay

RT-PCR detects influenza virus RNA or nucleic acid in the respiratory specimen.
Molecular assays like RT-PCR identify influenza A and B viruses by target conserved gene regions. Further, all Influenza A subtypes are defined by their surface HA and NA proteins, which is why primers and probes that specifically target the corresponding genes are effective for determining the subtype of these viruses.

Advantages of RT-PCR:

- Highly sensitive and specific in providing the definitive diagnosis of influenza infection.

Disadvantages of RT-PCR

- Expensive, requires highly specialized equipment and infrastructures
- The turnaround time is longer than rapid test

Currently, the diagnosis of avian influenza in humans at the field level presents significant challenges due to the unavailability of rapid diagnostic tests (RDTs) for confirmation. Diagnosis of AI for definitive confirmation using the RT-PCR technique can be performed in National Influenza Centers at Royal centers for Disease control.

1.8.5. Routine Investigation

- CBC
- ESR
- CRP
- RFT
- LFT
- Chest xray
- ILI and SARI
- Sputum Culture and GeneXpert
- ABG if necessary/available
- CT Chest - if available

The diagnosis is based on the case definitions given by CDC.

1.8.6. Case Definitions

Confirmed Case: A person with laboratory confirmed avian influenza A virus infection.
Suspected Case (also called Case Under Investigation): A person meeting clinical and epidemiologic criteria for avian influenza A virus infection and for whom confirmatory laboratory test results are unknown or pending.
Probable Case: A person meeting clinical and epidemiologic criteria for avian influenza A virus infection and for whom laboratory test results do not provide sufficient level of detail to confirm avian influenza virus infection.

1.8.7. Clinical Criteria for avian influenza

Patients with signs and symptoms of acute upper and lower respiratory tract infection

- Mild flu-like illness (cough, sore throat, fever or feeling feverish, rhinorrhea, fatigue, myalgia, arthralgia, headache) or conjunctivitis (red eye, discharge from eye)
- Moderate to severe illness including shortness of breath or difficulty breathing, altered mental status, seizures
- Complications including pneumonia, respiratory failure, acute respiratory distress syndrome, multiorgan failure, or meningoencephalitis

1.8.8. Epidemiological Criteria for avian influenza

Persons with recent exposure (within 10 days) to Avian influenza virus through one of the following:

1. **Exposure to infected birds** or other animals defined as follows;
   - Close exposure (within two meters) to infected birds or animals including Handling, slaughtering, defeathering, butchering, culling, or preparation of birds or other animals for consumption and consumption of uncooked or undercooked food (meat) or food products including unpasteurized milk or other dairy products.
   - Direct contact with surfaces contaminated by feces, secretions, excretions, unpasteurized milk or other dairy products or remains from infected birds or animals.
   - Visiting a live bird market with confirmed bird infection or associated with a case of human infection
2. **Exposure to an infected person**: Close (within two meters), and unprotected (without use of respiratory and eye protection) exposure to a person who is a confirmed, suspected, or probable case of avian influenza virus example in the household or healthcare facility.
3. **Laboratory exposure**: Unprotected exposure to the avian influenza virus in the laboratory

1.9. Management

Depending on the clinical condition and risk factors of the patient, they can be managed at outpatient and inpatient settings. Antiretroviral treatment to be started as early as possible, not waiting for laboratory confirmation. Treatment should be initiated even if more than 48 hours have elapsed since the onset of illness.

1.9.1. Groups at higher risk for influenzae complications (CDC- August 2023)

Children age < 5yrs but especially <2 yrs
Adults ≥ 65 yrs
Pregnant and up to 2 weeks postpartum
Residents of nursing home and long-term care facilities

People with medical conditions:

- Asthma
- Neurological and neurodevelopmental conditions (including disorders of the brain, spinal cord, and peripheral nerve and muscle such as cerebral palsy, epilepsy, stroke, intellectual disability, moderate-to-severe developmental delay, muscular dystrophy, and spinal cord injury)
- Chronic lung disease (eg - chronic obstructive pulmonary disease, cystic fibrosis)
- Heart disease (eg, congenital heart disease, congestive heart failure, coronary artery disease)
- Blood disorders (eg, sickle cell disease)
- Endocrine disorders (eg, diabetes mellitus)
- Kidney diseases
- Liver disorders
- Metabolic disorders (eg, inherited metabolic disorders and mitochondrial disorders)
- Weakened immune system due to disease (eg, HIV, AIDS, cancer) or medication (eg, chemotherapy or radiation therapy, chronic glucocorticoids)
- Children <19 years of age who are receiving long-term aspirin therapy
- People with Class III obesity (body mass index [BMI] ≥40)

1.9.2. Outpatient Treatment

- Oseltamivir (75mg) orally twice a day for 5 days is the preferred regimen regardless of time of onset of symptoms.
- Supportive treatment – encourages oral hydration, analgesics and antipyretics.

1.9.3. Inpatient Treatment

- Oseltamivir (75mg) orally twice a day for 5 days is a preferred regimen. However, it can be extended up to 10 days in severe cases depending on clinician judgment.
- Supportive management
- Oxygen therapy
- IV fluids
- Analgesics
- Management of complications - may need to refer to higher centre for further management

1.9.4. Treatment recommendations in children

- Oseltamivir is the preferred antiviral medication for patients with influenza A and B because of the cumulative experience of this drug in children, relative cost, and ease of administration.
- Although best results are observed when the child is treated within 48 hours of symptom onset, antiviral therapy should still be considered beyond 48 hours in:

    - Any child hospitalized with suspected or confirmed influenza disease
    - Any child with severe, complicated, or progressive influenza disease, regardless of health care setting (ie, inpatient or outpatient)
    - Any child with suspected or confirmed influenza disease of any severity if they are at high risk for influenza complications, regardless of health care setting (ie, inpatient or outpatient)

Treatment may be considered for the following individuals in the outpatient setting, after discussing benefits and risks with parents/guardians:

- Any child with suspected or confirmed influenza disease who is not at high risk for influenza complications, if treatment can be initiated within 48 hours of illness onset.
- Any child with suspected or confirmed influenza disease whose siblings or household contacts are either younger than 6 months or at high risk for influenza complications.
- Initiation of antiviral therapy should be based on signs and symptoms consistent with influenza infection and epidemiologic factors. Provision of antiviral therapy does not require a positive test for influenza.

Table 1. Dosing of oseltamivir for treatment of influenza in children

Age	**Body weight**	**Dose of oseltamivir for post exposure prophylaxis of seasonal influenza**
Adults and those 13 years and over	> 40 kg	75 mg twice daily for 5 days
	10 kg to 15 kg	30 mg twice daily for 5 days

Children from 1 year up to 13 years	> 15 kg to 23 kg	45 mg twice daily for 5 days
	> 23 kg to 40 kg	60 mg twice daily for 5 days
Children under 1 year		3mg/kg twice daily for 5 days

1.10. Prophylaxis in Influenza

For asymptomatic persons, who are at extremely high risk of severe illness if they develop influenza and who are exposed to seasonal influenza viruses in the prior 2 days, administration of the following antivirals is recommended, they are Baloxavir, laninamivir, and or oseltamivir.

a. Antiviral post-exposure prophylaxis does not replace influenza vaccination.

b. Extremely high-risk patients are considered those patients over 85 years old with or without risk factors for severe disease or younger patients with multiple risk factors.

The doses of the antivirals are given below:

1.10.1. Baloxavir

Baloxavir is given orally as a single dose, based on body weight, see Table 1 below.

Body weight	**Dose of baloxavir**
< 20 kg	2 mg/kg (as suspension)
20 kg to 79 kg	40 mg (tablet)
80 kg and over	80 mg (tablet)

Table 1. Dosing of baloxavir by weight

- Prophylaxis should be administered as early as possible, and within 2 days of symptom onset.
- Not recommended in pregnancy.
- Not recommended who are NOT at extremely high risk of severe illness if they develop influenza and who are exposed to seasonal influenza viruses in the prior 2 days.

1.10.2. Laninamivir

Laninamivir is given inhaled dry powder as a single dose, based on body weight, as below.

Table 2 Dosing of laninamivir for post-exposure prophylaxis of seasonal influenza

Age group	Dose of laninamivir for post-exposure prophylaxis of seasonal influenza
Adults	20 mg once daily for 2 days
Children < 10 years	20 mg single dose
Children ≥ 10 years up to 18 years	20 mg once daily for 2 days

- Treatment should be administered as early as possible, and within 2 days of symptom onset.
- Insufficient data in pregnancy and lactation.
- For asymptomatic persons, who are NOT at extremely high risk of severe illness if they develop influenza and who are exposed to seasonal influenza viruses in the prior 2 days, administration of laninamivir is not suggested.

1.10.3. Oseltamivir

Table 3. Dosing of oseltamivir for post-exposure prophylaxis of seasonal influenza

Age	Body weight	Dose of oseltamivir for post exposure prophylaxis of seasonal influenza
Adults and those 13 years and over	> 40 kg	75 mg once daily for 10 days
Children from 1 year up to 13 years	10 kg to 15 kg	30 mg once daily for 10 days
	> 15 kg to 23 kg	45 mg once daily for 10 days
	> 23 kg to 40 kg	60 mg once daily for 10 days
Children under 1 year		3mg/kg once daily for 10 days

- Prophylaxis should be administered as early as possible, and within 2 days of exposure.
- Oseltamivir is safe in pregnancy but limited information is available for lactation.
- For asymptomatic persons, who are NOT at extremely high risk of severe illness if they develop influenza and who are exposed to seasonal influenza viruses in the prior 2 days, we suggest not administering oseltamivir

A person exposed to zoonotic influenza virus is associated with high mortality or unknown risk of severe disease.

For asymptomatic persons exposed to zoonotic influenza viruses associated with high mortality in humans or with an unknown risk of causing severe disease in the prior 2 days, we suggest administering the following drugs like baloxavir, laninamivir, and or oseltamivir.

- Zoonotic influenza A viruses that have been associated with high mortality in humans when they become infected include HPAI A(H5N1), HPAI A(H5N6) virus; and HPAI and LPAI A(H7N9).
- It is likely there will be uncertainty with any novel influenza A virus as to the potential clinical consequences or virulence.
- It is likely that there is variable susceptibility of antiviral medications to novel influenza A viruses so in vitro and clinical studies will remain necessary.

The dose of baloxavir and laninamivir is similar to seasonal influenza virus as above.

1.10.4. Oseltamivir

The dose of oseltamivir is similar to seasonal influenza as above with the duration of 10 to 14 days as shown below:

Table 4. Dosing of oseltamivir for post-exposure prophylaxis of zoonotic influenza

Age	Body weight	Dose of oseltamivir for post-exposure prophylaxis for novel influenza A virus exposure
Adults and those 13 years and over	> 40 kg	75 mg twice daily for 10-14 days
Children from 1 year up to 13 years	10 kg to 15 kg	30 mg twice daily for 10-14 days
	> 15 kg to 23 kg	45 mg twice daily for 10-14 days
	> 23 kg to 40 kg	60 mg twice daily for 10-14 days
	> 40 kg	75 mg twice daily for 10-14 day
Children under 1 year		3 mg/kg twice daily for 10-14 days

Prophylaxis should be administered as early as possible, and within 2 days of exposure. Patients with chronic kidney disease dose adjustment is necessary.

1.10.5. Zanamivir

Zanamivir is given as a dry powder inhaler. The dose for adults and children over 5 years is 10mg once daily (that is two 5mg inhalations) for 10 days.

- Antiviral post-exposure prophylaxis does not replace influenza vaccination.

- Extremely high-risk patients are considered those patients over 85 years old with or without risk factors for severe disease or younger patients with multiple risk factors.

1.11. Infection Prevention and Control

1.11.1. Healthcare workers

- Healthcare workers and their employers who treat known or suspected persons with avian influenza should take specific precautions to prevent the spread of avian influenza.
- Install and properly maintain appropriate air-handling systems in healthcare facilities. Acute care facilities should already have appropriate heating, ventilation, and air conditioning (HVAC) systems (including appropriate exhaust and filtration) to help control exposure to avian influenza viruses.
- Place patients with suspected avian influenza infection in an Airborne Infection Isolation Room (AIIR). If an AIIR is not available, place a facemask on the patient (if the patient can tolerate wearing one) and isolate the patient in an examination room with the door closed. Transfer the patient as soon as possible to a facility where an AIIR is available.
- Implement policies and practices to minimize potential exposures to avian influenza before arrival, upon arrival, and throughout an affected patient's presence in the healthcare setting. Avoid transporting patients outside the isolation room unless necessary and limit the numbers of healthcare workers caring for patients and visitors allowed to see the patients.
- Track all healthcare workers and support staff who care for or enter the rooms of confirmed or suspected avian influenza patients. Implement a policy for healthcare workers who develop respiratory symptoms after an exposure to avian influenza. Workers should notify their supervisor, receive prompt medical evaluation, and comply with work exclusion (i.e., stay home) until they are no longer contagious. Implement sick leave policies for healthcare workers that are non-punitive.

1.11.2. Laboratory workers

Exposure to avian influenza viruses in clinical, research, and production laboratory workers can occur through inhalation and direct contact. Inhalation may occur from aerosols generated by manipulating viral cultures, handling avian influenza virus-infected samples, or inoculating research animals. Direct contact of the eyes, nose, and mouth with bare hands or gloves contaminated with avian influenza may occur after handling viral cultures or infected human and animal samples including nasopharyngeal and oral secretions, blood, tissues, and excrement. As a general principle:

- Use Biosafety Level 2 (BLS-2) practices for diagnostic, research and production activities involving LPAI viruses and Animal Biosafety Level 2 (ABSL-2) practices for work with these viruses in animal models.

- Use BSL-3 and ABSL-3 practices, procedures, and facilities for work involving HPAI viruses, with rigorous adherence to additional respiratory protection, showering and clothing change protocols.

1.11.3. Laboratories Working with HPAI Viruses

- All work with HPAI virus must be conducted in a USDA-approved Biosafety Level 3 or 4 enhanced containment facility.
- Follow appropriate BSL precautions. Work must be conducted under BSL 4 and ABSL 4 or BSL 3 conditions. Laboratory work with HPAI conducted in BSL 4 and ABSL 4 laboratories does not require additional provisions. All FSAP requirements must be met for BSL 3 conditions

1.11.4. Laboratories Working with LPAI viruses

- Use engineering controls including the use of a biosafety cabinet (BSC). Ensure that laboratory entrances and exits have self-closing doors. Conduct aerosol-generating procedures in a properly installed, maintained, and certified Class II BSC. If possible, conduct all work with infectious samples in the BSC. The OSHA Fact Sheet, Laboratory Safety Biosafety Cabinets, provides guidance on training and effective use of BSCs.
- Restrict access to the laboratory during work operations and post appropriate biohazard warning signs at the entrance.
- Enroll laboratory workers in a medical surveillance program.
- Minimize work tasks that contribute to the generation of bioaerosols or droplet sprays. These may include using syringes, pipetting, vortexing, centrifuging, and opening/closing sample tubes.
- Dispose of contaminated material in appropriate biohazard containers and autoclave, incinerate, or inactivate it using an alternative method of decontamination.
- Use respiratory protection and PPE appropriate for the work tasks performed. In laboratories, the BMBL guidance can help employers select appropriate respiratory protection and PPE. All PPE worn in the laboratory should be considered contaminated.

1.12. Reporting and Surveillance

Suspected avian influenza is an immediate notifiable disease under NEWARS. Hence, the health facilities detecting the suspected case fulfilling the case definition must be notified immediately through NEWARS or inform the officials concerned at RCDC through available means of communication channels.

The National Influenza Center (NIC) is established at the Royal Centre for Disease Control (RCDC). It provides diagnostic and surveillance services for influenza diseases including technical support to regional and district laboratories. The RCDC laboratory is well-equipped

with a Biosafety Level 3 (BSL-3) facility to safely handle highly pathogenic influenza virus samples and Real-Time PCR including gene sequencing to detect different strains of the virus.
In the field, suspect influenza cases are first tested using a rapid diagnostic kit, and positive samples are then confirmed using RT-PCR at the national level.
RCDC also sends virus isolates and selected clinical samples to the WHO Collaborating Centre or WHO Reference Laboratories for advanced molecular testing and confirmation. In addition to the RCDC lab in Thimphu, four regional labs in Mongar, Phuentsholing, Dewathang, and Gelephu are also equipped with RT-PCR machines to support influenza diagnostics.

RCDC initiated ILI surveillance in 2008 and SARI surveillance in 2012. Based on geography and population coverage, seven hospitals for ILI and eleven hospitals for SARI were designated as sentinel sites. These hospitals submit data daily (for SARI) and weekly (for ILI) via a web-based system.
This data is used to monitor illness trends and trigger response actions when needed. During a pandemic, sentinel surveillance will be expanded to include all health centers across the country, as well as points of entry (airports and ground crossings) for early case detection and control.
Bi-weekly reports from these surveillance systems are published on the RCDC website and also shared electronically.

1.13. Response to Avian Influenza outbreak

In the event of a Highly Pathogenic Avian Influenza (HPAI) outbreak in animals with suspected spillover to humans, a coordinated One Health response is essential. The Royal Centre for Disease Control (RCDC), animal health counterpart, and local stakeholders, will conduct active surveillance among communities in affected areas. This includes joint risk assessments, contact tracing, and timely management of potential human infections to minimize transmission and safeguard both human and animal health.

1.14. Prevention and Control measures for HPAI in **Humans**

Preventing and controlling avian influenza in humans requires a combination of personal protective behaviors, community awareness, and strong healthcare measures to minimize exposure, detect cases early, and stop further transmission.

1.14.1. Prevention Measures

- Promote regular handwashing with soap and water, especially after handling poultry or being in live bird markets.
- Educate the public to avoid direct contact with sick or dead birds, and to report unusual poultry die-offs.
- Encourage thorough cooking of poultry meat and eggs to safe temperatures.
- Provide personal protective equipment (PPE) and training for poultry workers, farmers, and healthcare providers.

- Issue health advisories and risk communication for travelers, poultry handlers, and communities in affected areas.

1.14.2. Control Measures

- Rapid identification and isolation of suspected human cases.
- Implement infection prevention and control (IPC) in healthcare facilities (PPE, patient isolation, safe specimen handling).
- Provide antiviral treatment to confirmed cases and prophylaxis for exposed high-risk contacts, as per guidelines.
- Conduct active monitoring and follow-up of close contacts of confirmed cases.
- Strengthen cross-border and community-level response to prevent human-to-human transmission.

1.14.3. Control of HPAI in animal health sector

When the HPAI is detected in the animal population, the animal health sector's preparedness and response is guided by the National Influenza Preparedness and Pandemic Plan and Standard Operating Procedures 2020 (NIPPP&SoPs 2020).

1.15. References

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2.Leptospirosis

2.1. Introduction

Leptospirosis is a bacterial zoonosis caused by pathogenic *Leptospira* spp, transmitted mainly through contact with water or soil contaminated by the urine of infected animals. The bacteria Leptospira interorgan is pathogenic to both humans and animals (commonly rodents, cattle, and dogs) predominantly found in tropical and subtropical areas. It affects a wide variety of animal species, both wild and domestic, which serve as sources of infection for humans (WHO).

2.2. Epidemiology

The disease is found around the world. About 1 million cases occur in people globally each year, with nearly 60,000 deaths. It often has a seasonal distribution and with increased rainfall or higher temperature. However, the disease can occur throughout the year. Epidemics may be associated with changes in human behavior, animal or sewage contamination of water, changes in animal reservoir density, or follow natural disasters such as cyclones and floods.

In the South-East Asia Region (SEARO), most of the countries have reported varying numbers of leptospirosis cases depending on their surveillance system. Human cases have been reported mostly from India, Indonesia, Thailand and Sri Lanka during the rainy season. Major outbreaks in SEARO were reported in the past in Jakarta (2003), Mumbai (2005) and Sri Lanka (2008). Seasonal outbreaks are reported in northern Thailand and in Gujarat, India following heavy rainfall and flooding. A few cases have been reported from Maldives. As per the latest available reports, incidences range from approximately 0.1–10 per 100 000 per year globally. During outbreaks and in high-exposure risk groups, disease incidence may reach over 50 per 100 000.

2.3. Leptospirosis in Bhutan

Leptospirosis remains a sporadically reported zoonotic disease in Bhutan, with cases detected through the AUFI surveillance system. According to data from the RCDC, Bhutan reported 76, 97, 57, and 63 cases in 2018, 2019, 2023, and 2024 respectively.

A 2021 seroprevalence study conducted in southern Bhutan revealed leptospiral antibodies in both humans and livestock, particularly among farmers and animal handlers, indicating significant exposure risks and likely underdiagnosed community transmission. The study also highlighted poor awareness about leptospirosis and limited diagnostic capacity at the peripheral health centers as contributing factors to underreporting.

In 2025, a leptospirosis risk mapping exercise was conducted using environmental, climatic, and population exposure data. The findings identified southern districts especially areas with frequent flooding, poor sanitation, and high livestock density as high-risk zones. These insights have guided

targeted public health interventions, strengthened surveillance, and intersectoral collaboration for future outbreak preparedness and response.

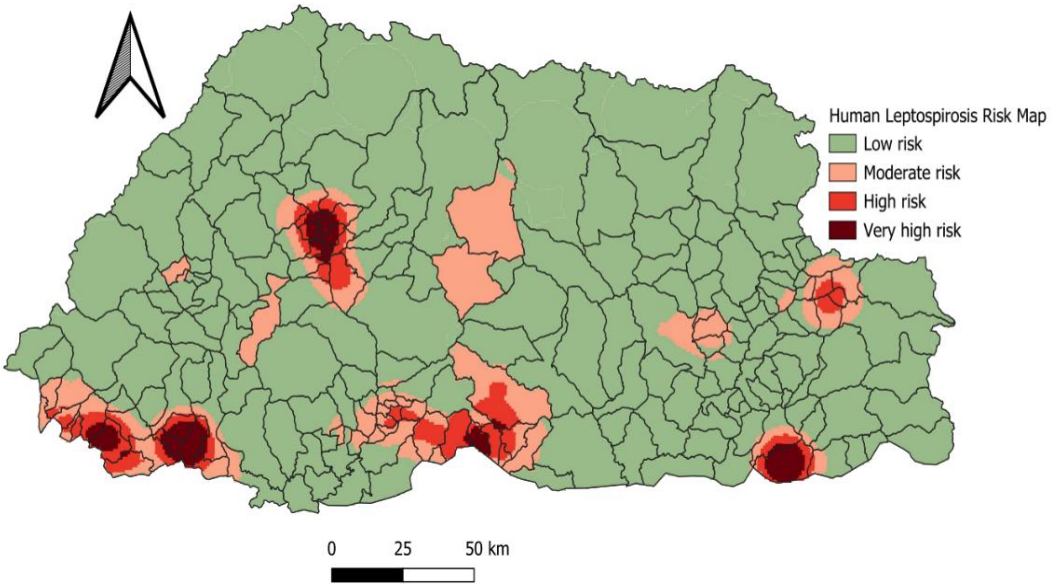


Fig 1. Map showing risk of leptospirosis in the country

2.4. Risk factors

- Occupational exposure: Farmers, ranchers, slaughter house workers, livestock handlers, animal caretakers, veterinarians, pet traders, loggers, sewer workers, landscapers, pet traders, military personnel, laboratory workers, Outdoor recreational participants Hiking, trail biking, or camping near potentially contaminated streams, rivers, or mud.
- Recreational activities: Freshwater swimming, canoeing, kayaking, trail biking, trekking, gardening.
- Household exposure: Pet dogs, domesticated livestock, rainwater catchment systems, infestation by infected rodents
- Low socioeconomic status: Living in overcrowded urban areas with poor sanitation
- Environmental risk- Seasonal Flooding, Warmer and humid climates, travel to endemic areas

2.5. Causative Agent

It is caused by Leptospira, spiral-shaped motile gram-negative bacteria.

There are 10 pathogenic species, and more than 250 pathogenic serovars. Disease severity can vary depending on the infecting serovar, making it important to identify the specific strains involved in outbreaks. Identifying serovars also supports epidemiological tracking by helping to determine

transmission routes and animal reservoirs. Additionally, vaccines are often developed to target the most common or regionally relevant serovars, enhancing their effectiveness in preventing disease.

2.7. Animal reservoirs

About 160 mammalian species serve as natural carriers of pathogenic *Leptospira*. Bacteria reside in the renal tubules and are shed in the urine of infected animals.

Rodents are the most important reservoirs for transmission. They are the first recognized carriers of leptospirosis and the only major animal species that can shed Leptospira throughout their lifespan without clinical manifestations, i.e., prolonged carrier state.

Other mammals, including cattle, pigs, dogs, horses, sheep, and goats, cats are rarely affected. Animals may be asymptomatic carriers or may develop clinical illness.

2.8. Mode of transmission

Leptospira are spread by the urine of infected animals (rodents, dogs, livestock, pigs, horses, wildlife. The bacteria can survive for weeks to months in urine-contaminated water and soil.

- People can be infected through:
    - » Direct contact with the urine, blood and tissues from infected animals.
    - » Contact with urine-contaminated water (floodwater, rivers, streams, sewage) and wet soil.
    - » Ingestion of food or water contaminated by urine or urine-contaminated water.

Transmission occurs through mucous membranes, conjunctiva, and skin cuts or abrasions. Human-to-human transmission is very rare but has been documented through sexual intercourse and breastfeeding. Transmission has also rarely occurred through animal bites.

2.9. Incubation Period

The average incubation period is 5–14 days, with a range of 2–30 days.

2.10. Clinical features

Most cases are mild and self-limited or asymptomatic. Approximately 10% progress to severe, potentially fatal illness with multi-organ dysfunction. Leptospirosis is typically classified into two forms: Anicteric and Icteric. While complications can occur in both forms, they are more commonly associated with the icteric form.

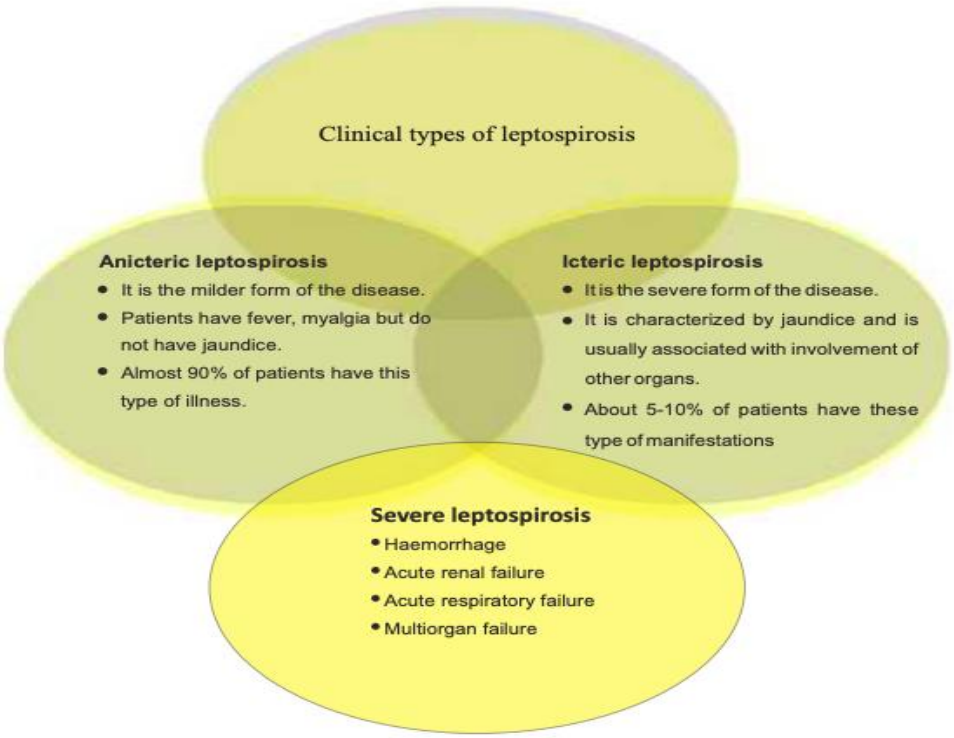
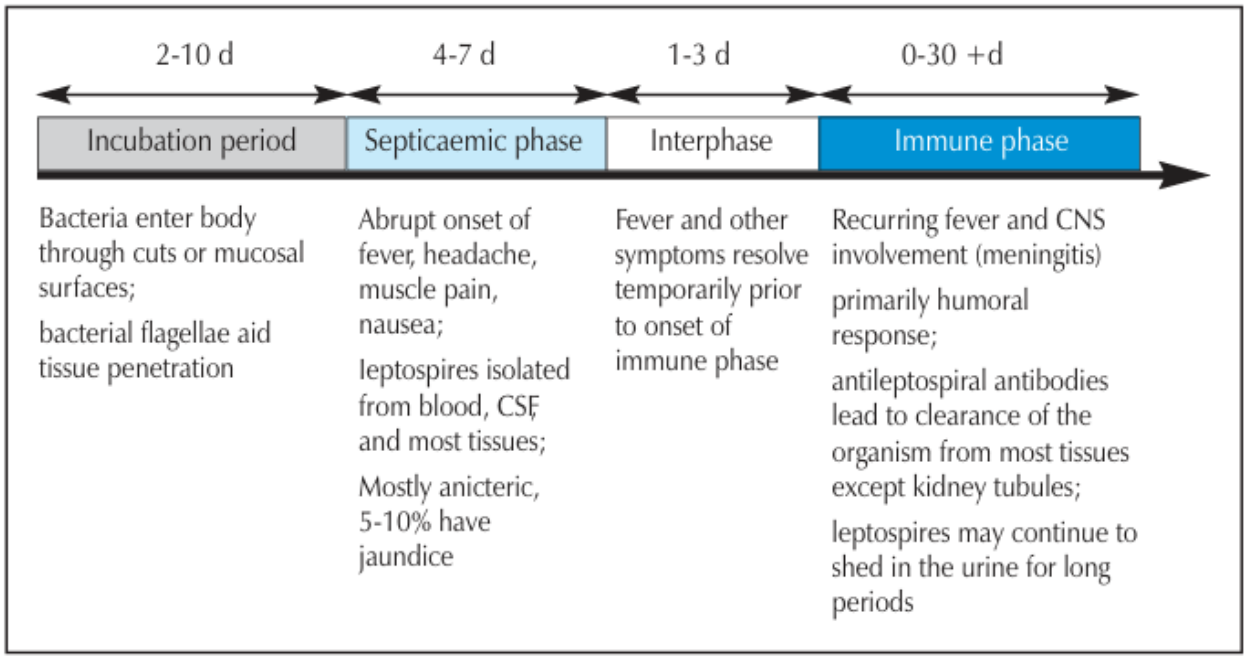


Figure 1. Showing clinical Spectrum of Leptospirosis

2.10.1. Anicteric leptospirosis

This form of the disease occurs in the majority of patients (90%), most cases are mild and self-limited and is typically described as a biphasic illness, comprising an acute phase and a subsequent immune phase. However, in most cases, patients do not progress to the immune phase, and in some instances, the clinical features of both phases may overlap. In those who develop the immune phase there is often a period of improvement between the end of the acute phase and the beginning of the immune phase.

Figure 1: Typical course of leptospirosis



	Symptoms	**Examination**	**Investigation**
Acute phase	-Abrupt onset fever, associated with rigors, myalgias (especially in the calves and lower back) and headache -50% of cases can have nausea, vomiting and diarrhea -25% to 35% cases can have nonproductive cough. -Rarely, rapidly progressive pulmonary hemorrhage can occur.	-Conjunctival suffusion(common) -Subconjunctival hemorrhage may occur with or without conjunctival suffusion. -Muscle tenderness and muscle rigidity -splenomegaly, hepatomegaly -lymphadenopathy -pharyngitis	-Neutrophilia -Thrombocytopenia -Anemia can also be seen, but are less common. -Pancytopenia -Mild elevation of liver aminotransferases -Elevated ESR, CRP and creatine kinase may occur. -Routine urine examination frequently shows proteinuria, pyuria, granular casts, and occasionally microscopic

		-skin rashes (non-puritic)	hematuria, however acute kidney injury rarely occurs.
Immune phase	**Systemic symptoms**: • Recurrence of fever, headache, and myalgia. • Nausea, vomiting, and abdominal pain may also occur. **Aseptic meningitis**: • Hallmark of the immune phase. • Symptoms: headache not responding to analgesics, neck pain or stiffness. • Occurs in ~50% of patients. • Symptoms usually resolve within 1–2 days, rarely persisting up to 3 weeks. **CSF findings**: • Neutrophilic or lymphocytic pleocytosis. • Mildly elevated protein. • Normal glucose. • Pleocytosis may persist up to 3 months. **Uveitis**: • Most common form: **anterior uveitis** (iritis, iridocyclitis); can be unilateral or bilateral. • Less common: **posterior uveitis**, presenting as painless visual disturbances (e.g., floaters, reduced visual acuity).		

