National guideline for the prevention, diagnosis and management of Leishmaniasis in Bhutan, 2023

Vector-borne Disease Control Programme, Department of Public Health
Ministry of Health, Royal Government of Bhutan
Second Edition, April 2023

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Foreword

The Department of Public Health, Ministry of Health is delighted to bring out this revised and updated national leishmaniasis prevention, diagnosis and management guidelines 2023. It is a timely initiative to review the current World Health Organization recommendations and advances in technology and treatment of leishmaniasis and incorporate them into the existing guideline that was last developed in 2012. Leishmaniasis transmission continues with reporting of sporadic cases with widespread distribution in most districts in the country. The sand fly vectors have also been confirmed in most districts and at altitudes as high as 2000 metres above sea level. In view of the public health risks posed by leishmaniasis transmission and to work towards achieving and sustaining leishmaniasis elimination, it is imperative that Ministry of Health develop a comprehensive and up-to-date guideline. This guideline is intended to address the public health burden of leishmaniasis through the provision of timely diagnosis and effective treatment for the patients.

Prevention and control of vectors also play a key role in leishmaniasis control. To strengthen the prevention of disease, the guideline has included measures that can be used by health workers and community members to prevent and control the disease. The Ministry of Health continues to enhance leishmaniasis diagnostic capability through the adoption of new diagnostic techniques and improved treatment regimens.

I acknowledge the expert panel members for their contributions to updating this guideline and extend my sincere appreciation and gratitude to all the working members and officials at Vector-borne Disease Control Program. I hope this revised guideline will be of great benefit to both the service providers and receivers across the country.

I would like to urge all the relevant health professionals to make use of this revised guideline to guide their efforts in the prevention, surveillance, diagnosis and holistic management of leishmaniasis.

Tashi Delek!

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List of abbreviations

AIDS	Acquired Immunodeficiency syndrome
ART	anti-retroviral therapy
CDC	Centers for Disease Control and Prevention
CL	cutaneous leishmaniasis
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
GIS	geographic information system
HIV	human Immunodeficiency Virus
IM	intramuscular
IRS	Indoor Residual Spraying
ITN	Insecticide Treated net
IV	intravascular
IVM	integrated vector management
JDWNRH	Jigme Dorji Wangchuck National Referral Hospital
LLIN	long-lasting insecticidal nets
ML	mucosal leishmaniasis
NEWARS	National Early Warning, Alert Response and Information System
NTD	Neglected Tropical Disease
OD	once daily
PCR	polymerase chain reaction
PKDL	Post-kala-azar Dermal leishmaniasis
RCDC	Royal Centre for Disease Control
rK39	recombinant kinesin 39
SAE	serious adverse event
SOP	standard operating procedure
VDCP	Vector-borne Disease Control Programme
VL	visceral leishmaniasis
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme

Method of guideline development

The first edition of the guideline on the prevention, diagnosis and management of leishmaniasis was developed by a panel of experts in 2012 under the coordination of the Vector-borne Disease Control Programme, Ministry of Health. This guideline primarily focussed on early diagnosis using the rapid test kit (rK39) and management of kala-azar at different levels of health facilities. The manual was endorsed during the 6th Annual Malaria Review Meeting. Subsequent to this, training on the use and implementation of the guideline were provided by the programme.

The second edition of the guideline was developed by a panel from 02 – 04 November, 2022 at the Vector-borne Disease Control Programme office, Gelephu, Bhutan. All relevant clinical guidelines published by agencies in the countries in the region and the World Health Organization were reviewed against the practicality and appropriateness of implementation in Bhutan. This was reviewed by a panel of experts from the World Health Organization and the Faculty of Medicine, University of Colombo, Sri Lanka. The comments received were deliberated by an expanded panel involving all relevant stakeholders from 04 – 06 January 2023 at Gelephu, Bhutan. The final version of this guideline was approved by the Department of Public Health, Ministry of Health, Royal Government of Bhutan.

Disclaimer

This guideline on the prevention, diagnosis and management of leishmaniasis was developed based on a review of the currently available evidence and best practices, and may be revised in light of future developments in the field.

The contributors mentioned in this guideline have made considerable efforts to ensure the information upon which they are based is accurate and up to date. Users of these guidelines are strongly recommended to confirm that the information contained, especially drug doses, is correct by way of independent and standard sources. The panel members accept no responsibility for any inaccuracies, information perceived as misleading, or the success of any treatment regimen detailed in the guidelines and disclaim all liability for the accuracy or completeness of a guideline, and disclaim all warranties, expressed or implied to their incorrect use. Ultimately, health care professionals must make their own treatment decisions about care on a case-by-case basis, after discussion with their patients, using their clinical judgement, knowledge and expertise. A guideline is not intended to replace a physician's judgment in diagnosing and treatment of particular patients.

Summary Practice Points

- The transmission of leishmaniasis is sparse but widely distributed across 13 districts out of 20 in Bhutan. The annual incidence of leishmaniasis per endemic district is below 1 per 10,000 population.

- The risk factors include poor living conditions, malnutrition, immunosuppression and environmental changes.

- Leishmaniasis occurs in three broad clinical categories: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML).

- Probable VL: A person living in or having travelled to endemic areas showing clinical signs and symptoms of leishmaniasis (fever lasting more than two weeks and/or splenomegaly, hepatomegaly, anaemia, loss of weight).

- Confirmed VL: A probable VL case with laboratory confirmation through either serological, parasitological or molecular techniques.

- Serological diagnosis of visceral leishmaniasis can be done using the rK39 test.

- All cases of VL must be evaluated with ELISA and PCR at the Royal Centre for Disease Control, Thimphu.

- The recommended treatment regimens VL are:
    - Preferred treatment: Liposomal amphotericin B or amphotericin B deoxycholate or Miltefosine
    - Alternative treatment: Sodium stibogluconate or paromomycin

- Combination therapy with liposomal amphotericin B and miltefosine is recommended for patients with HIV co-infection.

- Post-kala-azar dermal leishmaniasis (PKDL) occurs in those with or without history of VL.

- The recommended treatment for PKDL is miltefosine. Liposomal amphotericin B is recommended as an alternative treatment.

- Cutaneous leishmaniasis: A person showing clinical signs (skin or mucosal lesions) with parasitological confirmation of the diagnosis (positive smear or culture) and/or, for mucocutaneous leishmaniasis only, serological diagnosis.

- The laboratory evaluation for CL and ML include tissue aspirate, imprint smear, skin biopsy for microscopy, histopathology and PCR.

- Simple CL may be managed with physical treatment (cryotherapy), thermotherapy, intralesional antimonials and topical paromomycin.

- The management of complex CL may include local and systemic therapies.

- Mucosal leishmaniasis presents with lesions on the nasal and buccal mucosa or the pharynx and may cause respiratory obstruction in some situations.

- Mucosal leishmaniasis is managed with parenteral therapy.

- All cases with leishmaniases are recommended provider-initiated testing for HIV and tuberculosis co-infection.

- All cases of VL, PKDL, CL and MCL must be follow up for at least one year.

- All forms of diagnosed leishmaniasis must be reported to the VDCP and RCDC. Case-based surveillance and vector control response should be initiated immediately by the district health authorities.

- Sand fly vector control programmes include indoor residual spraying, use of personal protection using insecticide-treated nets, long-sleeve clothing and removal of vector breeding sites in the environment.

- Community awareness and mobilization for behavioural change of citizens are essential components in efforts towards prevention and elimination of leishmaniasis.

Chapter 1
Introduction

1.1 Global situation of leishmaniasis

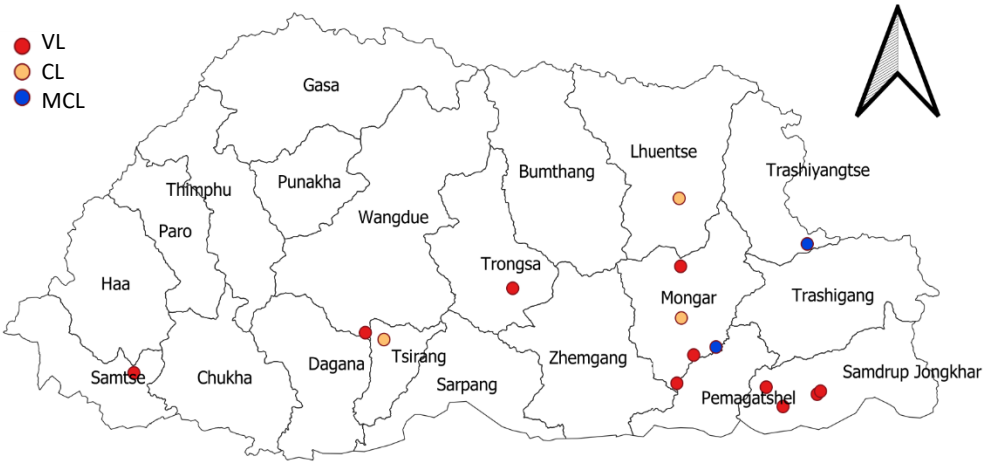
Leishmaniasis is one of the neglected tropical diseases (NTD) that affect the poorest of the poor, mainly in developing countries. Globally, 0.7 – 1 million new cases are estimated every year while >1 billion people are at risk of contracting leishmaniasis infection. An estimated 0.6 – 1 million cutaneous leishmaniasis (CL) and 50,000 – 90,000 visceral leishmaniasis (VL) cases occur annually [1]. In 2021, 11743 new VL and 221953 CL cases were reported to World Health Organization (WHO) with 12% coming from Indian subcontinent (Bangladesh, India and Nepal). About 65% of the VL cases were reported in males in high burden countries. The case fatality rate for VL ranged from 2.8% Ethiopia to 10% in Venezuela. In 2021, among the countries that reported VL, 47% of the cases were in people aged ≥15 years, 29% in those aged 5–14 years and 24% in those aged <5years. For CL, 57% of cases were in people aged ≥15 years, 24% were in children aged 5 – 14 years, and 19% were in children aged <5 years. About 5.5% had HIV-VL co-infection in WHO Southeast Asia Region.

In the South-East Asian Region, VL (also known as kala-azar) occurs in Bangladesh, India and Nepal where more than 200 million people are at risk. In 2018, 93% of cases were reported from India and 7% from Bangladesh and Nepal. These countries have launched the VL elimination programme since 2005 which was recently renewed with the Regional Strategic Framework for Elimination of Kala-azar from the South-East Asia Region by 2020, and again updated in 2023. Through these regional initiatives, the disease burden in the region declined from over 50,000 cases in 2007 to 1577 in 2021 [2]. The World Health Organization Neglected Tropical Diseases roadmap 2021-2030 has set targets to achieve elimination of VL as a public health problem globally (defined as <1% case-fatality rate due to primary visceral leishmaniasis) and achieving enhanced national capacities for case detection, treatment and reporting of CL and post-kala-azar dermal leishmaniasis (PKDL) cases [3].

1.2 Leishmaniasis situation in Bhutan

As per hospital register review, the first case of VL in Bhutan was detected from Neykalog, Monggar and was diagnosed at the then Thimphu General Hospital in 1994. However, the disease was not accorded much public health attention although sporadic cases were reported since the detection of the first case. A detailed investigation commissioned in 2007 and 2011 with support from the WHO confirmed the existence of local kala-azar transmission in diverse areas in Bhutan. As sporadic cases are reported from different parts of the country and the existence of sandfly vectors confirmed at altitudes above 2000 metres above sea level, the majority of the districts in the country are now considered potentially endemic for kala-azar [4].

From 2005 – 2021, a total of 47 cases (predominantly VL) were reported from 13 districts in the country. Disease transmission in Bhutan is sparse but widely distributed (Figure 1). Monggar district alone accounted for more than 45% of these reported cases.



This map is not authoritative on its international boundary.

Figure 1. Spatial distribution of various forms of leishmaniasis reported in Bhutan, 2018 – 2022. VL = visceral leishmaniasis, CL = cutaneous leishmaniasis, MCL = mucocutaneous leishmaniasis. *Source: VDCP, Ministry of Health, 2023*

On average, there are three cases detected in the country every year; however, annual incidence per 10,000 population remains below 1 as shown in Figure 2.

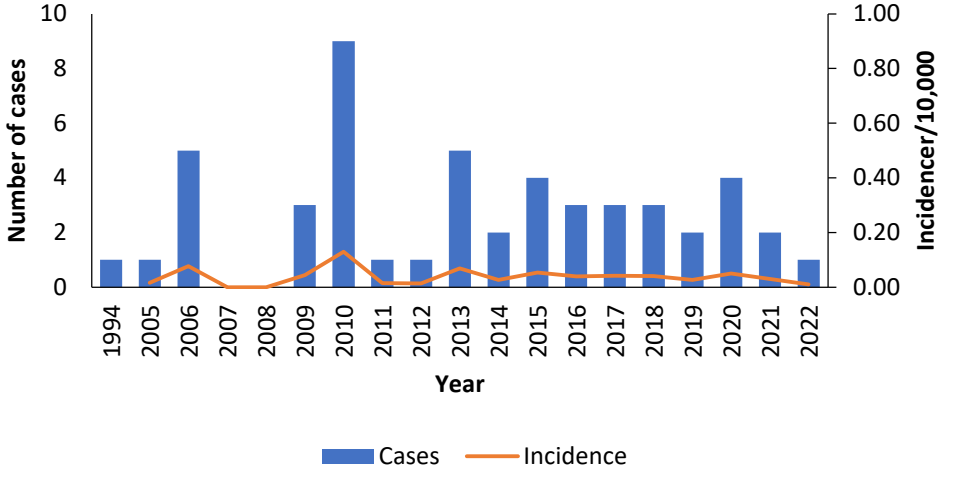


Figure 2. The annual trend of detection and the incidence of leishmaniasis cases in Bhutan, 1994 – 2021. *Source: VDCP, Ministry of Health, 2023*

Bhutan is a signatory to the Memorandum of Understanding on strengthening collaboration for regional VL elimination efforts along with Bangladesh, Nepal, India and Thailand since 2014 [5]. In alignment with updated global NTD roadmap 2021 – 2030 and regional strategic framework for accelerating and sustaining kala-azar elimination 2022-2026, the national programme has set a goal to achieve validation of elimination of VL as public health problem.

The targets for elimination of leishmaniasis in Bhutan are to reduce the:

1. **Annual incidence of kala-azar at the district-level to less than one per 10 000 population;**
2. **Case-fatality rate due to primary visceral leishmaniasis (VL) to less than 1%.**

1.3 Leishmaniasis overview

Leishmaniasis is a vector-borne disease caused by the trypanosomatid parasite belonging to the genus *Leishmania*. There are an estimated 21 species of *Leishmania* out of which 17 have been found in humans. The disease is transmitted by sandflies with specific species of sandflies involved in the transmission of specific species of *Leishmania-Phlebotomus* species in the Old World and *Lutzomyia* species in the New World [6].

