National Kala azar Elimination Program-Nepal

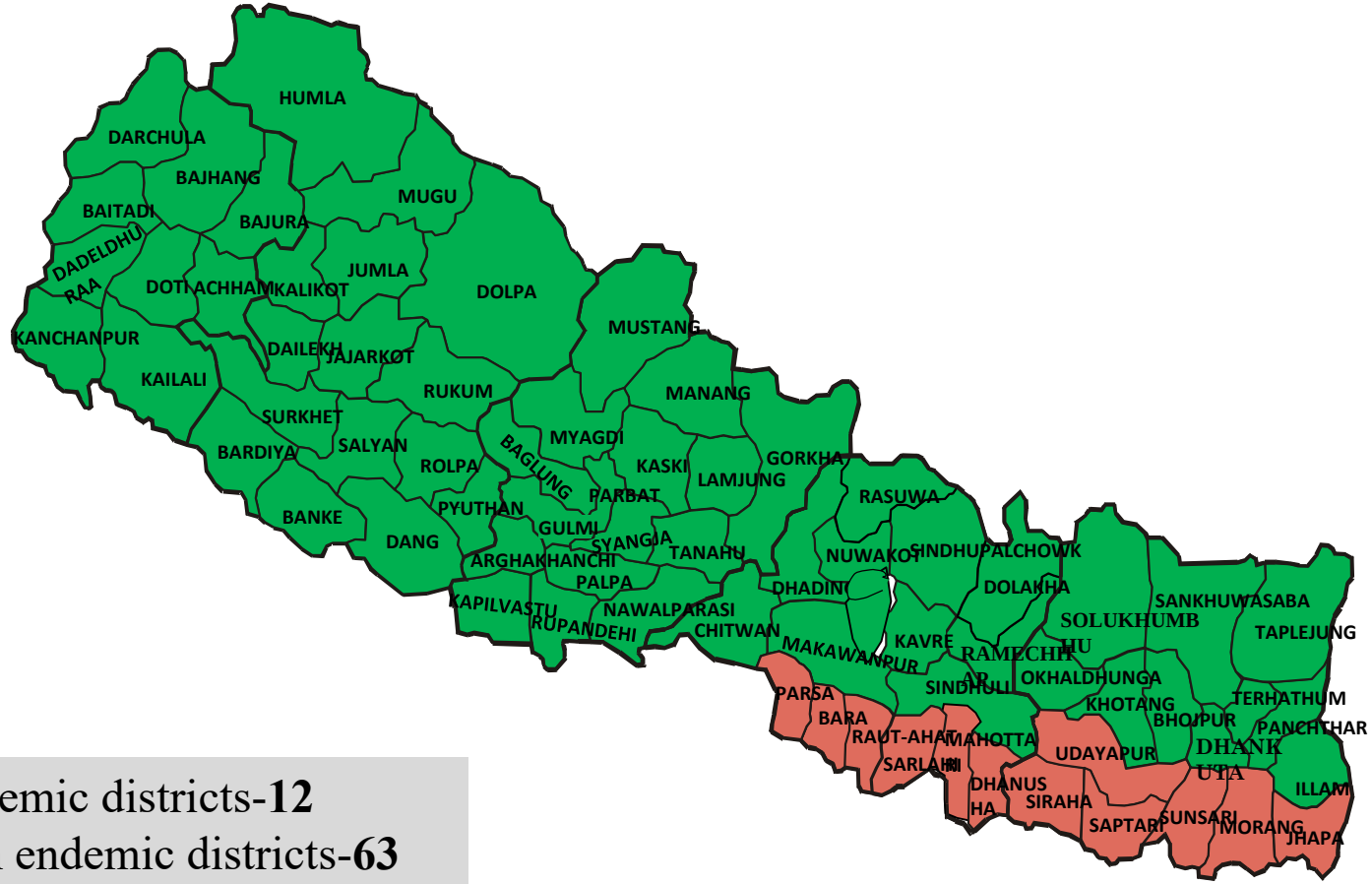
Kala azar situation-Nepal

- Slated for **elimination as a public health problem** **(achieving annual incidence of < 1 case/10,000 population at the district level.**
- GoN is committed to the WHO regional strategy to eliminate kala-azar.
- Nepal is also signatory to the MOU on strengthening collaboration in the regional elimination efforts along with Bangladesh and India during WHA 2005 which was renewed in 2014 with inclusion of Bhutan and Thailand.
- In Nepal parasite species for Kala azar is **Leishmania donovani** and **P. argentipes** is the main vector.