Table 1 showing Acute vs. Immune Phase – Symptoms & Signs

2.10.2. Icteric leptospirosis (Weil's disease)

Icteric leptospirosis also known as Weil's disease occurs in approximately 5 to 10 percent of symptomatic leptospirosis cases and is a rapidly progressive multisystem illness associated with mortality rates of 5 to 15 percent. The 2 phases of illness are often continuous and indistinguishable in this form and the classic triad of Weil's disease consists of bleeding manifestation, profound jaundice and renal failure.

Hepatic findings:

-Conjugated hyperbilirubinemia.

-Normal or mildly elevated aminotransferases.

-Serum bilirubin may reach 60–80 mg/dL.

-Liver failure is rare.

Renal findings:

-Acute kidney injury (oliguric or non-oliguric).

-Electrolyte abnormalities: hypokalemia &hyponatremia.

-Renal recovery is typical.

2.10.3. Complication of leptospirosis

Complication can be seen in both forms of leptospirosis but more commonly with icterus form. Complications are:

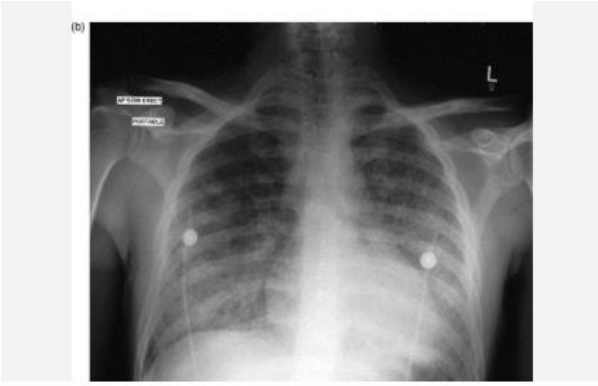
1. Pulmonary hemorrhage with ARDS
2. Myocarditis
3. Rhabdomyolysis
4. Acalculous cholecystitis
5. Pancreatitis

2.10.4. Pulmonary Complications of Leptospirosis

Pulmonary involvement is a significant prognostic factor in leptospirosis, with a high case **fatality rate (50%–70%)**. The incidence of pulmonary involvement varies, but ranges from 20-70% and depends upon the frequencies of exposure to pathogenic leptospirosis, differing pathogenicity of serovars present in the environments, focal emergence of pulmonary tropism, or varying levels of infecting Leptospira in environmental water sources of infections. Leptospira interrogens bataviae is the most common serotype seen in patients with pulmonary involvement. It primarily manifests as hemorrhagic pneumonitis and in advanced cases, adult respiratory distress syndrome, diffuse alveolar hemorrhage (DAH).

The syndrome of diffuse alveolar hemorrhage (DAH) consists of hemoptysis, anemia, bilateral peripheral patchy infiltrates with a "snowflake" appearance on chest radiographs, and a decreased hematocrit secondary to bleeding from the pulmonary microvasculature into the alveolar space.

High-resolution computed tomography (HRCT) is superior to chest radiography for the demonstration of subtle features, such as ground-glass opacities, and the extent of abnormalities.



Chest X-ray (CXR) image showing **Diffuse Alveolar Hemorrhage** in a patient with **Leptospirosis**:

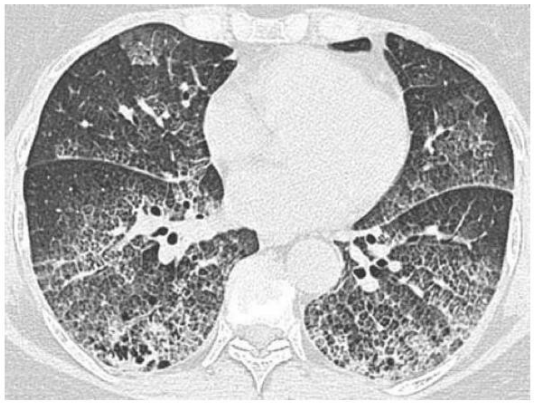


FIGURE 6. A 44-year-old man with leptospirosis. High-resolution CT at the level of the lower lobes demonstrates bilateral areas of ground-glass attenuation with superimposed interlobular septal thickening (crazy-paving pattern).

1.What are the indicators of pulmonary involvement?

Pulmonary symptoms usually appear between the 4th and 6th day of disease. Tachypnea (Respiratory Rate > 30/min) is the first sign of pulmonary involvement in most cases. One should consider lung involvement with the onset of cough, hemoptysis or dyspnea. Some patients may present with pleuritic chest pain. Pulmonary examination may be normal or presence of crackles at the lung bases maybe noted in some cases.

1. What are the predictors for the development of pulmonary complications in leptospirosis?

Significant risk factors for pulmonary complications are delayed antibiotic treatment, thrombocytopenia at the onset of the disease and serum creatinine > 2mg/dl and bilirubin > 2mg/dl.

3. What are risk factors associated with of pulmonary involvement

Cigarette smoking increases the risk of pulmonary involvement by promoting lung capillary permeability, alveolar damage, and inflammation. Pulmonary complications are also linked to hypotension and renal failure, and are associated with high mortality. Independent mortality predictors include hemodynamic instability, serum creatinine > 265.2 μmol/L, and serum potassium > 4.0 mmol/L. Early identification of these factors is recommended in severe respiratory failure cases.

2.3.2. Myocarditis: Patients present with one or more of the following features:

- Shock: Patients develop severe hypotension, cold clammy extremities, and tachycardia. Echocardiography reveals normal systolic function of the left ventricle hence hypotension is due to either dehydration or peripheral vasodilatation.
- Arrhythmias: Patient presents with palpitations, syncope and irregular pulse. Common arrhythmias seen are supraventricular tachyarrhythmia and various degrees of A.V. blocks. Ventricular tachy-arrhythmias are infrequent. Segment depression and T wave inversion may be present in some patients

2.10.5. Differential Diagnosis

- Scrub typhus
- Murine typhus
- Typhoid and paratyphoid fever
- Hantavirus
- Acute viral hepatitis, especially hepatitis A
- Ehrlichiosis
- Acute viral illness
- TTP/HUS
- Vasculitic pulmonary-renal syndrome

2.11. Laboratory Diagnosis of Leptospirosis

2.11.1. Case Definition as per WHO Guidelines

Suspected case:

- evocative epidemiological context; and
- clinical signs and symptoms consistent with leptospirosis: abrupt onset of fever, chills, conjunctival suffusion, headache, myalgia, jaundice, cardiac or renal failure, and pulmonary hemorrhage.

Probable case:

- suspected case and the presence of *Leptospira* immunoglobulins type M (IgM) in one serum sample detected by serology (e.g., Immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA)).

Confirmed case:

- a suspected case confirmed by laboratory test as follows:
    - seroconversion or a four-fold or higher rise in titre detected by serological techniques (e.g., microscope agglutination technique (MAT) or IgM ELISA) in consecutive serum samples; **or**
    - detection of *Leptospira* DNA from a clinical specimen by polymerase chain reaction (PCR); **or**
    - demonstration of *Leptospira spp.* in tissue.

2.11.2. Sample collection for leptospirosis

1. **Blood**

   While collecting blood and separating serum properly, procedures should be followed to avoid lysis or contamination.
   - Collect 5 ml blood
   - Transfer to 3 ml to the clot activator tube and 2 ml into EDTA tube.
   - For clot activator, centrifuge at 3500 rpm for 5 min to collect serum sample.
   - Transfer the serum to sterile cryovial.

2. **CSF**
   - CSF should be collected in a sterile container by lumbar puncture under aseptic conditions before the institution of antibiotics.
   - Preferably CSF should be collected in sterile container
   - CSF should be transported immediately to the laboratory without delay.

3. **Urine**
   - Urine should be collected in sterile wide-mouth container
   - Carefully clean the peri-urethral area with soap and plenty of water.
   - Discard the first voided sample and subsequent midstream urine is collected in a sterile wide-mouth container.
   - Transport the sample immediately to avoid multiplication of contaminants

Note: Each sample should be properly labeled mentioning name, date of collection, and accompanied with a duly filled case investigation form (CIF) with relevant clinical details should be included.

2.11.3. Laboratory diagnostic test

Diagnosis of Leptospirosis can be done either by direct and indirect method depending on the laboratory test facilities available in the different field settings. Direct method includes detection of leptospira pathogens using microscope, culture and PCR techniques. On the other hand, indirect methods include detection of leptospira specific antibodies with use of techniques such as rapid test, microscopic agglutination test and ELISA.

2.11.4. Laboratory diagnostic investigations to be carried out at different health facilities

2.5.3.1. At Primary Healthcare centers (PHCs)

- No lab facilities equipped for the diagnosis.

2.5.3.2. At Districts laboratories (on few selected hospitals based on disease burden)

- Immuno-chromatographic test (RDTs)
- Shipment of sample to RCDC for serological and molecular assay for further investigation

2.3.5.3. At Regional & National laboratories

- Immuno-chromatographic test (RDTs)
- Shipment of sample to RCDC for serological and molecular assay for further investigation

2.3.5.4. At Reference laboratory (Royal Centre for Disease Control)

- Immuno-chromatographic test (RDTs)
- ELISA
- MAT
- PCR

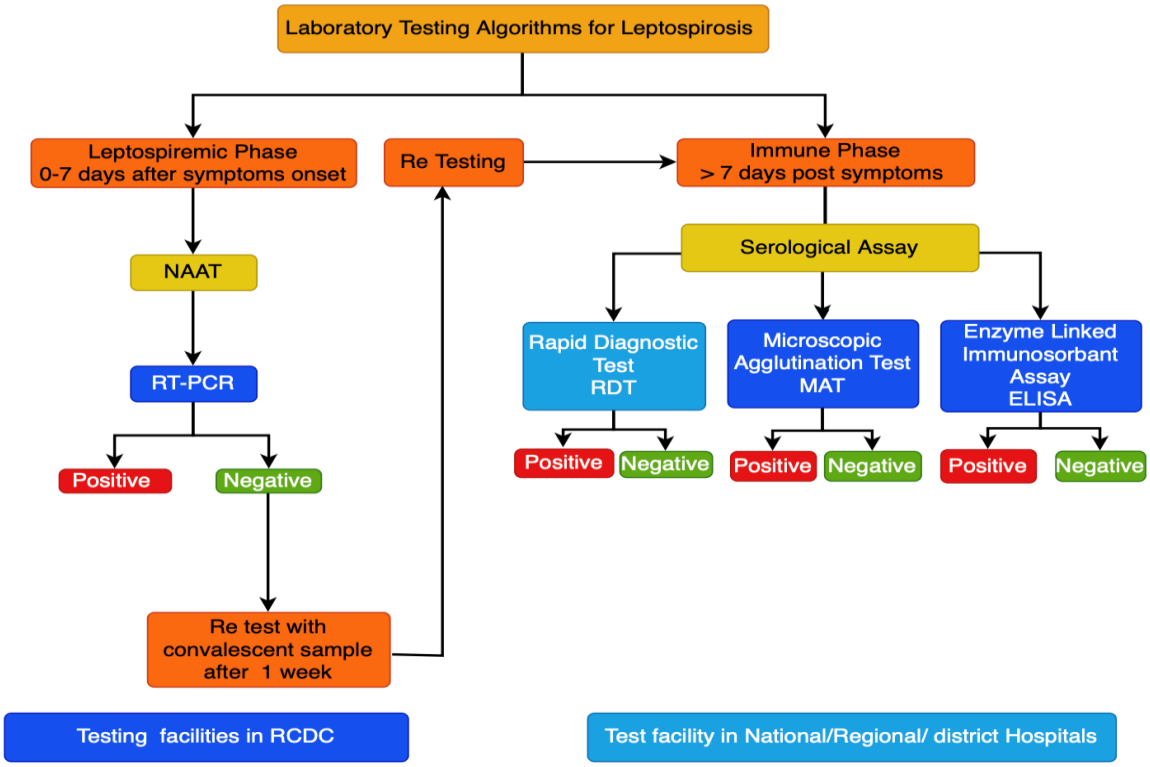


Fig.1. Laboratory testing algorithm for leptospirosis

2.12. Laboratory diagnosis

2.12.1. Isolation of Leptospira

Culture: Isolation of Leptospira in blood

At the acute phase of leptospirosis, blood is the choice of sample for the diagnosis. Leptospira in the blood can be detected either using Blood culture or PCR techniques.

For Blood culture, applicable during the acute phase of infection. few 2-3 drop of whole blood is directly added into culture medium and is incubated at 37*C. Isolation of Leptospira using selective culture media and direct examination under dark field microscope but may require prolonged incubation (up to several days to weeks) therefore, culture does not contribute to rapid diagnosis in the early phase of disease.

For urine culture is done on the immune phase of the infection. Add a few drops of urine sample directly into the culture media and incubate for 6 to 8 weeks and directly examine under a dark field microscope.

NOTE:
Culture remains GOLD standard BUT is time consuming, labor-intensive, requires 6-8 weeks incubation.

Advantages of Culture;

- Culture can be performed during acute phase from blood sample as well during the immune phase from the urine sample for isolation of Leptospiral
- It is the gold standard method for definitive diagnosis.
- Provides information on locally circulating lepto serovar.

Disadvantages of culture;

- Blood culture more than 10 days after disease onset is not worthwhile as Leptospira has mostly disappeared from the blood.
- Urine culture should be cultured into appropriate medium not more than 2 hours after voiding. Leptospira die quickly in acid urine environment
- Culture methods are tedious, complicated, technically demanding, and time consuming.
- Require prolonged incubation period

2.12.2. Serology

Rapid diagnostic Test (RDTs)
Leptospira IgM/IgG combo rapid test detects IgM and IgG antibodies against antigen of Leptospira species. The sensitivity rates are between 63% -72% and specificity rates between 93% - 96% when tested in illnesses of less than 7 days. If the serum is taken beyond 7 days, sensitivity improves to > 90%. Therefore, false negative results can be a problem if the tests are performed during the early stage of the illness. A second sample should be obtained for suspected cases with initial negative or doubtful results.

Advantages of RDTs

- Can be used as point of care test to be performed under field setting.
- Simple to perform, non- laboratory health personnel can perform the test with adequate PPEs.
- Provide results within 15 - 30 minutes.

Disadvantages of RDTs

- Cross reactivity with other diseases providing false positive results.
- Issue with sensitivity and specificity of the test kit. Need other diagnostic assays for confirmatory

2.12.3. Enzyme Linked Immunosorbent assay (ELISA)

ELISA is one of the techniques commonly used for the diagnosis. The test can detect specific antibodies earlier than MAT. The advantage of the test is that it can differentiate between recent and past infection by detecting the type of antibodies (IgM or IgG) present in the clinical specimen. In this test, broadly, reactive antigen is used. The antigen antibody reaction is visualized or measured by ELISA reader using a conjugate (enzyme conjugated to anti-IgM or IgG) and a color reagent.

IgM antibodies can be detected from day 6 of illness. However, these antibodies can persist for 2–3 months and may reflect either an acute or recent infection. To confirm an acute infection, testing of convalescent samples is recommended.

Advantages of ELISA;

- Commonly used assay performed where PCR and MAT are not available

Disadvantages of ELISA;

- Cross reactivity with other diseases providing false positive results.
- Issue with sensitivity and specificity of the test kit. Need other diagnostic assays for confirmatory

2.12.4. Microscopic Agglutination Test (MAT)

MAT is the gold standard test for detection of serovar/ serogroup specific antibodies. One of the critical issues of MAT is the cut off or a significant titer for diagnosis, when the test is done on a single sample. A battery of antigens covering the range of serovars that are expected or likely to be circulating in a particular geographical area, where the patient becomes infected, should be used.

Criteria for definite diagnosis of acute leptospirosis infection in diagnostic setting:

- A Cut off MAT titre of 1:400 or more in a single serum sample.
- Four - fold or greater rise in MAT titre between acute and convalescent samples.
- Seroconversion -where the first serum sample shows no detectable antibody titre, and the second sample is positive (above the diagnostic cut-off).

Criteria for definite diagnosis of leptospirosis infection in surveillance setting:

- A Cut off MAT titre of 1:80 or more in a single serum sample considered as positive to enhance the surveillance.

Advantages of MAT

- Leptospira serovar can be determined in the clinical specimens
- Provides information on locally circulating lepto serovar.

Disadvantages of MAT;

- Maintenance of live antigen challenges-
- Required trained laboratory personnel to perform the assays

2.12.5. Polymerase Chain Reaction (PCR)

PCR method involves an amplification of genus-specific target DNA sequence, if present, in clinical samples. A pair of short DNA fragments, known as primers, is used for specific amplification of DNA fragments from the pathogen in blood, urine or CSF. Positive diagnosis results from the amplification of the target sequence whereas negative samples fail to produce amplified DNA in PCR. PCR can be used to detect leptospiral infection especially during the first few days of the disease when antibodies are not fully detectable in serological tests.

1. **PCR from blood sample:**
   Leptospiral target DNA from blood sample is amplified and detected during acute phase of infection
2. **PCR from urine sample:**
   Leptospiral target DNA from urine sample is amplified and detected during immune phase of infection

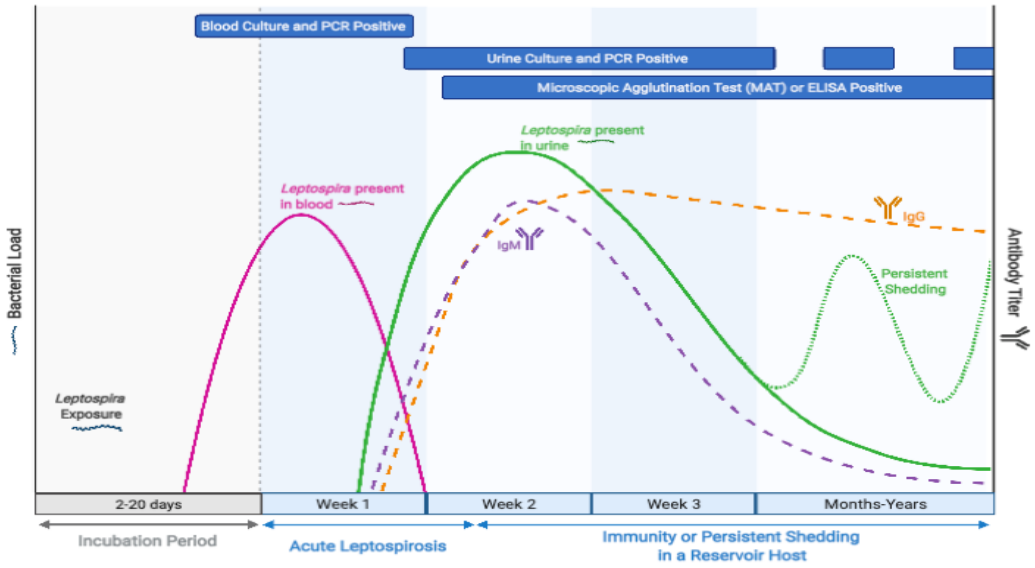
Advantages of PCR;

- Widely used method for diagnostic assay due to highly sensitivity and specificity.

Disadvantages of PCR;

- Do not provide information on leptospira serovar
- Expensive assays- cost of reagents and primers
- Required trained laboratory personnel to perform the assays

This graph illustrates the timeline of infection progression, bacterial presence, immune response, and diagnostic test utility in leptospirosis:



2.12.6. Other Routine Investigation

- CBC, ESR, CRP
- RFT, LFT, PT/INR
- Chest X-ray
- Sputum CS and GeneXpert
- ABG if necessary/available
- CT Chest – if indicated

2.13. Management of Leptospirosis

- Treatment should be initiated immediately with the onset of symptoms in order to reduce the risk of progression to disease.
- Treatment depends on severity of the cases - Mild and severe disease.

2.13.1. Case severity classification:

1. **Mild disease:** Presenting only with flu like symptoms without organ dysfunction
2. **Severe disease:** Presenting with organ dysfunction, shock, myocarditis, bleeding manifestation, hemoptysis, ARDS, hypoxia or imaging findings showing bilateral lung infiltrates

Treatment of Mild disease	**Adults:** • Oral Doxycycline 100 mg BD for 7 days OR • Oral Azithromycin 500 mg OD for 3 days. **Children:** • Oral Doxycycline 2mg/kg/day in two divided dose (BD) for 7 days OR • Oral Azithromycin 10mg/kg on day one and 5mg/kg on subsequent days (total 3 days) **Pregnancy:** • Oral Azithromycin 500mg OD for 3 days NOTE: **Doxycycline can be given to a child under 8 years if the duration of treatment is less than 21 days.**
Treatment of Severe disease (Duration of therapy is seven days)	**Adults:** • IV Penicillin G 1.5 million units IV q 6 hourly for 7 days (DOC) OR • IV doxycycline 100 mg BD for 7 days OR • IV ceftriaxone 2gm OD for 7 days **Children:** • IV penicillin 250,000 units/kg q 6 hourly for 7 days OR • IV Doxycycline 4mg/kg/day in two divided dose (BD) for 7 days OR • IV ceftriaxone 100mg/kg OD for 7 days **Pregnancy:** • IV Penicillin OR Ceftriaxone • **Supportive care -** In the setting of severe illness, supportive care with renal replacement therapy, ventilatory support, and blood products may be required • Refer to higher centers for further management

	NOTE: **Doxycycline can be given to a child under 8 years if the duration of treatment is less than 21 days** **Role of corticosteroids and plasmapheresis** limited evidence and recommendations but some studies suggested potential benefits of early methylprednisolone administration for severe leptospirosis patients with pulmonary complications. Therapeutic plasma exchange (TPE) shows promise as a rescue therapy in severe, treatment-resistant leptospirosis, helping reduce bilirubin and improve outcomes. Clear initiation criteria are lacking, and further research is needed to guide its optimal use

2.14. Chemoprophylaxis

Chemoprophylaxis may be considered for high-risk individuals exposed to leptospirosis during peak transmission seasons, particularly in endemic areas. Doxycycline is the drug of choice for chemoprophylaxis. Prophylaxis is typically reserved for short-term use during periods of high-risk exposure, such as for military personnel, disaster relief workers. It is recommended only for adults and is not advised during pregnancy or for children under the age of 8.

Additionally, doxycycline offers concurrent protection against Rickettsial diseases and malaria in areas with chloroquine-sensitive Plasmodium falciparum or P. vivax. However, the routine use of doxycycline for prophylaxis should be carefully weighed against the risk of antimicrobial resistance and individual health factors.

Regimen - Doxycycline 200 mg orally once weekly.

To be started 1-2 days before the exposure and to continue till the end of high-risk exposure.

2.15. Surveillance

2.15.1. Surveillance in humans

- Design risk-based passive (hospital-based surveillance) or community-based active surveillance to estimate the incidence or burden of leptospirosis in a community.
- Use indicator-based surveillance to report probable or confirmed cases of leptospirosis in high-risk populations such as farmers, slaughterhouse workers, sewage and sanitation workers, disaster relief workers.

- Use hospital-based testing of probable cases of leptospirosis for occupational exposure in hotspot areas during the high-risk seasons.
- Use community-based surveillance during flooding or outbreaks in high-risk occupational groups.
- Screening for anti-Leptospira antibodies in blood donors may indicate the prevalence of leptospirosis.
- Testing all probable cases for most prevalent serovars using appropriate laboratory diagnostic tests and confirmation using MAT/PCR.

2.15.3. Surveillance in animals

Risk-based surveillance of domestic and feral animals may be necessary to complement surveillance of human populations. Screen animals like livestock (cattle, pigs, etc), dogs, and rodents based on the serovars identified in humans to identify major species of animals responsible for human exposure.

2.16. Reporting

- Use RCDC's event-based or indicator-based surveillance reporting system.

2.17. Prevention and control

Because of the large number of serovars and infection sources and the wide differences in transmission conditions, the control of leptospirosis is complicated and will depend on the local conditions. Control can be achieved by controlling the reservoir or reducing infection in animal reservoir populations such as rodents, dogs or livestock. Control of wild animals may be difficult. Preventive measures must be based on a knowledge of the groups at particular risk of infection and the local epidemiological factors. Prevention and control should be targeted at:

1) The infection source;
2) The route of transmission between the infection source and the human host; or
3) Infection or disease in the human host

2.17.1. Control of infection source

It is important to establish what animal species are the infection sources in a particular area and targeted to the local reservoir species of animals. Such measures include:

- Reduction of certain animal reservoir populations, e.g., rats;
- Separation of animal reservoirs from human habitations by means of fences and screens;
- Immunization of dogs and livestock;
- Removal of rubbish and keeping areas around human habitations clean;

- Encouraging people not to leave food around, especially in recreational areas where rats may be present.

2.17.2. Individual level

If you take part in water recreation activities like swimming, boating, fishing, and adventure racing, some tips to avoid leptospirosis include:

- Research the location you'll be in the water for possible leptospirosis infections for that area.
- Cover scrapes and wounds with waterproof bandaging and wear shoes if leptospirosis or other diseases are known in the area.

If you may be exposed as a result of your job (veterinarians, veterinary staff, raising farm animals, dairy workers, animal control, butcher or slaughterhouse workers, sewage and sanitation workers, military and first responders):

- Wash hands frequently
- Use personal protective equipment (gloves, footwear, eye protection)
- Clean and disinfect surfaces and equipment
- Vaccinate animals against leptospirosis, and isolate sick animals

2.17.3. Community level

- Improving living conditions – housing condition to reduce flooding risk and contact with contaminated water, ensure proper drainage to avoid water logging, clean drinking water supply, adequate waste control as Leptospira bacteria thrive in water or soil contaminated with the urine of infected animals
- Control the rodent population around work area
- Provide communities with treated water during flooding
- Conduct awareness to avoid contact with rodents and rodent urine and contaminated water
- Vaccinate main livestock maintenance hosts like dairy cattle (but not relevant to Bhutan as no particular animal source of exposure has been identified yet).
- Human vaccine is not available

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3.Brucellosis

3.1. Introduction

Brucellosis is one of the most widespread zoonoses transmitted by animals and in endemic areas, human brucellosis has serious public health consequences. Expansion of animal industries and urbanization, and the lack of hygienic measures in animal husbandry and in food handling, partly account for brucellosis remaining a public health hazard. Worldwide, *Brucella melitensis* is the most prevalent species causing human brucellosis, owing in part to difficulties in immunizing free-ranging goats and sheep.

Human-to-human transmission is very rare. Human brucellosis is related to rural poverty and inadequate access to medical care. Failure of veterinary control programs due to conflicts or economic reasons contribute further to emergence and re-emergence of this disease.

3.2. Epidemiology

The true global prevalence of brucellosis is unknown. However, the recent studies suggest that at least 1.6-2.1 million new cases of human brucellosis likely occur annually. There has also been a significant change in human brucellosis over the past decades as compared to its status in 2006. The territorial distribution of human brucellosis has expanded noticeably from 53 countries to at least 97 countries including 18 additional countries in Europe, 12 in the Americas, 9 in Asia and 4 in Africa. The highest incidence of the disease has been reported in the Eastern Mediterranean region including Syria, Turkey, Iraq, Saudi Arabia, Oman and Algeria while some Asian countries have also observed significant rise in the number of brucellosis cases. Among the continents, Asia has the highest human brucellosis burden especially in Eastern, Central and Western Asian countries posing a serious public health problem. There has been increased recognition of brucellosis in India, Pakistan, Sri Lanka, China and importations to countries in Oceania such as Fiji and in Asia such as Thailand and Vietnam. However, the disease burden of human brucellosis in South-east Asia remains relatively low.

In Bhutan, though the actual prevalence of human brucellosis is not known, brucellosis in animals, mainly cattle, was detected through a series of surveys conducted by the National Centre for Animal Health, Ministry of Agriculture and Livestock. The sero-prevalence conducted in 2017 found the prevalence rate at 2% (21/1099). More cases were detected in the succeeding years in 2018 (11 cases) and 2019 (50 cases). Sporadic human cases have also been detected through Acute Undifferentiated Febrile Illness (AUFI) surveillance by Royal Centre for Disease Control.

3.3. Causative agent

Five of the six currently recognized *Brucella* species cause infection and clinical signs in one or more animal hosts. Four of these also cause human disease:

1.Brucella melitensis: It is the most virulent species in humans and the most common cause of human brucellosis worldwide. Endemic in South Asia, Middle east and Mediterranean.

2.Brucella abortus: Causes human brucellosis which is less severe than B. melitensis

3.Brucella suis: Causes chronic and localized infections in humans. Common in pig farming

4.Brucella canis: Causes widespread infection of dogs in many countries. It is infrequently associated with human disease. Reported cases have usually been mild.

Species	Animal Host	Virulence
Brucella melitensis	Goats, sheep, camels	++++
Brucella abortus	Cows, other Bovidae animals and camels	+++
Brucella canis	Dogs	+
Brucella suis	Pigs	+

Table 1. showing common Brucella species and their level of virulence in human

3.4. Risk factors

- Occupations at risk are: Farmers, veterinarians, slaughterhouse workers, butchers, laboratory workers, wild life reservoir workers and animal breeders or animal shelter staff
- Individuals at risk are; those with weakened immune system, travelling to endemic areas if they consume local dairy products or have contact with infected animals, accidental injection of brucella vaccine

3.5. Mode of transmission

It primarily transmits from animals to humans through several routes, with rare instances of human-to-human transmission.

Humans acquire infection through the following routes:

1. Direct contact of breaks on the skin or mucous membranes with infected animal tissue (such as placenta or miscarriage products) or infected animal fluids (such as blood, urine, or milk)

2.Ingestion of unpasteurized dairy products (milk, soft cheese, butter, cream) and undercooked or raw meat.

- In soft cheeses, brucella can persist for 38 days to over 180 days, In Cheddar cheese, for a year and for over a month in ice cream stored below freezing temperature

- Hard cheeses, sour milk, and yogurt are less likely to transmit the disease because fermentation inhibits the survival of the causative organisms)

3. Inhalation of aerosols bacteria in slaughter houses and laboratory

4.Human to human transmission (Rare): Vertical transmission, sexual transmission, breast milk transmission, blood transfusion, organ donation has been occasionally reported as potential routes of infection.

3.6. Incubation period

The incubation period typically ranges from 2 to 4 weeks but can vary from as early as 5 days to as long as several months.

3.7. Clinical Features

Brucellosis manifests as a systemic infection encompassing a wide clinical spectrum, ranging from asymptomatic cases to severe and potentially fatal illnesses. The symptoms of brucellosis are predominantly nonspecific, with the primary clinical presentation being an acute febrile illness, with or without indications of localization (focal complication). In many cases, the initial presentation is classified as a fever of unknown origin.

The classic general symptoms comprise:	**Physical findings:**
• Headache • Myalgia or bone pain • Anorexia and weight loss • Profuse sweating • Peculiar moldy odor • Depression or mood disorders • Fever • Arthralgia or arthritis	• Fever • Relative bradycardia • Arthritis • Hepatomegaly • Splenomegaly • Meningismus • Miscellaneous findings like skin rashes, cervical lymph node enlargement, drowsiness, periorbital swelling, and ataxia.

3.7.1. Clinical Signs of Brucellosis in Animals

Animal species	Clinical signs
cattle	- Abortion, premature birth, retained placenta - Hygromas, abscesses (esp. in Africa, *B. abortus* biovar 3) - Reduced milk production - Temporary infertility (usually abort once) – Persistent udder infection → frequent shedding in milk
Sheep and Goat	- Abortion - Orchitis, epididymitis (*B. melitensis*, *B. ovis*) - Occasional arthritis (*B. melitensis*)
Pigs	-Reproductive complications due to *B. suis*
Dogs	-Reproductive complications due to *B. Canis*
Horses	- Often asymptomatic - Localized bursae abscesses
Camels	-- Rare clinical signs - Shed *B. melitensis* in milk → major public health risk in some regions

3.8. Classification

Brucellosis can be classified based on the duration of illness into **acute**, **subacute**, or **chronic forms**:

- **Acute brucellosis**: Symptoms present for **less than 2 months**.
- **Subacute brucellosis**: Symptoms last for **2 to 12 months**.
- **Chronic brucellosis**: Symptoms persist for **more than 12 months**, often with relapsing or lingering manifestations.

3.8.1. Acute Brucellosis

- It typically begins with acute onset fever, which may be spiking, relapsing, or undulating in nature, and is often accompanied by chills, rigor and arthralgia
- Constitutional symptoms include night sweats with moldy odor, loss of appetite, generalized weakness, fatigue, malaise, and unintentional weight loss.