There are different clinical forms of leishmaniasis – VL, CL, MCL and PKDL. There are mainly two forms known to occur in the Indian subcontinent: VL and PKDL caused by *Leishmania donovani* and transmitted by the sandfly species, *Phlebotomus argentipes*. However, CL has also been described in the region, caused by *L. tropica*. Human beings are the only known host for the parasite. PKDL occurs in all areas endemic to *Leishmania donovani* and up to 5 – 15% of patients with kala-azar develop the condition.

1.4 Life cycle, transmission and human infection

The life cycle of *Leishmania* starts after a female phlebotomine sandfly gets infected with the amastigote stage of the parasite through a blood meal of an infected person. The sandfly ingests macrophages infected with amastigotes during a blood meal. Amastigotes penetrate into the midgut of sand flies where it changes into motile promastigotes (flagellated stage) and begins intensive multiplication. Slender and long forms of promastigotes appear in large numbers in the midgut and attach themselves to the epithelium of the midgut. It then migrates to the buccal cavity after about 4 – 6 days. During subsequent blood meal taken by the female sandfly, infective promastigotes are injected and transmitted into the next human victim. In the human body, the promastigotes are taken up by macrophages, where they transform into amastigotes form and continue to multiply, rupturing the macrophages and infecting the reticuloendothelial system, e.g., spleen, liver and bone marrow (Figure 3).

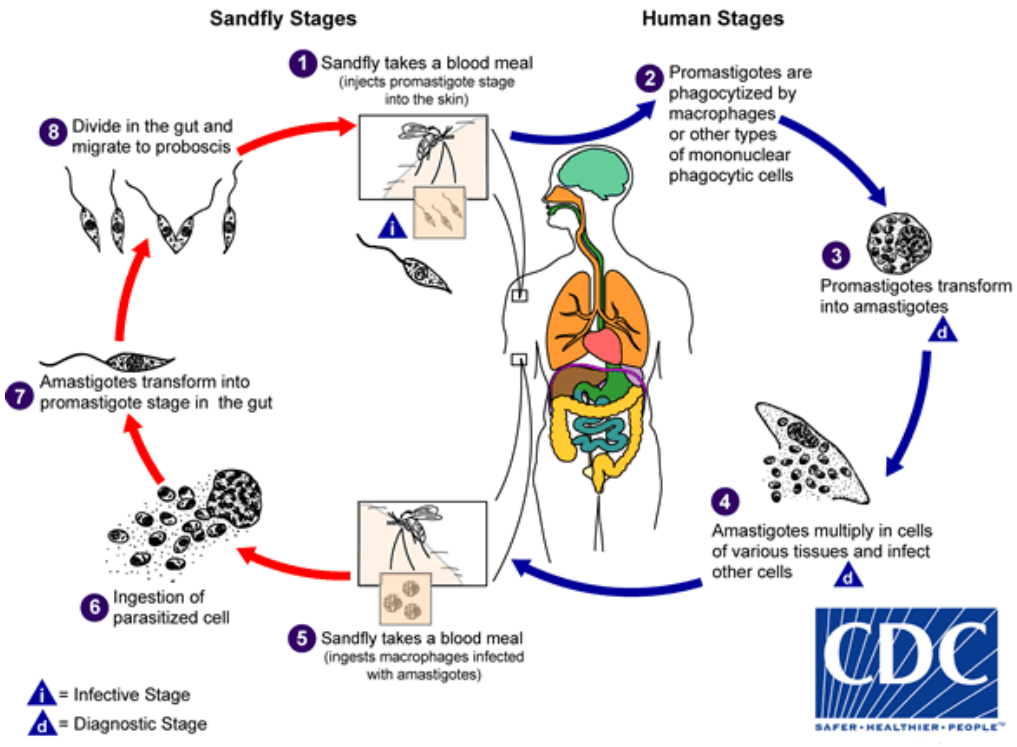


Figure 3. The life cycle of Leishmania
Source: Courtesy of the United States Centers for Disease Control and Prevention

In Bhutan, the probable causative agent of VL is *Leishmania donovani* [4, 7]. It is likely transmitted by the sandfly species *Phlebotomus argentipes*, however the vactor has not been confirmed. *Leishmania* parasites transmission to humans starts with the bite of an infected female sandfly that injects parasites into a susceptible host.

Most individuals infected by *L. donovani* will not develop the disease (only 10 – 25% of those infected develop the disease). When the host immune system is not able to suppress the parasite, it develops into VL.

1.5 Major risk factors

Leishmaniasis is mostly associated with poverty, poor living conditions and environmental changes. The following factors pre-disposing transmission and development of diseases:

- Poverty associated with poor housing and domestic sanitary conditions,
- Malnutrition,
- Immune compromised conditions,

- Climate and environmental changes (deforestation, construction of water reservoirs, roads, etc. that may affect sandfly distribution).

1.6 Pathophysiology

In VL, following infection with *Leishmania donovani* or *L. infantum*, reticuloendothelial hyperplasia takes place in the spleen, liver, mucosa of the small intestine, bone marrow, lymph nodes and other lymphoid tissues. Many of these cells are heavily parasitized and the lifespan of blood cells is reduced leading to granulocytopenia and anaemia. Liver function is altered resulting in decreased prothrombin production. This along with thrombocytopenia may result in severe mucosal haemorrhage. Hypoalbuminaemia is associated with oedema and other features of malnutrition. Diarrhoea may occur due to intestinal parasitization and ulceration or secondary enteritis. In the advanced stage, infections are frequent, especially pneumonia, dysentery and tuberculosis, and these are common causes of death.

The pathogenesis of PKDL is triggered by an immunological response and usually follows VL. Histologically, the hypopigmented macules consist of isolated areas, with a granulomatous reaction and few parasites. The erythematous nodular forms show histiocytic infiltration, oedema, proliferation of capillaries and numerous parasites.

Chapter 2
Diagnosis and management of leishmaniasis

Leishmaniasis is a complex group of disorders caused by protozoa, *Leishmania*. Its incubation period ranges from 10 days to over 1 year (typically 2 – 6 months) and the onset of the disease is gradual. It primarily affects the host's reticuloendothelial system and causes varying clinical syndromes ranging from asymptomatic cases to self-healing cutaneous ulcers and fatal visceral disease. These syndromes fall into three broad clinical categories: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML).

2.1 Visceral leishmaniasis case definitions

We recommend the following case definitions:

Probable VL: A person living in or having travelled to endemic areas showing clinical signs and symptoms of leishmaniasis (fever lasting more than two weeks and/or splenomegaly, hepatomegaly, anaemia, loss of weight).

Confirmed VL: A probable VL case with laboratory confirmation through either serological, parasitological or molecular techniques.

New case: Those with no previous history of VL.

Relapse case: Those with previous history of treatment of VL.

2.1.1 Clinical manifestations of visceral leishmaniasis

Asymptomatic infection

Many leishmaniasis infections are asymptomatic, reflecting the ability of the host immune system to control the parasite. Most patients with subclinical infection harbour viable parasites lifelong and can develop reactivation disease in the setting of immunosuppression.

Visceral leishmaniasis

The most important clinical manifestation of VL is the syndrome known as kala-azar (Hindi) or black fever as the skin turns black in the later stages of the disease. The incubation period is usually two to six months but can range from a few weeks to several years. The onset of symptoms is usually insidious or subacute, with slow progression of malaise, fever, weight loss, and splenomegaly (with or without hepatomegaly) over a period of weeks to months. Patients may complain of abdominal discomfort and fullness that may be localized to the left upper quadrant due to splenomegaly. The spleen is usually firm and minimally tender, but in some

patients, palpation is quite painful. Some patients may have hepatomegaly and lymphadenopathy.

In later stages, VL can be associated with anaemia, marked cachexia, oedema, jaundice and ascites. There may be hypoalbuminemia, thrombocytopenia and hepatic dysfunction. Rarely, chronic diarrhoea and malabsorption can occur as a result of parasitic invasion of the intestine.

Kala-azar is nearly always fatal without treatment. Immunosuppression and co-infection with human immunodeficiency virus (HIV) and tuberculosis increase the risk for secondary bacterial infections like pneumonia, otitis media, sepsis and mortality. Kala-azar during pregnancy can lead to spontaneous abortion or congenital leishmaniasis.

Differential diagnosis of visceral leishmaniasis

The differential diagnosis of VL includes:

- Malaria
- Tropical splenomegaly
- Amoebic liver abscess
- Lymphoma
- Extrapulmonary tuberculosis.

2.1.2 Laboratory evaluation

Laboratory findings

Nonspecific laboratory findings of VL include normocytic and normochromic anaemia and low neutrophil, eosinophil and thrombocyte counts. An elevated neutrophil count is uncharacteristic of VL and should prompt a search for secondary bacterial infection. There may be raised liver enzymes and bilirubin, and low albumin levels.

Provider-initiated testing for HIV, Hepatitis B, Hepatitis C and tuberculosis is recommended in all VL patients.

Definitive diagnosis and referral pathway of VL

Definitive diagnosis of VL is confirmed by histopathological examination or molecular and serological testing.

The diagnostic algorighm for VL is given in Figure 4.

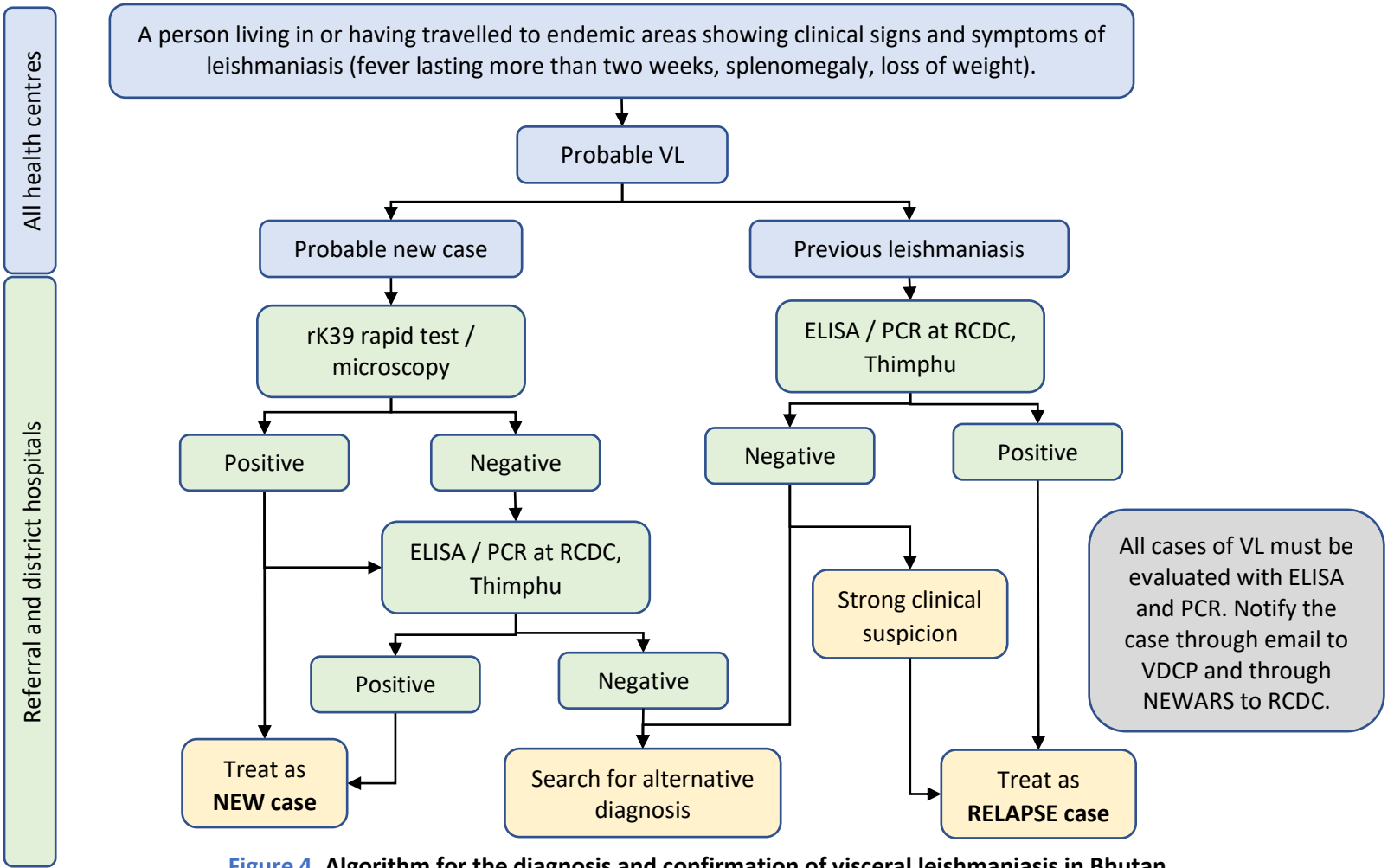


Figure 4. Algorithm for the diagnosis and confirmation of visceral leishmaniasis in Bhutan

Leishmania serology

rK39 test

In Bhutan, the recombinant kinesin (rK39) antigen test is the mainstay in the serological diagnosis of VL. rK39 test kits are available at all levels of health care facilities. Cases that are rK39 positive should be referred to the National or Regional Referral Hospitals or centres where further testing with tissue aspirate can be performed. The details on rK39 testing and interpretation are given in Annexure 1.

All serological tests suffer from two limitations: first, specific antibodies remain detectable up to several years after cure. Therefore, relapse cannot reliably be diagnosed by serology; secondly, a significant proportion of healthy people living in endemic areas with no history of visceral leishmaniasis are positive for antileishmanial antibodies owing to asymptomatic infections. Antibody-based tests must therefore always be used in combination with a standardized clinical case definition for visceral leishmaniasis diagnosis. rK39 can be false negative in immunocompromised status (HIV, immunosuppressant therapy and severe malnutrition).

ELISA for leishmaniasis

Enzyme-linked immunosorbent assay on serum sample can be performed at the Royal Centre for Disease Control, Thimphu. Refer Annexure 2 for sample collection and transport.

Molecular techniques

Polymerase chain reaction (PCR) can be performed at the Royal Centre for Disease Control, Thimphu on samples from the bone marrow, spleen or peripheral blood. Refer Annexure 3 for sample collection and Annexure 7 for sample storage and transport. The request forms for tests at the RCDC is given in Annexure 6.