Kala azar situation-Nepal

- **First cases** of kala azar were reported as early as **1960s** in Nepal.
- National program **initially** identified **12** districts as kala azar endemic districts.
- **6** other districts were included in the list in 2016 because sporadic cases were consistently being reported by these 6 districts.
- Currently **18 districts** are considered endemic.

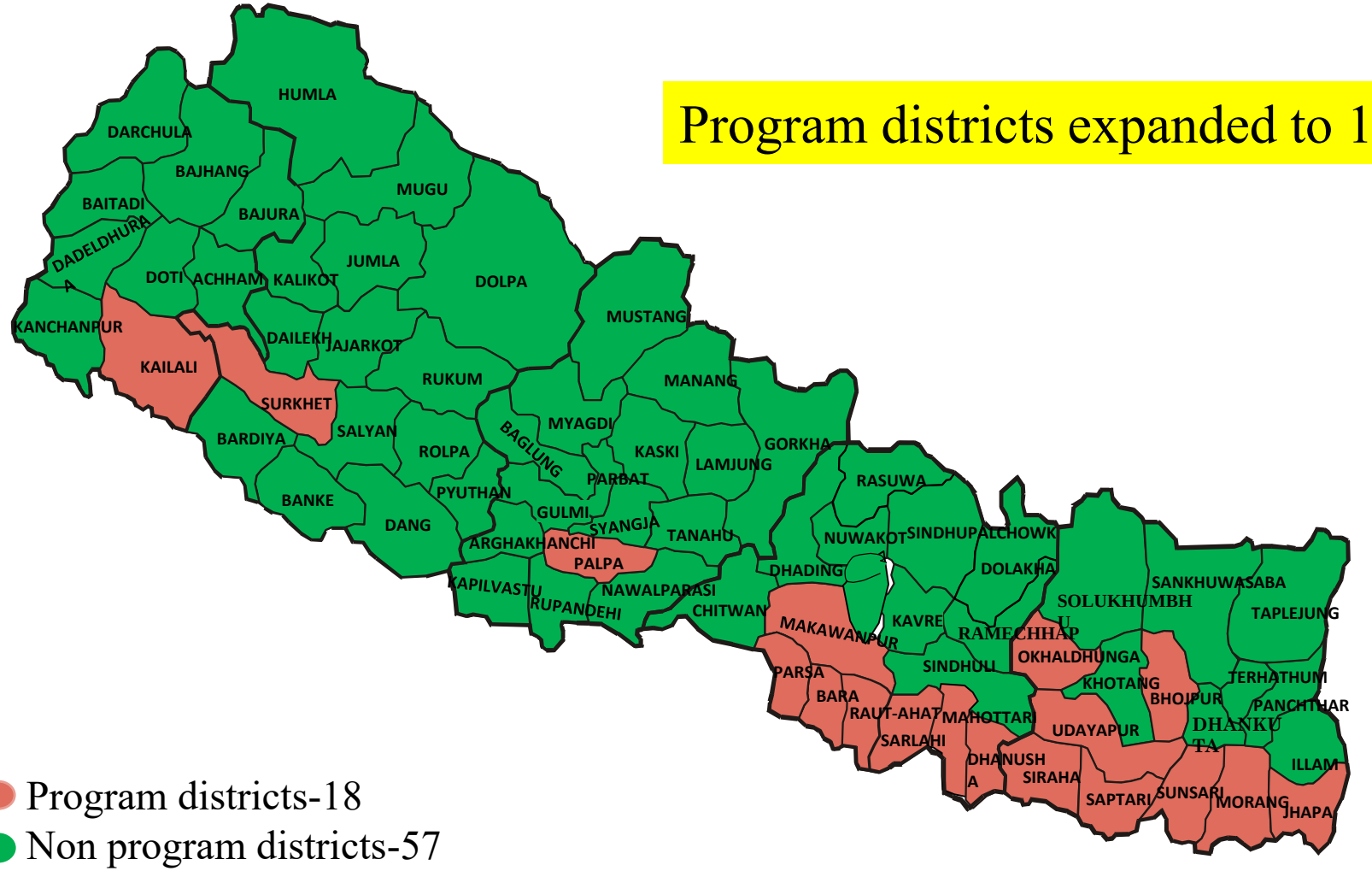
Kala-azar endemic districts- previously



Endemic districts-**12**

Non endemic districts-**63**

Kala-azar endemic districts- expanded



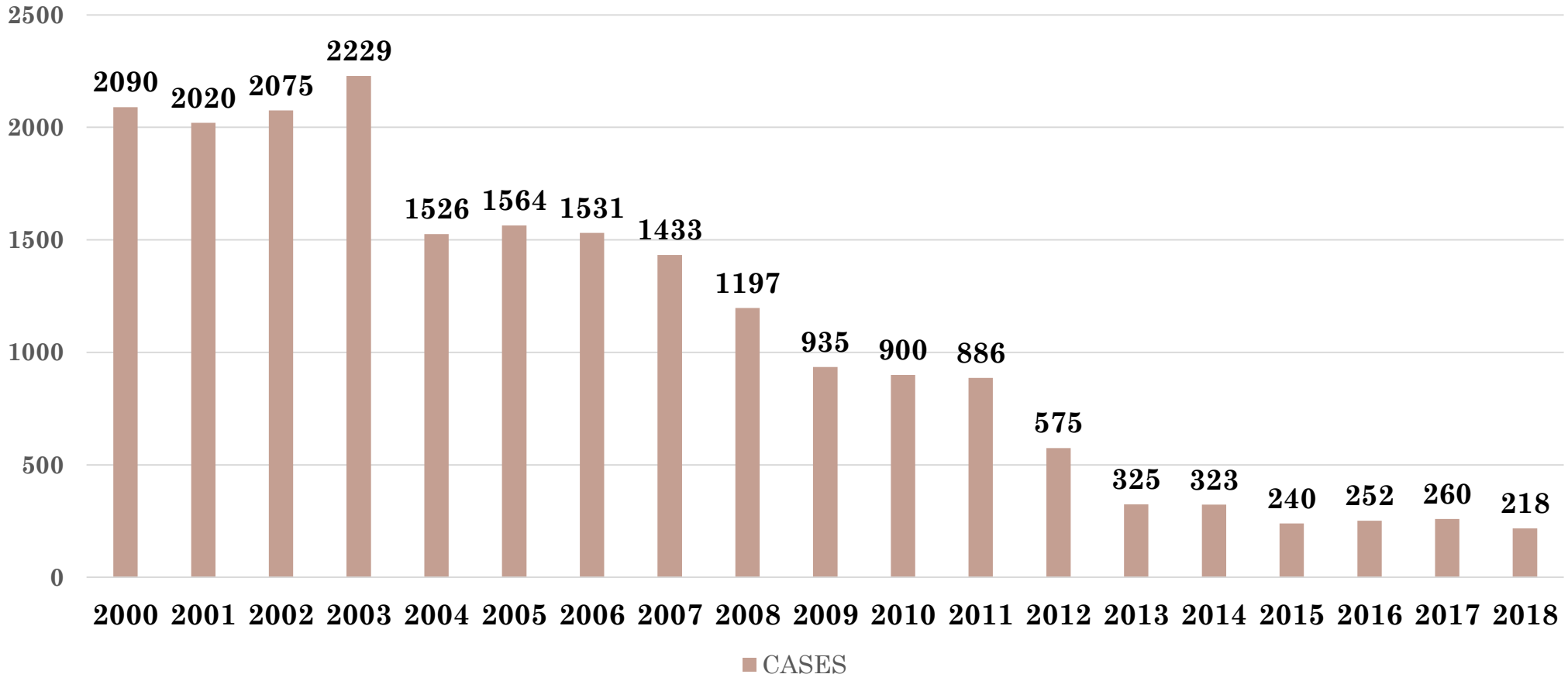
Program districts expanded to 18 districts