- Less frequently, patients may present with dyspepsia, mouth ulcerations, jaundice, and abdominal pain. Other reported manifestations include coughing, dyspnea, epistaxis, hemoptysis, lymphadenopathy, testicular pain, and scrotal swelling.

3.8.2. Chronic brucellosis

The term "chronic brucellosis" should be reserved for patients whose clinical symptoms persist for 12 months or more from the time of the diagnosis. Using this criterion, patients fall into three categories:

(1) relapse:

- Relapse is defined as the recurrence of characteristic signs and symptoms occurring at some time after the completion of a course of treatment
- Patients with relapse characteristically have objective signs of infection, such as fever, and persistently elevated titres of IgG antibodies in their serum (with or without a positive culture)
- The rate of relapse following treatment is 5% to 15% and it usually occurs within the first 6 months following completion of treatment but may occur up to 12 months later.
- Causes include an inadequate duration of antibiotic therapy and inadequate antibiotic regimen
- It can usually be treated by repeating the course of therapy with the same drugs.

(2) chronic localized infection:

- Defined as recurrence of characteristic signs and symptoms, with or without a positive blood culture.
- Caused by failure to eliminate a deep focus of infection (e.g., osteomyelitis, deep tissue abscess).
- Symptoms may recur intermittently over long periods.
- Associated with **persistent elevation of IgG antibodies** in serum.
- often requires **surgical intervention** (e.g., drainage of infected foci) in addition to antimicrobial therapy.

(3) delayed convalescence:

- Persistence of symptoms **without objective signs of infection** (e.g., fever) after completing therapy and its cause is unknown
- **Serology:** Antibody titres have declined or disappeared.
- **Treatment:** Repeated courses of antimicrobial therapy **do not provide benefit**.

3.9. Complications of Brucellosis

Brucellosis infection has the potential to affect one or more focal sites, with the likelihood of focal involvement varying widely. Almost any organ can be impacted by brucellosis, with the probability of focal involvement ranging from 6% to 92%.

These focal complications are more commonly seen in adults than in children. The major risk factor for the development of focal complications is symptom duration greater than 30 days before diagnosis.

The most common focal complications are:

3.9.1. Osteoarticular

- Bones and joints constitute the predominant sites of brucellosis, accounting for up to **80%** of cases.
- Sacroiliitis and spondylitis are more prevalent in adults, spondylitis especially in elderly, may involve paraspinal abscesses.
- In children brucellosis commonly affects large peripheral joints like knees, hips, and ankles. Sacroiliitis is an uncommon manifestation in children, but when it does occur, it is unique to Brucella. Patients typically present with fever and back pain, often radiating down to the legs (sciatica). Children may exhibit reluctance to walk and bear weight on the extremity.
- Other manifestations:
    - Peripheral arthritis (knees, hips, ankles, shoulders; mono-/polyarticular).
    - Osteomyelitis, bursitis, tenosynovitis.

Radiography -Finding involves small erosions, joint space narrowing, and occasional destruction. The spine, especially the lumbar region, is frequently involved in spondylitis, MRI stands out as a superior diagnostic tool, providing a comprehensive view of the extent of destruction and any associated collections.

3.9. 2. Neurobrucellosis

- Neurobrucellosis occurs more frequently in endemic regions and develops in approximately 5% of cases. It can also be the initial presentation of brucellosis.
- Symptoms such as headache, mental inattention, and/or depression are common complaints
- Meningitis, meningoencephalitis, and/or seizures represent the most prevalent manifestations of neurobrucellosis.

Neurobrucellosis can manifest as either acute or chronic

(a)Acute Neurobrucellosis:

- Meningitis, meningoencephalitis, and/or seizures are common manifestations
- Symptoms appear rapidly, usually within <7 days of onset:
- They respond well to early and aggressive antibiotic therapy; residual deficits are rare

(b)Chronic Neurobrucellosis: Develops after more than 3 months of gradually progressive symptoms. Clinical presentation may include:

- Papilledema
- Optic neuritis
- Radiculopathy
- Peripheral neuropathy
- Stroke-like episodes
- Intracranial hemorrhage
- CNS abscess

Cerebrospinal fluid (CSF) findings:

- Lymphocytic pleocytosis, elevated protein and low to normal glucose
- **Serological testing** (e.g., SAT, ELISA) often positive
- **Culture of Brucella** from CSF is definitive but has **low sensitivity (10–15%)** in the early stages of disease.
- **MRI/CT scan** may show: Meningeal enhancement, White matter lesions and Spinal cord involvement (in radiculopathy

3.9.3. Cardiovascular

Cardiovascular involvement in brucellosis is rare but can be life-threatening.

- **Endocarditis**:

  The most serious complication, occurring in 1–2% of cases but causing up to 80% of brucellosis-related deaths. Aortic valve is most commonly affected, followed by the mitral valve. Large, antibiotic-resistant vegetations may form, leading to valvular destruction and often requiring surgical replacement.

- **Pericarditis**: Presents with chest pain and pericardial effusion. It is rare and usually responds well to antibiotics.
- **Myocarditis**: An uncommon complication that can lead to arrhythmias or heart failure. Early treatment is essential.

- **Mycotic Aneurysms**: Rare arterial wall infections that can lead to aneurysm formation and rupture. The abdominal aorta is the most commonly affected segment, though the thoracic aorta and cerebral arteries can also be involved. Surgical intervention is often required.

3.9.4. Gastrointestinal

- Brucellosis, particularly when attributed to B. melitensis, is frequently transmitted through food.
- Systemic symptoms typically take precedence over gastrointestinal symptoms. Some patients may experience nausea, vomiting, and abdominal discomfort.
- Liver is commonly involved and manifest as hepatitis and less commonly hepatic abscess
- Other manifestations include acute cholecystitis. Ileitis, colitis, pancreatitis and spontaneous peritonitis.

3.9.5. Genitourinary

- Genitourinary involvement is the second most common form of focal brucellosis; In males, orchitis and/or epididymitis are the most common presentation; prostatitis and testicular abscess occur less commonly
- In females, tubo-ovarian abscess has been described and is associated with first-trimester abortion.

3.9. 6.Dermatologic manifestation

- Cutaneous lesions occur in approximately 5–10% of brucellosis cases. These skin manifestations are diverse and may result from direct inoculation, hypersensitivity reactions, immune complex deposition, or hematogenous spread of the organism to the skin.
- Findings may include macular, maculopapular, scarlatiniform, papulonodular, urticarial, erythema nodosum-like eruptions, ulcerations, petechiae, purpura, granulomatous vasculitis, and abscesses

3.9.7. Pulmonary

- Pulmonary involvement occurs in up to 2% of cases. Bronchitis, Interstitial pneumonitis, lobar pneumonia, lung nodules, pleural effusion, hilar lymphadenopathy, empyema, or abscesses may be observed

3.9.8. Hematologic

- Usually mild, reversible with treatment.
- Disseminated intravascular coagulation (DIC), hemophagocytic syndrome may be seen

3.9.9. Differential diagnosis

- Tuberculosis, Visceral leishmaniasis
- Infective Endocarditis
- HIV infection
- Enteric fever
- Malaria
- Malignancy
- Spondyloarthritis
- Reactive arthritis
- Autoimmune disease

3.9.10. Routine Investigation

- CBC, ESR, CRP, RFT, LFT,Viral marker
- Blood CS, Urine CS
- Sputum profile
- X-rays and CT/MRI if indicated
- Ultrasound: For hepatosplenomegaly and abscesses
- Echocardiogram: If endocarditis is suspected.

3.10. Laboratory Diagnosis for Brucellosis

3.10.1. Case definition

The case definition and classification of brucellosis by WHO are classified as suspected, probable and confirmed cases as shown in table below.

Case classification	Definition
Suspected case	Epidemiologic exposure + clinical manifestations
Probable case	Suspected case + presumptive diagnosis (RBT+SAT>160)
Confirmed cases	Probable case + confirmatory diagnosis (ELISA, Culture, PCR)

Suspected case:

A clinically compatible illness with at least one of the following:

- Epidemiologically linked to a confirmed human or animal brucellosis case

Probable case:

A clinically compatible illness with at least one of the following:

- Epidemiologically linked to a confirmed human or animal brucellosis case
- Presumptive laboratory evidence, but without definitive laboratory evidence, of Brucella infection

Presumptive laboratory evidence

- Brucella total antibody titer of greater than or equal to 1:160 by standard tube agglutination test (SAT) or Positive by Rose Bengal test in serum specimens obtained after onset of symptoms

Confirmed case:

A clinically compatible illness with **definitive laboratory evidence** of Brucella infection

Definitive laboratory evidence

- Culture and identification of Brucella spp. from clinical specimens
- Detection of Brucella DNA in a clinical specimen by PCR assay
- Evidence of a four-fold or greater rise in Brucella antibody titer between acute and convalescent phase serum specimens obtained greater than or equal to 2 weeks apart

3.10.2. Collection of specimens

Collection of clinical samples will depend on disease pathogens and types of laboratory tests available in the laboratory setting will determine what types of samples to be collected. The following clinical samples were collected for the brucellosis diagnosis;

1. **Blood sample**

   Blood sample is the most commonly collected sample for the brucellosis diagnosis. Blood culture and serology and PCR can be performed

Blood culture

- Collect the required volume of blood as per the blood culture bottle requirement before administration of antibiotics
- Aseptically transfer blood into culture bottles.
- Send it to the laboratory.

Note:

Blood culture facility is only available in laboratories equipped with microbiological facilities.

Serology and PCR

- Collect 5 ml blood
- Transfer to 3 ml to the clot activator tube and 2 ml into EDTA tube.
- For clot activator, centrifuge at 3500 rpm for 5 min to collect serum sample.
- Transfer the serum to sterile cryovial.

2. **Bone Marrow sample**

Bone marrow culture has higher sensitivity than blood culture. However, due to invasiveness, procedure is not routinely practiced for diagnosis (if needed)

- Collect the required volume of blood as per the blood culture bottle requirement
- Aseptically transfer the blood into culture bottles.
- Send it to the laboratory.

Note:

Blood culture facility is only available in laboratories equipped with microbiological facilities.

3. **CSF**

- CSF should be collected in a sterile container by lumbar puncture under aseptic conditions before the institution of antibiotics.
- Preferably CSF should be collected in three different vials, one for cell count, one for biochemical examination and one for culture.
- CSF should be transported immediately to the laboratory without delay.

General note:

- All samples should be properly labeled mentioning name, date of collection, and accompanied with a duly filled case investigation form (CIF) with relevant clinical details should be included.

- The clinician should notify the clinical laboratory personnel when considering a diagnosis of brucellosis to ensure that appropriate safety precautions are implemented by the laboratory staff

Sample type	Volume collected	Collection timing	Test Assay	Remarks
Primary serum sample	Collect 4 ml of whole blood in serum separator tube	Collected early after symptom onset (Acute phase of illness)	RBT, SAT & ELISA	Samples are best collected before antibiotics during acute phase
Convalescent serum sample	Collect 4 ml in serum separator tube	Collected 2-3 weeks later	SAT & ELISA test	To confirm recent infection by demonstrating a rise in antibody titers
Whole blood	Collect 2ml in EDTA tube	Collected early after symptom onset (Acute phase of illness)	PCR	Samples are best collected before antibiotics during acute phase
CSF, not commonly collected.	Collect in sterile container	Collected early after symptom onset (Acute phase of illness)	RBT, SAT, ELISA & PCR	Samples are best collected before antibiotics during acute phase

Table 2. Collection of specimens for brucellosis

3.10.3. Brucellosis diagnostic investigations carried out at different health facilities

3.10.3.1. At Primary Healthcare centers (PHCs) & District laboratories

- No lab facilities equipped to test for the diagnosis.
- Shipment of sample to RCDC for serological and molecular assay for further investigation

3.10.3.2. At Regional & National laboratories

- Microbiological assay for culture and isolation of brucella equipped with bacteriology facilities, but for such pathogens, it is recommended to handle at least BSL 3 or higher for biosafety concern.
- JDWNRN laboratory has MALDI-TOF MS, similar to molecular based assay can also be used in testing suspected brucellosis.
- All suspected sample should be shipped to RCDC for serological and molecular assay for further investigations

3.10.3.3. At Reference laboratory (Royal Centre for Disease Control)

At RCDC;

- ELISA
- SAT
- PCR

Laboratory Test	Health facilities	Specimen
Microbiological (Culture & isolation)	JDWNRH/CRRH/ERRH/PLG **(BSL-2)**	-Blood, bone marrow, cerebrospinal fluid (3ml), wounds and pus (3-5ml) -For blood culture collection, inoculate into BacT/Alert or BACTEC aerobic and anaerobic bottles, using 10 mL per bottle for adults and 3 mL per bottle for children.
Molecular (MALDI-TOF MS)	JDWNRH**(BSL-2)**	-It can be applied directly to colonies of Brucella on solid media or to positive blood culture broth. -It is known for its rapidity, accuracy, and cost-effectiveness and is capable of identifying Brucella isolates at the sub-species level.
PCR	JDWNRH/ERRH/CRRH/Cluster hospitals with PCR**(BSL-2)** RCDC **(BSL-3)**	Blood (3-5ml) in EDTA tube

Serological (SAT & ELISA)	RCDC	Serum(3-5ml), CSF (1-3ml)

Table 3. showing facilities with the types of testing available and their corresponding BSL levels.

3.11. Laboratory diagnosis

A laboratory diagnosis is essential for the identification of human brucellosis, given the variability of clinical symptoms. Diagnosis is based on two diagnostic assay approaches:

1. Direct diagnosis by culture, and PCR based method
2. Indirect diagnosis by serological tests

Due to the variable and limitation with diagnostic assay and nonspecific clinical symptoms in humans, a strong clinical suspicion, coupled with the patient's history of exposure to animals or consumption of raw dairy products, can significantly aid in diagnosing the infection.

3.11.1. Culture

A culture is considered the “gold standard” for the laboratory diagnosis of brucellosis. Since human brucellosis often begins with a bacteremic phase, blood cultures (BC) should be performed as soon as brucellosis is suspected. This method confirms the disease, though its sensitivity ranges from 10% to 90%. Factors affecting BC positivity rates include microbial (i.e., *Brucella* species), patient-related (e.g., age, duration of symptoms, acute or chronic disease, first infection or relapse, and antibiotic use), and method-associated variables (e.g., blood volume, number of BC bottle, BC system used and incubation time.

During the initial stage of brucellosis, patients present a low bacterial load in the blood, which can only be detected through the collection of multiple samples specifically, by drawing at least two or three separate blood culture sets and avoiding single collections.

As the infection progresses, the organism spreads to other organs, reducing circulating bacteria and making isolation difficult in 25–35% of patients, leading to focal infections. Brucella can be isolated from specimens other than blood, including bone marrow, urine, bone tissue, pleural and

synovial fluid aspirates, liver biopsy specimens, lymph nodes, cerebrospinal fluid, and abscesses, following inoculation of these specimens into culture bottle media used in blood cultures.

The conventional culture method requires a long incubation time (6 weeks) and its yield variability ranging from 40-90% in acute cases and 15-20% in chronic, focal, and complicated cases. The use of an automated blood culture system has improved the sensitivity of blood cultures and decreased the incubation time required for the detection of Brucella.

In addition to conventional methods, the identification of Brucella species can also be achieved using Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry; however, its accuracy may occasionally produce inconsistent results. Therefore, presumptive identification of Brucella should be confirmed by two or more diagnostic methods.

For the routine handling of clinical specimens suspected of brucellosis, it is recommended to utilize Biosafety Level 2 (BSL-2) containment equipment and facilities. In contrast, Biosafety Level 3 (BSL-3) practices, containment equipment, and facilities are recommended for all manipulations of cultures of pathogenic *Brucella* species.

Routine bacteriological procedures, such as homogenizing tissues, centrifuging and vortexing bacterial suspensions, performing subcultures, and conducting biochemical tests, can generate hazardous aerosols and spillage of viable bacteria, increasing the risk of unintentional transmission to personnel.

Advantages:

- Gold standard test for definitive diagnosis.
- Automated blood culture systems and other advanced technology aids in providing the report for a shorter period of time.

Disadvantages:

- Turnaround time (TAT) in culture methods is prolonged due to the long incubation period.
- Bone marrow culture has higher sensitivity than blood culture. However, the invasiveness of the procedure should be considered.
- Requires Biosafety Level 3 laboratory since *Brucella* is highly infectious via aerosols Laboratory-acquired brucellosis while handling especially during the manipulation process.

Note: All suspected brucellosis samples should be shipped to RCDC for further confirmation. Prior information to be communicated to RCDC focal.

3.11.2. Serology

Indirect diagnosis is based on serological tests aimed at detecting specific antibodies in patient serum. Interpretive criteria are clearly defined, including specific titers in agglutination assays, cutoff values in enzyme-linked immunosorbent assays (ELISA), and distinct bands in lateral flow immunoassays. However, these criteria can be controversial due to laboratory variations and clinical factors such as age, duration of illness, occupational risks, disease history, endemicity, and repeated exposure.

The primary disadvantages of serological tests include low specificity and challenges in result interpretation particularly in patients with repeated exposure to *Brucella* as well as difficulties in distinguishing between active and past infections. Additionally, serology exhibits low sensitivity in the early stages of the disease and suboptimal specificity due to cross-reactivity with other bacterial species. Despite these limitations, serological tests remain the primary diagnostic method for brucellosis in endemic and low-to-middle-income countries due to their affordability, ease of use, and high negative predictive value

3.11.3. ELISA

The most commonly used antigen based immuno assay which detects the presence of antibodies (IgM & IgG) in the blood samples. However, interpretation of these assays is often difficult because: i) a high proportion of the population in endemic regions may have persistent antibody titres due to exposure to Brucella; ii) antibodies can remain detectable despite successful therapy; and iii) cross-reaction with Gram-negative bacteria (e.g., Salmonella, Yersinia) may occur. Serology results should be interpreted in combination with clinical signs and symptoms.

Advantages:

- ELISA can be used in diagnosis of infection especially when culture facilities are not feasible in the laboratory due to biosafety concerns.

Disadvantages:

- Seronegative in the acute phase of the disease, which necessitates serological testing of paired sera or performing more than one serological test
- ELISA comes positive due to persistent antibody titres which cannot differentiate recent infection from past infection or can be cross reaction especially in autoimmune disease like SLE, multiple sclerosis and cystitis.

3.11.4. Standard agglutination test (SAT)

The serum agglutination test (SAT) for brucellosis is a diagnostic blood test that mixes a patient's serum with a Brucella antigen to detect antibodies. If the patient's antibodies are present, they cause the antigen to clump together (agglutinate), a reaction that is graded by the highest serum dilution, or titer, where agglutination occurs.

- Titer ≥1:160 is usually considered diagnostic in conjunction with compatible clinical presentation
- Rising titer on repeat testing supports active infection.
- The sensitivity and specificity of SAT are high

Advantages

- SAT can be used in diagnosis of infection especially when culture facilities are not feasible in the laboratory due to biosafety concerns.

Disadvantages

- The test can produce false-positive results due to cross-reactivity with antibodies from other bacteria, such as Yersinia enterocolitica O9, Francisella tularensis, and Vibrio cholerae.
- Very high antibody concentrations (hyperantigenemia), especially in the early stages of acute infection, can sometimes lead to false-negative results in lower dilutions (e.g., 1:10 to 1:80). This is why serial dilutions are essential.
- Antibodies, particularly IgG, can persist for months to years after successful treatment and clinical cure, making the SAT less useful for monitoring treatment success or detecting relapse in the early post-treatment period.
- The standard test often uses B. abortus antigen and may not effectively detect infections caused by all Brucella species, such as B. melitensis.

3.11.5. Molecular Methods

3.11.5.1. Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) detects the target of brucella DNA in the blood. PCR can be diagnosed from pure cultures and clinical specimens (i.e., serum, whole blood, urine samples, various tissues, etc.). PCR is more sensitive than blood cultures and more specific than serological tests. PCR is only useful for acute but not chronic Brucellosis since bacteraemia is present mainly in the acute stages of infection.

Advantages:

- PCR is highly sensitivity and specificity as compared with other assay

Disadvantages:

- The assay is useful in the acute phase of infection but not in chronic brucellosis.
- PCR positivity usually declines after effective antibiotic treatment.
- May remain positive for weeks to months if bacterial DNA persists.
- Negative PCR does not completely rule out brucellosis, especially in chronic or localized cases.

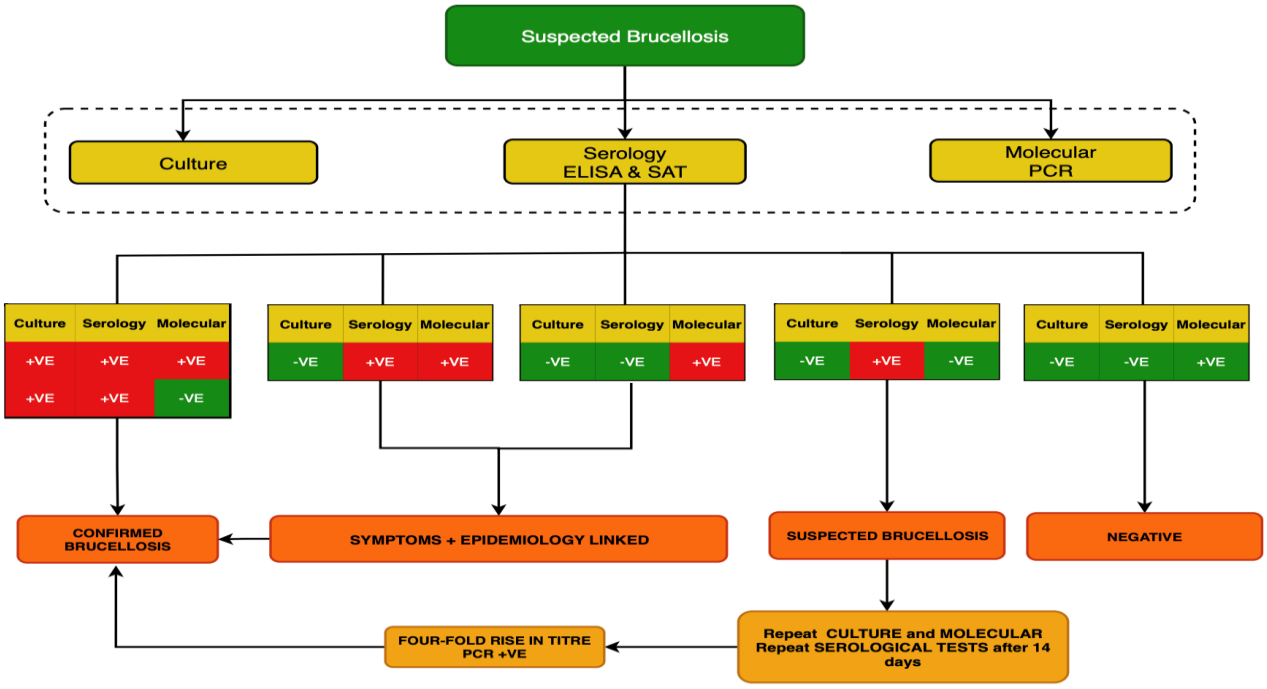

Figure 1. showing the diagnostic algorithm for brucellosis in human

3.12. Management of Brucellosis

The general principles of brucellosis treatment include use of antibiotics with activity in acidic intracellular environments (such as doxycycline and rifampin) and use of combination therapy with prolonged duration to prevent relapse and complications.

3.12.1. Treatment of Brucellosis in the absence of focal disease (complications)

Nonpregnant adults	• Oral Doxycycline 100 mg BD for 6 weeks **PLUS** Gentamicin 5 mg/kg IM/IV OD for 7-10 days **OR** • Oral Doxycycline 100 mg BD **PLUS** Rifampicin 600 to 900 mg OD for 6 weeks
Pregnant women	• <36 weeks gestation: rifampin **Plus** TMP- SMX 960mg BD (with folate supplementation), both for 6 weeks • ≥36 weeks gestation: rifampin monotherapy until delivery and after delivery continue combination therapy as in nonpregnant adults
Children < 8 years	• Oral TMP-SMZ (trimethoprim, 10 mg/kg per day, maximum 480 mg/day; and sulfamethoxazole, 50 mg/kg per day, maximum 2.4 g/day) divided in 2 doses for 4 to 6 weeks. Combination therapy: consider adding rifampicin. **Note: Tetracyclines (such as doxycycline) should be avoided in children less than 8 years of age as it is given for a longer duration of time.**
Children > 8 years	• Oral doxycycline (2–4 mg/kg per day, maximum 200 mg/day, in 2 divided doses) **PLUS** Rifampin (15–20 mg/kg per day, maximum 600–900 mg/day, in 1 or 2 divided doses) • Recommended for a minimum of 6 weeks. • Combination therapy with trimethoprim-sulfamethoxazole (TMP-SMZ) can be used if tetracyclines are contraindicated.

3.12.2. Treatment for Brucellosis with complication

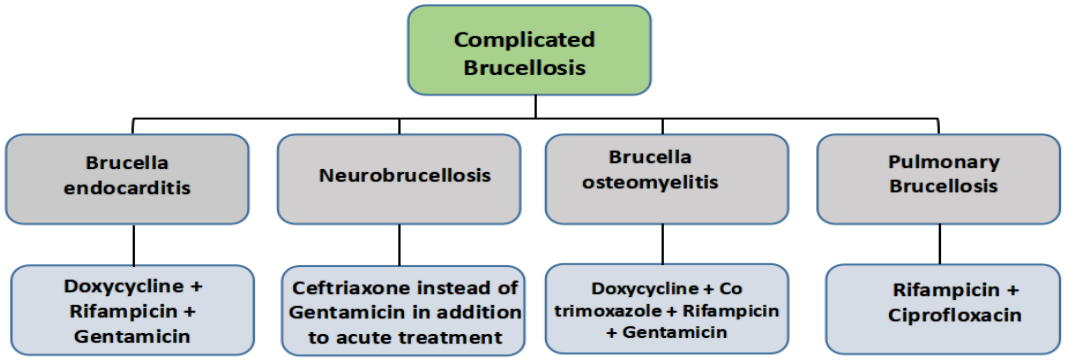

Figure 2. showing the treatment algorithm for complicated brucellosis

A. Brucella Spondylitis:

Nonpregnant adults	Gentamicin (for first 7 to 14 days) **PLUS** Doxycycline PLUS Rifampin for 12 weeks
Pregnant women	Ceftriaxone 2g IV OD for 4 to 6 weeks **PLUS** Rifampin and TMP-SMX for 12 weeks. **Note:** For pregnant women ≥36 weeks gestation: ceftriaxone and rifampicin until delivery, after delivery continue as in nonpregnant adults for 12 weeks.
Children >8 years	Doxycycline 4mg/kg/day (maximum 200mg/day) orally in two divided doses for at least 12 weeks **PLUS** Rifampin 15-20mg/kg/day (maximum 900mg/day) orally once daily for at least 12 weeks **PLUS** Gentamicin 5mg/kg/day IM or IV in one dose for the first 7-14 days

Children <8 years	TMP-SMX (TMP 10 mg/kg per day (maximum 320 mg/day) and SMX 50 mg/kg per day (maximum 1.6 g/day) divided in 2 doses for at least 12 weeks **Plus** Rifampin 15 to 20 mg/kg per day (maximum 900 mg/day) orally once daily for at least 12 weeks **Plus** Gentamicin 5mg/kg/day IM or IV in one dose for the first 7-14 days

Note: Treatment of osteoarticular disease (such as peripheral arthritis and sacroiliitis) should be as brucellosis without complication.

Brucella Osteomyelitis:

Non pregnant adults &children >8yrs	Doxycycline + TMP/SMX + rifampicin for 4 to 6 months **plus** Gentamicin for 2 weeks
Children <8 yrs	TMP/SMX + rifampicin + ciprofloxacin for 4 to 6 months **plus** Gentamicin for 2 weeks

C. Neurobrucellosis:

Non pregnant adults	Ceftriaxone 2gm OD for the 4 to 6 weeks, PLUS oral rifampin 600mg od Plus oral doxycycline 100mg bd for 12 weeks;
Pregnant women	< 36 weeks gestation: Ceftriaxone for the first 4 to 6 weeks, **Plus** Oral rifampin 600mg OD for at least 12 weeks **Plus** Oral TMP-SMX 960mg BD both for at least 12 weeks.

	≥36 weeks gestation: ceftriaxone and rifampin until delivery and after delivery continue as in nonpregnant adults for at least 12 weeks. **Note:** TMP-SMZ should not be used after 36 weeks of pregnancy because of the risk of kernicterus caused by elevated levels of bilirubin
Children <8 yrs	Ceftriaxone 100 mg/kg/day divided every 12 hours (maximum 2 g per dose) for the first 4 to 6 weeks **Plus** Rifampin 15 to 20 mg/kg per day (maximum 900 mg/day) orally once daily for at least 12 weeks **Plus** TMP 10 mg/kg per day (maximum 320 mg/day) and SMX 50 mg/kg per day (maximum 1.6 g/day) divided in 2 doses for at least 12 weeks
Children >8 years	Ceftriaxone 100 mg/kg/day divided every 12 hours (maximum 2 g per dose) for the first 4 to 6 weeks **Plus** Rifampin 15 to 20 mg/kg per day (maximum 900 mg/day) orally once daily for at least 12 weeks **Plus** Doxycycline 2-4mg/kg per day (maximum 200 mg/day) orally in 2 divided doses for at least 12 weeks

D. Endocarditis:

Non pregnant adults	Doxycycline 100 mg orally twice daily for at least 12 weeks **plus** Rifampin 600 mg orally once daily for at least 12 weeks **plus** Gentamicin 5 mg/kg/day intramuscularly or intravenously in 1 to 3 doses for the first 4 weeks
Children >8 yrs	Doxycycline 2-4mg/kg per day (maximum 200 mg/day) orally in 2 divided doses for at least 12 weeks **Plus** Rifampin 15 to 20 mg/kg per day (maximum 900 mg/day) orally once daily for at least 12 weeks **Plus** Gentamicin 5 mg/kg/day intramuscularly or intravenously in 1 to 3 doses for the first 4 weeks
Children < 8yrs	TMP 10 mg/kg per day (maximum 320 mg/day) and SMX 50 mg/kg per day (maximum 1.6 g/day) divided in 2 doses for at least 12 weeks **Plus** Rifampin 15 to 20 mg/kg per day (maximum 900 mg/day) orally once daily for at least 12 weeks **Plus** Gentamicin 15 to 20 mg/kg per day (maximum 900 mg/day) orally once daily for at least 12 weeks
Pregnant	Ceftriaxone 2 g intravenously once daily for first 4 to 6 weeks **Plus** Rifampin 600 to 900 mg orally once daily for at least 12 weeks **Plus** Septran 1 double-strength tablet (160 mg TMP/800 mg SMX) orally twice daily for at least 12 weeks

3.12.3. Other therapeutic considerations

Surgical intervention may be required when medical therapy is insufficient or complications arise, such as:

- Abscesses affecting vital functions (e.g., spinal, paraspinal with neurological deficits)
- Brucellosis endocarditis with large vegetations, valvular dysfunction, annular abscess, mycotic aneurysm, or septic emboli
- Early surgery in adults is associated with reduced mortality

Corticosteroids can be used as adjuncts in children with Brucella meningitis, myelitis, radiculopathy, cranial nerve palsy, optic neuritis, uveitis, and autoimmune complications (e.g., hemolytic anemia, thrombocytopenia, pancytopenia).

Immunomodulators (Levamisole, interferon-α) show inconsistent benefits in adults and are not recommended.

3.12.4. Monitoring clinical response

- Improvement is commonly observed within 3-7 days of initiating therapy.
- Close monitoring and follow-up are strongly recommended to ensure the sustained therapeutic response and adherence to brucellosis therapy. It is crucial to pay special attention to medication side effects, as these can potentially lead to premature discontinuation of therapy.

3.12.5. Tests During Follow-up of Brucellosis Patients

Follow up testing primarily involves serological monitoring to track antibody levels and assess for possible relapse or treatment failure. This typically includes quantitative serological tests such as ELISA or agglutination assays conducted at defined intervals following exposure or treatment. In some cases, further investigations like PCR, blood cultures, or imaging may be needed

- **Culture:**
    - If initial blood culture is positive, repeat after 7 days of therapy.
    - Persistent positivity warrants hospitalization and IV therapy and assess for focal infection and test antibiotic susceptibility.