Histopathology

Histopathologic demonstration of parasite requires needle aspiration or biopsy of affected organs, usually bone marrow or spleen. Bone marrow aspirates are generally safer than splenic aspirates. Histopathologic diagnosis requires visualization of amastigotes which are commonly found within the histiocytes (Leishman-Donovan bodies). The grading methods of Leishmania parasitic load on bone marrow aspirate (and bone marrow biopsy) and splenic aspirates is given in Annexure 3.

Culture facility for *Leishmania* is not available in the country. Treatment decisions for VL usually do not require species identification since they are based on disease severity, geographic origin and the presence of HIV and other coinfections.

2.1.3 Management of visceral leishmaniasis

The objectives of the treatment of VL are to cure the patient, prevent complications, reduce the risks of relapse and PKDL, minimize the side effects of antileishmanial medicines, contain the risk of development of drug resistance and reduce the risk of spread of the disease to others.

The principles of management include counselling the patient and family members to ensure good compliance to treatment; initiating treatment only after confirming the diagnosis; providing treatment under appropriate medical supervision, providing supportive therapy to address nutritional status, anaemia, haemorrhagic complications and secondary infections; and reporting the case to the Vector-borne Disease Control Programme, Ministry of Health.

Given the elimination efforts of leishmaniasis as a public health problem in Bhutan, it is important to diagnose and provide appropriate and adequate treatment for all diagnosed cases of leishmaniasis.

Refer all suspected cases to the National Referral Hospital, Regional Referral Hospitals or centres capable for confirmation of diagnosis and treatment.

All forms of leishmaniasis must be reported to the Vector-borne Disease Control Programme (email to: vdcp@health.gov.bt), Ministry of Health using the Case Reporting Form given in Annexure 8.

All forms of leishmaniasis must be notified to the Royal Centre for Disease Control using the National Early Warning, Alert Response and Surveillance Information System (NEWARS).

Management in an immunocompetent patient

The drugs available for the treatment of leishmaniasis are Miltefosine, Sodium stibogluconate, Liposomal amphotericin B and Paromomycin.

For patients with VL, liposomal amphotericin B is the preferred antileishmanial agent.

Due to high levels of resistance to antimonial drugs noted in India and Nepal, miltefosine may be used as an alternative preferred antileishmanial agent in Bhutan.

The preferred antileishmanial agents are Liposomal amphotericin B, Amphotericin B deoxycholate or Miltefosine. The alternative antileishmanial agents are Sodium stibogluconate or Paromomycin. The drug dosage and duration of therapy are

shown in Table 1. The side effects and monitoring during therapy is shown in Table 2.

Table 1. Drug dosage and duration of therapy for the management of leishmaniasis

Preferred treatment	Alternative treatment
Liposomal amphotericin B* 3 mg/kg OD on Days 1 to 5, Day 14 and Day 21 up to a total dose of 21 mg/kg by infusion, or 10 mg/kg as one or two doses by infusion	**Sodium stibogluconate** 20 mg/kg IV or IM OD for 30 days
OR	OR
Amphotericin B deoxycholate 0.75 – 1 mg/kg OD for 15 days by infusion	
OR	
Miltefosine Body weight <25 kg: 50 mg OD per oral for 28 days Body weight ≥25kg: 100 mg OD per oral for 28 days For children below 12 years: 2.5 mg/kg daily in two divided doses per oral for 28 days	**Paromomycin** 15 mg/kg IM OD for 21 days

IM = intra muscular, IV = intra venous, OD = once daily dosing
*Check for specific contra-indications of liposomal amphotericin B; administer test dose; follow manufacturer's instructions; monitor renal functions at baseline and at appropriate intervals

Table 2. Monitoring of side effects during the treatment of leishmaniasis

Therapeutic agent	Side effect monitoring
Liposomal amphotericin B*	Infusion related reactions (fever, rigor, nausea, vomiting, hypotension), malaise, nephrotoxicity, electrolyte abnormalities (K, Mg), anaemia
Amphotericin B deoxycholate	
Miltefosine	Nausea/vomiting, teratogenic. Rare risk of hepatic, renal impairment. Ocular adverse events may occur with prolonged treatment
Sodium stibogluconate	Myalgia, headaches, fatigue, nausea are common, elevated LFT, lipase, amylase (usually reversible), QT Prolongation, ST-T wave changes, cytopenias.
Paromomycin	Pain at injection site, reversible transaminitis, reversible ototoxicity and renal toxicity

Liposomal amphotericin B is available in the National Referral Hospital and the Regional Referral Hospitals under the National Essential Medicines List 2021 [8].

Miltefosine is made available through the leishmaniasis control initiative under the Vector-borne Disease Control Programme, Ministry of Health. The other medicines recommended are available on case-by-case basis at the referral hospitals or centres with specialists through Form II of the drug supply system.

A probable VL case with a high clinical suspicion but not confirmed by any laboratory test may be initiated on therapy upon specialist consultation.

Management in special sup-population

Situations where liposomal amphotericin B is contra-indicated

If the use of liposomal or lipid amphotericin B formulations is not possible, administer amphotericin B deoxycholate, with strict monitoring of toxicity. The following are conditions where the use of liposomal amphotericin B is not advised: age over 50 and under 1 year old, kidney failure, liver failure, heart failure, corrected QT interval >450 ms, concomitant use of drugs that alter the QT interval, hypersensitivity to or therapeutic failure to pentavalent antimonials or other medication used for the treatment of VL. The risk of nephrotoxicity may be reduced with adequate hydration of the patient.

Pregnancy and lactation

The suggested medication, when indicated, is liposomal or other formulations of amphotericin B. *The use of pentavalent antimonials and miltefosine is contraindicated.*

Patients with kidney disease, liver disease, heart disease

May use liposomal amphotericin B with frequent monitoring.

Comorbidity with tuberculosis

Take special care in monitoring adverse events, especially when deciding to use the two treatments concomitantly (tuberculosis and leishmaniasis).

Patients with HIV and other causes of immunosuppression

In patients with VL and HIV co-infection, combination therapy with liposomal amphotericin B and miltefosine is preferred over liposomal amphotericin B monotherapy [9].

Dual therapy: Liposomal amphotericin B 5 mg/kg on days 1, 3, 5, 7, 9 and 11 (up to a total dose of 30 mg/kg) + Miltefosine 100 mg/day for 14 days

If miltefosine is not available, consider using monotherapy with liposomal amphotericin B at 5 mg/kg on days 1 – 4, 8, 10, 17 and 24 (up to a total dose of 40 mg/kg).

In patients with HIV, antiretroviral therapy (ART) should be initiated or optimized as soon as the patient is sufficiently able to tolerate it. VL that becomes clinically manifest or worsens after initiation of ART should be treated with antileishmanial therapy.

Patients with therapeutic failure

Administer any of the recommended treatments other than the one initially used or a combination of liposomal amphotericin B and miltefosine.

Treatment-related definition in visceral leishmaniasis

The following are the treatment-related definition in VL:

- **Treatment completed**: The patient has completed the full-course of the treatment as per the national protocol, and the clinician's prescription. The length of treatment depends on the drug regimen.
- **Treatment stopped for medical reason**: The treatment was stopped by decision of the clinician (e.g. patient suffering from side effects, treatment failure) or after the death.
- **Defaulter**: The patient does not complete the full-course treatment.
- **Treatment completion unknown**: the patient completion of treatment is unknown (unrecorded). This is different from default, where the clinician knows that the patient has not completed the treatment.

2.1.4 Follow-up of visceral leishmaniasis

Response to treatment is assessed clinically based on the improvement in fever, splenomegaly, anaemia and other clinical parameters like weight gain and improvement in general condition. Fever may take about two weeks to subside while splenomegaly may persist for a month. There is no need for performing any tests to assess the response to treatment as the conventional tests may remain positive for months or years after the clinical recovery.

The clinician may share the clinical follow up schedule to the VDCP, MOH.

Treatment outcome definitions in visceral leishmaniasis

Treatment outcomes for VL cases have to be assessed twice:

- **Initial assessment**: At the end of the treatment, or 15 days after treatment starts for short-course regimen (less than 5 days);
- **Final assessment**: Six months after the last drug was taken (final outcome).

At the **initial assessment**,

- **Initial cure**: A full course of drugs has been completed AND the patient has clinically improved. The clinical criteria for initial cure is defined as "no fever + regression of splenomegaly (even partial) + return of appetite and/or gain in body weight".
- **Failure (non-response)**: Signs and symptoms persist or recur during treatment or up to initial treatment outcome assessment
- **Lost-to-Follow-up/Unknown**: The patient does not present for initial assessment after completion of treatment, or the patient status was not recorded.
- **Death**: Death of any person having been diagnosed of VL regardless of the treatment status and the cause of death within the standard post-treatment follow-up period.
  Any death should be notified with specification of the cause of death, as follows:
    - Death due to VL
    - Death due to HIV
    - Death due to other disease or medical condition(s)
    - Death due to serious adverse events (iatrogenic)
    - Death due to non-medical condition (accident)
    - Death due to unknown cause.

At the **final assessment**:

- **Final cure**: A patient who after initial cure remains symptom-free at six months after the end of treatment.
- **Relapse**: A patient who experiences recurrence of VL symptoms with parasitological confirmation at any time point after initial cure.
- **Death**: Death of any person having been diagnosed of VL regardless of the treatment status and the cause of death within the standard post-treatment follow-up period
- Any death should be notified with specification of the cause of death, as follows:
    - Death due to VL
    - Death due to HIV
    - Death due to other disease or medical condition(s)
    - Death due to serious adverse events (iatrogenic)
    - Death due to non-medical condition (accident)
- **Loss to follow-up**: Patient does not present for assessment at six months.

At least one year follow-up is recommended to exclude clinical relapse. A longer follow-up period, sometimes lifelong, is recommended for immunocompromised patients. In patients with immunocompromised conditions, a repeat PCR is recommended after the completion of treatment course. If positive, another course of treatment or secondary prophylaxis should be initiated.

Relapse of visceral leishmaniasis

The median duration of relapse of VL reported in India is between 6 to 10 months [10, 11].

A case of relapse is a confirmed case of VL who experiences recurrences of VL symptoms with parasitological confirmation at any time point after initial cure.

In cases of relapse of VL, administer any of the recommended treatments other than the one initially used or a combination of liposomal amphotericin B and miltefosine.

2.2 Post-kala-azar dermal leishmaniasis

A case of post-kala-azar dermal leishmaniasis (PKDL) is a patient with multiple hypopigmented macules, papules or nodules who is rK39 positive or parasite positive and who has been treated for kala-azar in the past.

PKDL is characterized by skin lesions that appear in patients with treated kala-azar patients. However, in some cases, PKDL occurs without the preceding history of kala-azar (Figure 5). Usually, patients do not have any signs of kala-azar but have multiple hypopigmented skin lesions (macules, papules, nodules) with no loss of sensitivity. It usually appears 6 months to 1 or more years after the apparent cure of visceral leishmaniasis but may occur earlier or even concurrently with VL. In about 15% of PKDL cases, there may be no previous history of leishmaniasis.

Case classification of post-kala-azar dermal leishmaniasis is as follows:
Probable PKDL: A patient from an area endemic for kala-azar with multiple hypopigmented macules, papules or plaques or nodules with no sensitivity loss

Confirmed PKDL: A patient from an area endemic for kala-azar with multiple hypopigmented macules, papules, plaques or nodules who is parasite- or PCR-positive in a slit skin smear or biopsy.

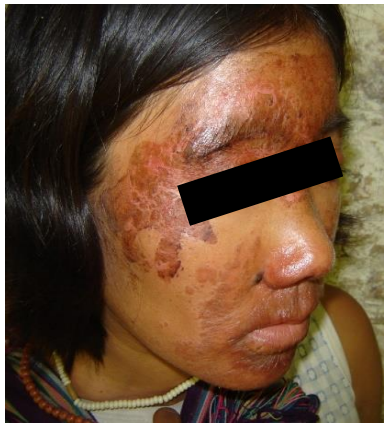


Figure 5. Erythematous patches and plaques on the face in a patient with PKDL
Source: VDCP, Ministry of Health, Bhutan

The features to identify the differential diagnoses are described in Table 3.

Table 3. The differential diagnoses of post-kala-azar dermal leishmaniasis

	Vitiligo	Leprosy	PKDL
Family history	Yes (genetic)	Yes (exposure)	Yes (exposure to VL)
Sites	Face, acral, central back	Any skin site	No acral, sparing of central back
Clinical features	Depigmented patches	Macular, papular, nodular, plaques	Macular, papular, nodular, plaques
Symmetrical lesions	Yes, but not in segmental type and few lesions	yes- lepromatous	Yes
Single lesion	Yes	Yes - tuberculoid or borderline tuberculoid	No
Neurological involvement	No	Yes	No
Lobulation of ears	No	Yes	Yes
Madarosis	No	Yes	No
Predilection for sun exposed areas	No	No	Yes
Slit skin smear	-	Modified ZN stain - acid fast bacilli	Giemsa stain - Leishmania amastigotes

The diagnosis of PKDL is confirmed by identification of the parasites in skin biopsy or scraping of skin slit. Skin smears are stained the same way as bone marrow or splenic aspirate. Tissue samples from skin biopsy may be tested with PCR. The procedure for slits skin smear sampling is given in Annexure 4.

Serological tests like rK39 are usually positive but of limited value as a positive result may be due to antibodies persisting after a past episode of VL. rK39 test may be negative in patients with only macular lesions. Patients with PKDL are an important reservoir of infection.

All forms of leishmaniasis must be reported to the Vector-borne Disease Control Programme (email to: vdcp@health.gov.bt), Ministry of Health using the Case Reporting Form given in Annexure 8.

All forms of leishmaniasis must be notified to the Royal Centre for Disease Control using the National Early Warning, Alert Response and Surveillance Information System (NEWARS).

The recommended treatment for PKDL is miltefosine 2.5 mg/kg/day for 12 weeks. Some PKDL patients may require up to 12 weeks of miltefosine.

An alternative treatment with amphotericin B deoxycholate 1 mg/kg/day up to 60 to 80 doses over four months may be recommended.

After the completion of treatment, the patient should be followed up for at least 12 months for reoccurrence.

Treatment outcome definitions in post-kala-azar dermal leishmaniasis

The following are the treatment outcome definitions in PKDL:

- **Initial cure**: Clinical improvement at the end of treatment defined as a considerable reduction in the number and size of skin lesions
- **Final cure**: Clinical cure 12 months after the end of treatment defined as a complete resolution of macules, papules, plaques and nodules, no new lesion, and near total repigmentation of maculae.