- **Serology:**
    - Repeat 6 weeks after starting the treatment and then at 12, 18, and 24 weeks
    - Monitor IgG decline; IgM may persist for 1–2 years.
    - Use ELISA or SAT with 2-mercaptoethanol to differentiate IgG.
    - If IgG remains elevated but the patient is clinically well, continue follow-up every 3 months for 1 year.
- **PCR (Molecular Testing):**
    - Not routinely used for follow-up due to prolonged DNA persistence.
    - Consider for symptomatic patients with low titers or undiagnosed persistent fever.

3.12.6. Management of Relapse, Therapy Failure, and Reinfection in Brucellosis

- Refer to infectious disease specialists.
- Thoroughly evaluate potential infection sites, especially CNS and heart.
- Repeat serology and blood cultures; rising titers suggest relapse.

- Prefer separate IgG and IgM estimation via ELISA or SAT with 2-mercaptoethanol (2-ME); IgG rise is typical in relapse.
- Imaging (e.g., CT, MRI, PET-SCAN) is useful to detect hidden infection foci.

3.13. Antimicrobial Post-Exposure Prophylaxis (PEP)

PEP is recommended based on Occupational and Laboratory Exposure.

High-Risk Activities

- Handling Brucella cultures outside a biosafety cabinet (open bench) especially in labs not initially aware of the organism).
- Mucous membrane or non-intact skin exposure to infectious material which includes splashes to eyes, mouth, or open wounds.
- Accidental inhalation of contaminated dust or aerosols from infected animal products (e.g., wool, hides, unpasteurized dairy). Rare but possible in occupational or field settings.
- Unintentional exposure during sampling of infected livestock and collecting biological materials without PPE
- Aerosol-generating procedures without proper protection
- Veterinarians/animal handlers with direct contact

Low-risk activities

- The person remained at a distance of over 5 feet away from the specimen handler working on an open bench.
- There were no incidents involving spills or procedures likely to generate aerosols, such as unsealed centrifugation, vortexing, or splashing.

Post-Exposure Prophylaxis (PEP)

Exposure	PEP
High risk	Oral Doxycycline 100mg BD + Oral Rifampin 600mg OD X 21 days **OR** Oral doxycycline 100mg bd + Septran (160mg/800mg) BD X 21 days (exposed to brucella isolates abortus RB51 strain
Low risk	Consider PEP if immunocompromised/pregnant **Note:** In cases where doxycycline or rifampin is contraindicated, trimethoprim-sulfamethoxazole (TMP-SMZ) or another antimicrobial agent effective against Brucella should be selected for a minimum of 21 days

Note:

- Monitoring: Weekly symptom review and daily fever checks for 24 weeks; serology at weeks 0, 6, 12, 18, and 24
- If clinical symptoms develop at any point while on PEP and brucellosis infection is confirmed by culture and isolation or serology, PEP is no longer appropriate and treatment and monitoring is required

3.14. Prevention and Control Measures

3.14.1. Prevention and Control in human

Education campaigns

- Create public awareness about the endemicity of brucellosis
- Avoid consuming unpasteurized milk and milk products
- Avoid consuming raw or undercooked meat and meat products.
- Practice good hygiene and wear protective equipment when handling anima and animal products (fresh meat particularly).
- Avoid contact with birthing fluids, blood, or tissues of animals.
- Wash your hands with soap and water after touching animals or animal products.

Occupational safety practices

- Utilize clean, sharp knives for field dressing and butchering.
- Wear eye protection and nonporous, disposable gloves (e.g., rubber, nitrile, or latex) when handling carcasses.
- Avoid direct (bare skin) contact with fluids or organs from the animal.
- Steer clear of direct (bare skin) contact with hunting dogs that may have had contact with hunted animals.
- After butchering, dispose of disposable gloves and parts of the carcass that will not be consumed by burning or burying.
- Refrain from feeding dogs raw meat or other parts of the carcass.
- Wash hands promptly with soap and warm water for at least 20 seconds. Dry hands with a clean cloth.
- Clean all tools and reusable gloves with a disinfectant, such as dilute bleach (following safety instructions on the product label).
- Be aware that freezing, smoking, drying, and pickling do not eliminate Brucella.

3.14.2. Prevention and control measures in animals

- Responsible animal ownership, animals must be individually identified (brand, tattoo, ear tag).
- Movement or sale from infected areas should be prohibited.
- Avoid contact between susceptible animals and infected ones or their discharges/tissues
- Proper disposal (burial or burning) of placentas and non-viable fetuses. Disinfection of contaminated areas should be performed thoroughly.
- Import into disease-free areas allowed only from certified brucellosis-free herds with recent negative test.
- Breeding your animals with disease free animals
- Periodic milk ring tests in cattle (at least four times per year), and testing of slaughtered animals with simple screening serological procedures such as the RBT
- Vaccinating your livestock against brucellosis (not practiced in Bhutan)
- Since the prevalence of brucellosis (Brucella abortus) was recorded to be low ~0.2%, the Department of Livestock adopts a "test and cull" method over the conventional vaccination and control method.

3.15. Bio-terrorism

B. *melitensis* and B. *suis* have been weaponized in state-sponsored programs due to their stability in aerosol form and extremely low infectious dose, making them effective for targeting humans and animals. The consequences of such use would be most severe in regions where brucellosis is not endemic. Health and veterinary authorities must maintain heightened awareness of their potential misuse.

3.16. References

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4. Scrub Typhus

4.1. Introduction

Scrub Typhus (ST) is an acute febrile illness caused by *Orientia tsutsugamushi*, a rickettsial infection, transmitted to humans through the bite of infected chiggers (larval mites). It is a vector-borne zoonotic disease prevalent in the Asia-Pacific region, including Bhutan. The illness ranges from mild to severe and, if left untreated, can lead to complications or death. Early detection and timely treatment are essential for reducing morbidity and mortality.

4.2. Epidemiology

Geographically, ST is endemic in the "tsutsugamushi triangle," which includes Bhutan, India, Nepal, Srilanka, Thailand, and parts of China. ST was first detected in Bhutan in 2008 and since then the southern districts have been identified as endemic to scrub typhus with sporadic cases in central districts. However, the disease is likely to be under-diagnosed and under-reported, and the true impact is difficult to estimate. Small rodents such as rats serve as natural hosts and incidence peaks during monsoon and post-monsoon seasons (June to November).

4.3. Risk factors

Farming and gardening, and behavioral factors such as not changing garments after returning from work are associated with higher risk in the endemic areas. In Bhutan, harvesting cardamom was the major risk factor, traditional housing, largely caused by an outside toilet location, as well as owning a goat and frequently sitting on grass.

4.4. Causative agent

Scrub Typhus (ST) or tsutsugamushi disease is a vector-borne rickettsial disease that is caused by the obligate intracellular bacterium *Orientia tsutsugamushi*. The primary reservoir is a trombiculid mite of the genus *Leptotrombidium*, which maintains the infection within populations through both transovarial and transtadial means of transmission.

4.5. Mode of transmission

Transmission to humans and other mammals occurs through the bite of infected chigger mites. A high risk of exposure to ST is associated with outdoor activities, agricultural work in particular, or living near grasslands or fields. Humans are dead-end hosts, with no evidence of horizontal transmission of *O. tsutsugamushi* between people.

4.6. Incubation period

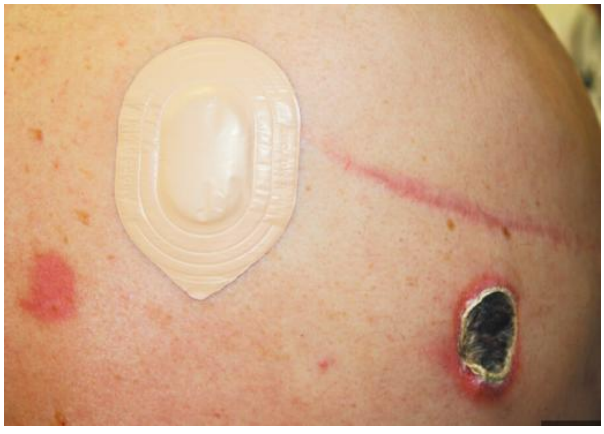
Incubation period is typically from 6-10 days but it can range from 6-21 days.

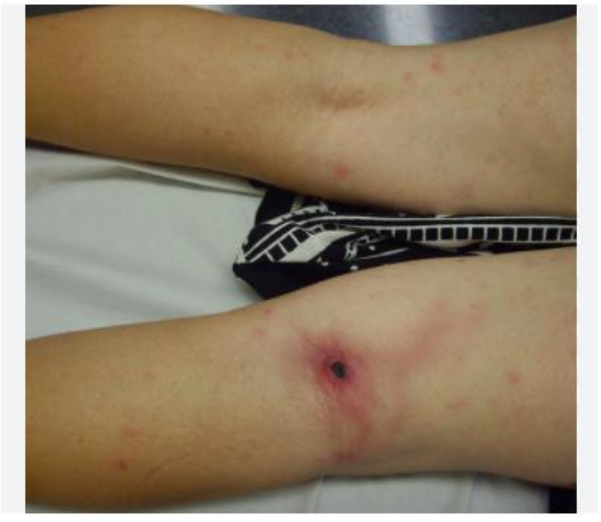
4.7. Clinical feature

Symptoms	Signs
• Fever and chills • Myalgia • Headache • Arthralgia • GI symptoms: nausea, vomiting &diarrhea	• Generalized Lymphadenopathy • Hepatomegaly, splenomegaly • Rash(Nonpruritic,macular,or maculopapular rash typically begins on the abdomen and spreads to the extremities) • Relative bradycardia

Eschar is a pathognomonic feature of scrub typhus but its absence does not rule out scrub typhus. The presence of an eschar in patients with scrub typhus can vary greatly between studies, with reported rates ranging from as low as 9.5% to as high as 86%. Lower rates are often reported in Indian and Southeast Asian populations. It begins as a painless transient localized itch with subsequent central necrosis and is surrounded by pink areola. Eschars are mostly seen in hidden areas of the body: axilla, groins and under the breasts, but it can occur in any part of the body (lower extremities, neck and chest region, abdomen).

Picture showing an eschar in a patient with scrub typhus.





4.7.1. Mild – Moderate scrub typhus

- Acute febrile illness
- Rash with or without eschar on the 5th day of the bite/exposure
- Lymphadenopathy

4.7.2. Severe Typhus (COMPLICATIONS) **–** may present with fever associated with any of the following multi organ dysfunctions

- Pneumonitis/Acute respiratory distress syndrome (ARDS)
- Acute Kidney Injury
- Hepatitis or acute liver failure
- Meningo-encephalitis
- Hypotension
- Septic shock
- Myocarditis
- DIC (Disseminated Intravascular Coagulation)

4.7.3. Risk factors for developing complication

1. Delay in treatment
2. Patients of older age (>65 yrs.)

4.7.4. Differential Diagnosis

- Leptospirosis
- Enteric fever
- Other *Rickettsial* infections such as Murine Typhus
- Dengue
- Malaria
- Leptospirosis
- Chikungunya
- Japanese encephalitis

4.8. Laboratory Diagnostic Approach for Scrub Typhus

4.8.1. Case Definition

Suspected /Clinical case

Acute undifferentiated febrile illness of 5 days or more with or without eschar should be suspected as of Rickettsial infection (if eschar is present, fever of less than 5 days duration should be considered as scrub typhus)

Probable case

A suspected clinical case with positive on any one of the serology test assays either by RDT or optical density >0.5 cutoff by ELISA.

Confirmed case

The one in which

- Scrub typhus DNA is detected in eschar samples or whole blood by PCR or
- Four-fold increase of titers between primary and convalescent sample
- High titre of IgM antibodies in ELISA

4.8.2. Routine Investigation

- CBC, ESR, CRP, RFT, LFT
- Viral marker
- Chest xray

4.8.3. Collection, Storage and Transport of Clinical samples

Blood for molecular tests should be collected as early as possible, ideally within the first 4 to 7 days of illness. Additionally, a 3 mm diameter punch tissue/biopsy of a skin lesion, preferably a maculopapule containing a petechiae or the margin of an eschar should be collected in a sterile container.

For serology diagnosis, blood is collected within the first 7-10 days of illness in a plain colt activator vacutainer tube.

Clinical specimens collected	Volume collected	Remarks
Serum sample for serology assay	2 ml collected in clot activator vial	• Ideally collected within the first 7-10 days of illness (Acute sample)

Whole blood for molecular assay	2ml collected in EDTA vial	• Collect as early as possible after onset, ideally within the first 4 to 7 days of illness
Eschar Tissue	Collected in sterile container	• Collect where applicable

4.8.4. Handling, Storage & Transport

- Collect blood specimen with minimum PPEs such as hand gloves
- Store serum and whole blood samples at 2-8*C if testing within week or freeze at 20*c or lower for longer storage
- Transport samples under cold chain condition as per

4.9. Laboratory test available for diagnosis of Scrub Typhus

Serological assays are the most commonly used method for confirming cases of scrub typhus. The indirect fluorescent antibody (IFA) test is generally considered the reference standard but is usually not available in most laboratory settings. Other serological tests including ELISA and RDT were available.

4.9.1. Rapid Diagnostic Tests (RDT): Available at National/Regional/District hospital

Detects IgM and IgG antibodies against the antigen protein of Orientia tsutsugamushi in serum/ plasma samples. They are simple, easy to perform and results are provided rapidly within 15 min and have been used extensively in field settings and hospitals. Negative RDTs **do not exclude** scrub typhus, especially in early illness; follow up with other serology or PCR testing may be required.

4.9.2. IgM ELISA: Available at RCDC/ National/Regional Hospital

Commonly used for screening and confirmation assay. It detects IgM antibodies to the antigen protein of Orientia tsutsugamushi, indicating recent infection.

4.9. 3. Polymerase Chain Reaction (PCR): RCDC

This technique is highly sensitive and specific and can detect the pathogen early, before antibodies are detectable through serological assays. PCR detects Orientia tsutsugamushi DNA in blood or eschar samples, especially within the first week when rickettsemia is present.

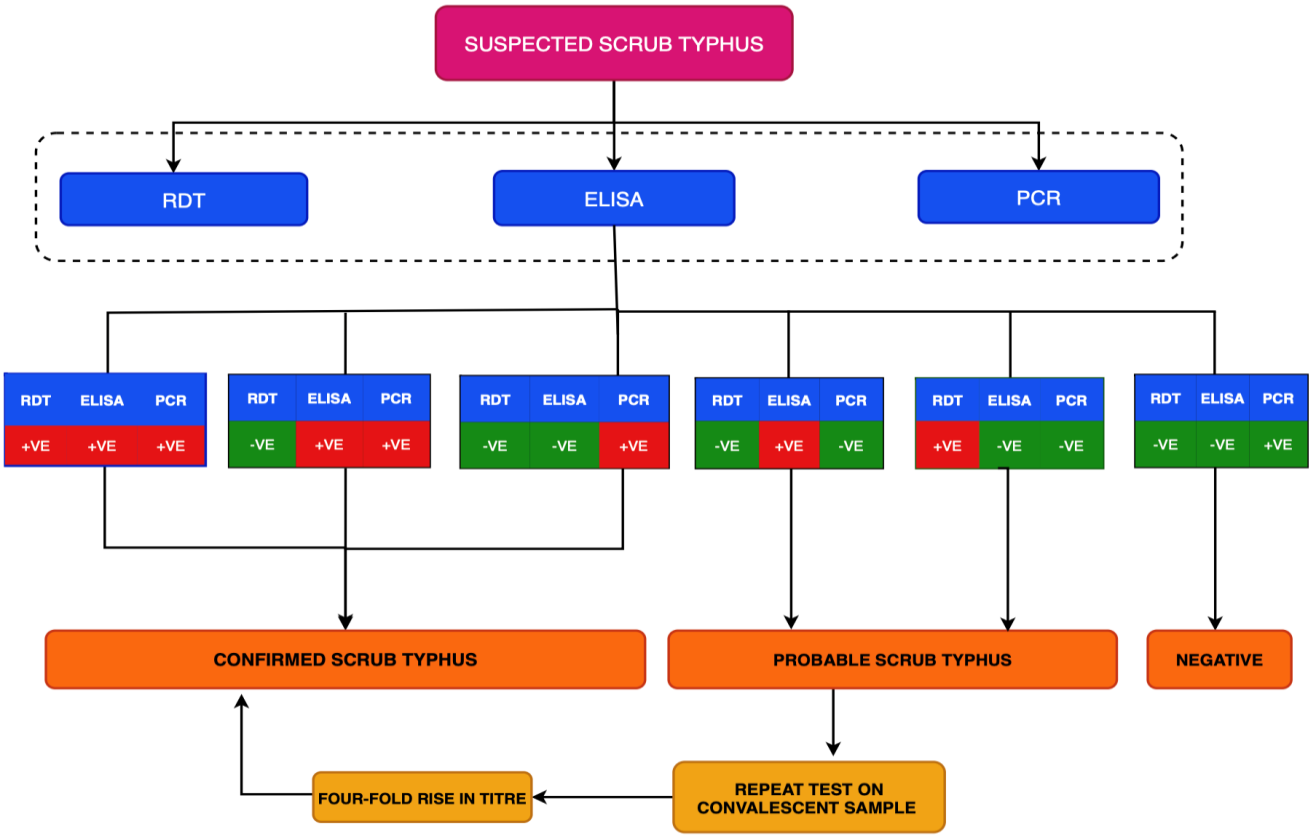


Figure 1. Laboratory testing algorithm for Scrub Typhus

4.10. Management

Treatment should be initiated as soon as possible. The approach to the treatment depends on the severity of the disease as follows. Doxycycline is the preferred treatment for scrub typhus.

	Adults	Children	Pregnancy
Mild - Moderate Disease (Oral or Intravenous)	Doxycycline 100mg twice daily for 7-10 days **OR** Azithromycin 500mg once daily for 7 days	Doxycycline is the drug of choice for all ages If Weight (>45kg) Doxycycline 100mg twice daily for 5-7 days If Weight (<45kg) Doxycycline (2.2mg/kg) twice daily 5-7 days	Azithromycin 500mg once daily for 7 days

		OR Azithromycin 10mg/kg/day (maximum dose 500mg) once daily for 5-7 days	
Severe Disease (Intravenous)	Dual therapy: Doxycycline 200mg twice daily on Day 1, followed by 100-200mg twice daily for 7-10days **AND** Azithromycin 500mg once daily for 7 days	If Weight (>45kg) Doxycycline 100mg twice daily for 7-10days days If Weight (<45kg) Doxycycline (2.2mg/kg) twice daily 7-10days AND Azithromycin 10mg/kg/day (maximum dose 500mg) once daily for 7-10 days	Azithromycin 500mg once daily for 7 days
Alternate Regimen	Fluroquinolone Rifampicin 600mg orally once daily for 7days Chloramphenicol (250 - 500mg IV every 6 hourly for 7 days)	Chloramphenicol (50-100mg/kg/day) every 6 hourly for 5-7 days (Most common side effect is agranulocytosis) Fluoroquinolones not recommended in pediatric age group	

NOTE: Patients should be treated for minimum 7 days of antibiotic and treatment should be continued for at least three days after the patient becomes afebrile (total duration 7-14 days)

4.11. Chemoprophylaxis

Chemoprophylaxis may be considered for high-risk individuals exposed to scrub typhus during peak transmission seasons, particularly in endemic regions with significant chigger (mite) activity. Doxycycline is the drug of choice for chemoprophylaxis. Prophylaxis is typically reserved for short-term use during periods of high-risk exposure, such as for military personnel, disaster relief workers. It is recommended only for adults and is not advised during pregnancy or for children under the age of 8.

Additionally, doxycycline offers concurrent protection against leptospirosis and can serve as malaria chemoprophylaxis in areas with chloroquine-sensitive Plasmodium falciparum or P. vivax. However, the routine use of doxycycline for prophylaxis should be carefully weighed against the risk of antimicrobial resistance and individual health factors.

Regimen - Doxycycline 200 mg orally once weekly.

To be started 1-2 days before the exposure and to continue till the end of high-risk exposure.

4.12. Prevention and Control

4.12.1. Public Health Measures

The public health intervention for prevention and control of scrub typhus is mainly aimed at advocating hygienic personal protection and eliminating the carriers and reservoirs of *O. tsutsugamushi*. As scrub typhus is common among the rural farming population and other occupational groups such as students, army personnel, housewives and cardamom workers at large. The public health intervention is targeted at humans, environmental and rodent/animal levels.

Human Level

Strategy	Phase	Targeted Audience	Favorable time of intervention	Materials and methods	Contents

Public and communities are informed on how they can prevent themselves from scrub typhus	Prevention and Control	General public (farmers, students, army personnel, housewives, cardamom workers)	May-June every year coordinated by DHOs and spearheaded by the Zoonoses Program, DoPH, MoH	Posters, leaflets, media, audio-visual, radio jingles, video clips, panel discussions	signs and symptoms, personal protection, environmental management, rodent and vector control, early reporting
Health Workers are informed on what they can do to prevent and treat scrub typhus through training and supervision	Prevention, treatment and control	All health workers [Medical Officers, Specialists, COs/ HAs, Nurses (Clinical/ staff) & Laboratory personnel]	Throughout the year	Training materials: workshops, Power points, posters, leaflets in Dzongkha & English	Background on scrub typhus, public health importance, clinical presentation early management, as per the national guideline

4.12.2. Personal protection

4.12.2.1. Protective clothing and personal hygiene

- Wear full clothing while visiting bushy/scrubby areas or undertaking general agricultural practices like working in cardamom and paddy fields. Long trousers (tucked into socks/ gumboots) are preferable when “bushwalking”.
- Changing and washing the clothes worn at work.
- Taking a shower after visiting bushy areas or agricultural work.
- Avoid walking barefoot outdoors.
- Use a suitable mat or other ground cover while sitting or lying (including resting babies) on the grassy grounds.

4.12.2.2. Repellants

- Apply insect repellent (containing dibutyl phthalate, benzyl benzoate, diethyl toluamide) to all exposed skin areas on the legs, onto socks and bottom half of trousers.
- Avoid diethyl-3-methylbenzamide (DEET) if under 12 months.

4.12.3 Environmental management

The favored ecotype of the chigger and rodents of *O. tsutsugamushi* are scrubby vegetation consisting of low-lying trees and bushes and also specific habitats such as rice fields, cardamom plantations, poorly maintained kitchen gardens, abandoned plantations, overgrown forest clearings, shrubby fringes of fields and forests, grassy fields and river banks as it provides optimal conditions (mites prefer warm, moist, and shady places) for the infected mites and rodents to thrive. These ecological patches which attract the natural host of mite vectors are called 'mite islands.

The environmental management strategies that reduce or eliminate vector breeding grounds are:

- Clearing bushes and vegetation around settlements by cutting the grasses close to the ground to destroy mite islands.
- Judicious use of insecticides (spraying chemicals/fogging/fumigation and dusting with chemical insecticides) e.g., Lindane or Chlordane to soil and vegetation.
- Maintaining good environmental sanitation and proper toilet facilities
- Proper garbage disposal to control rodent population and reduce 'mite islands'
- Avoiding piling of woods inside and/or against the wall of the houses
- Avoiding stacking hay or fodder near the human dwellings
- Keeping animals away from human dwellings

4.12.4. Rodent and vector control

The vector for transmission of scrub typhus is the larval stage of trombiculid mite; chigger phase. These larvae feed on wild rodents which are key to maintenance of population density of chiggers. A clean living-environment and control of rodents decreases the incidence of scrub typhus significantly.

4.12.5. Control strategies for rodents

- Good sanitation by regular cleaning of inside and outside of buildings
- Trapping of rodents using baits and disposal of dead rodents appropriately by placing them in plastic bags that are sealed tightly.
- Natural predators (cats).
- Management of food and kitchen waste.

Habitat modification (make habitat unsuitable for rodents):

- Storing grains and vegetables at a height above 0.5 m and away from the wall
- Fill holes and cracks in the walls.
- Remove trash every day to decrease food for rodents.

4.12.6. Control strategies for mites

- Remove overgrown weeds/grasses near houses, toilets and along the roads
- Apply lindane and chlordane on ground and vegetation

4.13. References

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5. Bovine Tuberculosis(bTB)

5.1. Introduction

Bovine tuberculosis (bTB) is an infectious disease caused by *Mycobacterium bovis*, which is part of the *Mycobacterium tuberculosis* complex (MTBc). It is a chronic condition in animals, marked by the development of granulomas mainly in the lungs, lymph nodes, intestines, and kidneys. *M. bovis* has the broadest host range, and can be easily transmitted to humans as well as various domestic and wild animals (Fitzgerald et al).

5.2. Epidemiology

Bovine tuberculosis is found globally, with the highest occurrence observed in countries across Asia and Africa. According to WHO Global Report 2018 *M. bovis* was responsible for 143,000 new human TB cases worldwide and 12,300 deaths in 2018, and has caused 1.4% of 10 million incidents of pulmonary TB globally. A large majority (over 91%) of the deaths were from African and Asian regions (OIE). However, determining the exact incidence of bovine tuberculosis in humans caused by *M. bovis* is difficult due to the technical challenges involved in isolating the bacterium. Since humans and animals often share the same living spaces, particularly in rural areas, bovine tuberculosis is increasingly becoming a significant issue in developing countries *(Malama et all, 2013*).

The primary reservoir for *M. bovis* is cattle. It is also commonly found in other animals such as bison, elk, and deer.

Currently, Bhutan has no recorded confirmed human M.bovis TB cases. A 2021 sero-survey in cattle across a few districts in eastern Bhutan showed that the overall apparent seroprevalence of bTB was 2.6%.

5.3. Risk groups

Farmers, veterinarians, slaughterhouse workers, and others who are in close contact with infected animals or animal products are at increased risk.

5.4. Etiology and Pathogenesis

Causative agent

M. bovis is a gram-positive, acid-fast, facultative intracellular, non-motile curved or rod-shaped bacillus, and does not create spores. Its dimensions are 1–10 µm in length and 0.2-0.6 µm in breadth, and it takes 15–20 hours to generate.

5.5. Mode of transmission

Mycobacterium bovis infections primarily occur in humans who consume raw milk and dairy products, undercooked meat products, while they can also occur in the air (Ortiz et al, 2021). Infection can also occur from direct contact with a wound, such as what might occur during slaughter or hunting, or by inhaling the bacteria in air exhaled by animals infected with *M. bovis.* People who spend extended periods in close contact with cattle or other animals that might carry *M. bovis,* such as dairy workers (CDC) can get the infection.

5.6. Incubation period

Variable; often months to years depending on exposure and host immunity. Most exposed individuals typically present with symptoms between 2-12 weeks.

Latent TB can persist silently for years.

- are infected with TB bacteria but do **not** feel sick.
- Have no symptoms
- Cannot spread TB to others.

However, if not treated, latent TB can turn into active TB later in life, especially if the immune system is compromised or in the elderly or young.

5.7. Clinical Features

Unlike M.tuberculosis, not everyone infected with M.bovis becomes sick. They can present as inactive TB (latent TB) or active TB.

5.7.1. Active TB Disease

In humans, bovine TB can resemble human TB and can involve the lungs, lymph nodes, or organs of the digestive system.

Initial symptoms of bovine TB disease can present as;

- Productive cough
- Fever
- Night sweats, and
- weight loss
- Other symptoms might occur depending on the part of the body affected by it.

5.7.2. Complication

- Hemoptysis
- Bronchiectasis
- Firbosis
- Cavitation

5.7.3. Differential Diagnosis

- Mycobacterium tuberculosis disease
- Nontuberculous mycobacterial infection
- Fungal infection
- Sarcoidosis
- Malignancies

5.8. Diagnosis

5.8.1. Laboratory Diagnostic Approach for Bovine Tuberculosis

Laboratory diagnosis of *Mycobacterium bovis* in humans can be challenging as it mimics *Mycobacterium tuberculosis* infection in terms of diagnostic findings. Similar diagnostic techniques can be applied for confirmation of bovine tuberculosis.

5.8.2. Baseline routine Investigation

- CBC
- RFT
- LFT
- ESR and CRPQ
- Chest xray
- Viral marker
- Blood culture

5.8.3. Clinical samples collected for diagnosis of Bovine Tuberculosis

5.8.4. Sample Collection

- **Sputum (Expectorated)**

Ideally, early morning samples obtained on 3 consecutive days of 5 to 10 mL containing recently discharged material from the bronchial tree with minimal saliva content.

- **Induced Sputum**

Sputum specimen obtained by inducing the production must be clearly marked “INDUCED” since induced sputum is watery in consistency and could be mistaken for saliva

- **Gastric Lavage**

A specimen volume of 5 to 10 mL neutralized with 100 mg of sodium carbonate is to be collected early in the morning before eating and at least 8 hours after the patient has eaten or taken oral drugs.

- **Sterile Fluids**

Body fluids (spinal, pleural, pericardial, synovial, ascetic, blood, pus, and bone marrow) to be collected aseptically.

- **Tissue**

Tissue samples must be collected into sterile containers without fixatives or preservatives unless it is for histopathological procedures.

5.8.5. Biosafety recommendations

Mycobacterium bovis is classified as a risk group 3 agent and requires BSL 3 for culture, drug susceptibility testing and other laboratory examinations.

Clinical Specimens Collected	Remarks
Sputum, tracheal aspirates, BAL, Body fluids, fine needle aspirates, CSF, synovial fluids, peritoneal fluids, pericardial fluids for microscopy	Preferably morning samples
Sputum, tracheal aspirates, BAL, Body fluids, fine needle aspirates, CSF, synovial fluids, peritoneal fluids, pericardial fluids for culture	Collect in sterile wide-mouth container
Sputum, tracheal aspirates, BAL for Line Probe Assay (LAP)	
Tissue biopsy for histopathological tests	

Laboratory Test	Health facilities	Remarks
Microscopy: Ziehl-Neelsen stain	DH/Regional Referral Hospital/National referral Hospital /RCDC	
Microscopy: Auramine stain	RCDC/National referral Hospital/Regional referral hospital / Few selected district hospital (Phuntsholing & Samtse)	Required fluorescent microscope
Gene-Xpert	Selected DHs/Regional/Referral/RCDC	To rule out human TB
Culture & DST	RCDC	
Line Probe Assay (LPA)	RCDC	
TST (PPD)	DH/Regional Hosp/Referral	

Table 1. showing the testing facilities available at different health facilities level

5.9. Laboratory test available for diagnosis of Bovine Tuberculosis

The laboratory diagnosis of bovine tuberculosis involves a combination of clinical, microscopy, microbiological, molecular and immunological methods. Confirmatory diagnosis in human bovine TB often requires a combination of these approaches due to overlapping clinical and laboratory features with human TB. Radiological tests will also further aid in diagnosis of mycobacterium infection.

5.9.1. Microscopy

Detection of Acid-Fast Bacilli (AFB) in clinical samples is key initial steps indicating the presence of mycobacterial infection. Microscopy is a rapid, cost-effective test used worldwide especially in low- and middle-income countries. However, a positive AFB smear does not distinguish between mycobacterium bovis from other complexes. Further molecular or culture -based tests assays are required for species identification.

5.9.2. Culture

Mycobacteria are slow growing and require specialized culture media. Culture is a traditional method for detecting mycobacterial infection. However, it is time consuming and requires biosafety level 3 containment. Culture can be either performed solid culture which is old techniques and liquid culture system with use of technology such as BACTEC MGIT 960 reduce detection time and improve sensitivity.

5.9.3. Immunological test

Immunological test focus on detecting the host's immune response to mycobacterial antigen rather than the pathogen directly. Tuberculin skin test (TST) is the most commonly used method which measures a delayed type hypersensitivity reaction to purified protein derivative (PPD) antigens

derived from mycobacteria. It cannot differentiate between M.bovis from other complexes but indicates mycobacterial exposure.

5.9.4. Gene Xpert MTB/RIF

Gene Xpert MTB/RIF assay is a rapid molecular diagnostic test for tuberculosis (TB) that detects DNA from Mycobacterium tuberculosis complex (MTBC) and mutations associated with resistance to rifampicin (RIF). While the assay accurately detects MTBC, it does not specifically differentiate M. bovis from M. tuberculosis.

5.9.5. Line Probe Assays (LPAs)

LPAs are molecular based diagnostic assays for rapid detection of mycobacterium complexes. They utilize PCR amplification followed by hybridization of amplified products to specific oligonucleotides probes on membranes to detect bovine tuberculosis (bTB).

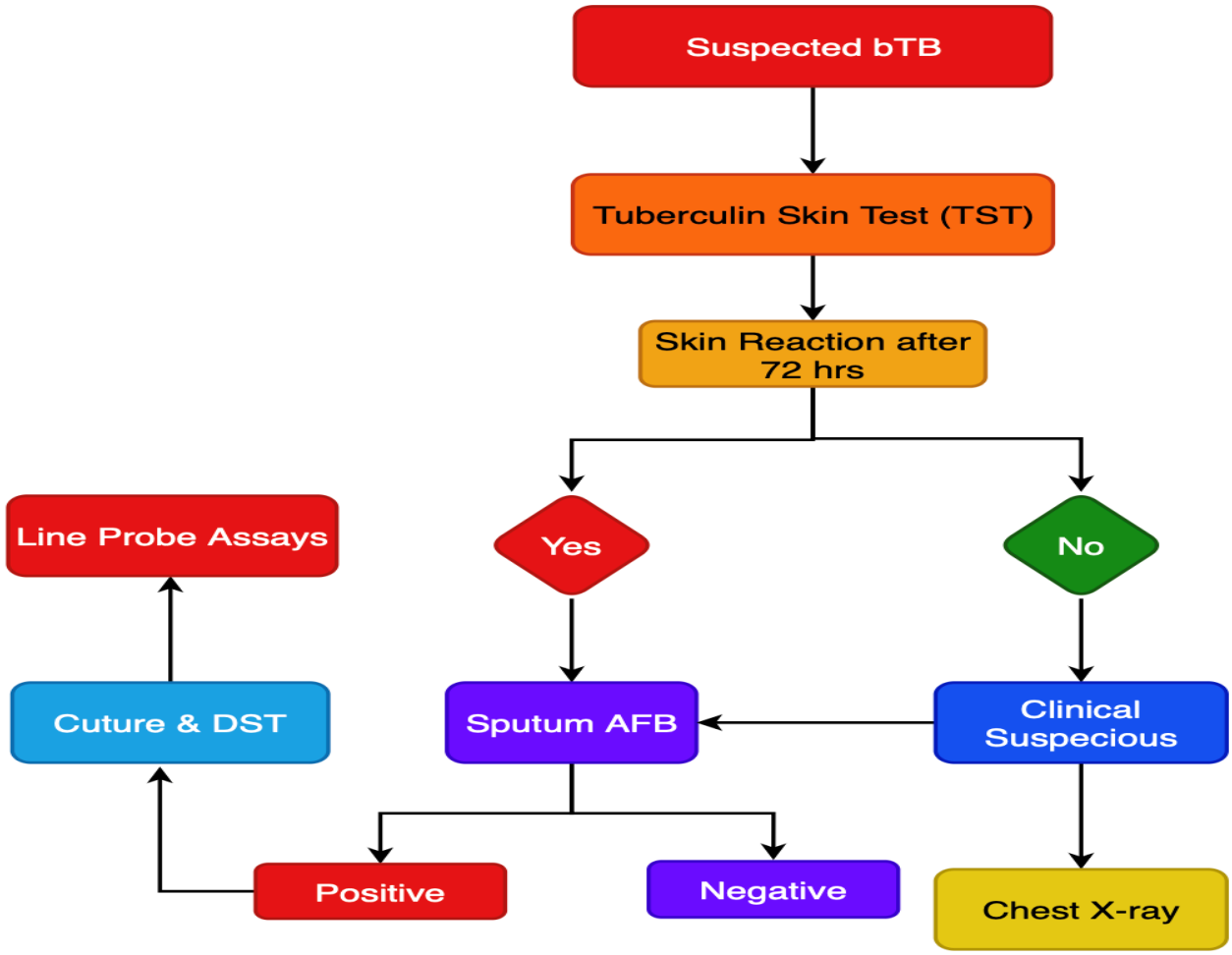

Figure 1. Laboratory testing algorithm for Bovine TB

5.10. Treatment and Management

M. bovis is intrinsically resistant to PZA; therefore, the approach to treatment of disease due to *M. bovis* depends on the expertise.

1. Drug- susceptible disease

For patients with disease due to drug-susceptib*le M. bovis*, treatment consists of two months of isoniazid, rifampin, and ethambutol followed by seven months of isoniazid and rifampin [uptodate - M bovis]. The duration of therapy for pulmonary and most extrapulmonary disease should be nine months, although the optimal duration for meningitis has not been established; some experts recommend at least 12 months for such cases.

2. Drug-resistant disease

For patients with disease to an *M. bovis* strain that is resistant to drugs (apart from PZA), or for patients who are intolerant of first-line drugs, the treatment regimen should be chosen according to susceptibility testing results with expert consultation.

5.11. Prevention and control of bovine tuberculosis (bTB)

5.11.1. Prevention measures in humans

Prevention of bTB in humans requires comprehensive measures that involve animal health management, public health interventions, and community awareness.

5.11.1.1. Safe handling of animal products

5.11.1.2. Pasteurization of Milk

- Ensure all milk and dairy products are pasteurized to kill *M. bovis*.
- Avoid consuming raw or unpasteurized milk, especially in endemic areas.

5.11.1.3. Meat Inspection

- Conduct a thorough inspection of carcasses in slaughterhouses to prevent contaminated meat from entering the food chain.

5.11.1.4. Occupational safety

Farmers, veterinarians, and slaughterhouse workers should use gloves, masks, and protective clothing when handling animals or animal products.
Provide training to at-risk occupational groups on safe practices to reduce exposure.

5.11.1.5. Public Awareness and Education

- Educate communities on the risks of consuming raw milk and improperly cooked meat.
- Raise awareness about the symptoms of bTB in humans and the importance of seeking medical care.

5.11.1.6. Surveillance and reporting

- Monitor bTB in cattle and human populations to identify and respond to outbreaks.
- Encourage reporting of suspected cases to veterinary and public health authorities.

5.11.1.7. Early diagnosis and treatment in Humans

- Conduct screening for individuals at high risk, such as farmers and dairy workers, in endemic areas.
- Administer appropriate antibiotic therapy for confirmed cases of bTB in humans.

5.11.1.8. Cross-sectoral collaboration (One Health Approach)

- Strengthen collaboration between veterinary and public health sectors to control bTB taking the One Health approach.

5.11.1.9. Prevention and control of bTB in the Animal Health Sector

- Prevent contact between cattle and wildlife reservoirs of Mycobacterium bovis.
- Maintain good hygiene practices in farms, including disinfecting equipment and facilities.
- Implement regular testing of cattle herds for *Mycobacterium bovis* infection.
- Remove and cull infected animals to prevent further spread (not practiced currently)
- Research and deploy vaccines for cattle in endemic regions to reduce disease incidence (not practiced currently)

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6. Anthrax

6.1. Introduction

Anthrax is primarily a zoonotic disease in herbivores caused by a bacterium called *Bacillus anthracis*. Sheep, goats, cattle and other herbivores are primarily affected. Humans are secondarily infected through contact with infected animals or contaminated animal products such as meat or hides, and rarely from injection drug use. Anthrax is a seasonal disease and its occurrence is affected by temperature, rain or drought. Climate probably acts directly by influencing the way in which the animal comes into contact with the spores (for example, grazing closer to the soil in dry periods when grass is short), or indirectly by affecting the general status of health of the host and thereby affecting the level of resistance to infection.

Anthrax in humans usually depends on the route of *B. anthracis* inoculation. Cutaneous anthrax which results from direct inoculation through the skin is the most common form and accounts for > 95% of human cases. Ingestion of anthrax usually results from consumption of infected meat. Inhalation anthrax results from inhalation of aerosolized spores. Injection anthrax, which is relatively new, results from injection of heroin contaminated with B. anthracis spores. Anthrax meningitis can complicate any form or occur alone.

Prior to the development of the vaccine in the 1930s, anthrax was regarded as a disease of major health or economic importance and was the foremost cause of uncontrolled mortality in cattle, sheep, goats, horses and pigs worldwide. The disease is still enzootic worldwide and *B.anthracis* has always been high on the list of potential agents with respect to biological warfare and bioterrorism.

6.2. Epidemiology

Worldwide, the estimated incidence of human anthrax decreased from between 20,000 - 100,000 cases per year in 1958, to 2,000 per year during the 1980s. The progressive global reduction in livestock anthrax cases over the past decades has been attributed to successful national preventive strategies. The majority of the cases (~95%) are cutaneous anthrax, which is treatable with antibiotics if identified early. Untreated fatality rate for cutaneous is 20-20%, gastrointestinal is 25-60% and inhalational with 45-80%.

Regionally, China and Vietnam reported cutaneous anthrax till 2021, while Orissa state in India has reported 1208 human cutaneous anthrax cases between 2008-2023.

In Bhutan, sporadic anthrax outbreaks have been reported among animals, posing risks to persons who come into contact with the infected animals. From 1998-2012, 34 anthrax outbreaks in animals have been reported from Samtse, Dagana, Chukha, Zhemgang, Wangdue, Mongar, Trashigang, Tsirang, Trongsa, Sarpang and Haa with 11 outbreaks of anthrax reported in 2012 alone. A total of 9 cases of human cutaneous anthrax were recorded in 2010 alone.

6.3. At-risk groups

At-Risk Group	Risk Factor
Workers handling animal products	Exposure to contaminated wool, meat, hair, skin, bones, or bone products
Veterinarians	Close contact with infected animals or animal products
Livestock workers in endemic areas	High exposure due to handling sick or dead livestock
Households/breeders consuming dead livestock	Eating meat contaminated with anthrax spores
Military personnel	Possible exposure during field operations, especially in endemic or conflict zones
Laboratory workers	Handling anthrax samples in lab settings
Emergency response workers	Response to bioterrorism incidents involving anthrax
People without footwear outdoors	Walking/playing barefoot in fields increases contact with anthrax spores in soil

Table 1. Risk factors and at-risk groups

6.4. Causative agent

6.4.1. Causative Agent

Caused by ***Bacillus anthracis***, a gram-positive, rod-shaped, spore-forming encapsulated bacterium.

Forms of the Bacteria:

- Vegetative form: Found within the host in low-oxygen environments.
- Spore form: Sporulation develops when exposed to air outside the host and are extremely resistant to heat, cold, pH, desiccation, chemicals, irradiation and other adverse conditions, and can survive for years in soil, wool, and hair of infected animals.

6.4.2. Pathogenesis

B. anthracis possesses three primary virulence factors namely extracellular capsule and two exotoxins. The capsule prevents phagocytosis of the vegetative bacteria by macrophages, allowing it to evade the immune system. Two toxins also evade the immune system by disrupting various immune cell functions.

Anthrax infection is initiated by spore forms of bacillus anthracis. Upon entering the host through inhalation, ingestion and cuts in the skin, the spores are phagocytosed by macrophages and carried

to regional lymph nodes. The spores germinate inside the macrophages and become vegetative bacteria which multiplies in the lymphatic system and enters the bloodstream. During the multiplication of vegetative bacteria two toxins; Lethal toxin (LT) and Edema toxin (ET) are produced which are the main virulence factors of the bacteria. These toxins are primarily responsible for tissue oedema, necrosis, ulceration and septicaemia and shock.

Type of Anthrax	Mode of Transmission	Key Characteristics
Cutaneous	Spores enter through cuts or abrasions on the skin	Most common form; causes black eschar (necrotic ulcer)
Inhalation	Inhalation of spores (e.g., handling contaminated animal products or bioterrorism)	Most severe form; primarily affects lymph nodes rather than the lung parenchyma; often fatal if untreated
Ingestion	Eating undercooked or raw meat from infected animals	Two forms exist; Oropharyngeal and gastrointestinal anthrax may cause massive gastrointestinal bleeding with high fatality rate if left untreated
Injection	Intravenous drug use involving contaminated substances	Rare; reported among drug users; causes deep tissue infection

Table 2. Types of anthrax, transmission mode and clinical characteristics

6.5. Transmission

- Cutaneous Anthrax which is the most common, is transmitted by entry of spores through cuts or abrasion of skin, which results in black eschar (necrotic ulcer)
- Inhalation Anthrax (most severe), is through inhalation of spores, often associated with handling contaminated animal products or bioterrorism.
- Gastrointestinal Anthrax is acquired through consumption of uncooked or undercooked meat from infected animals.
- Injection anthrax is acquired through intravenous routes which are reported among intravenous drug users.
- The records of person-to-person transmission exist but such instances are very rare.

6.6. Incubation period

The incubation period for Anthrax depends on the route of transmission.

Type of Anthrax	Incubation period
Cutaneous	Typically, 2-6 days but it can range from few hours - 21days
Inhalation	Typically, from 1-7 days but it can be as long as 2 months
Ingestion	1-7 days after consumption of contaminated food.
Injection	1-4 days after exposure

Table 3. Incubation period for different forms of anthrax

6.7. Clinical Features

There are four major anthrax syndromes: cutaneous, inhalation, ingestion (gastrointestinal) and primary anthrax meningitis (rare).

6.7.1. Cutaneous anthrax

- It is the most common form of Anthrax and accounts for over 95% of human cases worldwide.
- It occurs when Bacillus anthracis spores enter the skin through cuts or abrasions.
- Greater than 90% of lesions are found on exposed areas like Face, neck, arms and hands
- People who handle infected animals or contaminated products like wool, hides or hair are at risk of acquiring cutaneous anthrax.
- Without treatment, up to 20% of people with cutaneous anthrax die whereas almost all patients with cutaneous anthrax will survive with appropriate treatment

6.7.1.1. Clinical Progression

Begins as a small, painless, often itchy (pruritic) papule and rapidly enlarges and forms a central vesicle or bulla which progresses to erosion, leading to a painless necrotic ulcer with a black, depressed eschar.

Although antibiotic treatment will rapidly kill the infecting bacteria, the characteristic lesion will take several days to evolve and possibly weeks to fully resolve, presumably reflecting toxin-induced damage and repair. Clinicians need to be aware of the delay in resolution and not prolong treatment unnecessarily or resort prematurely to surgery.

6.7.1.2. Associated Findings

- Extensive edema of surrounding tissues due to toxin release
- Regional lymphadenopathy and lymphangitis are common
- A black eschar with extensive lymphedema is highly suggestive of anthrax

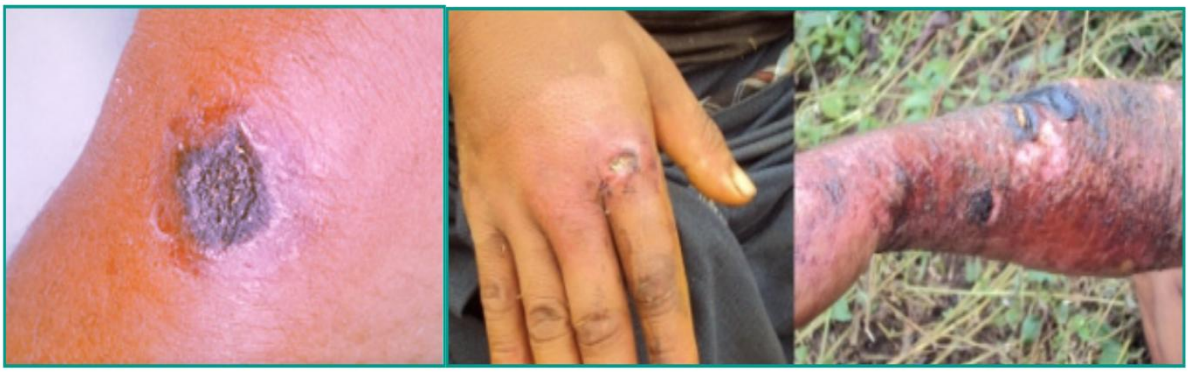


Figure 1: Cutaneous form of anthrax in humans with eschar and extensive skin reaction

6.7.1.3. Differential Diagnosis of Severe Cutaneous Anthrax

- For facial, neck, or anterior chest wall lesions, consider:
    - Orbital cellulitis
    - Dacryocystitis
    - Deep neck tissue infections
- Other conditions to rule out:
    - Necrotizing soft tissue infections, especially from Group A Streptococcus
    - Gas gangrene
    - Severe cellulitis caused by Staphylococcus species
- Key distinguishing features:
    - No gas formation in cutaneous anthrax
    - No abscess formation unless secondary bacterial infection is present (e.g., with streptococci or staphylococci)

6.7.2. Inhalation anthrax

- It is also known as **Woolsorter's Disease**, is the most severe and life-threatening form of anthrax infection.
- Humans acquire infection by inhaling *Bacillus anthracis* spores.
- High-risk groups include individuals who work in places such as wool mills, slaughterhouses and tanneries
- Inhalation anthrax starts primarily in the lymph nodes in the chest before spreading throughout the rest of the body.

- The term “inhalational anthrax” has replaced older terms like “pulmonary anthrax” since the infection primarily affects lymph nodes rather than the lung itself.
- Without treatment, inhalation anthrax is almost always fatal. However, with aggressive treatment, about 55 percent of patients survive.

6.7.2.1. Pathogenesis

- Spores are inhaled into the lungs, where no active infection develops.
- Macrophages engulf the spores and transport them to the lymphatic system.
- Germination and initial bacterial multiplication begin inside macrophages during transit.
- In the lymph nodes:
    - Spores become vegetative cells.
    - These cells kill the macrophages and are released into the bloodstream.
- The bacteria multiply rapidly in the blood, resulting in fatal septicaemia if untreated.

6.7.2.2. The clinical features of inhalation anthrax consist of two phases

1. Prodromal
2. Fulminant

Phases	Prodromal	Fulminant
Clinical features	• Fever • Body aches, • Malaise, fatigue, • Cough • Sore throat, • Chest pain/discomfort. It may mimic a viral respiratory infection. The symptoms last for 4-5 days.	Rapidly progressive respiratory symptoms: • Severe dyspnea • Hypoxemia • High fever • Shock Often results in high mortality within hours Non-thoracic symptoms may include: • Nausea, vomiting, abdominal pain • Headache, sweating, altered mental status Chest X-ray findings: • Infiltrates, pleural effusion Mediastinal widening (mediastinal lymphadenopathy)

Table 4. Clinical features of Inhalational anthrax

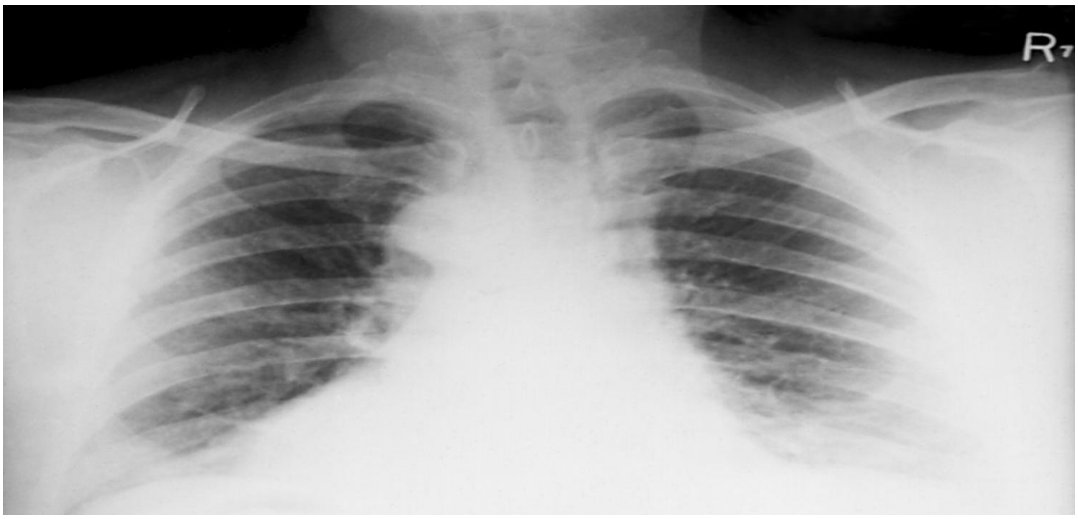


Fig. A classic finding is widening of mediastinum secondary to mediastinitis

6.7.2.3. Differential diagnosis of inhalation Anthrax

- Mycoplasmal pneumonia, viral pneumonia
- legionnaires' disease,
- Psittacosis, tularaemia,
- Q fever
- Histoplasmosis and coccidiomycosis
- Malignancy.

6.7.3. Ingestion anthrax

- There are two forms of Ingestion anthrax:
    - Oropharyngeal (less common)
    - Gastrointestinal form.
- Acquired by consuming food/ drinks contaminated by bacillus anthracis or by consumption of undercooked meat of infected animals
- The spectrum of disease ranges from asymptomatic to severe sepsis, septic shock and death within 2 - 3 days.
- Mild cases with gastroenteritis attract little attention and may go unreported.
- The suspicion of alimentary canal anthrax depends largely on physician's awareness and alertness paying particular attention to patient's history of consuming contaminated food/drink or meat of likely infected animal
- Without treatment, more than half of patients with gastrointestinal anthrax die but with proper treatment, 60 percent of patients survive.

6.7.3.1. Pathogenesis

Bacillus anthracis spores are ingested through consumption of raw or undercooked meat of an infected animal. These spores germinate in the intestines transforming into vegetative bacteria which multiply particularly in the intestinal mucosa. During its replication, two key toxins- lethal toxin (LT) and oedema toxin (ET) are produced. These toxins cause damage to the intestinal lining leading to inflammation and formation of ulcers.

The lesions occur commonly in the ileum and caecum, although it can affect anywhere within the gastrointestinal tract. The character of lesions are usually multiple, superficial ulcers surrounded by oedema. Pathological examination shows mucosal ulceration with oedema, enlarged and hemorrhagic regional lymph nodes and necrosis is sometimes present.

	Oropharyngeal Form	**Gastrointestinal Form**
Main clinical symptoms	• Fever • Sore throat • Dysphagia • Hoarseness • Painful regional lymphadenopathy in the involved side of the neck Swelling around the lymph node may progress and result in extensive swelling of the neck and anterior chest wall compromising airway.	• Fever • Nausea & vomiting • Diarrhea • Abdominal pain Anorexia & Asthenia • Malena • Hematemesis After 24 hours of initial non-specific symptoms some may progress to develop more severe forms with: • Bloody diarrhea. • Massive GI bleed

Clinical signs	-The lesions are generally localized on the buccal mucosa, tongue, tonsils or posterior pharynx wall. - The oral lesion is generally 2–3 cm in diameter and covered with a grey pseudo membrane surrounded by extensive oedema. -Lessions especially on tonsils may be ulcerated. - Regional lymphadenopathy with extensive oedema of neck and anterior chest wall - Signs of respiratory distress may be present	- Ulcers can occur in the esophagus, stomach and intestines, with the ileum and cecum being the most commonly affected sites -Signs of bowel perforation or obstruction may be present - Ascites can be massive - Signs of sepsis and shock
Differential diagnosis	• Diphtheria • Complicated tonsillitis • Streptococcal pharyngitis • Vincent angina • Ludwig angina • Para- pharyngeal abscess	• Food poisoning (in the early stages) • Acute surgical abdomen • Haemorrhagic gastroenteritis especially necrotizing enteritis caused by *Clostridium perfringens* • Dysentery (amoebic or bacterial)

Table 5: Clinical Features of Ingestion anthrax

6.7.4. Primary anthrax meningitis

- Meningitis due to anthrax is a serious clinical development which may follow any of the other three forms of anthrax.
- Anthrax meningitis is a haemorrhagic leptomeningitis. The prognosis is extremely poor as there is markedly elevated CSF pressure and the appearance of blood in the CSF.

- The presence of following clinical symptoms and signs should have high suspicion of anthrax meningitis:
    - Severe headache
    - Altered mental status
    - Presence of meningeal signs
    - Seizures
    - Cranial nerve palsies
    - Limb weakness
    - Papilloedema
- Differential diagnosis should include bacterial meningitis, cerebral malaria and diffuse subarachnoid hemorrhage.
- CSF finding includes hemorrhagic CSF with increased number of white blood cells and Low glucose levels.
- The definitive diagnosis is obtained by visualization of the capsulated bacilli in the CSF and/or by culture.
- CSF PCR should also be considered.

6.7.5. Complications of Anthrax

- Sepsis with shock
- Respiratory failure and ARDS
- Meningitis
- Renal failure
- Coagulopathy and severe bleeding
- Arrhythmias
- Fluid collections such as pleural effusions, pericardial effusions, ascites

6.8. Laboratory Diagnosis of Anthrax

Laboratory diagnosis for anthrax should be attempted only by a laboratory well trained to do so. A high index of suspicion of the disease is important. Collection and transportation should be carried out under strict aseptic conditions.

Diagnosis of anthrax from suspected clinical samples of humans and either dead/live 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.

6.8.1. Collection of specimens

Note: Bacillus anthracis is a risk group 3 micro-organism which needs very cautious handling especially during sample collection, processing, transport and testing in the laboratory or in the field. Therefore, practice of bio-safety guidelines, infection prevention measures and proper waste disposal should be ensured at all times.

6.8.1.1. Cutaneous Anthrax

Collection of swabs from the lesions depend on the stage of the lesion - collect two swabs as follows;

a. **Lesion exudates** and **skin swabs** are collected from individual suspected cutaneous anthrax. Sample should be taken prior to antibiotic treatment. The specific site of the sampling will depend on the stage of the lesion
b. **Vesicular stage:** In a sterile manner, soak sterile dry swabs in vesicular fluid from previously unopened vesicles. Transport the swab at room temperature.
c. **Eschar stage:** Rotate sterile swabs for 2-3 seconds beneath the edge of eschar without removing it (pre-moistened with sterile saline)
d. **Ulcer stage:** If no vesicle or eschar is present, swab the base of the ulcer using a sterile moist swab (pre-moistened with sterile saline).

Blood culture sample is collected for the inhalation and gastrointestinal anthrax with highly suspicious sepsis. Approximately, 10 ml of whole blood is collected aseptically in commercial blood culture bottles. For molecular detection, whole blood is collected in an EDTA tube.

6.8.1.2. Inhalation anthrax: respiratory samples like sputum, pulmonary effusion fluid and bronchial biopsy are collected in sterile containers.

6.8.1.3. Gastrointestinal anthrax: samples such as ascitic fluid, feces, vomit or rectal swab may be collected if **gastrointestinal anthrax** is suspected.

Cerebrospinal fluid samples can also be obtained by lumbar puncture in anthrax meningitis cases.

6.8.2. Storage, Packaging and Shipment

- Store the samples at 2-8°C if testing within a week or freeze at -20°C or lower for longer storage
- Avoid repeated freeze-thaw cycles.
- Clinical specimens of probable and suspected Anthrax should be shipped as Category A, using proper packaging (triple packaging), labelling, markings, and documentation as per UN approval

- Sample collection, packaging, and transport of suspected anthrax specimens should be done in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations (Refer National guideline for sample collection, packaging and transport of biological specimens).

6.8.3. Infection Control and Biosafety during specimen collection

1. Collect samples using full PPE (N95 mask or equivalent, gown, gloves, eye protection)
2. Follow strict infection control, decontamination, and biohazard protocols when handling all specimens

6.8.4. Infection Control and Biosafety during testing

1. Handle all samples using a biosafety cabinet (BSC, Class II or higher) until inactivation.
2. Non-inactivated samples are handled in BSL-3 or higher, if BSL- 3 is not available, inactivate under strict BSL-2 or within gloveboxes.
3. Decontaminate all surfaces and equipment after handling samples.

6.9. Laboratory Diagnostic Test

6.9.1. Microscopy

In resource-limited settings, a rapid diagnosis can be established by direct microscopy, which can demonstrate the presence of Gram-positive bacilli with a characteristic boxcar-shaped morphology

Direct examination of anthrax bacilli can be done by staining of lesion swabs with laboratory stains under the compound microscope.

6.9.2. Culture

Culturing *B. anthracis* from clinical specimens is the gold standard for diagnosing anthrax. Depending on the form of disease, *B. anthracis* can be cultured from the following specimens as stated below. Samples should be ideally taken before the patient starts antibiotic treatment. The type of samples will depend on the exposure and/or symptoms the patient has. Blood can be cultured under Biosafety level III or higher level to isolate Bacillus anthracis

6.9.3. PCR

Specimens like whole blood, biopsy/ tissue collected from papule or vesicle, body fluids and skin lesion including drain fluid can be sent for PCR test from suspected anthrax for confirmation through detection of B. anthracis DNA.

******Any suspected case of anthrax should be tested only in a Biosafety Level 3 (BSL-3) laboratory. The only BSL-3 laboratory in the country is the RCDC. Although the test for anthrax is available at the NRH and RRH, they only have BSL-2 laboratories, where such tests should not be performed. Testing in BSL-2 laboratories should only be considered after proper inactivation, conducted under strict BSL-2 conditions or within certified gloveboxes**

Laboratory Test	Testing centers	Biosafety measures
Microbiological: Blood culture with application of automatic bacteriological analyzer Blood culture system, MALDI-TOF MS **Microscopy:** Direct examination of bacillus under microscope.	• Blood culture facilities are available at national and regional health centers equipped with BSL-2. • Primary clinical specimens (such as blood, cerebrospinal fluid, and other body fluids) can be handled at BSL-2 using standard microbial practices, provided that procedures that generate aerosols are conducted inside a certified Class II biological safety cabinet (BSC) or certified gloveboxes to minimize risk of airborne exposure • Any further manipulation, especially once B. anthracis is suspected or confirmed, should be shipped to RCDC for further confirmation.	• Always use a certified biological safety cabinet for potential aerosol-generating procedures. • **If Bacillus anthracis is suspected or confirmed, escalate to BSL-3 for all culture manipulation.** • Decontaminate surfaces and use appropriate personal protective equipment (PPE) at all times.

Molecular: Detection of anthrax DNA	• **RCDC has BSL 3-laboratories to handle and process the suspected anthrax samples**	

Table 6. Facilities with the types of testing available and their corresponding BSL levels.

6.9.4. Routine Investigation

- CBC, ESR, CRP
- RFT, LFT
- Viral marker
- Blood CS, CXR

6.10. Management

Early diagnosis of anthrax and early initiation of appropriate treatment are critical to improve the outcome. Antimicrobial drug susceptibility testing (DST) should be done whenever possible and the choice of antimicrobial drug might need to be modified based on the DST results.

Penicillin-class antibiotics are generally effective for post-exposure prophylaxis and treatment of anthrax. However, **<10% of B. anthracis isolates show natural resistance. Therefore, penicillin should be used only if susceptibility is confirmed.**

Every patient with cutaneous anthrax should be assessed for modes of exposure, signs and symptoms of systemic infection and meningitis.

All patients with systemic anthrax should be hospitalized and should be treated urgently with IV antimicrobial combination therapy and antitoxin (raxibacumab, obiltoximab or anthrax immunoglobulin).

Treatment duration and choice of antibiotic depends on the route of exposure and type of Anthrax:

6.10.1. Antimicrobial therapy for adults with cutaneous anthrax without signs and symptoms of meningitis

Persons with cutaneous anthrax without signs and symptoms of meningitis, the antibiotics can be administered orally and the treatment regimen should continue for 7–10 days, or until clinical criteria for stability are met.

Table 7. Empiric treatment regimens for adults aged ≥18 years with cutaneous anthrax without signs and symptoms of meningitis, by descending order of preference

Doxycycline	**100 mg every 12 hours orally**
or	
Ciprofloxacin	**500 mg every 12 hours orally**
or	
Levofloxacin	**750 mg every 24 hours orally**
For penicillin susceptible strains	
Amoxicillin	**1 g every 8 hours orally**
or	
Penicillin V	**500 mg every 6 hours orally**

Note: If an aerosol exposure has occurred, patients should transition from a treatment to a PEP regimen and continue for a total 60 days from exposure.

Table 8. Empiric treatment regimens for children aged ≥1 month to <18 years with cutaneous anthrax without signs and symptoms of meningitis, by descending order of preference

Antibiotics	**Dose and Routes**
Ciprofloxacin	15 mg/kg every 12 hours orally (maximum 500 mg/dose)
or	
Levofloxacin	<50 kg: 8 mg/kg every 12 hours orally (maximum 250 mg/dose) ≥50 kg: 750 mg every 24 hours orally
or	
Doxycycline	<45 kg: 2.2 mg/kg every 12 hours orally (maximum 100 mg/dose) ≥45 kg: 100 mg every 12 hours orally
For penicillin sensitive strains	
Amoxicillin	25 mg/kg every 8 hours orally (maximum 1 g/dose)

Penicillin V	12.5–18.7 mg/kg every 6 hours orally (maximum 500 mg/dose)

Table 9. Antitoxins options for adults ≥ 18 years with cutaneous anthrax without signs and symptoms of meningitis.

Raxibacumab	40 mg/kg single IV dose
or	
Obiltoxaximab	16mg/kg IV single dose
AIGIV (Anthrax immunoglobulin intravenous)	420 units IV (840-unit can be considered for severe cases)

Note:

- Antitoxin for cutaneous Anthrax should only be used if antimicrobial drugs are not available or not appropriate.
- Hypersensitivity and anaphylaxis have been reported after raxibacumab and obiltoxaximab administration, therefore premedication should be administered before administering antitoxin.