2.3 Cutaneous leishmaniasis

Cutaneous leishmaniasis constitutes the majority of all incident cases globally and is the mildest form of disease. The range of cutaneous manifestations may be attributed to the variability in the parasite virulence and the variability in the host's immune response. Cutaneous involvement includes a range from localized cutaneous leishmaniasis, the most common presentation, to mucosal leishmaniasis and diffuse cutaneous leishmaniasis.

The diagnosis of CL should be considered in patients with one or more nodules, ulcero-crusted nodules or plaques in a person living or coming from an endemic district.

The definitive diagnosis requires appropriate clinical examination and laboratory tests.

2.3.1 Clinical manifestations of cutaneous leishmaniasis

CL occurs on the exposed areas of the skin which is accessible to the sand fly. Sand fly mouthparts generally cannot penetrate through clothing. Sand fly bite reactions are pruritic but do not enlarge, and may resolve slowly over weeks.

Localized cutaneous leishmaniasis

Localized CL begins as a pink-coloured papule that enlarges and develops into a nodule or plaque-like lesion leading to a painless ulceration with an indurated border (Figure 6). The lesion can be located anywhere on the body including the face and scalp and is often covered with an adherent crust. In some cases, the size of the ulcers may be ≥6 cm while in others it may be 1 – 2 cm. There may be multiple lesions and the clinical appearance may be variable taking multiple shapes and forms. Small satellite lesions may develop outside the plaque or ulcer. It spreads along the draining lymphatics causing nodular lymphangitis. Regional lymphadenopathy may become prominent in some cases.

It must be noted that secondary bacterial infection of the ulcer may be associated with fever, purulent drainage and local cellulitis. Superinfection with bacteria leads to the enlargement of the lesions and their persistence.

Localized CL heals with the formation of an atrophic or depressed scar or as a keloid over months to years. However, following the resolution of the initial lesions, it may get reactivated later as either cutaneous or mucosal leishmaniasis.

Diffuse cutaneous leishmaniasis

Diffuse CL is a rare entity where the amastigotes disseminate to areas of the skin other than the primary site and may involve entire limbs or the body. This is reported in infections by *L. amazonensis* and *L. aethiopica* or other species in patients with Acquired Immunodeficiency Syndrome WHO Stage 4.

Cutaneous leishmaniasis in immunocompromised hosts

Patients with immunosuppressive conditions are at increased risk for more severe CL. This includes patients on chronic corticosteroids, biologic modulators, or other immunosuppressive drugs; organ transplant recipients; pregnant patients; and

patients with HIV/AIDS. These conditions have been associated with the reactivation of CL and dissemination or progression to mucosal leishmaniasis.

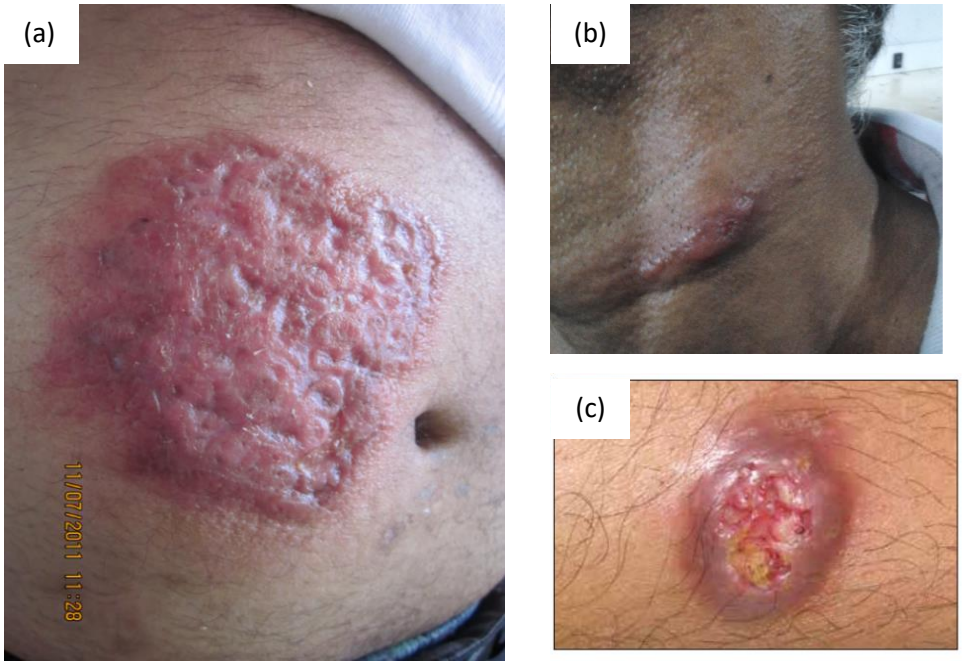


Figure 6. Manifestations of cutaneous leishmaniasis (a) A large erythematous plaque on the abdomen. (b) Erythematous plaque with central erosion and mild crusting on the anterolateral neck. (c) Ulcer with slough and central granulation
Source: (a) & (b) Dr Ambika Pradhan, JDWNRH, Bhutan

Differential diagnosis of cutaneous leishmaniasis

The differential diagnoses of localized CL include:

- Bacterial infection
- Cutaneous myiasis
- Pyoderma gangrenosum
- Ecthyma
- Impetigo
- Prurigo nodularis
- Lichen simplex chronicus
- Cutaneous tuberculosis
- Skin malignancy
- Sarcoidosis
- Fungal infections.

2.3.2 Laboratory evaluation

The laboratory tests for diagnosis of CL include direct microscopy, histopathology or molecular diagnosis. The specimens include press imprint smear, slit skin smear, fine needle aspirate and skin biopsy. Refer Annexure 4 for slit skin smear sampling, Annexure 5 for skin smear aspirate smear and Annexure 7 for storage and transportation of tissue aspirates for microscopy and PCR. The request forms for tests at the RCDC is given in Annexure 6.

Smears

Smears can be obtained from fine needle aspirates and imprint smear. The samples are air-dried, fixed with methyl alcohol, and stained with Giemsa stain. The procedure for skin aspirate smear is described in Annexure 5.

Skin biopsy

Take the skin biopsy from the infiltrated margin for an impression smear, histologic examination, molecular studies and culture (if available). Visualization of the kinetoplast is essential for a histologic diagnosis. This may require viewing several different sections in fixed tissue samples.

Histopathology

Histopathology samples exhibit mixed inflammatory cell infiltrate composed of histocytes and scattered multinucleated giant cells, lymphocytes, and plasma cells, sometimes with Russel bodies. Histopathologic diagnosis requires visualization of amastigotes which are commonly found within the histiocytes (Leishman-Donovan bodies).

Molecular techniques

Molecular techniques for diagnosis include polymerase chain reaction that can be performed at the Royal Centre for Disease Control, Thimphu.

Other diagnostic methods include serologic testing which can be considered in cases with mucosal leishmaniasis before the initiation of treatment. **Serologic tests for VL may be cross-reactive in the setting of CL.**

2.3.3 Management of cutaneous leishmaniasis

The objective of CL treatment is a clinical cure. Simple CL lesions may resolve without treatment but it is prudent to provide treatment. Not all patients who undergo treatment demonstrate elimination of parasitic infection.

Decision-making about the therapeutic strategy to be used in patients diagnosed with leishmaniasis should be individualised and shared with patients based on a clear explanation of the risks and benefits of the available alternatives.

It is not imperative to identify the species to initiate treatment, but if the most prevalent species in the region is known, treatment should be initiated according to the clinical condition, availability of the medication, and considering risk-benefit balance.

All forms of leishmaniasis must be reported to the Vector-borne Disease Control Programme (email to: vdcp@health.gov.bt), Ministry of Health using the Case Reporting Form given in Annexure 8.

All forms of leishmaniasis must be notified to the Royal Centre for Disease Control using the National Early Warning, Alert Response and Surveillance Information System (NEWARS).

Based on the clinical characteristics, CL is classified into simple and complex infection (Infectious Diseases Society of America, 2016) as follows (Table 4) [12]:

Table 4. Classification of cutaneous leishmaniasis into simple and complex cases based on the Infectious Diseases Society of America criteria, 2016 [12]

Simple cutaneous leishmaniasis	Complex cutaneous leishmaniasis
No mucosal involvement	Local subcutaneous nodules
Single or a few skin lesions	>4 skin lesions of substantial area (>1 cm)
Small lesion size (diameter <1 cm)	Large individual skin lesion (diameter ≥5 cm)
Location of lesion feasible for local treatment	Size or location of lesion such that local treatment is not feasible
Non-exposed skin (ie, not cosmetically important)	Lesion on the face including ears, eyelids or lips*; fingers, toes or other joints; or genitalia
Immunocompetent host	Immunocompromised host (impaired cell-mediated immunity)
Lesion resolving without therapy	Clinical failure of local therapy
	Unusual syndromes: leishmaniasis recidivans, diffuse CL or disseminated CL
*Consider the diagnosis of muco-cutaneous leishmaniasis	

The management of simple cutaneous leishmaniasis with lesions includes ***wound care*** with one of the following:

- ***Physical treatment*** with cryotherapy
- Thermotherapy
- **Intralesional** antimonials
- Topical paromomycin.

The indications for the above therapy include:

- Lesions <4 cm
- Number of lesions <4
- Lesions located on nose, lips, and close to the eyes.

The management of complex CL and simple CL not responding to topical therapy includes ***systemic therapies*** (same agents recommended for VL in Table 1).

Management in an immunocompetent patient

The topical agents available for cutaneous leishmaniasis are shown in Table 5.

Table 5. Intralesional and topical drug regimens for the treatment of cutaneous leishmaniasis in adults

Therapeutic agent	Dosage and duration
Sodium stibogluconate	0.5 to 2 mL of 100 mg/mL pentavalent antimony intralesionally every 3 to 7 days until healed (treat up to 3 weeks)
Paromomycin ointment	Apply topically to lesions twice daily for 10 days, withhold for 10 days, then reapply for 10 days
Paromomycin cream (15%)	Apply topically to lesions once daily for 20 days
Fluconazole	200 to 400 mg orally once daily for 6 weeks
Ketoconazole	600 mg orally once daily for 28 days

Local therapy

Local therapy is indicated for patients with simple CL. It is recommended to provide one of the following local therapies for CL:

- Cryotherapy with liquid nitrogen weekly for six weeks depending on the resolution of skin lesion. During cryotherapy, it is important to treat into 1 – 2 mm of normal-appearing tissue around the lesions.
    - If cryotherapy is given alone, two to three freeze-thaw cycles are recommended with freezing time of 10 seconds.
    - If cryotherapy is combined with intralesional therapy, only one freeze-thaw cycle is recommended with freezing time of 10 seconds.
- Thermotherapy under local anaesthesia for 30 seconds in a grid-like pattern extending 1 – 2 mm of normal skin. Usually one session, sometimes up to 3 sessions may be recommended.
- Intralesional antimonial every three to seven days ± cryotherapy weekly for six weeks
- Paromomycin ointment/cream.

Topical application of Paromomycin ointment or Paromomycin cream (15%) may be used for the treatment of ulcerative lesions and is not recommended to be used with cryotherapy.

Adverse effects of cryotherapy include erythema, oedema of the perilesional area, occasional blistering, permanent hypopigmentation (in dark-skinned individuals), hyperpigmentation, and scarring.

The contraindications for intralesional antimonials include lesions on the face or having multiple lesions. Avoid cryotherapy or thermotherapy on eyelids, tip of the nose, lips, mucous membranes, cartilaginous structures and superficial nerves.

Oral systemic therapy

Oral azoles (Fluconazole or Ketoconazole) alone or in combination with cryotherapy may be used in CL.

Parenteral systemic therapy

Pantavalent antimonial 20 mg/kg/day IM or IV for 10 – 20 days is recommended in complex CL or simple CL not responding to local therapy.

Refer Table 2 under the section on VL on the side effects and monitoring of side effects.

2.3.4 Follow-up of cutaneous leishmaniasis

Patients should be followed for 6 to 12 months to evaluate for relapse. Treatment response is generally assessed by physical appearance. In some cases, a paradoxical increase in the local inflammatory response may be seen in the first two to three weeks of treatment. By four to six weeks following treatment, the lesion size decreases by >50%, with improvement in oedema and inflammation. Lesion sampling for parasitologic assessment is not necessary if the lesion appears to be healing clinically. In some cases, there may not be complete resolution of the lesion at the end of the treatment course.

Treatment outcome definitions in cutaneous leishmaniasis

Treatment outcomes for CL cases have to be assessed twice:

- Initial assessment: Between 2 to 4 weeks after initiating the treatment.
- Final assessment: Between 45 and 90 days after initiating treatment, or longer depending on the parasite.

At the **initial** assessment,

- **Initial cure (improvement)**: Decrease in the size of the lesion or signs of reepithelization
- **Failure**: Increase in the size of a nodule or a plaque or an ulceration
- **Death** with specification of the cause of death:
    - Death due to CL

    - Death due to HIV
    - Death due to other disease or medical condition(s)
    - Death due to severe adverse events (iatrogenic)
    - Death due to non-medical condition (accident)
- **Death due to unknown cause**
- **Unknown**: Patient does not present for assessment or the outcome was not recorded.

At the **final** assessment,

- **Final cure**: Total re-epithelization
- **Failure**: Lack of complete re-epithelization
- **Death** with specification of the cause of death:
    - Death due to CL
    - Death due to HIV
    - Death due to other disease or medical condition(s)
    - Death due to serious adverse events (iatrogenic)
    - Death due to non-medical condition (accident)
    - Death due to unknown cause
- **Lost to follow up – unknown**: Patient does not present for assessment or the outcome was not recorded

A **relapse of CL** is a confirmed case who experiences recurrence of typical CL lesions with parasitological confirmation at any time point after cure.

2.4 Mucosal leishmaniasis

Mucosal leishmaniasis (ML) presents with nasal congestion, mucosal bleeding, increased secretions, pain, deformity, and inflammation. There may be an erosion of mucosal surfaces in the nose, mouth, or nasal septum. Other involved areas may include the cheek, pharynx, palate, epiglottis, larynx, trachea, and genitalia. Hoarseness and/or a brassy cough are suggestive of laryngeal involvement. Mucosal involvement of leishmaniasis is shown in Figure 7.

The disease is considered mild in the setting of isolated nasal stuffiness, moderate if there is associated odynophagia or dysphonia, and severe if there is respiratory distress or severe dysphonia.