6.10.2. Antimicrobial therapy for adults with systemic anthrax with or without meningitis

- Systemic anthrax treatment (with or without meningitis) should include:
- Two bactericidal drugs from different antimicrobial classes

  - Plus

- a protein synthesis inhibitor (PSI) or an RNA synthesis inhibitor (RNAI)

- Adjust or continue therapy based on antimicrobial susceptibility results once available.
- All patients with systemic anthrax should be given single dose antitoxin as adjunctive therapy in addition to antibiotics.
- Oral formulations of ciprofloxacin and doxycycline can be considered for patients with an intact gastrointestinal tract where absorption is expected to be complete after oral administration

6.10.3. Duration of the treatment

- Minimum of 2 weeks or longer.
- May be shortened and switched from IV to oral based on clinical improvement and medical judgment.

Post-exposure prophylaxis (PEP) considerations in aerosol exposure following completion of treatment:

- Immunocompetent patients treated for systemic anthrax do not require additional PEP, as they likely develop natural immunity.
- Immunocompromised patients should transition to oral PEP and the total duration of antimicrobial therapy (treatment + PEP) should be 60 days from illness onset.

Table 10. Empiric treatment regimens for adults aged ≥18 years with systemic anthrax with or without meningitis

Antibiotics	Dosage and route
Meropenem (Bactericidal)	2 g IV every 8 hours
plus	
Ciprofloxacin (Bactericidal)	400 mg IV every 8 hours
plus	
Doxycycline (protein synthesis inhibitor)	200 mg IV once followed by 100 mg IV every 12 hours
Plus	
Any of the following antitoxins	
Raxibacumab	40 mg/kg IV
or	
Obiltoxaximab	16 mg/kg IV
or	
AIGIV	420 units IV (840-unit can be considered for severe cases)

Table 11: Empiric treatment regimens for children aged ≥1 month to <18 years with systemic anthrax with or without meningitis.

Antibiotics	**Dose and Routes**
Meropenem	40 mg/kg every 8 hours IV (maximum 2 g/dose)
plus	
Ciprofloxacin	10 mg/kg every 8 hours IV (maximum 400 mg/dose)
plus	
Doxycycline	<45 kg: 2.2 mg/kg loading dose IV (maximum 200 mg/dose), then 2.2 mg/kg every 12 hours IV (maximum 100 mg/dose) ≥45 kg: 200 mg IV loading dose, then 100 mg every 12 hours IV
plus	
Antitoxin (single dose as an adjunct to antimicrobial drug)	
Raxibacumab	≤10 kg: 80 mg/kg as a single dose IV >10 to 40 kg: 60 mg/kg as a single dose IV >40 kg: 40 mg/kg as a single dose IV
or	
Obiltoxaximab	≤15 kg: 32 mg/kg as a single dose IV >15 to 40 kg: 24 mg/kg as a single dose IV >40 kg: 16 mg/kg as a single dose IV
or	
AIGIV	<10 kg: 1 vial (approximately 60 units) IV 10 to <18 kg: 2 vials (approximately 120 units) IV 18 to <25 kg: 3 vials (approximately 180 units) IV 25 to <35 kg: 4 vials (approximately 240 units) IV 35 to <50 kg: 5 vials (approximately 300 units) IV 50 to <60 kg: 6 vials (approximately 360 units) IV ≥60 kg: 7 vials (approximately 420 units) IV

6.11. Post Exposure Prophylaxis (PEP)

After exposure to anthrax, post exposure prophylaxis antimicrobials (PEPAbx) should be administered as early as possible.

PEP regimens for adults (≥18 years) exposed to *B. anthracis* may include:

- A single oral antimicrobial drug (preferred), or
- A single anthrax antitoxin (if antimicrobials are unavailable or unsuitable).

Both antimicrobial drugs and antitoxins are highly effective at preventing disease in animals however oral antimicrobial drugs are preferred over antitoxins due to their higher efficacy. Antitoxins also carry a risk of allergic reactions. Anti-toxins can be used as PEP only if antimicrobial drugs are not available or not appropriate.

Table 12. Post exposure prophylaxis antimicrobials duration

Population	**Type of exposure**	**Vaccine**	**PEPAbx duration**
Adults (18-65yrs)	Non-aerosol (Cutaneous or ingestion exposure)	Not recommended	7 days
Adults >65 yrs or immunocompromise	Non-aerosol /Aerosol	No	60 days
Adults (18–65 yrs)	Aerosol	If vaccine not Received	60 days
Healthy non pregnant adults (18 -65 yrs)	Aerosol	If Vaccine received	Upto 42 days after first dose or 2 weeks after last dose of vaccine, whichever is later

Note: Anthrax vaccine use in older adults (aged >65 years), pregnant or lactating persons, and children (aged <18 years) would be guided by data available at the time of an anthrax event.

If coadministration of anthrax vaccine and antitoxin is indicated, the only antitoxin that should be used is raxibacumab.

6.11.1. Post exposure antimicrobial regime

Table 13. Post exposure antimicrobial prophylaxis for adults (including pregnant and lactating) aged ≥ 18 years after exposure to bacillus anthracis by descending order of preference

Doxycycline	100 mg 12 hourly orally
or	
Ciprofloxacin	500 mg 12 hourly orally
or	
Levofloxacin	500 mg 24 hourly orally
For penicillin susceptible strains	
amoxycillin	1g 8 hourly orally
or	
Penicillin VK	500 mg 6 hourly orally

Table 14. Empiric postexposure antimicrobial prophylaxis for children aged ≥1 month to <18 years after exposure to Bacillus anthracis, by descending order of preference

Antibiotics	**Dosage and routes**
Ciprofloxacin	15 mg/kg every 12 hours orally (maximum 500 mg/dose
or	
Doxycycline	<45Kg: 2.2 mg/kg every 12 hours orally (maximum dose 100mg /dose) ≥45 kg: 100 mg every 12 hours orally
or	
Levofloxacin	<50 kg: 8 mg/kg every 12 hours (maximum 250 mg/dose) ≥50 kg 500mg every 24 hours
For Penicillin sensitive strains	

Amoxicillin	25 mg/kg every 8 hours orally (maximum 500 mg/dose)
Penicillin	12.5–18.7 mg/kg every 6 hours orally (maximum 500 mg/dose)

Table 15. Post exposure antitoxin prophylaxis for adults (including pregnant and lactating) aged ≥ 18 years after exposure to bacillus anthracis by descending order of preference

Raxibacumab	40 mg/kg as a single dose IV
or	
Obiltoxaximab	16 mg/kg as a single dose IV

Table 16. Table 6 Empiric postexposure antitoxin prophylaxis for children aged ≥1 month to <18 years

Antitoxin	**Dosage and route**
Raxibacumab	≤10 kg: 80 mg/kg as a single dose IV >10 kg to 40 kg: 60 mg/kg as a single dose IV >40 kg: 40 mg/kg as a single dose IV
or	
Obiltoxaximab	≤15 kg: 32 mg/kg as a single dose IV >15 kg to 40 kg: 24 mg/kg as a single dose IV >40 kg: 16 mg/kg as a single dose IV

Note: Anthrax Antitoxins should be given only if antimicrobial drugs are not available or not appropriate. Earliest administration of antitoxins after exposure has demonstrated greatest benefits. Premedication should be given prior to its administration due to risk of allergic reactions.

6.12. Prevention and Control

Preventing anthrax in humans requires a multi-sectoral approach involving public health, veterinary, and environmental interventions. Below are key strategies

6.12.1. Vaccination of at-risk groups

For persons with aerosol exposure: The incubation period for inhalation anthrax might be up to 60 days. Vaccination is recommended to prevent anthrax after completion of PEPAbx for adults aged 18–65 years with aerosol exposure.

Anthrax vaccine is given **subcutaneously** at **0, 2, and 4 weeks** after exposure (Subcutaneous or intramuscularly)

In **July 2023**, the **FDA approved** a **second-generation anthrax vaccine**, *anthrax vaccine adsorbed, adjuvanted,* for **post-exposure prophylaxis (PEPVx)** against **inhalation anthrax**.

It is given **intramuscularly** as a **2-dose series**, administered **2 weeks apart**.

To be used **along with antimicrobial prophylaxis (PEPAbx)** in **adults aged 18–65 years**.

Anthrax vaccine use in older adults (aged >65 years), pregnant or lactating persons, and children (aged <18 years) would be guided by data available at the time of an anthrax event.

However, vaccine is currently not available in the country.

6.12.2. Treatment and Vaccination in animal

In endemic areas, or if there is concern that the out- break may spread, the herd should be vaccinated. Further anthrax deaths can be expected to cease within 8 to 14 days of vaccination.

Decontamination of the site(s) where the index case or other case(s) died should be carried out.

Early recognition of signs of anthrax in animals and early initiation of treatment will prevent further spread of the disease.

6.12.3. Safe handling of animals and animal products

- Avoid direct contact with sick or dead animals, especially in areas where anthrax is known to occur.
- Wear personal protective equipment (PPE) such as gloves, masks, and protective clothing when handling animal carcasses or hides.
- Ensure proper disposal of animal carcasses by incineration or deep burial to reduce environmental contamination.

6.12.4. Food safety measures

- Do not consume meat from animals that died suddenly or under suspicious circumstances.
- Cook meat thoroughly to kill anthrax spores.
- Educate communities in endemic areas about the risks of consuming contaminated animal products.

6.12.5. Environmental management

- Decontaminate soil and areas exposed to infected animal carcasses.
- Monitor and restrict access to anthrax-contaminated sites to prevent human exposure.
- Implement controlled grazing and rotational farming to reduce soil spore exposure for animals.

6.12.6. Public awareness and education

- Conduct public health campaigns to educate at-risk populations about anthrax transmission, symptoms, and prevention.
- Disseminate materials on safe animal handling practices and the importance of seeking medical care promptly.
- Inform communities about the risks of handling raw hides, wool, or hair from animals in endemic regions.

6.12.7. Rapid detection and response

- Strengthen surveillance systems to detect and report anthrax outbreaks in humans and animals.
- Train healthcare providers to recognize the symptoms of anthrax (cutaneous, inhalational, and gastrointestinal forms) for early diagnosis and treatment.
- Implement isolation and treatment protocols to manage suspected or confirmed human anthrax cases.

6.12.8. Cross-sectoral collaboration (One Health Approach)

- Foster collaboration between public health authorities, veterinary services, and environmental agencies to address anthrax risks comprehensively.
- Share data and expertise across sectors to ensure a coordinated response to outbreaks.

6.12.9. Control in Animal Health Sector

Once an outbreak is detected in the animal population, the response measures to control the outbreak should be initiated as per the Guideline for prevention, surveillance and control of anthrax in humans and animals of Bhutan 2013.

6.13. References

1. Centers for Disease Control and Prevention. (2023). *Guidelines for the prevention and treatment of anthrax, 2023*. MMWR Recommendations and Reports, 72(RR-06). https://www.cdc.gov/mmwr/volumes/72/rr/rr7206a1.htm CDC

2. World Health Organization. (n.d.). *Guidelines for the surveillance and control of anthrax in humans and animals* (WHO/EMC/ZDI/98.6). WHO. World Health Organization

3. Ministry of Health, Bhutan & Ministry of Agriculture and Forests, Bhutan. (2013). *Guidelines for preparedness, surveillance and control of anthrax in human and animals in Bhutan*. (First edition). moh.gov.bt

4. National Centre for Disease Control, India. (2016). *Zoonotic diseases of public health importance*. Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India.

For rabies, please refer the National Guideline for Management of Rabies and Anti-rabies prophylaxis, 3rd edition, 2023, Revised in September 2024

Link:
https://moh.gov.bt/wp-content/uploads/2025/01/B5_NRMG_FINAL_VERSION_Sept-2024.pdf

7. Shiga toxin-producing Escherichia coli (STEC)

7.1. Introduction

Escherichia coli (E. coli) is one of the many groups of bacteria that normally live in the intestines of healthy humans and most warm-blooded animals. E. coli bacteria help maintain the balance of normal intestinal bacteria against harmful bacteria.

Enterohemorrhagic E. coli (EHEC) or Shiga toxin-producing E. coli (STEC) causes a severe intestinal infection in humans.

7.2. Epidemiology

Children under 5 years are at increased risk of EHEC infection and are more likely to develop severe outcomes such as hemolytic uremic syndrome (HUS) (CDC 2025). Adults above 65 years are also identified as a high-risk group for severe disease and hospitalization.

The Centers for Disease Control (CDC) estimated that foodborne *E. coli* O157:H7 is responsible for over 63,000 illnesses per year that leads to more than 2100 hospitalizations in the United States (CDC, 2009). STEC/EHEC infections are primarily transmitted through contaminated food or water and are a major cause of foodborne illness worldwide, including SEARO.

The burden of STEC/EHEC in the SEARO region is significant, but the detailed epidemiological data specific to this region are limited.

A retrospective data analysis across sentinel sites done by RCDC, Bhutan revealed diarrheagenic E. coli (DEC) as major pediatric diarrhea. DEC cases, STEC/EHEC accounted for approximately 5-6% of total cases.

7.3. Risk factors

Age is a major risk factor, where children under 5 and older adults (65+) are particularly vulnerable to severe EHEC outcomes.

Table 1. Common risk factors associated with STEC infections

Category	Risk Factors
Foodborne Exposure	- Eating undercooked meat - Drinking raw (unpasteurized) milk - Eating food contaminated with animal wastes
Waterborne Exposure	- Drinking contaminated/dirty/unboiled water
Animal Contact / Occupational Exposure	- Working with cattle, and other livestock - Touching animals and not practicing proper hand-washing afterwards
Poor Hygiene and Sanitation	- Not washing your hands like after touching/petting animals

7.4. Causative agent and pathophysiology

The infections caused by *E. coli* O157:H7 range from asymptomatic to severe. Few individuals can develop potentially fatal complications like hemolytic uremic syndrome (HUS). Chronic renal pathology may persist at times among those that survive.

Cattle are considered as the principal reservoirs for *E.coli* O157:H7. Fecal shedding of *E. coli* O157:H7 varies among the cattle population; a seasonal pattern with increasing prevalence in warmer summer temperatures has been shown (Renter et al., 2002).

7.5. Mode of transmission

Humans can become infected through various routes, including:

- Consumption of contaminated food, such as:
  - Unpasteurized milk
  - Raw vegetables
  - Undercooked meat
- Drinking contaminated water, especially from runoff near cattle farms
- Direct contact with:
  - Infected animals
  - Animal waste, such as manure used as fertilizer
  - Infected humans, particularly in childcare settings

7.6. Incubation period

The incubation period for EHEC infections is typically 3-4 days after exposure but it can range for 1-10 days. The incubation period is determined by infectious dose and host factors.

7.7. Clinical features

The infections caused by STEC range from asymptomatic to severe cases. Patients may present with anyone of these symptoms or as a constellation of following symptoms

- Abdominal pain
- Diarrhea (usually bouts of bloody/blood-stained)
- vomiting
- With or without fever

7.7.1. Complications

1. Gastrointestinal complications- perforations, obstruction, bleeding
2. Hemolytic uremic syndrome (HUS)

Hemolytic uremic syndrome (HUS) - A thrombotic microangiopathy (TMA) Characterized by:

- Thrombocytopenia
- Microangiopathic hemolytic anemia
- Acute kidney injury (AKI)

HUS is classified into two from: Typical HUS and atypical HUS

a. Typical HUS (Shiga toxin-associated HUS)

- Most common cause: Shiga toxin (often from *E. coli* O157:H7)
- HUS develops 3 to 10 days after the start of diarrhea
- High-risk group: Children under 5 years (more than 10% progress to HUS) ·

b. Atypical HUS (aHUS)

- Accounts for 5–10% of HUS cases and is caused by genetic mutations affecting the alternative complement pathway. It often begins with nonspecific symptoms like fatigue, pallor, and somnolence, progressing to acute kidney injury (AKI) with signs such as oliguria, uremia, and fluid overload.
- **It carries a high risk of progression to chronic kidney disease (CKD)** or end-stage renal disease (ESRD). Without treatment, about 50% of cases require dialysis, and the mortality rate can reach 25%, as kidney recovery is unlikely without intervention.

7.7.2. Differential Diagnosis

- Other enteric bacterial infections - such as *Salmonella*, *Campylobacter*, *Shigella*, and *Yersinia* spp
- Viral infections - Rotavirus, Norovirus, Adenovirus, Cytomegalovirus
- Parasitic infections - Entamobea histolytica, Giardia lamblia

7.7.3. Routine Investigation

- CBC (hematocrit & platelet count)
- Erythrocyte sedimentation rate (ESR)
- C reactive protein (CRP)
- Renal function test (RFT)
- Liver function test (LFT)
- Serum electrolytes (SE)
- Stool routine examination (RE)

Clinical samples collected for diagnosis of EHEC

Clinical specimens collected	Volume collected	Remarks
Fresh unformed stool sample collected within first week of illness (preferably diarrheal stool)	Approximately 1 gram or 1 ml.	-Preferred, gold standard specimen for detecting STEC -Ideally within 1 week of illness
Rectal swabs (not able to collect stool sample, especially neonate/ Children)	Place the swab immediately in the transport medium.	
Gastric content / Vomit	3-5 ml	Not recommended routinely

7.8. Laboratory test available for diagnosis of EHEC

Diagnosis of EHEC infection primarily involves laboratory testing of stool samples to detect the presence of the bacteria. Key diagnostic tests include;

7.8.1. Bacteriological test

a. Culture & Isolation-

Samples are cultured and isolated using enrichment and selective media to support the growth of E. coli which is further subjected for pathogenic E. coli speciation by PCR

b. PCR assays-

Suspected EHEC isolates are further confirmed by using Multiplex PCR for speciation of pathogenic E. coli including EHEC

Table showing facilities with the types of testing available

Laboratory Test	Health facilities	Remarks
Microbiological (culture & isolation)	RCDC	Transport using appropriate medium
Multiplex PCR	RCDC	To detect the stx1 and stx2 genes (to confirm with RCDC)

7.9. Management

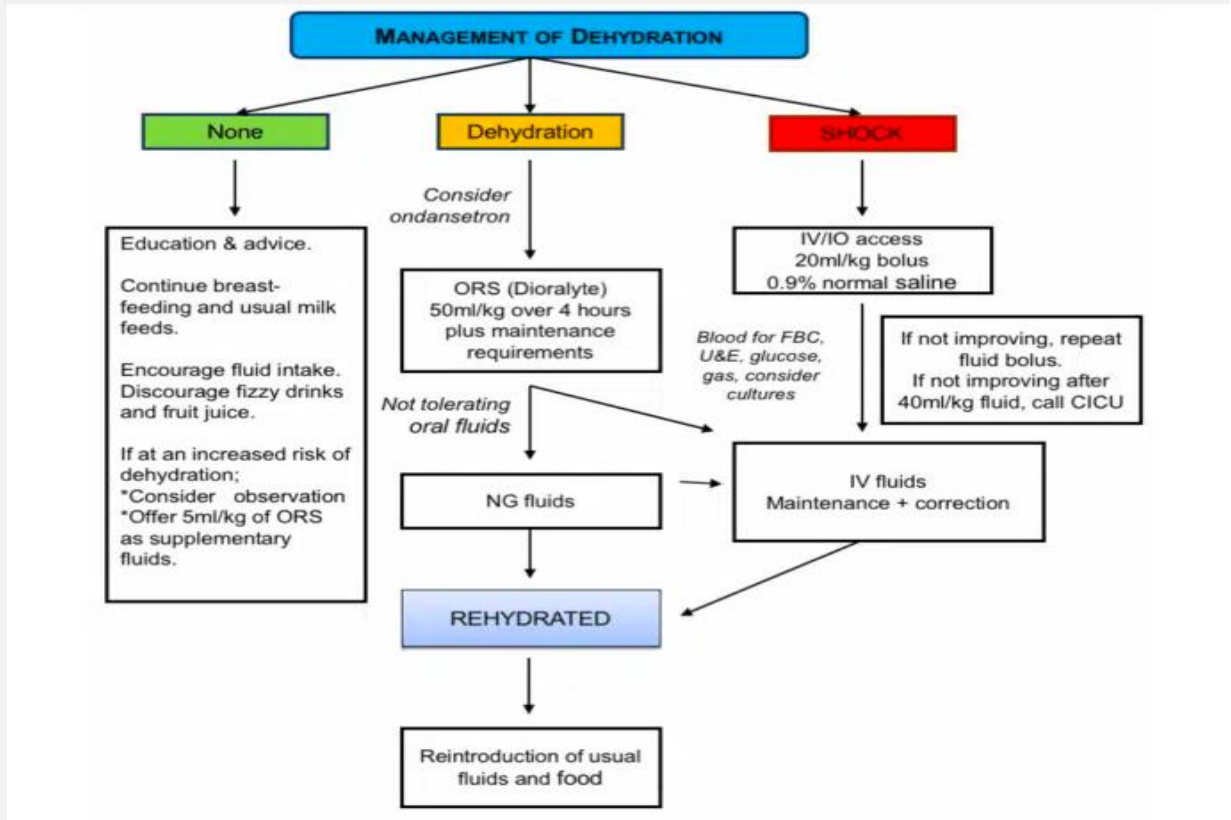
7.9.1. Mainly supportive care

- The mainstay of treatment is rehydration
- **Antimotility agents (eg. Loperamide) and Antibiotics are avoided**, as their use can trigger the bacteria to release more toxins and has been associated with worse outcomes

Assessment of dehydration

Increasing severity of dehydration			
Symptoms (remote and face-to-face assessments)	**No clinically detectable dehydration**	**Clinical dehydration**	**Clinical shock**
	Appears well	Appears to be unwell or deteriorating	–
	Alert and responsive	Altered responsiveness (for example, irritable, lethargic)	Decreased level of consciousness
	Normal urine output	Decreased urine output	–
	Skin color unchanged	Skin color unchanged	Pale or mottled skin
	Warm extremities	Warm extremities	Cold extremities
Signs (face-to-face assessments)	Alert and responsive	Altered responsiveness (for example, irritable, lethargic)	Decreased level of consciousness

	Skin color unchanged	Skin color unchanged	Pale or mottled skin
	Warm extremities	Warm extremities	Cold extremities
	Eyes not sunken	Sunken eyes	–
	Moist mucous membranes (except after a drink)	Dry mucous membranes (except for ‘mouth breather’)	–
	Normal heart rate	Tachycardia	Tachycardia
	Normal breathing pattern	Tachypnoea	Tachypnoea
	Normal peripheral pulses	Normal peripheral pulses	Weak peripheral pulses
	Normal capillary refill time	Normal capillary refill time	Prolonged capillary refill time
	Normal skin turgor	Reduced skin turgor	–
	Normal blood pressure	Normal blood pressure	Hypotension (decompensated shock)



7.9.2. Management of Hemolytic uremic syndrome

a. Supportive Management:

- IV fluids
- Avoid antibiotics
- Avoid antidiarrheal medications

b. Renal Replacement therapy (dialysis)

c. Blood transfusion: Packed red cells may be used as needed. Platelet transfusions are avoided unless there is active bleeding, since they may exacerbate microangiopathic clotting.

7.10. Prevention, control and surveillance of EHEC

The following measures can help reduce the risk of infection and transmission

7.10.1. Hygiene and Sanitation

- Practice proper handwashing with soap and running water, especially after using the toilet, after animal contact, and before eating or preparing food.
- Proper disposal of wastes especially of infected individuals
- Avoid exposure to dirty water and accidental exposure to water.

7.10.2. Food Safety

- Follow the four food safety steps: clean, separate, cook, and chill.
- Wash raw fruits, vegetables, and all utensils and surfaces after contact with raw meat.
- Prevent cross-contamination by using separate utensils for raw and cooked foods.
- Proper cooking of meat and safe storage
- Only consume pasteurized/boiled milk, water/beverages.

7.10.3. Animal Contact

- Wash hands after contact with animals or their environments.

7.10.4. Water and Swimming Safety

- Avoid swallowing water while swimming.
- Individuals with diarrhea should avoid swimming in public waters, sharing baths, and handling food for others.

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8. Non-Typhoidal Salmonellosis

8.1. Introduction

Salmonellosis is an infection caused by bacteria of the genus *Salmonella*, which are Gram-negative, rod-shaped, and facultative anaerobes. It is a major global public health concern, particularly in developing countries where sanitation and food safety standards are often inadequate. In many regions, cases peak during warmer months, which favor bacterial growth.

There are two main clinical forms:

- **Typhoidal salmonellosis** (caused by *Salmonella enterica* serotype Typhi or Paratyphi): causes enteric (typhoid) fever, a systemic illness.

- **Non-typhoidal salmonellosis (NTS)**: caused by other *Salmonella* serotypes (e.g., *S. Enteritidis*, *S. Typhimurium*), usually resulting in self-limiting gastroenteritis but can occasionally lead to invasive infections.

8.2. Epidemiology

Globally, *Salmonella* infections are among the most common causes of foodborne illness. The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2017 estimated that non-typhoidal salmonella resulted in 95·1 million cases, 50,771 deaths, and 3.1 million DALYs worldwide in 2017. It is ranked among the top contributors to foodborne disease burden.

In the WHO Southeast Asia Region including Bhutan-*Salmonella Typhi* and non-typhoidal *Salmonella enterica* account for approximately 20 million foodborne illness cases and over 56,000 deaths annually. Non-typhoidal Salmonella enterica attributed approximately 17,000 deaths. In South Asia, the incidence of non-typhoidal salmonella enterica is 881 per 100,000 population causing an estimated 0.9 deaths per 100,000 population, including 58 DALYs per 100,000.

South Asia has a particularly high burden, with Bhutan sharing similar risk factors due to food handling practices and high consumption of animal-source foods.

8.3. Causative agent

Salmonella species are gram-negative, flagellated facultative anaerobic bacilli in the Enterobacteriaceae family characterized by O, H, and Vi antigens. There are over 2,500 identified serotypes or serovars within the two species, Salmonella bongori and Salmonella enterica. This bacterium is widespread and resilient, capable of surviving for weeks in dry conditions and for months in water.

While all Salmonella serotypes can cause human infection, some are host-specific, like Salmonella enterica serotype dublin in cattle and serotype choleraesuis in pigs, often causing severe, invasive,

and life-threatening diseases in humans. Other serotypes, such as S. enteritidis and S. typhimurium, cause gastroenteritis in humans, which is usually mild but can be severe in young, elderly, or immunocompromised individuals. These two serotypes are the most common causes of animal-to-human transmission worldwide.

The common salmonella species prevalent in different animals include:

- Cattle- S. typhimurium, S. dublin, and S. newport;
- Sheep and goats- S. typhimurium, S.dublin, S.abortusovis, S.anatum, and S.montevideo
- Pigs- S.typhimurium and S.choleraesuis
- Poultry- S.enteritidis, S.typhimurium, S.gallinarum, and S.pullorum.

The most common animal reservoirs are chickens, turkeys, pigs, and cattle, including dozens of other domestic and wild animals. The major outbreaks are linked to mass gatherings or food festivals.

8.4. Source & mode of transmission

The ability of salmonellae to survive in meat and animal products that are not thoroughly cooked, it remains the main source of human infection.

The main routes of infection are:

Foodborne

- Undercooked meat, poultry, and eggs
- Contaminated dairy products and produce

Waterborne

- Drinking or using contaminated water

Fecal-Oral Route

- Poor hand hygiene after using the toilet or handling diapers

Animal Contact

- Handling infected animals (especially poultry, pig, cattles)

Person-to-Person

- Rare, but possible through close contact or shared contaminated surfaces

8.5. Risk Factors

The most vulnerable populations are:

- Children under 5 years and elderly over 65 years
- Individuals with malnutrition, immunodeficiencies, or chronic illnesses
- Food handlers and farmers in close contact with animals
- Individuals on acid suppressants or antibiotics

8.6. Incubation Period

The typical incubation period is 6–72 hours after exposure and most commonly within 12 to 36 hours.

8.7. Clinical features

Human salmonellosis manifests in three main clinical forms:

(1) Gastroenteritis

(2) Enteric fever

(3) Septicemia/extraintestinal infection.

The progression and severity of illness depend on host immunity and the virulence of the *Salmonella* strain. While gastroenteritis is the most common and often self-limiting, enteric fever and septicemia are more severe and may require prompt medical intervention. The severity of the infection and whether it remains localized in the intestine or disseminates to the bloodstream may depend on the resistance of the patient and the virulence of the Salmonella isolate.

1. Gastroenteritis

This is the most frequent form of salmonellosis. Symptoms typically begin **6 to 48 hours** after ingestion of contaminated food or water and include:

- Diarrhea (which may be bloody, especially in children) and is the cardinal manifestation
- Nausea and vomiting
- Abdominal cramps (reported in about two-thirds of cases)
- Fever (38°C–39°C) and chills
- Headache and myalgia

The illness is usually **self-limiting**, with fever and diarrhea lasting **2 to 7 days**. Dehydration is the most common complication.

2. Enteric fevers

Enteric fever refers to a **systemic, life-threatening illness**, classically caused by *Salmonella Typhi* (typhoid fever), but can also be caused by *S. Paratyphi* and occasionally by other *Salmonella* spp.

Key features include:

- Incubation period of **7 to 14 days**
- High, sustained fever
- Anorexia, headache, myalgia
- Abdominal discomfort; constipation more common than diarrhea
- Bradycardia (relative to fever) and hepatosplenomegaly in some cases

Enteric fever may be **preceded by a short-lived gastroenteritis phase**, and without treatment, can lead to serious complications including intestinal perforation or hemorrhage. **Prompt antibiotic therapy** is essential to reduce morbidity and mortality.

3. Septicemic form or extraintestinal infections

This form may occur **with or without gastrointestinal symptoms**, particularly in immunocompromised patients. Blood cultures may be positive even when stool cultures are negative.

Manifestations include:

- **Bacteremia and sepsis**, potentially leading to **shock and multi-organ dysfunction (MODS)**
- **Endovascular infections** (e.g., infected aortic aneurysms)
- **Endocarditis**
- **Musculoskeletal infections**: osteomyelitis, septic arthritis, reactive arthritis
- **Central nervous system infections**: meningitis, brain abscesses
- **Visceral abscesses** in the liver, spleen, or kidneys

Patients with salmonella bacteremia should be carefully assessed for **metastatic foci** of infection, particularly if febrile despite antibiotic therapy.

8.7.1. Relapsed infection

Relapse refers to the recurrence of symptoms or bacteremia after an initial recovery from Salmonellosis

Risk factors for relapse include:

- Immunocompromised states (e.g., HIV, malignancy, immunosuppressive therapy)
- Inadequate duration of antibiotic therapy (typically <7 days)

Key points:

- Relapse can occur **after an asymptomatic interval**
- May occur **without a detectable focus of infection**
- Often associated with persistent infection in the **reticuloendothelial system**

8.7.2. Asymptomatic Chronic Carriage

Chronic carriage is defined as continued shedding of Salmonella spp. in stool for more than one year after acute infection. Occurs in <1% of cases and is more frequent in individuals with biliary tract abnormalities, especially those with gallstones. Individuals may not exhibit any clinical symptoms.

Diagnosis is based on:

- An initial positive stool culture obtained at least one month after recovery from acute illness.
- Followed by repeated positive stool cultures over time.

8.7.3. Differential Diagnosis of non-typhoidal Salmonellosis

- Viral diarrhea- rota virus, adenovirus, norovirus
- Other bacterial diarrhea- Campylobacter, shigella, Vibrio, Clostridium difficile
- Protozoa – Cryptosporidium, Giardia, Cyclospora, Entamoeba

8.8. Laboratory Diagnostic Approach for Salmonellosis

8.8.1. Culture & Isolation

8.8.1.1. Stool

Stool is the primary specimen for diagnosing non-typhoidal Salmonella, especially in cases of gastroenteritis. Culture typically takes 24 to 72 hours. Multiple stool samples (1–3) may improve detection sensitivity.

8.8.1.2. Blood

Blood cultures are essential in suspected enteric fever (caused by Salmonella Typhi or Paratyphi) or **systemic salmonellosis(septicaemia)**. The time to detection ranges from 1 to 5 days, and sensitivity is approximately 40–80%. It is recommended to collect 2–3 blood culture sets before starting antibiotics to increase the likelihood of isolation.

8.8.1.3. Gastric content or vomit

Gastric content or vomit may help detect Salmonella early in infection, especially in children or when stool is unavailable, though they are not commonly used and have variable sensitivity and culture time.

8.8.1.4. Other samples (e.g., bile, urine, CSF, Bone marrow)

Other body fluids such as bile, urine, or cerebrospinal fluid may be cultured depending on the clinical presentation. These are not routine specimens but may help identify invasive or disseminated infections when Salmonella spreads beyond the gastrointestinal tract.

8.8.2. Clinical samples to be collected for diagnosis of salmonellosis

Clinical specimens collected	Volume collected	Remarks
Stool sample used mainly for diagnosing gastrointestinal salmonellosis.	1-2 gm or 1 ml	Collect in sterile, moisture-free containers without urine contamination.
Blood, essential for systemic infections	5-10 ml	Collected aseptically by venipuncture with multiple cultures improving diagnostic accuracy
Food sample, for outbreak investigations to identify sources of infection	Suspected food items are gathered using aseptic methods and stored in sterile receptacles or pouches.	Transport at cold chain at 2-8*C

8.8.2.1. Culture & Sensitivity

Automated blood culture systems are employed for the detection of bacteremia, with culture results typically becoming positive within 48 hours; nearly all positive results are identified within five days. Positive samples undergo further subculture, biochemical testing, and serotyping to identify the specific Salmonella serovar following culture growth.

For stool samples, initial is culture in enrichment medium to enhance and support the growth of salmonella before plating into the selective media

8.8.2.2.Biochemical identification

A panel of commonly used biochemical tests are performed for identification of salmonella species. If any suspected isolates, further confirmed by using bacteriological techniques such as MALDI-TOF MS and VITEK 2 system are performed to confirm diagnosis

8.8.2.3.Serotyping characterization

Salmonella serotyping determine the specific serovar of a Salmonella isolate by identifying the antigenic structures on the bacterial surface using specific antisera.

- Standard biochemical tests are used for confirming and identifying salmonella species

Laboratory Test	Health facilities	Remarks
Culture facilities to perform blood and stool C/S test	• Diagnostic laboratories at National, regional and selected distinct hospitals (Phuntsholing hospital) were equipped with culture facilities to perform microbiological testing including isolation and identification of salmonella species. • For RCDC, stool specimens were obtained for the purpose of Diarrheal surveillance through independent testing.	• Culture and Isolation is always performed under BSC to avoid contamination.
VITEK 2 system for rapid bacterial identification and antimicrobial susceptibility testing	• VITEK 2 system is available at JDWNRH and RCDC.	
Salmonella serotyping	• Salmonella serotyping is available at JDWNRH and RCDC.	
MALDI-TOF MS	• JDWNRH is established with MALDI-TOF MS technology which can supplement diagnosis at the genus and species level.	

Table 1. showing facilities with the types of testing available

8.8.3. Routine Investigations

- CBC, ESR, CRP, RFT, LFT, SE
- Blood culture
- Stool RE and CS
- ECHO (if indicated)
- USG of Abdomen (if indicated)

8.9. Management of Gastrointestinal Infection

It is usually self-limiting and resolves within 5-7 days. The treatment includes:

1. Hydration – Oral fluids and IV replacement if necessary
2. **Antibiotic Therapy - antibiotic therapy is not necessary** but is warranted for patients with severe illness or patients who are prone to high risk for invasive disease.

Severe illness in Salmonella gastroenteritis is characterized by:

1. Diarrhea (more than 9-10 stools per day)
2. High or persistent fever
3. Need for hospitalization

8.9.1. Treatment

Population	**Preferred Regimen**	**Alternative Regimen**
Non-pregnant and adolescents	Oral Ciprofloxacin 500mg BD or IV Ciprofloxacin 400 mg BD **OR** Ceftriaxone 1-2gm IV OD	Oral Cotrimoxazole (Septran) 480mg bd **OR** Oral Azithromycin 1gm STAT, followed by 500mg od or IV Azithromycin 500mg OD **OR** Oral Amoxycillin 1gm TID **OR** Ampicillin 1gm IV 4-6 hourly

Children	Azithromycin 10 mg/kg (max 500mg) Day 1, followed by 5 mg/kg (max 250mg) x 4 more days OR Ceftriaxone 50mg/kg (max 1gm) IV OD	• Ciprofloxacin • Ampicillin • Septran

8.9.2. Duration of Therapy - depending on the immune status of the patient and risk factors

Immunocompetent	5 - 7 Days
Immunocompromised	At least 14 days (weeks to months in some cases specially in the extraintestinal infections) with goal of preventing persistent or relapsing infection

8.10. Management of Extraintestinal infections

Extraintestinal infections caused by *Salmonella* occur when the bacteria spread beyond the gastrointestinal tract. While *Salmonella* typically causes self-limiting gastroenteritis, in certain populations or under specific conditions, it can lead to invasive or systemic infections.

Common Extraintestinal Manifestations of Salmonella Infection include:

- Bacteremia
- Endocarditis
- Osteomyelitis
- Septic Arthritis
- Meningitis
- Brain Abscess
- Urinary Tract Infections
- Lung Infections (rare)

1. Bacteriemia

For patients with salmonella bacteremia without other extraintestinal infections, the duration of therapy depends on the host immune status and risk factors.

Adults	Children
Preferred agents: Ceftriaxone 1- 2 gm IV OD **OR** Ciprofloxacin 400mg IV BD Oral: 500 – 750mg orally BD **Alternative:** Oral septran: 1 double strength BD **OR** Ampicillin 2gm IV 4-6 hourly **OR** Oral Amoxycillin 500mg TID	**Preferred agents:** Ceftriaxone – 50 - 75 mg/kg IV OD or in two divided doses (maximum 2 g/day) **OR** Ciprofloxacin 10 mg/kg IV every 8 to 12 hrs (max 400 mg per dose) Oral: 10 to 20 mg/kg per dose every 12 hrs (max daily dose 1.5 gm) **Alternative:** Ampicillin 50mg/kg IV or IM - QID (max 8g/day) **OR** Oral Amoxicillin 40-50mg/kg/day orally divided every TID (max 500mg/dose)

- For an immunocompetent host – duration of therapy 10–14days.
- For immunocompromised hosts - duration of therapy 4-6 weeks is given to prevent recurrence or relapse.

3. Endovascular infection, endocarditis, musculoskeletal infection, or central nervous system infection

Preferred agents	Adults	Children
Ceftriaxone **OR**	1-2 gm iv od	50-75mg/kg IV or IM OD or in two divided doses (maximum 2g/day)
Ciprofloxacin	400mg IV BD 500-750 mg oral BD	10mg/kg IV every 8 to 12 hours 10 mg-20 mg/kg oral every 12 hours

8.10.1. Duration of treatment depends on patients' immune status

- Immunocompetent: 4 to 6 weeks
- Immunocompromised:6 to 8 weeks

8.10.2. Suppressive Therapy

Suppressive therapy is given in cases of *extraintestinal Salmonella* infection following completion of the initial course of antibiotics. Antibiotics are continued for an **additional 10 to 14 days.** It is recommended for patients in the following situations:

- Immunocompromised individuals due to higher risk of persistent or relapsing infection.
- Patients with indwelling devices or hardware (Suppressive therapy is given due to the difficulty in fully eradicating infection and the risk of severe relapse) such as:
  - Vascular grafts
  - Prosthetic heart valves
  - Orthopedic implants
    Suppressive therapy is given due to the difficulty in fully eradicating infection and the risk of severe relapse.

8.10.3. Relapse

- Among immunocompromised patients, once infection becomes established within the reticuloendothelial system relapse may occur even after an asymptomatic interval and in the absence of a known focus of infection (such as osteomyelitis or an abnormal biliary or urinary tract)
- Relapses also occur in patients treated **with less than 10** days of antibacterial therapy

8.10.4. Management of Relapsing or persistent infections

- Decisions for further antibiotic therapy should be individualized in consultation with infectious disease experts.
- **Antibiotic therapy duration is generally 4- 6 weeks**
- Other therapies – like fecal transplant for eradication of antibiotic resistant salmonella in patients with relapsing gastroenteritis has been described.

8.10.5. Management of Asymptomatic carriage

No role for routine treatment for all asymptomatic carriers, treatment should be considered in selected cases like, health care workers who take care of immunocompromised patients, hosts or food handlers linked to outbreak.

Antibiotic Selection

Fluoroquinolone susceptible: Ciprofloxacin 500mg BD - for 4 weeks
Fluoroquinolone non susceptible: High dose amoxicillin (75 – 100 mg/kg per day) for 6 weeks OR Septran 480mg BD – 3 months

Follow up – Three stool cultures obtained over a week, beginning at least a week after stopping antibiotics, would provide reasonable documentation for eradication.

8.11. Prevention, Control, and Surveillance of salmonellosis (non-typhoidal)

Reducing the risk of *Salmonella* infection requires a combination of personal hygiene, food safety practices, animal handling precautions, and public health surveillance.

8.11.1. Hand Hygiene

Proper hand hygiene is critical to preventing the spread of *Salmonella* spp. Handwashing with soap and water is more effective than alcohol-based sanitizers against *Salmonella* spp.

8.11.2. Food Safety Practices

Safe food handling and cooking practices can significantly reduce the risk of infection.

- Avoid unpasteurized (raw) milk and foods made from unpasteurized milk
- Keep food preparation areas clean and sanitized

- Store raw meat and poultry separately from other foods
- Wash hands, cutting boards, countertops, and utensils thoroughly after handling raw meat or eggs
- Wash fresh fruits and vegetables before eating

8.11.3. Prevent cross-contamination

- Do not use the same knives or cutting boards for raw and ready-to-eat foods without cleaning in between
- Cooked foods can be re-contaminated by contact with raw foods or their drippings
- Keep raw poultry, meat, and seafood separate from ready-to-eat items

8.11.4. Cooking and Storage Temperatures

Ensure appropriate cooking and storage temperatures to eliminate bacterial contamination.

- Do not consume raw or undercooked eggs; use pasteurized eggs for uncooked dishes
- Cook poultry, meat, and hamburgers thoroughly until no longer pink inside
- Store food at safe temperatures:
    - Refrigerate at ≤ 4°C
    - Freeze at ≤ –18°C
- Defrost food safely in the refrigerator, cold water, or microwave—not at room temperature

8.11.5. Animal Contact Precautions

Direct or indirect contact with infected animals can be a source of *Salmonella*.

- Always wash hands after handling animals or cleaning animal environments
- Avoid contact with animals exhibiting signs of diarrhea
- Do not eat or prepare food in areas where animals are present

8.11.6. Recreational Water Safety

Ingestion of contaminated water during recreational activities can also pose a risk.

- Avoid swallowing water from lakes, rivers, or swimming pools
- Individuals with diarrhea should not:

    - Swim in public pools or natural water bodies
    - Share baths with others
    - Prepare or handle food for others

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9.Cystic Echinococcosis (CE)

9.1. Introduction

Echinococcosis is a zoonotic parasitic disease. It has been recognized as one of the neglected tropical diseases by the World Health Organization (WHO). It has also been recognized and listed by the Food and Agriculture (FAO) as one of the most important food-borne parasitic diseases. In general, there are three primary species of *Echinococcus* parasite that is of clinical and public health importance; cystic echinococcosis (CE) caused *E. granulosus s.l*, alveolar echinococcosis (AE), caused by *E. multilocularis*, and neotropical echinococcosis (NE), reported only in Central and South America, caused by the *E. vogeli* and *E. oligarthrus*. So far, only CE cases have been reported in Bhutan.

9.2. Background of the Disease

CE is one of the neglected tropical diseases (NTDs), caused by the larval stage of *Echinococcus granulosus s.l.* complex. **In Bhutan, currently only CE cases have been reported in hospitals and from communities especially from the Western and Central regions of the country.** Sporadic cases have been reported from almost every district in the country. CE poses a significant public health and animal health burden in endemic areas. CE is characterized by the development of cysts in the liver and other organs and is often diagnosed in advanced/late stages. In Bhutan, where livestock rearing and dog ownership/presence of free roaming dogs are widespread, CE remains a potential but underreported issue. Echinococcosis is categorized as a stage-2 zoonotic disease; transmission between humans does not occur.

9.3. Global disease burden

CE has a worldwide distribution, affecting many regions. WHO estimates an annual incidence of 188,000 cases, with more than 1 million people infected worldwide at any given time. Globally, many regions in South-America, Middle-East, Mediterranean and Eastern Europe, Central Asia, and wider Asia including China remain endemic/hyper-endemic for human CE. CE is among the most important food-borne parasitic diseases as per the World Food and Agriculture Organization of the UN.

In addition to its physical impact, CE also has substantial global socio-economic impact in disability-adjusted life years (approximately 1 million DALYS) and annual monetary losses of approximately $3 billion. In Bhutan, the epidemiological situation of CE remains poorly understood, with limited data available on its prevalence and transmission dynamics. Clinical data from hospital-based studies provide little insight into the burden of CE in Bhutan.

An average annual incidence of CE based on hospital records is 4.4 cases per 100,000 population, with a higher prevalence among females, adults aged 30–59 years, farmers, and residents of central and western districts. The same study estimated the CE burden of approximately 39 DALYs per year for treatment-seeking cases and up to 80 DALYs per year when including non-treatment seeking cases. A 2017 study at the National Referral Hospital found that most patients presented with non-specific abdominal symptoms, and over 90% underwent de-roofing and drainage via

laparotomy, often with minor postoperative complications. From the animal health sector, pilot and nationwide studies have demonstrated the presence of *Echinococcus granulosus* sensu lato (G1–3) and *E. ortleppi* (G5) in both stray dogs and livestock, including cattle and yaks, as well as in imported meat. The detection of infective genotypes in human cysts and environmental samples highlights ongoing transmission and widespread environmental contamination in the affected regions. Notably, the identification of *E. ortleppi* suggests a dog - cattle transmission cycle, particularly relevant given cattle's dominant role in Bhutanese livestock farming.

9.4. Risk Group and Risk Factor for CE Infection

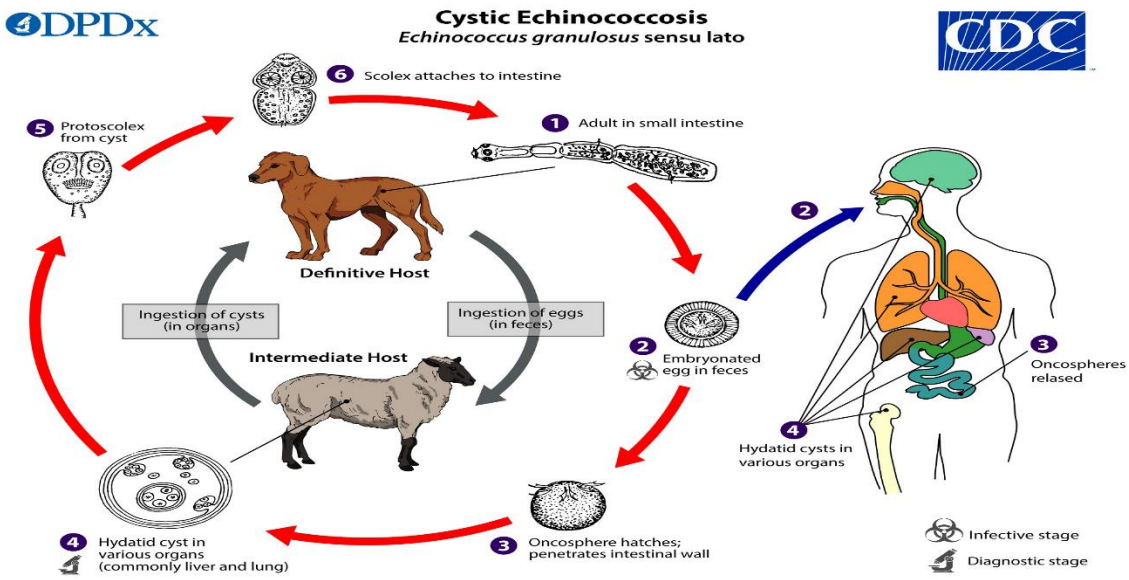
Risk factors for acquiring CE are diverse, including contacts with dogs, and livestock farming, occupational exposure to livestock or their products, and poor hygienic practices including unsupervised home slaughtering and feeding of dogs with viscera. Following are the important risk factors in Bhutan:

- Contacts with dogs (domestic/stray/community)
- Occupational exposure to livestock, herding practices
- Unsupervised or traditional home slaughtering and meat handling practices
- Feeding dogs raw offal/viscera
- Consumption of raw food, fruits, vegetables
- Drinking unboiled water or from open water sources
- Poor hand hygiene practices

9.5. Etiology and Pathogenesis

Etiology

Echinococcus is a 'true tapeworm', belonging to the Cestoda Class of Platyhelminthes Phylum. The adult tapeworm develops within the gut of the definitive host (dogs/canines) while the metacestode stage grows within the internal organs of intermediate hosts (livestock/ruminants). Adult worms release fertilized eggs that are expelled from the definitive hosts through their feces, resulting in contamination of the environment.



(From CDC, https://www.cdc.gov)

Figure 1. showing Life cycle of Echinococcus granulosus parasite

9.6. Transmission route

CE is transmitted to humans mainly through ingestion of contaminated food (unwashed vegetables/ fruits) and water, and by hand-to-mouth contact with contaminated objects and surfaces including touching of dogs/animals.

9.7. Incubation Period

Incubation period is variable and could range from many months to decades. Most patients remain asymptomatic until they show symptoms. Symptoms depend on the location of the cyst and are caused by mechanical compression or obstruction of the neighboring anatomical structures, as well as by anaphylactic reactions.

9.8. Clinical Presentation, diagnosis and management of CE

In CE, **approximately 70–80 % of hydatid cysts occur in the liver**, with the lungs being the second most commonly involved organ at around 10–20 % of cases. Less frequently, cysts may develop in the spleen, kidneys, central nervous system, bones, heart, muscles, thyroid, and other soft tissues, collectively accounting for only a few percent of cases. In roughly 80 % of patients,

CE affects a single organ (most often the liver alone), with multiorgan involvement being relatively uncommon.

Presenting symptoms of CE are mostly non-specific or vague. As cyst grows, mass effects are exerted. Symptoms and signs depend on the site (organ involved), and the rate at which the cyst grows.

9.8.1. Hepatic CE (most common site ~ 70-80%%)

- Chronic right upper quadrant or epigastric pain/discomfort
- Nausea or vomiting
- Fever and chills (in case of superinfection or rupture)
- Jaundice (mass effect on bile ducts are compressed)
- Palpable abdominal mass
- Hepatomegaly

9.8.2. Pulmonary CE (second most common site ~ 15-30%)

- Chronic cough
- Chest pain
- Dyspnea
- Hemoptysis or expectoration of cyst content
- Fever
- Failure to thrive in children (due to large cysts reducing lung capacity)

9.8.3. Cyst complications

CE cysts present as complicated cases in which immediate attention is required. Following cyst complications can occur:

- Cysts with fistulas (biliary/bronchial)
- Biliary/bronchial obstruction
- Bacterial infection/abscess formation
- Compression syndrome (brain, heart, blood vessels, etc…)
- Cyst rupture
- Venous/arterial embolism
- Anaphylactic reaction (in case of cyst rupture or leakage, but rare)

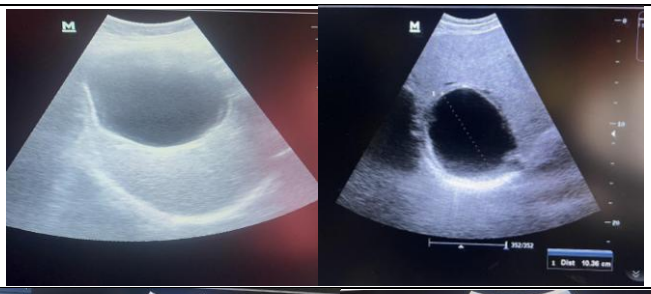
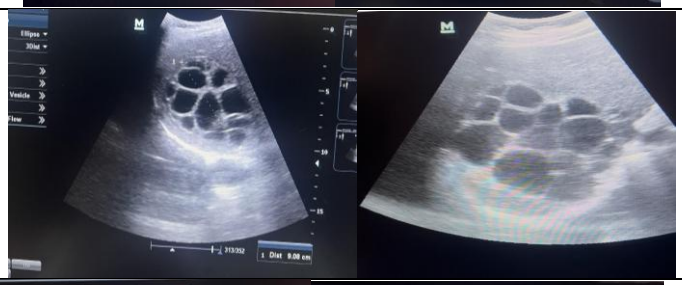
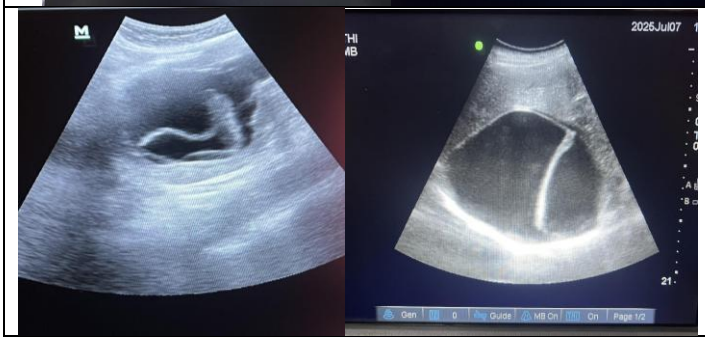
9.9. Diagnosis

9.9.1. Radio-imaging

Ultrasound (US) is **the most reliable, superior and first-line diagnostic tool** for cystic echinococcosis (CE) and is recommended for both clinical and field settings due to its ability to visualize characteristic features of CE cysts, such as the double-wall sign of active CE1 cysts. The sensitivity and specificity of US in CE diagnosis are 90-95% and 93-98%, respectively.

The WHO classification system, based on US imaging, categorizes CE cysts into active and inactive stages, guiding stage-specific treatment decisions, and is applicable to cysts in most organs except bone. For cysts located in areas inaccessible by US i.e., extrahepatic cases (brain, heart, bone, etc.,) and when complications are suspected, MRI and CT provide additional diagnostic value, with MRI being more effective in delineating cyst features. However, overuse of CT and MRI must be discouraged.

Table 1. Ultrasound Images of CE Stages and Suspected Cystic Lesions

Ultrasound Image	**WHO CE Cyst Stage**	**Sonographic Feature Description**
	CE1	Unilocular, anechoic cyst with visible double wall - Could visualize 'hydatid sand'
	CE2	Multivesicular, multiseptated cyst (rosette-like or honeycomb pattern)
	CE3a	Cyst with detached membranes, floating membrane, partial detachment, transitional cyst (Water - lily sign)

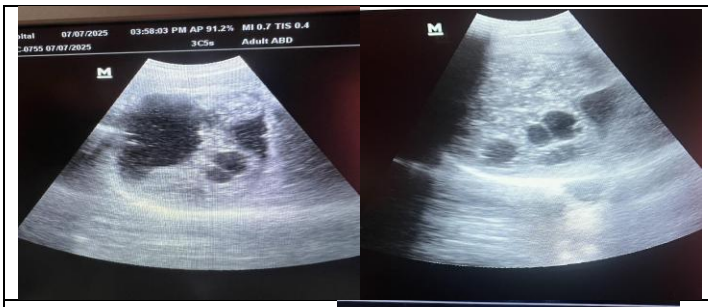
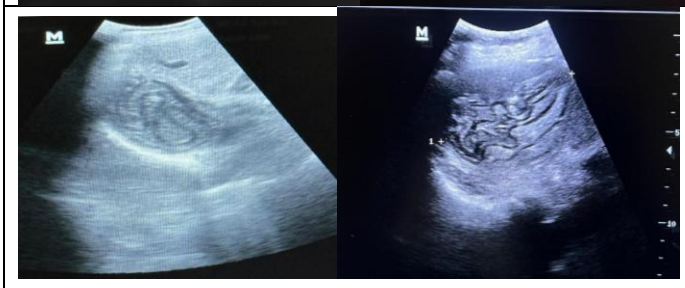
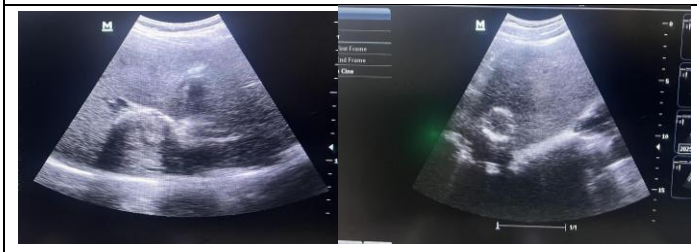
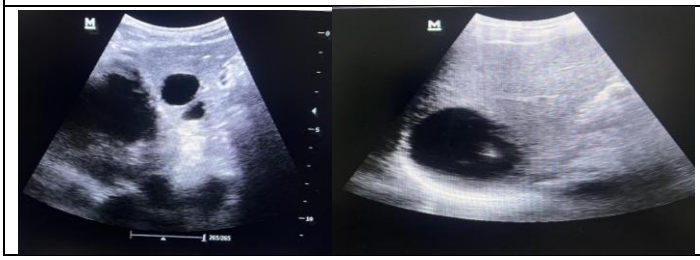
	CE3b	Cyst with daughter cysts in solid matrix.
	CE4	Cyst with heterogeneous hypoechoic/hyperechoic contents, in particular so-called ‘canalicular structures. No daughter cysts.
	CE5	Solid cyst content; always calcified wall (cotton-wool sign)
	CL	Cystic lesion with no pathognomonic CE features – ‘suspected CE’

Table 2. Differential diagnosis of Cystic and Solid Hepatic Space-occupying Lesions

Cystic/Pseudocystic Space-Occupying Lesions with Liquid Content	**Solid Space-Occupying Lesions**
Simple cyst	Hemorrhagic simple cyst
Hematoma	Hematoma/Hemangioma
CE (CE1, CE2, CE3a, CE3b (mixed cystic-solid))	CE (CE3b (mixed cystic-solid), CE4 and CE5)
AE with pseudocyst	AE
Abscess	Tuberculoma
Cystadenoma	Hepatic adenoma/Focal nodular hyperplasia

Cystadenocarcinoma	Metastatic liver tumours/Hepatocellular carcinoma/Cholangiocarcinoma

9.9.2. Laboratory Diagnosis

9.9.2.1. Serological tests

The role of serological tests in CE diagnosis is limited to supplementary roles. **Serological tests should only be used when imaging (ultrasound imaging) does not show pathognomonic signs of CE in the affected organ.** It is also useful for monitoring recurrence following surgical resection. Serological tests detect antibodies against hydatid antigens and may be used as adjuncts. Following serological tests can be used:

- Rapid diagnostic tests (RDTs)
- ELISA: overall sensitivity of 80% (90% in hepatic CE, 40% in pulmonary CE).

Note: The diagnostic performance of serological tests is influenced by the cyst stage (active versus inactive).

9.9.2.2. Confirmatory test

In cases of sero-negativity, confirming a presumptive diagnosis might involve demonstrating the presence of protoscoleces and/or hooks by microscopic examination of the cyst fluid, histology, polymerase chain reaction (PCR) of cyst material or observation of changes in the cyst ultrasound appearances on treatment, such as detachment of parasite layers in an unilocular cyst (suspected CE1) after percutaneous puncture or administration of ALB.

9.9.2.3. Routine baseline investigations

- CBC with eosinophil count where available
- LFT

Table 3. Specimen Collection and Handling

Test Types	Specimen Types	Test Center
Hydatid Serology	5 mL Whole blood/ 1 mL Serum	JDWNRH/RCDC
Histopathology	Cyst wall/tissue sample	JDWNRH

9.10. Treatment and Management

9.10.1. Cyst stage-based treatment

Treatment of uncomplicated CE is based on cyst stages, particularly for the hepatic and other abdominal cases. For the pulmonary CE, complicated cases, and other organs involvement, management of cases must be tailored to individual cases.

In summary, the clinical management of CE depends on the following categories.

1. CE cyst stage- based on ultrasound imaging findings **(WHO-IWGE staging)**
2. Assess cyst size and location
3. Determine level of hospitals and available resources

Management options include four main approaches:

- Medical therapy with albendazole (ABZ)
- Percutaneous techniques (PAIR-Puncture, Aspiration, Injection, and Re-aspiration)
- Surgery
- A wait and watch approach.

9.10.2. Medical therapy

- ABZ is the preferred benzimidazole, particularly effective for small CE1 and CE3a cysts (< 5 cm) and for inoperable hepatic or peritoneal CE. **Albendazole should not be prescribed in pulmonary CE prior to surgery.**
- Inactive CE4 and CE5 cysts without complications are best managed with a 'watch-and-wait' strategy, and with regular follow-up imaging.

Table 4. Recommended treatment modalities for 'Uncomplicated hepatic CE cysts'

Cyst Stage (size)	Recommended Treatment	Remarks	Health Facility Levels
CE1 / CE3a < 5 cm	Albendazole (ABZ)	*Follow-up imaging at 3–6 months and thereafter once a year for a minimum of 5 consecutive years after inactivation*	Any hospital
CE1 / CE3a 5–10 cm	Surgery or percutaneous method (PAIR) + ALB *(PAIR is only recommended if there is nobiliary communication)	*PAIR combined with ALB (PAIR should not be used if biliary communication is present)*	District Hospital with surgical facilities
CE1 / CE3a >10 cm * (*large cyst-higher risk of forming fistula and rupture*)	Surgery or percutaneous method (PAIR) + ALB	*Percutaneous treatment (PAIR) combined with ALB.*	District Hospital with surgical facilities

		*PAIR preferred over surgery or standard catheterization	
CE2 / CE3b ≤ 5 cm	Albendazole (ALB)	*Initial treatment with ALB alone.*	Any hospital
CE2 / CE3b > 5 cm	Surgery + ALB	*Surgery combined with ALB*	District Hospital with surgical facilities
CE4/CE5 (i.e., inactive cysts)	-	**Do not require treatment but may be monitored.**	Any hospital
Post-op spillage (as per surgeon's discretion)	ALB + Praziquantel		Referral Hospital with surgical facilities
Lung CE < 5 cm	Surgery (no pre-op ALB; post-op ALB if spillage)		National Referral Hospital (cardio-thoracic surgeon)

*Complicated CE cases should be referred to Hepato-biliary surgeon, Department of Surgery, JDNWRH

9.10.3. Anti-parasitic therapy (albendazole)

9.10.3.1. Albendazole (ABZ) - Primary Antiparasitic Drug

Albendazole is a benzimidazole compound and the drug of choice for CE due to its efficacy in inhibiting microtubule formation in *Echinococcus* larvae. It is used as:

Monotherapy (Albendazole Alone)

- Indications:
  - CE1 and CE3a cysts <5–6 cm in diameter.
  - Inoperable cases of liver or peritoneal CE.
  - Post-surgical or post-PAIR therapy (as adjunct).
- Dosage:
  - 10–15 mg/kg/day (up to 400 mg twice a day) in two divided doses.
  - **Co-administer with a fatty meal to improve absorption.**
- Regimen
  - **Albendazole should not be given in interrupted doses. It should be administered continuously, without monthly treatment interruptions.**
- Duration:
  - Minimum of 3 - 6 months depending on cyst response.
    - 'Cyst response' is defined as detachment of the parasitic layers from the outer cyst wall, size reduction, or stage modification after 3 months of treatment
  - Longer treatment may be needed for multiple or recurrent cysts.
- Use with caution:
  - Chronic liver disease
  - Patients with bone marrow suppression
- Contraindications:
  - Pregnancy (especially 1st trimester).

- Pulmonary CE (cysts at risk of rupture)
- Severe hepatic dysfunction.

- **Monitoring**
  - **Monitor albendazole toxicity:**
    - Baseline and monthly liver function tests (LFTs): LFT elevation occurs in 16% of cases, mostly less than 4-fold. **If elevation is >4-fold, stop albendazole until LFT normalizes, then reinitiate treatment**, which is mostly well tolerated.

    - CBC every 2–4 weeks (**due to risk of leukopenia**).
  - **Imaging:**
    - Follow-up imaging at 3–6 months and thereafter once a year for a minimum of 5 consecutive years after inactivation.
    - For inactive cysts i.e., CE4 & CE5 cysts, falling under the "wait and watch" category, long-term ultrasound follow-up should be conducted for at least 10 years at 6-month intervals. At any point during the follow up, if inactive cyst reactivates, cyst stage should be reassessed and stage-based management should be re-initiated accordingly.

9.10.3.2. Albendazole + Percutaneous Treatment (PAIR or Catheterization)

- Indications:
  - CE1 and CE3a cysts (typically >5 cm or symptomatic).
  - Catheter drainage in CE2 and CE3b (e.g., via MoCaT).
- Albendazole Use:
  - Start 1 week before the procedure.
  - Continue for at least 1 month after percutaneous treatments (PAIR or catheter drainage).
- Purpose:
  - Prevent secondary dissemination in case of leakage.
  - Improve parasite clearance.