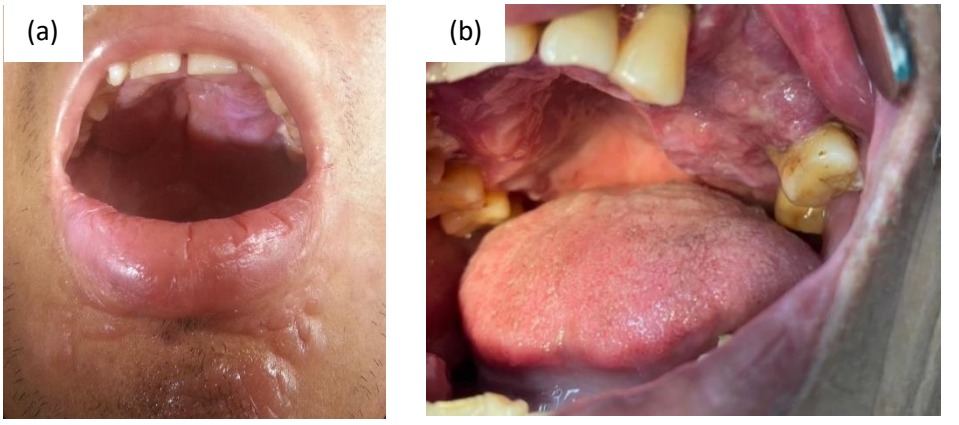


Figure 7. (a) Erythematous plaques on the chin and palate in a case of muco-cutaneous leishmaniasis. (b) Ulcerated irregular plaque over the palate in a case of mucosal leishmaniasis. *Source: (a) Dr Ambika Pradhan, JDWNRH, Bhutan; (b) Dr Gyan Prasad Bajgai, JDWNRH, Bhutan*

2.4.1 Management of mucosal leishmaniasis

The involvement of mucosal surfaces is a rare entity. However, it has a presentation similar to other sino-nasal lesions, most often like granulomatous nasal disease and carcinoma. A high index of suspicion is needed especially when the biopsy report is negative or inconclusive.

Mucosal leishmaniasis may occur concurrently or following partially treated or untreated CL. In rare instances, there may be only mucosal involvement. ML is characterised by mucosal destruction.

Diagnosis of mucosal leishmaniasis

The diagnosis is established through tissue biopsy for histopathological evaluation and PCR. Serological testing with rK39 and ELISA may be helpful in establishing the diagnosis. Refer Annexures for details on obtaining and transportation of relevant samples.

Management in an immunocompetent patient

It is difficult to achieve a cure unless ML is identified when mild. The goals of treatment include preventing morbidity (eg, disfigurement) and mortality (eg, from aspiration pneumonia or respiratory obstruction).

All forms of leishmaniasis must be reported to the Vector-borne Disease Control Programme (email to: vdcp@health.gov.bt), Ministry of Health using the Case Reporting Form given in Annexure 8.

All forms of leishmaniasis must be notified to the Royal Centre for Disease Control using the National Early Warning, Alert Response and Surveillance Information System (NEWARS).

The initial management of ML is Liposomal amphotericin B 3 mg/kg intravenously daily for up to a total cumulative dose of 20 – 60 mg/kg.

An alternative therapy includes Miltefosine and Amphotericin deoxycholate (refer Table 1 for dosing).

Management in special sub-groups

See the section on visceral leishmaniasis.

2.4.2 Follow-up of mucosal leishmaniasis

During follow up, patient will need thorough examination of the oral cavity followed by fibre-optic nasolaryngoscopy to visualize the upper respiratory tract up to the level of endolarynx every three months for one year or longer in the absence of resolution. Evidence of clinical response depends on the initial presentation; signs to follow include erythema, oedema, infiltration, ulceration, and tissue destruction. Septal perforation and granulomatous-appearing lesions are common. The response is usually seen during treatment, but relapses are common. In some cases, surgical reconstruction may be required; if possible, surgical reconstruction can be attempted after 1 year of full resolution.

2.5 Serious adverse event during treatment

Serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose results in:

- Death;
- Inpatient hospitalization or prolongation of existing hospitalization;
- Persistent or significant disability or incapacity;
- Life threatening situation;
- Congenital anomaly or birth defect.

The term “severe” is not synonymous with serious. Seriousness of an event is based on patient/event outcome or action criteria which serves as guide for defining regulatory reporting obligations. SAEs should be recorded in the patient form and the case register.

Prescribers should monitor the pharmacovigilance of antileishmanial drugs.

SAEs should also be notified as per the national adverse reaction reporting protocol to the National Pharmacovigilance Centre, Medical Products and Controlled Substances Division, Bhutan Food and Drug Authority, Thimphu, or to the nearest Regional Pharmacovigilance centre using the “Suspected Adverse Drug Reaction Reporting Form”.

SAEs consecutive to treatment with antileishmanial drugs should also be reported to WHO through the standardized pharmacovigilance form. Refer Annexure 14 on the WHO Adverse Event Reporting Form upon using antileishmanial agents.

Minor adverse events are not included in serious adverse events reporting. More definitions can be seen in Safety Monitoring of Medical Products reporting system for the general public, World Health Organization, 2012 [13].

Chapter 3
Prevention of leishmaniasis

Bhutan is signatory to kala-azar elimination initiative in South East Asia Region. The Regional Strategic Framework for accelerating and sustaining elimination of kala-azar in the South-East Asia Region 2022 – 2026, aims to achieve elimination of kala-azar as public health problem from all endemic implementation units in the region by 2026.

The targets for elimination are to achieve/sustain:

1. Annual incidence of kala-azar at the district or sub-district level to less than one per 10 000 population
2. Case-fatality rate due to primary visceral leishmaniasis to less than 1%.

3.1 Disease surveillance for leishmaniasis

A functional surveillance system is key a component of VL elimination programme for evidence-based planning, implementation and evaluation of control measures.

3.1.1 Objectives of kala-azar and PKDL surveillance

Kala-azar is targeted for elimination as a public health problem in Bhutan with the target to keep an annual incidence of kala-azar cases below 1 per 10000 population at the district level.

The objectives of kala-azar and PKDL surveillance are to:

- Monitor the incidence trends in time and space
- Follow-up every kala-azar case for development of PKDL
- Monitor progress towards elimination
- Evaluate elimination programs
- Identify at risk population for targeted interventions
- Conduct spot entomological surveys and control measures.

3.1.2 Objectives of cutaneous leishmaniasis surveillance

Though cutaneous forms of leishmaniasis are not targeted for elimination, there are growing incidence of reported cases in Bhutan. The resulting disfigurement and social stigmatization from unattended CL and MCL cases, often due to poor awareness among health workers and community, must be addressed. It therefore, needs to be closely monitored to with the objective to:

- Estimate CL and MCL burden
- Monitor the incidence trends in space and time
- Determine the distribution and potential extension of CL
- Identify population at risk for targeted interventions
- Conduct spot entomological surveys and control measures
- Evaluate impacts of control and intervention activities.

The surveillance system for leishmaniasis comprises of passive and active search of cases and vector surveillance.

The integration of pharmacovigilance and treatment outcome monitoring should be a part of the routine surveillance system. All forms of leishmaniasis (VL, PKDL, CL) must be reported to the Vector-borne Disease Control Programme, Ministry of Health.

3.2 Passive surveillance

Passive surveillance is the mainstay of VL surveillance which is hospital-based. It consists of timely, regular and accurate reporting of every case from different levels of health care centres using the form provided in Annexure 8 (Case Reporting Form). Any clinically suspected case that was provided treatment or laboratory-confirmed case must be reported for surveillance purpose.

Any health facility in the country that provides diagnosis and treatment is considered as the **reporting unit**.

Primary Health Centres and District Hospitals should refer suspected cases to the National Referral Hospital or Regional Referral Hospitals for diagnosis and treatment.

Any case detected must be notified to the Royal Centre for Disease Control (RCDC through National Early Warning Reporting System (NEWARS) platform and submit the Case Reporting Form / web-based reporting system to the VDCP, Ministry of Health.

The concerned clinicians/health facilities should inform VDCP and submit Leishmania Form 1 – Case Reporting Form (Annexure 8) to the Vector-borne Disease Control Programme at the email address: vdcp@health.gov.bt.

The VDCP will initiate reactive surveillance for finding additional cases (VL/CL/MCL) in the affected communities.

3.3 Active surveillance

The objective of the active surveillance is to find additional forms of leishmaniasis (VL, CL, MCL) in the affected communities for early detection and treatment and interrupt the transmission cycles. Therefore, the surveillance must try to find symptomatic VL, PKDL and other forms of cutaneous leishmaniasis.

Active surveillance requires an active search of cases of VL/PKDL/CL in the community outside of the hospital setting. However, routine active surveillance is

resource intensive and may not be feasible as up to 300 – 400 households are searched before finding a case of VL. This can also be challenging as it may take several days of effort to find a case of kala-azar. For the success of active surveillance, it is necessary to ensure that diagnostic and treatment facilities are provided. Therefore, the following active surveillance approaches are recommended:

3.3.1 Index case-based reactive surveillance

The VDCP, Ministry of Health will coordinate case-based surveillance in collaboration with RCDC and the local hospitals in the affected community. The clinician from the local hospital must be able to screen the signs and symptoms of VL, PKDL and MCL. A dermatologist may be consulted or invited to participate in the surveillance team.

The index case-based approach includes the search of all forms of leishmaniasis among the household members through house-to-house visits around the house (radius of 100 metres or 50 households) of a recently diagnosed case. The investigation must be completed within one month or program office may optimise the frequency of the index-case based surveillance based on the occurrence of the multiple cases from the same locality.

The index case-based approach is the preferred method for active case detection in endemic and non-endemic areas and in those areas where households are scattered.

3.3.2 Camp-based surveillance

Camp-based surveillance will be undertaken coinciding with the yearly follow-up, up to three years unless new cases are detected. The objectives of the camp-based surveillance are to:

- Screen for PKDL in the previously treated case(s)
- Symptomatic screening of the community for all forms of leishmaniasis
- Conduct awareness in the community.

The VDCP, Ministry of Health will coordinate the camp-based surveillance and the team should comprise of:

- Medical officer/specialist from the local health facility
- Dermatologist
- RCDC and local laboratory staff.

The line list of cases screened during both reactive and camp-based surveillance along with other response and surveillance measures implemented should be reported (Annexure 9). Submit these forms to the Vector-borne Disease Control Programme, Ministry of Health on the email address: vdcp@health.gov.bt.

3.4 Epidemiological stratification of kala-azar transmission areas

The epidemiological stratification of kala-azar transmission areas should be an annual activity to monitor the intensity of the transmission as well as to know the status and progress towards elimination of kala-azar. In line with the regional and global framework for elimination of kala-azar, transmission areas are stratified as endemic, endemicity doubtful and non-endemic at the district level.

The concept of endemicity is based on the demonstration of full cycle of transmission at any given time. The demonstration of full cycle of transmission includes confirmation of presence of competent vector, the parasites, both in human and the vector and the seropositivity of non-sick people. However, proving the existence of the whole transmission cycle may be quite challenging and costly. Until then the following standard definitions may be used to describe endemicity.

Endemic districts

Districts where full cycle of transmission has been demonstrated at any given time (maintained population of competent vector + parasite reservoir + locally acquired case) and at least one locally acquired case reported in the last 10 years.

Endemicity doubtful

Districts where full cycle of transmission has never been demonstrated but at least one locally acquired case reported in the last 10 years, or

Full cycle of transmission has been demonstrated, but no case has been reported in the last 10 years.

Non-endemic districts

Non-endemic districts can be categorized into:

- Previously reported cases: Full cycle of transmission has not been demonstrated at any point of time and no locally acquired case has been reported in the last 10 years, but locally acquired case has been reported earlier
- At risk: No locally acquired case has ever been reported but epidemiological risk factors are present (a competent vector population + a reservoir + and conducive environmental conditions)
- No autochthonous cases reported: No locally acquired case has ever been reported

Chapter 4
Vector control and surveillance

4.1 Vector surveillance and control

4.1.1 Bionomics of sand fly

The vector for VL transmission is *Phlebotomus* sand fly. Though the sand fly follows the same life-cycle pattern as mosquitoes, there are differences that have to be understood by all personnel involved in kala-azar control operations.

Phlebotomus argentipes is probably the only species amongst about 600 phlebotomine species that transmits kala-azar in humans in the Indian subcontinent. Its prevalence in Bhutan was confirmed in seven districts: Thimphu, Monggar, Wangdue Phodrang, Dagana, Tsirang, Trashigang and Trashi Yangtse. It is believed to be playing the vector role in *Leishmania* transmission in the country. The other species found in Tsirang and Monggar were *Phlebotomus longiductus*. *P. argentipes* was recorded even at altitudes of 2350 metres above sea level in Bhutan, especially in the summer months. Recent routine surveillance in incidental foci revealed *P. argentipes* to be prevalent only in Kalapang, Monggar, and that too in very low numbers. In these foci, other little known mountain species of sandfly are more common than *P. argentipes* and need to consider in the transmission and control (unpublished article). In total, Bhutan has recorded six species of *Phlebotomus* and four species of *Sergentomyia*. No *Phlebotomus* species were encountered in the southern plains where regular malaria control activities are in place. It is well documented that the sand fly is very susceptible to all insecticides.

4.1.2 Life cycle of sand fly

Phlebotomus sand fly is normally small, pale brown, hairy insect about 2.5 mm from the tip of the mouth to the last abdominal segment. Sand flies thrive in alluvial soil and in areas with relatively even temperatures, high humidity and an abundance of cattle. The sand fly has four stages in its life cycle: egg, larva (of which there are four additional stages), non-feeding pupa, and adult (Figure 8). Eggs and larvae of the sand fly can withstand immersion in water for a period of 5 days, while the larvae of the fourth stage can withstand for a period of 14 days. Breeding places are found within a radius of about 20 – 50 metres from a dwelling in dark and humid soil protected from sunlight. Inside the cattle sheds, the favoured breeding place is underneath cattle troughs. It is very difficult to locate breeding sites and find larvae and hence larval control cannot be implemented.

Indoor, adult sand flies thrive in damp cracks and crevices in the walls above the ground level where humidity is high due to the dampness of the ground floor.

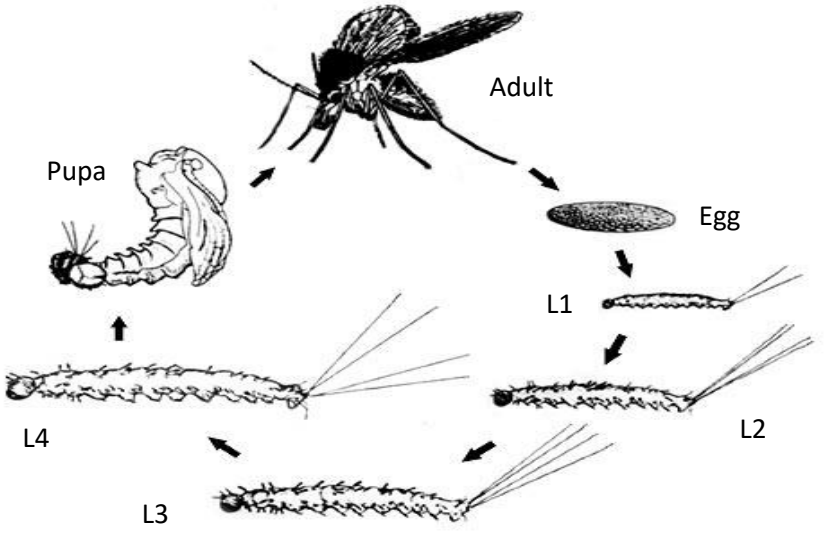


Figure 8. The stages of the life cycle of sandfly: egg, larva, non-feeding pupa and adult

4.1.3 Resting places of sand fly

An adult sand fly is active after dusk. During daytime, they escape into their resting shelters. Since sand fly is endophilic (indoor resting and feeding) with little variation in resting places, it can be easily targeted for indoor residual spraying (IRS). This fact is very important in view of vector control for kala-azar elimination. The presence of large numbers of *P. argentipes* on the lower margins of walls of mud houses is attributable to a lot of moisture on the lower side of dwellings. High humidity (75 – 85%) is a decisive factor in the life history of sand flies. This is the main reason why they are abundant during monsoon and post-monsoon seasons.

Figure 9. Sand fly resting places and indoor residual spraying in a typical household in southern Bhutan. (a) A sand fly positive cattle shed in Dagapela. (b) and (c) Sand fly surveillance in the floors of houses during the day

A typical Bhutanese village house is made of stones and mud. While some use the ground floor (*oka*) for keeping domestic animals, others use it for piling up wood

and for storage. These ground floors are ideal places for sand flies to rest. The use of mud makes these areas humid and damp, which promotes breeding. Such areas should not be missed during residual spraying for disease control (Figure 9).

4.1.4 Dispersal of sand fly

The dispersal range of *P. argentipes* is about 25 metres horizontal and 1.8 metres vertical. It is a poor flier, moving in short hopping flights.

4.1.5 Feeding habit of sand fly

The adults feed and mate within 24 – 48 hours of emergence from the pupal stage. The female sand fly requires a blood meal for egg development and maintenance of water balance. Only female sand flies are hematophagous, i.e., take a blood meal. During feeding, saliva is introduced into the host together with the parasite. The saliva enhances the growth of Leishmania in the human host.

4.2 Rapid assessment of vector prevalence

Vector surveillance will be conducted as part of case-based reactive surveillance within a radius of 500 metres around the index household. As larval surveillance is not applicable for sand fly; only adult surveillance can be done using any one of the methods mentioned below:

- CDC Light Trap set before dark for the whole night indoors.
- Oil-coated board (25 x 20 cm) on stand set before nightfall for the whole night indoor.
- Indoor resting collection early morning (6.00 – 8.00 AM) in ground floor wall, crevices and crack
- Cattle biting collection (7.00 – 12.00 PM) with mouth aspirator or test tubes.
- Human landing collection indoors and outdoors (7.00 – 12.00 PM) with mouth aspirator or test tubes.

Refer Annexure 13 for the SOP on setting CDC Light Trap for sand fly collection.

Site selection

There should be at least 5 sites and they should be selected depending on conditions receptive to sand flies around the index case house. The index case house should be one site for vector surveillance and the rest 4 houses should be randomly selected and conditions should be sand fly favourable.

4.3 Vector control options

Prevention of kala-azar requires the application of the right mix of interventions based on vector behaviour and bionomics. Integrated vector management (IVM) for the elimination of kala-azar comprises the following:

- Indoor residual spraying (IRS)
- Personal protection to prevent human vector contact, including the use of insecticide-treated nets (ITNs)
- Environmental management for source reduction.

4.3.1 Indoor residual spraying

In the context of a few sporadic cases reported in the country, reactive case-based IRS can be cost-effective vector control option in Bhutan.

All households within 500 metres radius of index case household(s). Cattle sheds and chicken coops owned by each household within the radius should be sprayed. The following are the criteria for reactive case-based IRS:

- There should be a confirmed notified indigenous case (VL, CL, ML or PKDL) from an area (village).
- Sand fly detected during current focus surveillance in the area.

After the transmission focus (indigenous/imported) is determined, a quick vector surveillance should be undertaken to assess the vector prevalence in that identified area.

The IRS programme plan and implementation should ensure the safe and correct application (uniform and complete) of a residual insecticide to indoor surfaces of houses and animal shelters in the target areas. This is critical for achieving a marked reduction in the populations of the sand fly vector in the target area. Further details on the implementation of indoor residual spraying for the control of sand fly vector are given in Annexure 12.

It is recommended to maintain all surveillance and vector control activities records for supervision purposes, and for planning till completion. All reports, as given in the annexure (Leishmania Forms 2, 3 and 4 given in Annexures 9, 10 and 11) should be forwarded in time to the VDCP, Ministry of Health.

4.3.2 Personal protection

Insecticide-treated materials such as long-lasting insecticidal nets (LLINs) and ordinary nets treated with chemicals are effective against sand flies. The mass distribution of LLINs or impregnation activities for communities will depend on the extent of the disease outbreaks and cost-effectiveness.

Use of repellents such as mosquito coils and topical lotions used against mosquitoes are also effective against sand fly bites. Clothing having long sleeves to cover legs and hands will have good protection from sand fly bites at night.

4.3.3 Environmental management

Environmental measures involve holistic approaches to vector-borne disease control. Sand fly breeding sites cannot be detected easily in damp soil. The environmental measures mentioned below are recommended for reducing sand fly breeding sites:

- General sanitation measures — such as having animal shelters away from human dwellings and removing decaying matters including cattle dung and poultry droppings around the house — reduce organic matter around residents and reduce sand fly breeding.
- Surrounding areas should be cleaned regularly, once per week and should be supervised by a health worker initially.
- Responsibility for regular cleaning should be given to the communities later under supervision by a selected supervisor among themselves.
- Garbage should be disposed of in an appropriate place far away from human dwellings.
- Cracks and crevices in house walls from the ground level to six feet provide ideal resting as well as breeding sites for sand flies; plastering those will limit the sand fly population.
- White-washing of walls will have an impact on the adult sand fly population but should not be done after spray operation.
- Since sand flies are very minute, using fans at night will obstruct their access to human hosts.

4.4 Behavioural change communication for community mobilization

Information and education is an effective tool for behavioural change communication. Communities living in endemic areas should be mobilized and sensitized on the local transmission risks and prevention of leishmaniasis. Individuals should be able to recognize disease signs and symptoms as well as take proper prevention and control measures. Communities should be made aware of the accessibility and availability of health services for disease prevention, case management and treatment.

With the appropriate level of knowledge and awareness, the community can participate in disease prevention and control. During Information, Education and Communication sessions, the symptoms of the disease, the importance of early diagnosis and complete course of treatment, environmental sanitation, as well as personal protection measures (repellents and proper clothing) should be covered. If IRS is planned, the need for community cooperation and acceptance have to be emphasized by all public health professionals.

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Annexure 1: rK39 testing and interpretation

Principle of rK39 Strip Test

rK-39 rapid test for kala-azar is a membrane-based immunoassay for the detection of antibodies to visceral leishmaniasis (kala-azar). It is based on antigen-antibody reaction. The membrane is pre-coated with a recombinant VL antigen (rK39) in the test line region and chicken anti-protein A in the control line region. The membrane is coated with the dye conjugate (protein A colloidal gold conjugate).

Serum, plasma or whole blood samples may be used for testing. During testing, the sample reacts with dye and the mixture migrates upward on the membrane by capillary action to react with the recombinant VL antigen in the test region and generates a red line presenting the **positive** result. Regardless of the presence of antibody to VL in the sample, the mixture continues to migrate across the membrane to the control region and reacts with chicken anti-protein A of the control line region and generates a red line indicating verification for sufficient sample volume, proper flow and **control** of the reagents (Figure A1).

The following are the advantages of the rK39 test strip:

- Test can be performed with one drop of blood drawn from a finger stick
- It is a rapid test and the result is obtained in 10 minutes
- The test can be performed in any setting by a trained health worker
- rK39 test is reliable and compares well with confirmatory tests. Therefore, there is no need to perform confirmatory tests in all cases of kala-azar
- The test is also positive in cases of post-kala-azar dermal leishmaniasis
- The rapid test rK39 is positive in 95 – 100% of patients of kala-azar

The following cautions are recommended while using the rK39 test:

- The test strips must be stored at recommended temperature and humidity to maintain their reliability. The test will lose its value if stored at temperatures <20 and >30 °Celsius for a long time (several months).
- It may be negative in patients who have HIV-kala-azar co-infections.
- A negative rK39 test does not rule out VL.
- The test cannot be used in patients who have a relapse or a re-infection
- It is not recommended to decide about the cure from kala-azar since the test continues to be positive even after the patient has clinically recovered from the disease.
- It might give a false positive result due to cross-reactivity in cutaneous leishmaniasis.

Materials required

The following materials are required rK39 test:

- Cotton

- Rectified spirit
- Disposable needle/Lancet
- rK39 test strip
- Chase buffer solution
- Test tube

How to perform the rk-39 test

- Remove the test strip from the pouch or the vial.
- With a new lancet, prick the fingertip of the patient suspected to be suffering from kala-azar. Used lancets should not be re-used because of the risk of transmitting HIV and Hepatitis B and Hepatitis C.
- Let the blood come out on its own. Do not use pressure or squeezing for obtaining blood.
- Place one drop of blood on the absorbent pad of the strip bottom.
- Place the test strip into a test tube so that the end of the strip is facing downwards. This would encourage the blood to migrate upwards by capillary action.
- Add 2 – 3 drops of buffer solution provided with the kit to the pad.
- Read the results in 10 minutes. Do not read the results before or after 10 minutes. If the time period of 10 minutes is not adhered to there are chances of error in its interpretation.
- Follow manufacturers instructions on the interpretation of test results.

How to interpret the results

Positive result

A red line is seen in both the test and control regions at the end of 10 minutes. A faint red line also is to be considered positive (Figure A1).

Negative result

A red line is seen only at the control region at the end of 10 minutes (Figure A1).

Invalid test

There is no red line at the control region. If the test is invalid, repeat the test by following the correct procedure recommended above.

Storage of rk-39 test strips

The test strips and the buffer should be stored safely at room temperature between 20 – 30 °Celsius. The temperature in excess of 30 °C can reduce the quality of the test. The test strips and the buffer should not be frozen since freezing deteriorates the quality of the reagent. The strip should be taken out from the vial or the pouch only at the time of performing the test. If the strip has not been used within one hour of taking it out from the vial or the pouch, it should not be used. The vial or the

pouch should be checked to ensure that the test strips have not exceeded their expiry date.

rK39 positive rK39 negative

Figure A1. Interpretation of the rk39 test result. Two red lines on the test and control are considered positive. Only one red line on the control is considered negative

Quality assurance of the test kits

The reference laboratory, the Royal Centre for Disease Control, Ministry of Health should network with other laboratories to maintain the quality of the test kits. As part of quality assurance, all tests may be cross-checked at the reference laboratory.

Advantages and disadvantages of the rK39 test

Advantages

- Simple to perform with minimal training.
- Does not require a laboratory.
- Can be performed with finger-prick whole blood, serum or plasma.
- Kits can be transported and stored at ambient temperature (up to 30 °C).
- Results are available within 10 – 20 min.

Disadvantages

- Cannot distinguish between active cases and relapse in previously
- treated cases. Therefore, interpretation must always be accompanied by clinical case definition.
- In patients with advanced HIV infection, a negative result does not rule
- out a diagnosis of visceral leishmaniasis.

Annexure 2: Serum sample collection for ELISA

Equipment and materials

- Plain Tubes
- Syringes
- Cotton wool
- Marker pens

Reagents and solutions

- Ethanol

Procedure

- Draw 3 mL venous blood
- Put 3 mL in the plain tube
- Serum should be separated from this tube and an aliquot should be made with the serum (2 mL microtube). Label the tube with the code number. This should be stored in –20 °C until transfer to the Royal Centre for Disease Control, Thimphu.

Sample collection for dried blood spot

Materials

- 5 mL sterile disposable syringe and needle
- Tourniquet
- Sterilizing swabs
- Specimen labels
- Band-aid
- Zip-lock plastic bags
- Whatman filter paper
- Disposable gloves
- Sharp container

Procedure

Venous blood collection

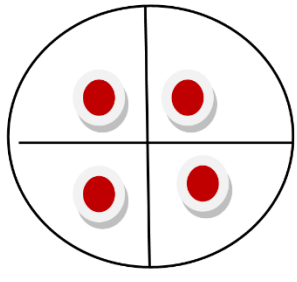
- Make sure the patient is seated comfortably.
- Lay out all blood collection supplies and necessary labels. Assemble needle and syringe.
- Examine both arms to find the best vein.
- Locate the puncture site; apply the tourniquet.
- Wipe the area in a circular motion making sure the area is thoroughly cleaned and allow it to dry.

- If it is necessary to feel the vein again, do so, but cleanse the area again with an alcohol wipe and dry with gauze.
- Fix the vein by pressing down on the vein about 1 inch below the proposed point of entry and pull the skin taut.
- Remove the needle shield.
- Approach the vein in the same direction the vein is running, holding the needle so that it is at an approximately 15° angle with the subject's arm.
- Push the needle, with bevel facing up, firmly and deliberately into the vein.
- **Withdraw 4 – 5 mL blood.** If the needle is in the vein, blood will flow freely into the tube. Collect samples in two separate vacutainers.
- After collection is completed, loosen the tourniquet.
- Withdraw the needle. When the needle is out of the arm, press gauze firmly on the puncture.
- Use a cotton swab to apply pressure to the venepuncture site until bleeding stops and apply a band-aid.
- Dispose the needle in the sharp container

Follow the steps shown in the following figures for sample collection and disposal of used sharps.