9.10.3.3. Albendazole in Surgical Treatment

- Indications:
  - All patients undergoing elective surgery for CE cysts.
- Timing:
  - Pre-operative: At least 1 week before surgery.
  - Post-operative:
    - Continue for 1 month if no spillage occurs.
    - Extend to 3 months if cyst rupture/spillage occurred intra-operatively.

9.10.3.4. Praziquantel (PZQ) – Adjunctive Use

Praziquantel is not a primary treatment for CE but is used in combination with albendazole in specific cases.

Indications:

- Intraoperative or PAIR-related spillage of cyst content.
- When there is risk of secondary dissemination.

- During liver transplantation or surgery involving multiple cysts.

Dosage

- 25–40 mg/kg/day, divided in three doses for 7–14 days.

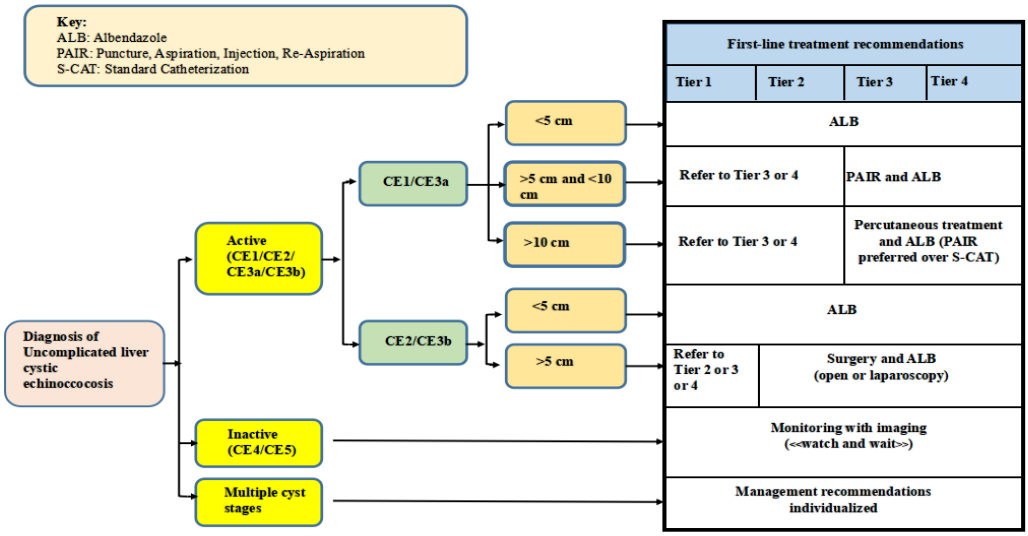
Timing

- Begin at the time of spillage or shortly after.
- Administered for 1–2 weeks.

Rationale

- PZQ is protoscolicidal and may reduce the viability of split scolices.
- Synergistic effect with ABZ improves larval killing rate.

Figure 2. Summary of the recommendations for the first-line treatment of uncomplicated hepatic cysts, relating this to the tiers specified in the recommendations for each option.



- Tier 1: Medical doctor, basic laboratory capacity, ultrasound referral availability.
- Tier 2: Tier 1 plus general surgeon, anaesthetic and operating theatre capacity, on-site ultrasonography.
- Tier 3: Tier 2 plus laparoscopic surgeon, physician trained in PAIR, S-CAT, CT and fluoroscopy capacity.
- Tier 4: Tier 3 plus thoracic surgery and interventional radiology capacity, MRI and MRCP capacity, advanced laboratory capacity.

9.10.4. Special Notes and Considerations (WHO, 2025)

- Combination therapy (ABZ + PZQ) is not routinely recommended unless there is high risk of spillage or confirmed rupture.
- Drug absorption of albendazole is enhanced with fatty meals.
- Prolonged therapy (>6 months) increases risk of hepatotoxicity; monitor LFTs regularly.

- **For patients with multiple or large cysts, long-term ABZ therapy (6–12 months) is often required.**
- PZQ alone is ineffective against CE and should not be used as monotherapy.

Note on Alveolar Echinococcosis:

Alveolar echinococcosis (AE) is a serious parasitic disease caused by *Echinococcus multilocularis* that behaves like a fast-progressing hepatic tumor. Early detection through imaging (ultrasound ± CT or MRI) complemented by serology is essential. According to WHO-IWGE recommendations, the cornerstone of management is radical (tumor-like) surgical resection of localized liver lesions whenever feasible, followed by continuous albendazole therapy for at least two years to minimize relapse. In in-operable or advanced-stage cases, long-term benzimidazole therapy alone - primarily with albendazole - is the pragmatic alternative, often lifelong, given the risk of disease progression. Lifelong clinical and imaging follow-up is strongly advised to monitor for recurrence or metastasis

9.11. Prevention and Control

In order to achieve echinococcosis control and elimination, both vertical i.e., programmatic interventions targeting the parasite directly in humans and animals using One Health approach, and horizontal control measures i.e., community-based, interdisciplinary engagement of local stakeholders should be adopted.

9.11.1. Human Health Interventions

- Strengthen national surveillance systems to detect and report human CE/AE cases
- Train healthcare workers on ultrasound diagnosis, WHO cyst staging, and clinical management

9.11.2. Community Education and Behavior Change

- Health education campaigns- raising awareness on transmission, risk factors, dog deworming, hand hygiene, and food safety including in schools

9.11.3. Environmental Measures

- Improved sanitation and water safety
- Safe dog feeding practices: Encourage cooking of animal viscera before feeding to dogs and prohibit feeding raw offal (*WHO/OIE Manual, 2001)*

9.11.4. Veterinary Interventions

- Regular deworming of dogs
- Maintaining standard slaughter house hygiene
- Meat inspection and safe offal disposal
- Vaccination of intermediate hosts (e.g., EG95 vaccine available in sheep)

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10.Ebola Virus

10.1. Introduction

Ebola virus disease (EVD) is a severe hemorrhagic fever caused by viruses of the genus *Ebolavirus* (Filoviridae family), first recognized in 1976 during outbreaks in the Democratic Republic of the Congo and Sudan.

10.2. Epidemiology

10.2.1. Global distribution

Ebola Virus Disease (EVD) outbreaks have predominantly occurred in Central and West Africa, with the most affected countries including the Democratic Republic of the Congo (DRC), Uganda, Guinea, Sierra Leone, and Liberia. The region continues to experience recurrent outbreaks due to the presence of natural reservoirs, primarily in forested areas where human-wildlife interactions are common.

Since its discovery in 1976, over 42 EVD outbreaks have occurred worldwide, with approximately 83% of them in sub-Saharan Africa (mostly Central and West Africa regions). The largest epidemic took place from 2013–2016 across Guinea, Sierra Leone, and Liberia, resulting in roughly 28,600 reported cases and over 11,000 deaths (~40 % fatality rate). From 2018–2020 the Kivu outbreak in the eastern DRC (with spread into Uganda) resulted in about 3,470 cases, ranking as the world's second-largest Ebola epidemic, with a case fatality rate around 60-67 %. Between 2017 and March 7, 2025, there were eight EVD outbreaks in sub-Saharan Africa, resulting in 3,892 cases and 2,471 deaths (overall ~53 % fatality).

The 2025 Sudan EVD outbreak in Uganda (Jan–Apr 2025) involved 14 confirmed and probable cases and 4 deaths before being declared over on April 26, 2025. Across outbreaks, the **average EVD fatality rate is around 50 %, though it has varied widely from as low as 25 % to as high as 90 %** depending on the strain and response context."

About **11 confirmed Ebola cases were detected outside Africa**, most related to importations or medical evacuations. Only a few resulted in limited onward transmission notably in Nigeria, Spain, and the US.

10.2.2. National burden

No cases have been reported in Bhutan. However, given many Bhutanese travel to highly endemic countries in Africa for the UN mission, there is potential for its introduction into Bhutan. Therefore, Bhutan needs to prepare to strengthen surveillance, isolation and management of potential Ebola cases.

10.3. Reservoirs and vectors

Fruit bats (Pteropodidae family) are the most likely natural reservoir hosts. Other wildlife, such as non-human primates, may serve as incidental hosts in zoonotic spillovers.

It is spread to humans via the body fluids such as secretions and blood of infected animals like chimpanzees, gorillas, monkeys, forest antelope or porcupines

10.4. Risk groups

- Healthcare workers
- Funeral and burial teams
- Individuals handling bushmeat or involved in wildlife contact
- Travelers to outbreak areas
- Laboratory personnel managing suspect specimens.

For Bhutan the high-risk groups are:

- Military contingents living for mission to endemic countries in Africa
- Non-military Bhutanese expatriates working in endemic countries in Africa
- People traveling to conferences, meetings, and for tourism purposes to the endemic countries in Africa.

Ebola virus disease should be considered for patients presenting with signs and symptoms consistent with Ebola in these groups of people, with a travel history to endemic countries within the incubation period of the disease.

10.5. Etiology and pathogenesis

Causative agent

Human-pathogenic *Ebolavirus* species include Zaire, Sudan, Bundibugyo, and Taï Forest viruses . The Zaire ebolavirus is the most severe species of Ebola virus, with a high mortality rate (up to 90%).

10.6. Transmission routes

- Zoonotic spillover resulting from contact with infected animals and their products like bush meat, etc
- Human-to-human transmission via direct contact with bodily fluids of symptomatic individuals or contaminated objects during the period of acute illness. Possible sexual transmission from survivors with persistent virus

10.7. Incubation period

Ranges from 2 to 21 days, typically 5–9 days. Individuals are not infectious until symptom onset.

10.8. Pathophysiology

After exposure, the virus replicates in immune cells and spreads systemically, causing vascular leakage, coagulopathy, multi-organ failure, and hemorrhage.

10.9. Clinical presentation

Table summarizing the clinical presentation and disease progression of Ebola

stage of illness	Time post symptom onset	Clinical	Laboratory
Early Febrile	Days 1-3	Fever, malaise, fatigue, body aches	leukopenia, lymphopenia, thrombocytopenia, elevated AST and ALT
Gastrointestinal	Days 3- 10	Primary: Epigastric and abdominal pain, nausea, vomiting, diarrhoea Associated: Persistent Fever, asthenia, headache, conjunctival injection, chest pain, dysphagia, odynophagia, arthalgias, myalgias, hiccups, deliriums, and rash.	Persistently elevated AST/ALT and thrombocytopenia. Elevated BUN and creatinine Hypokalemia, Hypomagnesemia, hyponatremia, hypoalbuminemia Elevated PT/PTT/INR/Fibrin-split products. leukocytosis (elevated neutrophils and band cells)
Shock	Days 7-12	Diminished consciousness or coma, thready pulse, oliguria, anuria, tachypnea	In addition to findings during gastrointestinal stage: Elevated lactate, decreased bicarbonate.
Other Complications	Day 10 and after	Gastrointestinal hemorrhage, respiratory failure associated with aggressive fluid resuscitation or lung injury	Finding may overlap with prior stages of illness. Decreased

		secondary infections. Neurocognitive abnormalities, seizures, syndrome consistent with meningoencephalitis	hemoglobin and hematocrit observed with gastrointestinal bleeding. Hypoxemia observed with respiratory failure.
Recovery	Day 7-12	Resolution of gastrointestinal symptoms, increased oral intake, increased energy.	Resolution of laboratory abnormalities
Convalescence	up to 12 months	Arthralgias, myalgias, abdominal pain, fatigue, persistent Neurocognitive abnormalities, uveitis, meningitis, hearing loss.	

The most common signs and symptoms reported during the 2014–2016 West Africa outbreak include fever (87%), fatigue (76%), vomiting (68%), diarrhea (66%), and loss of appetite (65%). While Ebola Virus disease (EVD) is often associated with the term "hemorrhagic fever," hemorrhage is not universally present and is not always the predominant clinical sign. While some patients with EVD experience bleeding, it's not a defining or consistent feature for all cases. The disease can present with various symptoms, and bleeding may manifest later in the illness or not at all.

10.9.1. Disease progression

Rapid deterioration is typical in severe cases, progressing from nonspecific febrile illness to shock and multiorgan failure within days.

In the West African Ebola outbreaks in 2014 to 2016, the mortality was observed between 6 and 16 days (with a mean of 7.5 days) from the symptom onset due to multiorgan failure and septic shock.

Case fatality rate ranged from 40 % to 90 %, depending on the viral species and care availability.

Individuals who recover may experience prolonged sequelae such as arthralgia, neurocognitive dysfunction, uveitis, sometimes followed by cataract formation, and clinical and subclinical persistent infection may occur in immune-privileged compartments

10.9.2. Ebola Disease and Pregnancy

Evidence of mother-to-child transmission of Ebola virus remains limited. Pregnancy increases the risk of severe outcomes from Ebola, including miscarriage and bleeding. Most infants born to infected mothers have not survived.

Ebola can cross the placental barrier, and the virus has been detected in various pregnancy-related tissues and fluids. EBOV RNA may persist up to 32 days after recovery, requiring continued infection control and precautions.

10.10. Diagnosis

10.10.1. Case definitions

Suspected

compatible symptoms with exposure history either via contact with a suspected, probable, or confirmed Ebola case, or with a dead or sick animal potentially carrying the virus and sudden unexplained death, particularly if associated with symptoms like fever or bleeding.

Probable

symptomatic individual with epidemiologic link to a confirmed or suspected case, contact with someone who was sick or died from Ebola.

Confirmed

A suspected or probable case with laboratory confirmation of Ebola virus infection, typically through RT-PCR (reverse transcription polymerase chain reaction) or detection of Ebola virus-specific antibodies in the blood.

10.10.2. Differential diagnoses

Viral diseases

- Marburg virus disease – Closely related filovirus; indistinguishable clinically without lab tests
- Lassa fever – Common in West Africa; fever, vomiting, and hemorrhagic signs
- Yellow fever – Jaundice, hemorrhage, fever
- Dengue fever – Fever, rash, retro-orbital pain, bleeding tendencies
- Crimean-Congo hemorrhagic fever (CCHF) – Tick-borne, causes hemorrhagic symptoms
- Rift Valley fever – Can have hemorrhagic and encephalitic forms
- Severe COVID-19 – Fever, fatigue, and systemic inflammation; now part of differential in endemic regions
- Influenza – Fever, myalgia, malaise

Bacterial diseases

- Typhoid fever – High fever, abdominal pain, relative bradycardia
- Leptospirosis – Jaundice, renal impairment, hemorrhage
- Meningococcemia – Fever, petechial rash, rapid progression
- Sepsis from Gram-negative/positive bacteria – Shock, organ failure
- Acute gastroenteritis.

Parasitic diseases
Malaria (esp. falciparum) - Fever, anemia, confusion, bleeding in severe cases
Trypanosomiasis (late stage) - Fever, CNS signs, wasting

Non-Infectious Conditions

- Acute leukemia – Fever, bleeding, hepatosplenomegaly
- Autoimmune diseases (e.g., lupus) – Fever, cytopenia, organ involvement
- Toxic shock syndrome – Fever, rash, hypotension, multiorgan failure
- Snakebite (hemotoxic venom) – Bleeding, shock, local tissue damage

10.10.3. Specimen Collection, Storage, and Transport

Ebola virus samples should be handled in compliance with Biohazard Level 4 pathogen. Samples should be collected using adequate PPE.

Clinical specimen	Volume Collected	Remarks
Whole blood samples (Living patient)	4 ml collected in EDTA tube	Collect after symptoms appear If collected less than 3 days after symptom onset and the test if the test is negative, collect a second sample after at least 48-72 hours after the first specimen.
Oral swabs	Collect in Viral transport medium	Deceased patient or when blood is not possible.

10.10.4. Storage, Packaging and Shipment

- Store the samples at 2-8°C if testing within a week or freeze at 20°C or lower for longer storage
- Avoid repeated freeze-thaw cycles.
- Clinical specimens of probable and suspected Ebola virus disease should be shipped as Category A, using proper packaging **(triple packaging),** labelling, markings, and documentation.

Sample collection, packaging, and transport of suspected Ebola specimens should be done in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations (**Refer National guideline for sample collection, packaging and transport of biological specimens).**

10.10.4.1. Infection Control and Biosafety during specimen collection

- Collect samples using full PPE (N95 mask or equivalent, gown, gloves, eye protection) due to aerosol risk.
- Follow strict infection control, decontamination, and biohazard protocols when handling all specimens

10.10.4.2. Infection Control and Biosafety during testing

- Handle all samples using a biosafety cabinet (BSC Class III or higher) until inactivation.
- Non-inactivated samples are handled in BSL-4, if BSL- 4 is not available, inactivated under strict BSL-3 or within gloveboxes.
- Decontaminate all surfaces and equipment after handling samples.

10.10.5 Molecular detection

10.10.5.1. RT-PCR

It is a gold standard used for the early detection of viral RNA in EVD suspects. False-negative results if tested too early, should be done 3 to 10 days after the onset of symptoms.

Safe transport of specimens under biosafety protocols

Limited access to BSL4 (Biosafety level-4) - level labs

Limited laboratory technicians

RCDC can either have a pre-identified referral lab and agreement to ship samples within 24 hrs. The feasibility of maintaining a limited buffer stock of PCR reagent for Ebola virus needs to be properly studied.

Repeat RT-PCR if the first test is negative within the first 3 days

Test Type	Testing Center	Sample Type	Biosafety measures
Polymerase Chain Reaction (PCR)	RCDC	Whole blood	Handling clinical samples and potentially infectious materials in Biosafety Level 4 (BSL-4) laboratories for virus isolation. For molecular testing (e.g., RT-PCR), samples should be inactivated first or processed in Biosafety Level 3 (BSL-3) labs using biological

			safety cabinets (class II or III). Inactivated samples may then be handled under BSL-2 conditions. Use of appropriate personal protective equipment (PPE) including gloves, N95 or higher respirators, impermeable disposable gowns, face shields or goggles, laboratory caps, and shoe covers. PPE must be worn by all personnel handling non-inactivated samples

10.11. Treatment and Management

10.11.1. Treatment protocols

Aggressive supportive care is the cornerstone

Oral/IV rehydration

- The volume of intravenous fluids required should be assessed based on clinical exam (i.e., level of dehydration, signs of shock) and fluid losses (i.e., volume of diarrhea and/or vomitus). Large volumes of fluid replacement (up to 10 L/day) may be required in febrile patients with diarrhea.
- Electrolyte correction in severe diarrhoea and vomiting
- Respiratory support, including mechanical ventilation
- Nutritional support enterally or parenterally
- Empirical antibiotics when bacterial co-infection is suspected
- Monoclonal antibody therapies approved for Zaire ebolavirus include *Inmazeb*(atoltivimab/maftivimab/odesivimab) and *Ebanga* (ansuvimab).

Supportive care

- Close monitoring of vitals, heart rate, blood pressure, urine output, gastrointestinal fluid loss
- Need frequent assessment of fluid status and to look for signs of shock,
- Monitor renal and hepatic function
- Manage complications such as electrolyte disturbances, shock, and secondary infections.

Complication management

- Empirical antibiotics for possible bacterial co-infection with reassessment after 48 hours.
- Organ support such as mechanical ventilation, dialysis and neurocognitive support
- Psychosocial supportive services for survivors and families

10.11.2. Case Isolation and Management

1. Screening and Triage

- Train healthcare staff to recognize suspected Ebola cases using a case definition considering clinical and epidemiological characteristics.
- Maintain at least **1 meter distance** between a patient and healthcare workers.
- Use physical barriers (e.g., plexiglass) during screening.
- Prevent crowding at the triage area with proper spacing.
- Follow **no-touch techniques** during patient evaluation.
- Use **contactless thermometers** (infrared/gun type).
- When distancing is not possible, **full PPE** must be used.
- Keep an **Ebola-specific questionnaire and algorithm** at the triage area.
- Ensure availability of **hand hygiene stations** with posters for instruction.
- Use **alcohol-based hand rub or soap**, or **0.05% chlorine solution** if those are unavailable.

Additional required items include:

- Disposable (single-use) paper towels.
- Cleaning and disinfection tools.
- WHO-compliant, color-coded waste bins.

Suspected cases must be safely transported to isolation wards using full PPE.

2. Dedicated Isolation Ward Requirements

Isolation rooms must have **negative air pressure** of at least **2.5 Pascals** compared to surrounding areas.

Air should be **filtered before being released** outside.

Benefits of negative pressure:

- Prevents airborne pathogens from escaping.
- Minimizes cross-contamination.
- Protects healthcare workers and other patients.

Patients with **severe Ebola disease or multi-organ failure** should be treated in a dedicated **isolation ICU**.

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Annexure 1. SOP for Safe and Dignified Handing and Burial of EVD disease.

Purpose

To ensure that the bodies of people who die from suspected or confirmed Ebola Virus Disease (EVD) are handled safely and respectfully, in a manner that protects health workers, the public, and honors local cultural and religious practices.

1. Guiding Principles

Minimize handling of the body.
Respect cultural and religious traditions, while ensuring safety.
Only trained personnel should handle the body.
Ensure families are informed, involved, and agree to the process before burial.

2. Key Roles

Burial Team Leader: Coordinates the process and liaises with the family.
Communicator: Engages with family and religious leaders.
Technical Team: Handles body preparation and burial.
Sprayer: Manages disinfection.
Faith Representative: Provides spiritual guidance (as requested by the family).

3. Preparation Before Departure

Assemble team (at least 3 people in PPE).
Prepare disinfectants:
- 0.5% chlorine for surfaces and equipment.
- 0.05% chlorine or alcohol-based solution for hand hygiene.

Pack all necessary PPE and burial kits, including:
- Body bags (2 per deceased – one transparent, one opaque).
- Disinfectants, gloves, goggles, gowns, aprons, boots, waste bags.

4. At the Deceased's Home

4.1. Inform the Family
- Offer condolences.
- Clearly explain the burial procedure.
- Obtain informed verbal agreement to proceed.

4.2. Body Preparation
- Wear full PPE before touching the body.
- Place absorbent material beneath the body.
- Wrap in the first (transparent) body bag and disinfect the outer surface.
- Place into a second (opaque) bag, disinfect, and label.

4.3. Environment Sanitation

- Disinfect room, objects, and any waste or soiled materials.
- Burn contaminated linens with permission.

5. Transportation and Burial

Once the body is properly prepared and packed by the health staff, it will be handed over to the Red Cross for further handling, following the protocol outlined below.

5.1. Transporting the Body

- Use designated vehicles.
- Rear space to carry the body; family may walk behind.
- No family members to sit in the cabin with the burial team.

5.2. At the Burial Site

- Respect rituals: first soil, prayers, grave marking.
- Lower body bag carefully using ropes.
- Place any agreed-upon items with the body (pre-disinfected or sealed).

6. After Burial

- Thank the family and community.
- Encourage hand hygiene for all attendees.
- The burial team removes PPE carefully, manages waste as infectious, and disinfects reusable items.
- Clean vehicle and return to base for final disinfection.

7. Prohibited Practices

- No embalming or washing of the body.
- Do not open or unzip body bags after sealing.
- No public touching of the body.

8. Special Notes for Bhutan Context

- Local Dzongkhag Health Officers should oversee implementation.
- PPE and chlorine stock should be prepositioned in outbreak-prone areas.
- Collaboration with monastic institutions and local religious bodies is key for acceptance.

Personal protective measures

WHO suggests that health care workers conducting triage for patients with suspected or confirmed Ebola disease wear:

- A Medical mask in combination with eye protection (face shield or goggles)
- A fluid-resistant coverall (versus a fluid-resistant gown)
- Two pairs of gloves
- Foot wears

Technical specifications on PPE

1.1 Medical mask

- Fluid resistant medical or surgical mask
- High fluid resistant
- Good breathability
- External and internal faces should be clearly identified
- Structured design that does not collapse against the mouth (e.g; Duckbill or Cup shaped)

(World Health Organization. (2023, August 11). *Infection prevention and control guideline for Ebola and Marburg disease, August 2023*)

1. 2. Goggles

- Fog and scratch resistant
- Adjustable band that can firmly secure and does not become loose during clinical activity
- Indirect venting to reduce fogging
- May be reusable (provided appropriate arrangements for decontamination are in place) or disposable

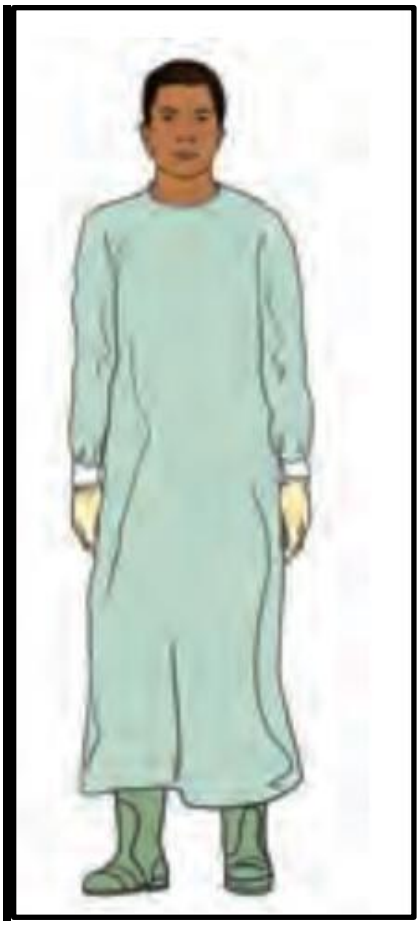
Goggles

1.3. Gloves

- 2 pairs of gloves of different sizes
- Nitrile
- Powder free
- Outer gloves should preferably reach mid forearm (minimum 280 mm total length)

1.4. Disposable gown

- Single use
- Mid-calf length, to cover the top of the boots
- Avoid colors that are culturally unacceptable (e.g; black)
- Prefer light colors to allow better detection of possible contamination
- Thumb or finger loops to anchor sleeves in place



1.5. Disposable coverall

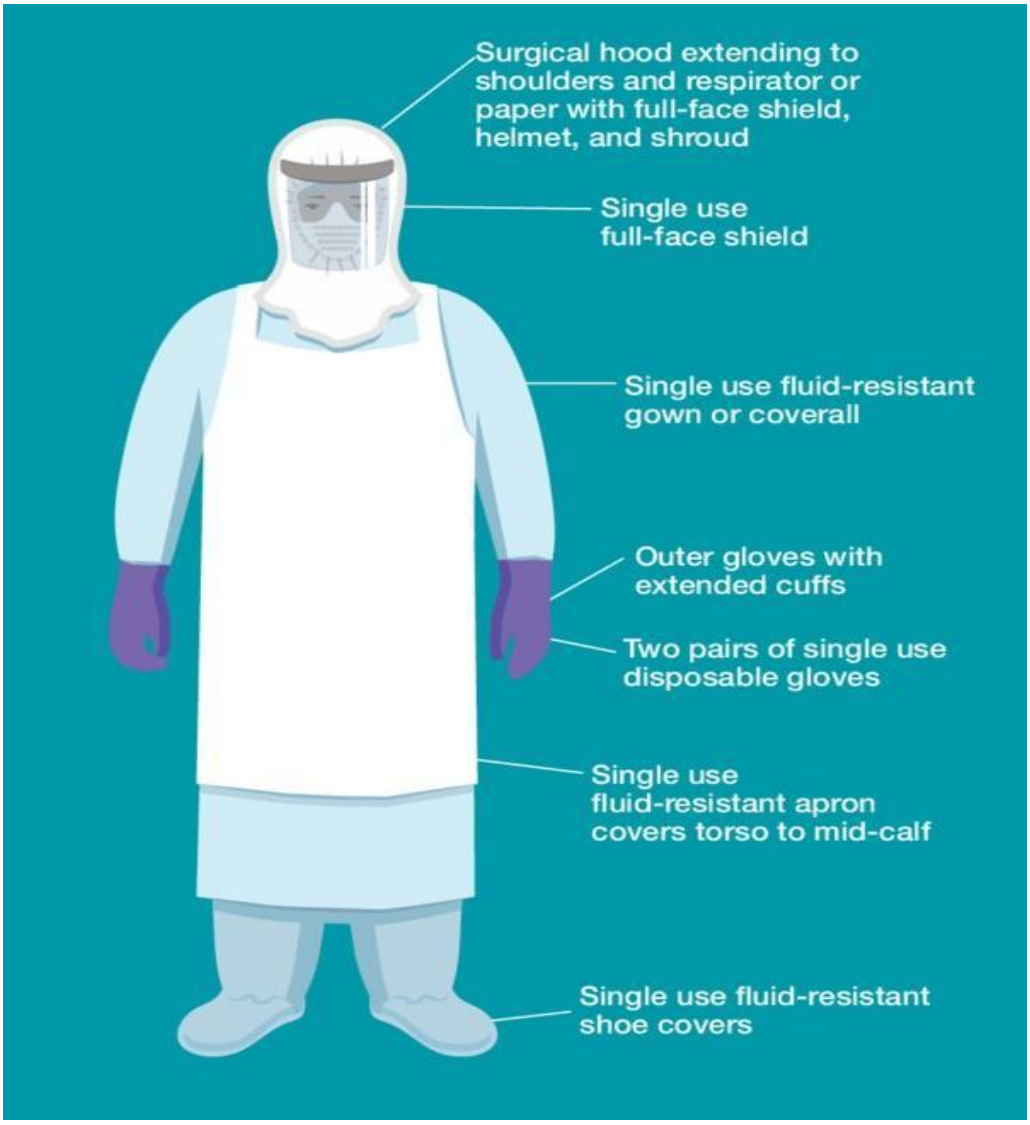
- Single use
- Avoid colors that are culturally unacceptable (e.g; black)
- Prefer light colors to allow better detection of possible contamination
- Thumb or finger loops to anchor sleeves in place
- In terms of sizes large seizes especially important
- Quality compliant with either of two international standards, depending on resistance of materials:

Option 1: tested for resistance to blood and body fluid penetration: meets or exceeds ISO 16603 class 3 exposure pressure,
Option 2: tested for resistance to bloodborne pathogen penetration: meets or exceeds ISO16604 class 2 exposure pressure,

Disposable coverall

1.6. Foot wears

- The boots should be knee-high, to provide sufficient coverage.
- Waterproof boots
- Nonslip, with a PVC sole that is completely sealed
- Optional light color, for better detection of possible contamination
- A variety of sizes, to improve comfort and avoid trauma to the feet



PPE for healthcare workers treating Ebola patients

1.7. Hand hygiene

- Ensure staff are trained on appropriate techniques for hand rubbing and handwashing
- Resources should be available for staff/patients and visitors at designated areas and points of care (e.g., alcohol-based hand rub, stations/sinks with soap and water) to perform hand hygiene.
- Posters depicting proper technique for hand rubbing and handwashing should be available in identified areas such as screening and triage and patient-care areas.

Identify separate donning and doffing areas under strict supervision by the trained personnel

Vaccination

Ervebo (rVSV-ZEBOV) is licensed for Zaire ebolavirus and recommended for at-risk frontline workers in high-risk settings.

Prevention and Control

Public Health Measures

- Effective Infection Prevention and Control (IPC) is essential to stop the spread of Ebolavirus.
- Without proper IPC, the virus can spread within healthcare settings (nosocomial infections), increasing risk to communities and potentially leading to outbreaks across borders.
- Failure to implement timely IPC can lead to more cases, higher death rates, and broader transmission.

Preventive measures through IPC Ring Approach in the 4 Key Steps

Step 1: Preparation

- Form an IPC Task Force.
- Activate the IPC ring immediately after a suspected case.
- Define the ring's geographic boundary around the case location.
- Identify health facilities, households, and public spaces within this area.
- Deploy field teams and coordinate logistics, security, and communication.
- Inform health workers, local leaders, and households.

Step 2: Healthcare Facility Interventions

- Clean and disinfect surfaces and equipment.
- Distribute IPC kits to healthcare staff.
- Conduct rapid IPC assessments using a scorecard.
- Provide briefings to healthcare workers and develop an improvement plan.

IPC Kits May Include:

- PPE (Personal Protective Equipment)
- Hand hygiene supplies
- Cleaning and disinfection materials

Step 3: Household Interventions

- Disinfect the household environment.
- Provide standardized hygiene and prevention kits.

- Carry out health education and promotion activities.

Step 4: Community/Public Place Interventions

- Disinfect relevant public spaces (e.g., markets, transport hubs).
- Engage communities to raise awareness.
- Perform a quick Water, Sanitation, and Hygiene (WASH) assessment.
- Provide hygiene kits to affected households and community sites.

Timeline

IPC ring activities around targeted facilities should begin within **24 hours** of identifying a suspected case.