Dried blood spot (DBS) preparation (please follow the accompanying figure)

- Use one filter paper for each individual patient.
- Label the Whatman filter paper provided with subject identification number.
- Place Whatman filter paper on the clean area (top of the tables, books or any hard surface)
- Drop venous blood from syringe (100 ul) on Whatman filter paper slowly
- Prepare at least **four spots** on the same Whatman filter paper from each subject blood sample



- Allow the dry the blood spots in the room temperature. To dry the blood spot completely, it may take more than one hour.
- After it is dried, put the Whatman filter paper in the Ziplock bag.
- Place the Ziplock bags in the envelop and transport it to the Royal Centre for Disease Control, Thimphu with sample collection form.

Annexure 3: Bone marrow and splenic aspiration and grading of parasite load

Bone marrow aspiration

Bone marrow aspiration can be performed on the iliac crest, sternum or tibia. We advise caution in performing procedures in case of severe anaemia (haemoglobin <5 g/dL), difference in prothrombin time between patient and control is >5 seconds and platelet count is <40,000/μL.

Bone marrow aspiration is performed as follows:

- The site of aspiration should be cleaned thoroughly with spirit and povidone iodine and local anaesthetic given over the area.
- The bone marrow aspiration needle is advanced with the stylet in position. Once the needle pierces the periosteum, there is a feeling of loss of resistance. At this point, the bone marrow is sucked out by negative suction.
- Withdraw the needle and apply dry dressing.
- Keep the patient under observation for 1 hour after the procedure.
- Place one drop of the material aspirated on a glass slide and with the help of a micropipette suck the blood out.
- Use the edge of another slide to make a thin smear of the bone marrow aspirate.
- Collect 0.5 mL of bone marrow aspirate in EDTA tube, or a Whatman filter paper for dried blood spot. Store the bone marrow aspirate in EDTA at 2 – 8 °C and transport it to RCDC at the earliest.

Splenic aspiration

Splenic aspiration is contraindicated if the patient has any signs of active bleeding, jaundice, is pregnant and the spleen is barely palpable. The two most important prerequisites for the safety of this procedure is the rapidity, so that the needle remains in the spleen for less than a second and precision so that the entry and exit axes of the needle are identical to avoid tearing the splenic capsule.

- Clean the aspiration site with alcohol spirit and povidone iodine and let it dry.
- Using a 21-gauge needle attached to a 5 mL syringe, penetrate the skin, midway between the edges of the skin, 2 – 4 cm below the costal margin. The needle should be aimed cranially at an angle of 45 degrees to the abdominal wall.
- The actual aspiration is done as follows: Pull the syringe plunger back to the 1 mL mark to apply suction and then push the needle into the spleen to the full needle length and then withdraw it completely, maintaining suction throughout.
- Put the material gently onto glass slides, holding the needle tip on the surface of the slide. Spread evenly with the needle, using a linear motion.

The smear should be slightly thinner than a thick blood film for malaria. Allow the slide to dry.

- Collect 0.5 mL of splenic aspirate in EDTA tube, or a Whatman filter paper for dried blood spot. Store the splenic aspirate in EDTA at 2 – 8 °C and transport it to RCDC at the earliest.
- After the procedure, the patient must be kept under observation for 12 hours. Monitor the pulse and blood pressure every half hour for 4 hours and then every hour for 6 hours.
- The slides are stained with Giemsa, using the same procedure as for thin malaria film and examined under oil immersion.

Grading of parasite load

Grading of parasite load increases the sensitivity of parasite detection, provides a measure of the speed of response to treatment, and distinguishes between slow responders and non-responders.

The average amastigote density when viewed with a 10x eyepiece and 100x oil immersion lens is graded as follows:

6+	>100 parasites per field
5+	10 – 100 parasites per field
4+	1 – 10 parasites per field
3+	1 – 10 parasites per 10 fields
2+	1 – 10 parasites per 100 fields
1+	1 – 10 parasites per 1000 fields
0	0 parasite per 1000 fields

Annexure 4: Slit Skin Smear Sampling

Skin sampling

- Clean the whole lesion and its border using 70% alcohol at least 3 minutes before injecting the anaesthetic.
- Inject 0.1 – 0.5 mL of lignocaine with adrenaline, using a short 23-gauge needle thereby creating a blanching area. It is not necessary to anaesthetize the whole lesion. For lesions on fingers or toes use lignocaine without adrenaline (necrosis risk).
- Pinch strongly the lesion to further prevent bleeding.
- Remove the crust, remove blood with a gauze, scratch firmly (using a sterile scalpel with a short-angle curved blade) the border and the centre of the lesion until tissue material is visible on the blade.
- Gently move the blade on the surface of a slide to deposit a thin layer of the scraped material. Repeat the procedure on different parts of the anaesthetized zone until at least half of the surface of each of three slides is covered with material.
- Dry the three slides at room temperature (>3 minutes).
- Fix the slides and stain them with Giemsa according to validated procedures.

Giemsa staining

The following are the materials required:

- Reagents: Giemsa stain, Giemsa buffer
- Supplies: Glass slides that are alcohol washed, glass marker
- Equipment: Microscope, binocular with mechanical stage; low (10×), high dry (40×) and oil immersion (100×) lens.

Procedure

- Fix air-dried slides in methanol by dipping the slides briefly (two dips) in a jar containing methanol.
- Remove and let air dry.
- Stain with diluted Giemsa stain (1:20 vol/vol) for 20 minutes. For a 1:20 dilution, add 2 ml of stock Giemsa to 40 ml of buffered water in a jar.
- Wash by briefly dipping the slides in a jar of buffered water (one or two dips).
- Let air dry.
- Examine the slides under the microscope (100× oil immersion lens).
- Read smears for at least 20 minutes (1000 fields) at 400× or 1000× magnification.

- A smear can be reported positive when at least two amastigotes are observed. For valid identification, an amastigote form must show a nucleus, a kinetoplast and a plasma membrane.

Annexure 5: Skin lesion aspiration

Equipment and materials

- 1 mL syringe
- Micro centrifuge tubes
- Cotton wool, gauze and plaster
- Bunsen burner

Reagents and solutions

- Normal saline
- Surgical spirit

Procedure

- The wound/lesion should be cleaned with surgical spirit with the aid of cotton wool
- 1 mL syringe is loaded with little less than 1 mL normal saline (~700 µL)
- The normal saline is injected into the epidermis of the lesion by horizontally inserting the needle.
- Withdraw the fluid back into the syringe. The content may contain some pus material mixed together with the saline.
- The liquid in the syringe will be collected to a labelled micro centrifuged tube under sterile conditions closer to the Bunsen burner.
- The wound should be wiped off with cotton wool and dressed with gauze and plaster.

Send the tissue aspirate to the Royal Centre for Disease Control, Thimphu for molecular diagnosis.

Annexure 6: Leishmaniasis test request form

Royal Centre for Disease Control, Thimphu

Patient details (please fill in the details)

Name: _____ Age/Sex: _____

CID: _____ Phone no: _____

Sample collection date: _____ Sample transport date: _____

Name of hospital: _____

Brief clinical description (please fill in the details)

……
……
……
……
……
……
……
……

Sample description (please tick)

- □ Serum (plain tube)
- □ Plasma (plain tube)
- □ Whole blood (EDTA)
- □ Bone marrow aspirate
- □ Splenic aspirate
- □ Skin lesion aspirate
- □ Dried blood spot (DBS)
- □ Tissue biopsy
- □ Bone marrow biopsy
- □ Others (please specify)

Test requested (please tick)

- □ ELISA (on serum and plasma)
- □ PCR (all samples except serum and plasma)
- □ Culture (tissue aspirate and biopsy)

Test requested by:

Clinician name: _____

Phone no: _____

Annexure 7: Transportation and storage of tissue aspirates

Transportation and storage of tissue aspirates for microscopy, PCR and culture

Materials

- Sample collection form
- Sample tubes for collecting tissue aspirations

Requests for diagnosis of leishmaniasis

- Requests for diagnosis of leishmaniasis are received from hospitals.
- For the samples received directly for diagnosis,
    - The request forms are checked by the laboratory personnel
    - Sample acceptance or rejection is done based on sample requirements.
        - Bone marrow/splenic aspirations should be sent for culturing within 6-8 hrs of samples collection.
        - The referring doctor is informed about sample rejection or for requesting another sample if required.
    - Accepted samples are processed for light microscopy, in-vitro culture and PCR.
    - Report is issued after completion of the test. If there is positivity in any test, the laboratory should inform the treating doctor immediately.

Transport conditions of samples

- Samples should be sent at +4 ^{0}C (on ice) if it is taken more than 2 hrs for sample sending.
- Samples in plain sterile tubes are used for light microscopy, culture and PCR (For light microscopy and culture, EDTA bottles are recommended for avoiding clotting of bone marrow/splenic aspirations).

Storage conditions of samples

- Samples should be stored at room temperature (about 24^{0}C) until they are processed for culturing. Microscopic slides also should be prepared at the same date of sample collection. Immediate sample processing is required and please minimize the sample storage time for better results.
- Samples should be stored at +4^{0}C refrigerator until they are processed for PCR. Please minimize the sample storage time and avoid any freeze-thawing before processing them for PCR.
- After samples are processed for light microscopy, culture and PCR, remaining samples can be aliquoted and stored at –20 ^{0}C for further use. Filter papers are blotted with each sample and labeled filter papers are stored properly (in separate packs) at –80 ^{0}C freezer.

Annexure 8: Case reporting form

LEISHMANIA CASE REPORTING FORM
MINISTRY OF HEALTH
VECTOR-BORNE DISEASE CONTROL PROGRAMME

Leishmania Form 1

A. Patient profile (Tick [✓] where appropriate)					
A.1	Name of the patient:	A.2	Age:	A.3	Sex: M / F
A.4	Occupation:	A.5	Residence:		
A.6	Gewog:	A.7	Dzongkhag:		
A.8	Contact Number:	A.9	Travel history: Yes [ ] No [ ]		
B. Clinical information					
B.1	Pregnancy status: Yes [ ] No [ ]	B.2	Breastfeeding: Yes [ ] No [ ]		
B.3	Date of diagnosis: _ _/ _ _/ _ _ _ _	B.4	Name of health centre:		
B.5	Fever: Yes [ ] No [ ]	B.6	If yes, duration of fever:		
B.7	Splenomegaly: Yes [ ] No [ ]	B.8	Hepatomegaly: Yes [ ] No [ ]		
B.9	Pallor: Yes [ ] No [ ]	B.10	Skin lesions: Yes [ ] No [ ]		
B.11	Others(specify):	B.12	Other co-morbidities (Specify):		
B.13	Macular lesion: Yes [ ] No [ ]	B.14	Maculo-papular lesion: Yes [ ] No [ ]		
B.15	Nodular lesion: Yes [ ] No [ ]	B.16	Ulcerative lesion: Yes [ ] No [ ]		
B.17	Symmetrical/Localized/Generalized/Facial involvement/Mucosal involvement [ ] [ ] [ ] [ ] [ ]				
C. Laboratory findings					
C.1	HIV test done: Yes [ ] No [ ]	C.2	rK39 test: Positive [ ] Negative: [ ]		
C.3	LD bodies: Positive [ ] Negative: [ ]				
C.4	Other findings:				
D. Treatment					
D.1	Treatment provided: Yes [ ] No [ ]	D.2	If yes, specify the drugs:		
D.3	Follow up at 12 months and status of patients: Cured [ ] Not cured [ ]				
	Any remarks:				

E. Suspected [ ] or Confirmed [ ] case of VL [ ], PKDL [ ], CL [ ], ML [ ]

Name of reporting official/clinician: …… Signature:……..
Contact number: ……

Annexure 9: Reactive screening form

Leishmania Form 2

LEISHMANIA REACTIVE SCREENING FORM
MINISTRY OF HEALTH
VECTOR-BORNE DISEASE CONTROL PROGRAMME

A. Focus information			
A.1	Name of the index case:		
A.2	Village:	A.3	Gewog:
A.4	Dzongkhag:	A.5	Date of screening:

B. Reactive screening details

Sl. no	Name	Age	Sex	Fever (Y/N)	Other signs/symptoms (Splenomegaly, hepatomegaly, Pallor etc.)	rK39 test result	Remarks

Annexure 10: Vector surveillance and control reporting form

Leishmania Form 3

VECTOR SURVEILLANCE AND CONTROL REPORTING FORM
MINISTRY OF HEALTH
VECTOR BORNE DISEASE CONTROL PROGRAMME

Village: …….. Gewog: ……..

Dzongkhag: …… GIS coordinates: ……..

Tick [✓] where appropriate:

A.1	Date of vector surveillance conducted:		
A.2	Method applied:		
A.3	*Phlebotomus* present: Yes [ ] No [ ]	A.4	If yes, species:
A.5	IRS recommended: Yes [ ] No [ ]	A.6	If yes, date of IRS:
A.7	Total households within 500 m radius: …….	A.8	Total population in the village: …….
A.9	Number of households sprayed: …….	A.10	Total population covered: …….
A.11	Number of cattle shed sprayed: …….	A.12	Number of chicken coops sprayed: …….

IRS = indoor residual spraying

Annexure 11: Indoor residual spraying operation form

INDOOR RESIDUAL SPRAYING OPERATION FORM
MINISTRY OF HEALTH
VECTOR-BORNE DISEASE CONTROL PROGRAMME

Leishmania Form 4

Gewog:	**Dzongkhag:**	**Name of health centre**	**Date of IRS:** **Insecticide Issued (kg or g):** **Insecticide consumed (kg or g):** **Stock balance:**

Targeted					**Sprayed**					
Village	Households	Rooms	Cattle shed	Chicken coops	Household s	Rooms	Refused locked	Locked	Cattle shed	Chicken coop

Annexure 12: Sand fly vector control – Indoor residual spraying

Adopted from VDCP/Ento/SOP No. 0/V1 – SOP on case-based indoor residual spraying for Leishmaniasis control, (2022)

Rapid planning and operations of indoor residual spraying

The success of indoor residual spraying (IRS) operations depends on planning and timely implementation of the plan. The plans for IRS operations should be developed right after the detection of vectors by the team.

The number of houses to be sprayed in the village(s) should be mapped using geographical information system (GIS) and demography enumerated together with information on the date of spray to residents and house arrangements. The number of cattle sheds and chicken coops owned by each household equally be enumerated in the same form.

In addition to the date of spray, the following information must be provided to the individual household:

- Importance of spraying their houses i.e., to prevent a disease called kala-azar by destroying sand flies that transmit the disease; other benefits against nuisance insects such as fleas, bed bugs, houseflies, cockroaches and termites which could irritate them should also be mentioned.
- House owners should cover their food items during spraying time.
- Outer walls do not require spray
- Mud plastering and white washing of sprayed walls should be avoided for 6 months after spraying as these will reduce the effectiveness of the insecticide.

Timing of indoor residual spray

IRS should be implemented immediately after the confirmation of transmission focus and vector prevalence survey. Hence, the surveillance team should be equipped with adequate logistics for IRS despite the seasonality of vector proliferation. Operation of IRS should be done during the daytime and the households should be informed of IRS program. To maximize the impact, IRS should be initiated in an area of the disease transmission only and two rounds should be carried out for 2 subsequent years. Cattle sheds and chicken coops owned by every household should be covered for IRS. However, poultry farms should be avoided by IRS.

Choice of insecticide for indoor residual spray

Through experiences so far in the country, sporadic leishmaniasis cases were reported from areas beyond malaria control areas and hence chemicals for malaria control IRS can be deployed for ease of annual procurement. If cases are reported from malaria IRS areas and based on evidence in sand flies, chemicals for IRS should be decided in advance.

The World Health Organization Pesticide Evaluation Scheme (WHOPES) recommends the judicious use of dichlorodiphenyltrichloroethane (DDT) but its use has been discontinued in Bhutan. Instead, a synthetic pyrethroid, Cyfluthrin 10% WP, is being used for malaria control and the same chemicals will be used for kala-azar control programme.

Table A2. World Health Organization Pesticide Evaluation Scheme recommendation for indoor residual spray for vector control for the prevention of leishmaniasis

Insecticide group	Insecticide agent	Dosage (gm/m^2)	Effectiveness (months)	Insecticidal action
Organo-chlorine	Dichloro-diphenyl-trichloroethane (DDT)	2	3	Contact
Synthetic pyrethroids	Alphacypermethrin	0.03	2 – 3	Contact
	Cyfluthrin	0.025	3 – 5	Contact
	Cypermethrin	0.5	2 – 3 or more	Contact
	Deltamethrin	0.05	2 – 3 or more	Contact
	Lamadacyhalothrin	0.025 – 0.05	2 – 3	Contact
	Permethrin	0.5	2 – 3	Contact

Dosage for indoor residual spraying

The dosage of insecticide application for kala-azar control is same as for malaria. E.g., use 100 g of cyfluthrin 10% WP in 8 litres and 125 g in 10 litres Hand Compression Sprayer pump formulate 0.025gm/m^{2}dose.

Insecticide quantification

For IRS in kala-azar elimination, the insecticide requirement is more than that required for malaria. This is due to the fact that in addition to the walls, the ground floor (*oka*) and cattle sheds must be covered. In rural settings, a 10-litre pump is sufficient to cover 5 houses when sprayed for malaria, but for kala-azar, only 3 households will be covered.

For instance, the insecticide (Cyfluthrin) quantification for anti-kala-azar IRS done in Kalapang, Monggar in 2006 with 30 households is shown below:

Village: Kalapang, Monggar
Population: 210
Number of households: 30
Insecticide: Cyfluthrin 10% WP
Dosage of active ingredient/m^2: 0.025 g
Required quantity per round of IRS (kg): 1.250 kg
How was the requirement calculated: 30 households / 3 houses by a 10-litre pump * 125 grams per pump.

Equipment needed for indoor residual spraying

The hand compression pumps used for existing malaria control may be used for IRS in leishmaniasis prevention. The use of this equipment requires one operator and its nozzle should be fan-shaped.

Each spray squad would need the following equipment:

- Hand compression pumps (3 Nos.)
- Spare nozzle tips (2 Nos.)
- Bucket, 10-litres (3 Nos.)
- Measuring mug (1 No.)
- Straining cloth (1 metre)
- Register for records (1 No.)
- Writing material (marker pen) to identify households covered by IRS
- Tools for minor repairs

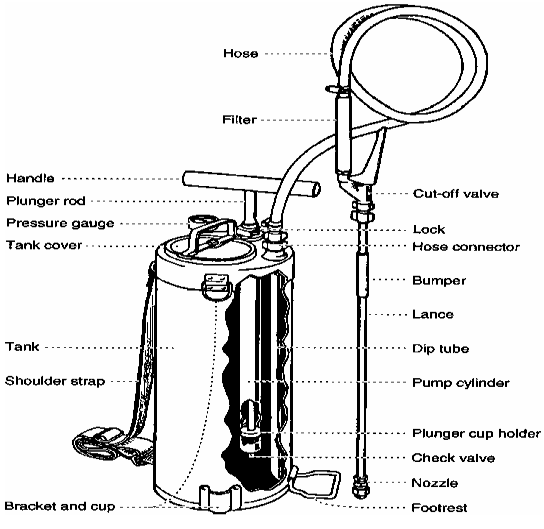


Figure A2. Hand compression insecticide spray pump

Each spray squad must be provided with personal protection gear, including a pair of hand gloves, goggles, cap, apron, and gum boots for each member of the squad and soap for hand-washing.

Routine maintenance of equipment and minor repairs

The pumps and other equipment are subject to wear and tear because of the corrosive action of the insecticide. Hence, it is essential that spray equipment is maintained with due care. The following maintenance activities should be completed every day in the evening:

- The spray pump should be thoroughly rinsed with clean water.
- Filter assembly should be rinsed and cleaned. The filter should be removed from the valve by grasping it at its screen and slightly twisted on pulling it out.
- Reassemble all clean parts except the nozzle. Put clean water in the tank, seal the tank and pump air into it. Open the control valve and let the water flow from the lance to flush the hose, filters, control valve and lance. Remove the tank cover and dry the inside of the tank.
- Clean the nozzle tip by washing thoroughly with water. Remove any dirt from the orifice with a fine bristle/a brush. Never use metal wire or nails.

Manpower for indoor residual spraying

The spray operations should be completed within the stipulated schedule. For this reason, the relevant public health professional in coordination with the respective in-charge at the health centre should plan and deploy adequate spray squads depending on spray target area.

Each spray squad should consist of three-five spray men and one supervisor. Labourers on daily wages may be employed as spray men if employed sprayers are not available. The hiring of labourers should be planned and reported in advance. The spray squads should be supervised adequately to ensure the quality (correct dose, uniformity and completeness of application) of IRS operation. The number of houses to be sprayed by each squad depends on the terrain in which the team is operating.

Training may be provided to the spray squads prior to actual spraying. The training modules include the following components:

- Importance of uniform and complete spraying
- Obtaining cooperation from the community
- Safe storage of the insecticide
- Preparation of insecticide suspension
- Correct use of the equipment
- Maintenance of the equipment

- Safety precautions and personal protection measures to be observed during the spraying operations
- Safe disposal of insecticide waste and packets.

The training should include hands-on sessions about the correct use of spray equipment and observance of all the steps needed from the preparation of the suspension to safe disposal of left-over insecticide suspension. Training of the spray squads should be conducted just prior to the spray operations.

The training schedule must be ready at the health centre levels just after confirmation of the prevalence of vectors in the focus area.

Pre- and post-spray activities

The investigating health centre or surveillance team must draw up a spray program so that the households in focus area is covered within the recommended schedule.

The post-spray activities include taking stock of the work completed, preparing a report, disposal of the material that could not be used, maintenance of the equipment, estimating the requirements for the next day and planning spraying in the households that have not been covered.

Safe handling of insecticide

It is essential to follow the following safety measures during the transportation, storage and handling of insecticides:

- The containers in which the insecticide is transported should be well-sealed and properly labelled.
- The insecticide should not be transported along with food items.
- In consultation with the community, the insecticides should be stored in a safe place where the chances of contact with humans and animals are minimal.
- Make sure that the insecticide is properly labelled with the name of the insecticide, the name of the manufacturer, the date of manufacture, the date of expiry and the danger sign that it is a poison.
- There should be written guidelines with each container/sac regarding what to do in case of exposure to the insecticide.
- The insecticide should be stored in a well-ventilated room, not exposed directly to sunlight, and away from the walls.
- The place where the insecticide is stored should be away from the reach of children and animals.
- It is important that no food items are stored in the vicinity of the place where the insecticide is stored.
- The storage should be carefully done so that there is no spillage of the insecticide.

- The stocks that arrive first are to be used first and it should be confirmed that the expiry date has not been exceeded prior to use.
- Expired insecticide can only be used after permission from the Vector-borne Disease Control Programme which will confirm that the active ingredient tested conforms to the minimum specifications.
- Stock registers should be carefully maintained to keep a track of their use. No unauthorized person should have access to the insecticides.
- The storeroom where the insecticide is stored should be kept locked and danger signs should be displayed to indicate the storage of hazardous material. Eating, drinking and smoking are not permitted in the place where the insecticide is stored, nor are they permitted during the spray operations.

The following are recommended for the disposal of insecticides:

- The unused insecticides or the washings should be disposed of safely to ensure that they do not mix with water or food.
- Prepare only the required quantity of insecticide suspension that is likely to be consumed in one day. Do not carry over any unused insecticide to the next day.
- Never put any left-over insecticide into a river, pond, well or source of drinking water.
- Any spilled insecticide in solid or liquid form as well as the washings from the spraying should all be emptied into a pit that is dug away from the source of drinking water and covered with mud.
- Do not use the empty sacks or containers in which the insecticide was stored for any other purpose. These must be buried safely away from the drinking water source.
- All empty containers/sacks should be returned to the supervisor. The supervisor must check carefully that all empty sacs/containers have been received.

Annexure 13: SOP for setting CDC Light Trap for sand fly collection

Scope

The purpose of this SOP is to outline the materials and processes required to set up CDC light traps to collect host-seeking adult sand flies

Materials

- CDC miniature light trap (at least 12 numbers)
- 6 V rechargeable battery (≥4.5 Ah)
- Battery charger
- Data collection forms/digital device
- Head torch
- Oral aspirator
- Dissecting microscope
- Collection cups, Nets & rubber bands
- Ethyl alcohol 80%
- Vials/ Microcentrifuge tubes
- Trays
- Forceps
- Pipettes
- Labelling paper
- Pencil/Pen/marker pen
- Instruction Manual

Selection of households for setting up the trap

Before selecting households for setting up light traps seek consent from the households.

- Identify the index case household
- Identify at least 5 additional households away from index case in concentric circle
- Determine where to set up the light traps. CDC miniature light traps are highly efficient if they are located close to resting or breeding places such as dark corners or tree holes, rock crevasses, other high humidity areas, e.g., rock piles, old wood piles, organic debris, buttress roots/banyan trees; also near penned blood sources—chicken coops, cow pens
- Since transmission to humans for many forms of Leishmania occurs in wooded or forest settings, woods, large trees along the rivers and edges of thick forest may produce previously unidentified species that may be responsible for the sporadic Bhutan cases. The above places may also have recognizable and potential larval habitats, with characters of organically rich

moist soils—human defecation areas, edges of animal pens, in denser forest undergrowth

- Traps can be set indoor of houses, outdoor or animal sheds and/or in forested areas
- Indoor light traps should be place only in sleeping areas open to free fly entry

Setting up CDC Light Traps

Traps should be placed before dusk and collected in the morning after dawn.

- In the selected households place at least an indoor and outdoor light trap based on the above considerations. The net has a label inside on with the site code and date of capture
- Additionally, light traps can be set in forested/vegetation areas
- Traps should be placed close to the ground or adjacent to vertical surfaces (walls, tree trunks), preferably far from an external light source
- Ensure the cable connections are right
- Check the speed of the fan and intensity of the light for any problems
- The catch of sand flies can be augmented by adding a carbon dioxide source such as dry ice
- The battery should be protected from rain
- Traps are generally collected within two hours after sunrise. First, the net should be tied, then removed from the trap, and only then the battery should be disconnected. Nets should be stored in conditions preventing the desiccation of sand flies
- Anaesthetize each trap collection with ethyl acetate before opening net. Use large black garbage bag, place wad of ethyl acetate impregnated cotton on bottom; hold one to three trap nets by top at top of garbage bag –taking care not to crush specimens in net. Check, but 5 min minimum anaesthetization time necessary
- Before the trap contents are physically sorted, keep trap contents in high humidity (i.e., not in air-conditioned room) to slow desiccation of specimens
- After finding a fly, place it immediately in ethanol before it dries. Fine forceps are necessary to move specimens into ethanol vials
- Label vials with field code
- Charge the battery after a single night's use to avoid complete discharge and to be used during next collection

Annexure 14: WHO Adverse Event Reporting Form upon using antileishmanials

Adverse Event Reporting Form

A. Patient and Health Facility Information			
Patient ID number:			Treatment Centre:
Date of Birth (*or* Age):			Province:
Sex:	☐ Male	☐ Female	☐ Other
HIV status:	☐ Non-reactive	☐ Reactive	
Pregnancy:	☐ No	☐ Yes	Trimester:
Weight (kg):			

B. Adverse events experienced by patient					
Adverse event	Onset date	End date	Severity grade	Seriousness *	Outcome §

* Please select:	***D*** *died*	***LT*** *life threatening*	***HA*** *caused or prolonged hospital admission*		***PD*** *permanent disability*
	OS *other medically serious*		***CA*** *congenital abnormality*		***NS*** *not serious*
§ Please select:	***A*** *recovered*	***B*** *recovering*	***C*** *recovered with residual effects*	***D*** *died*	***E*** *not recovered* ***F*** *unknown*

Detailed description of adverse event(s):

Was treatment of adverse event required? ☐ No ☐ Yes (please specify):

C. Laboratory assessment: Results of tests if any

Test performed	Test date	Result	Unit	Reference range

D. Medicines: List all medicines used for the treatment as well as other commitment medications if any

☑ *Tick if medicine suspected of causing adverse event*

Medicine	Dose	Frequency	Route	Start date	Stop date	Reason for use	Action taken †	Response ‡
☐								

☐								
☐								
☐								
☐								
☐								

† *Action taken in response to AE:*	***DW** drug withdrawn*	***DR** dose reduced*	***DI** dose increased*	***DNC** dose not changed*	***UK** unknown*	***NA** not applicable*
‡ *Response to action taken:*	***RA** recovered*	***NE** no effect on AE*	***FA** fatal AE*	***UN** unknown*	***NA** not applicable*	

E. Other relevant information

Name and Brand name of the Drug used:

Batch number:

Expiration date:

Any other information on the drug:

G. Reporter Information

Name:	Phone number:
Email:	

Occupation:	☐ Doctor	☐ Nurse	☐ Paramedics	☐ Other (please specify):
Signature:			Date	

Submit form to:

Give email address of national programme

MoH Use Only:

Date received:		
Causality assessment:	☐ Likely	☐ Unlikely
Comment:		