- Program districts-18
- Non program districts-57

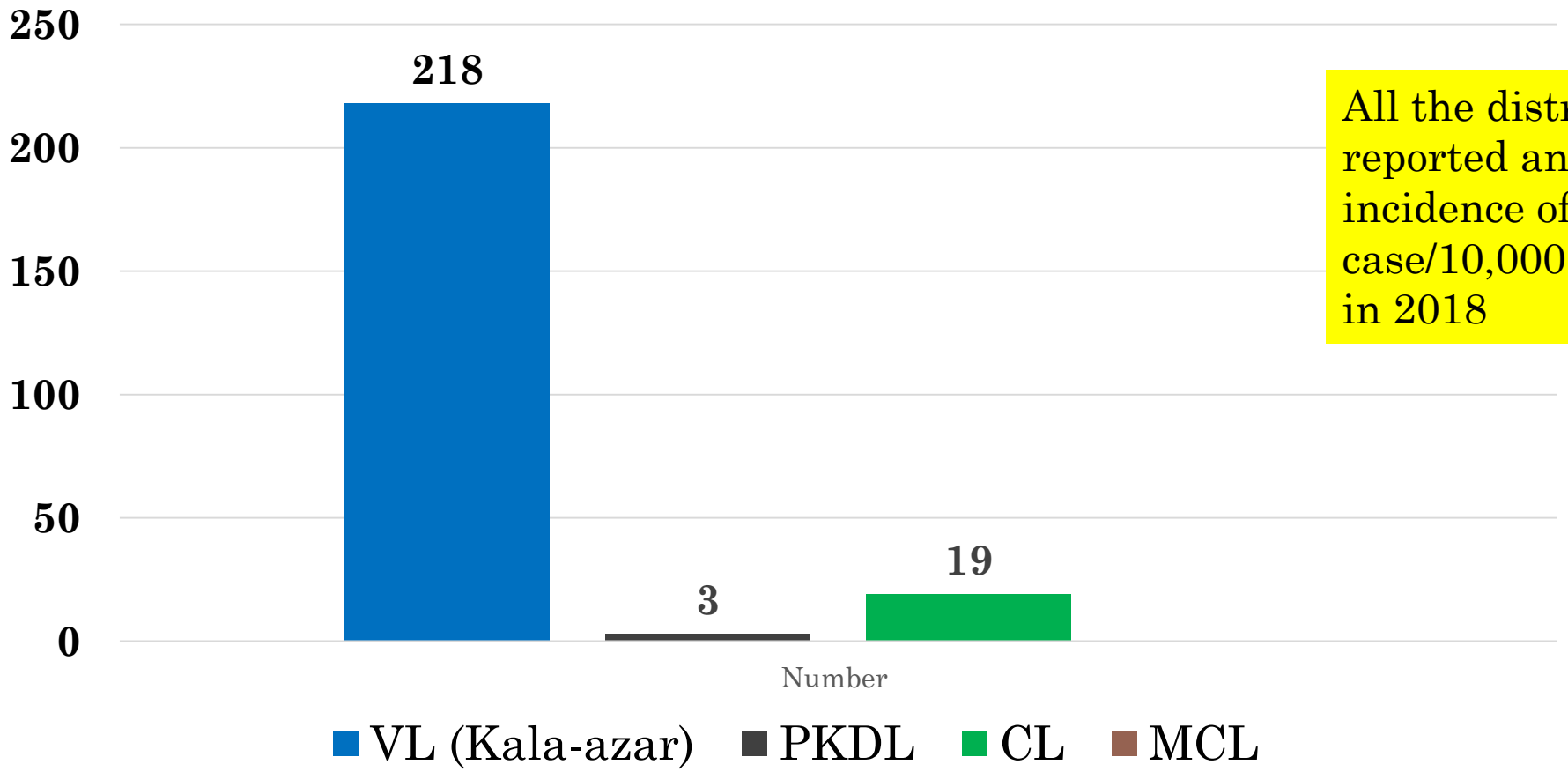
Kala azar situation-Nepal (contd..)

- Increasing number of cases are being reported by districts not included in the endemic list.
- In 2018, **32** districts considered as non endemic have reported at least 1 kala-azar case.
- All the endemic districts achieved annual incidence to < 1 case/10, 000 population by 2013, however in 2017 one district Dolpa considered non endemic, crossed the elimination threshold.
- In 2018, all districts reported annual incidence of <1/10,000 population.

KA Cases 2000-2018

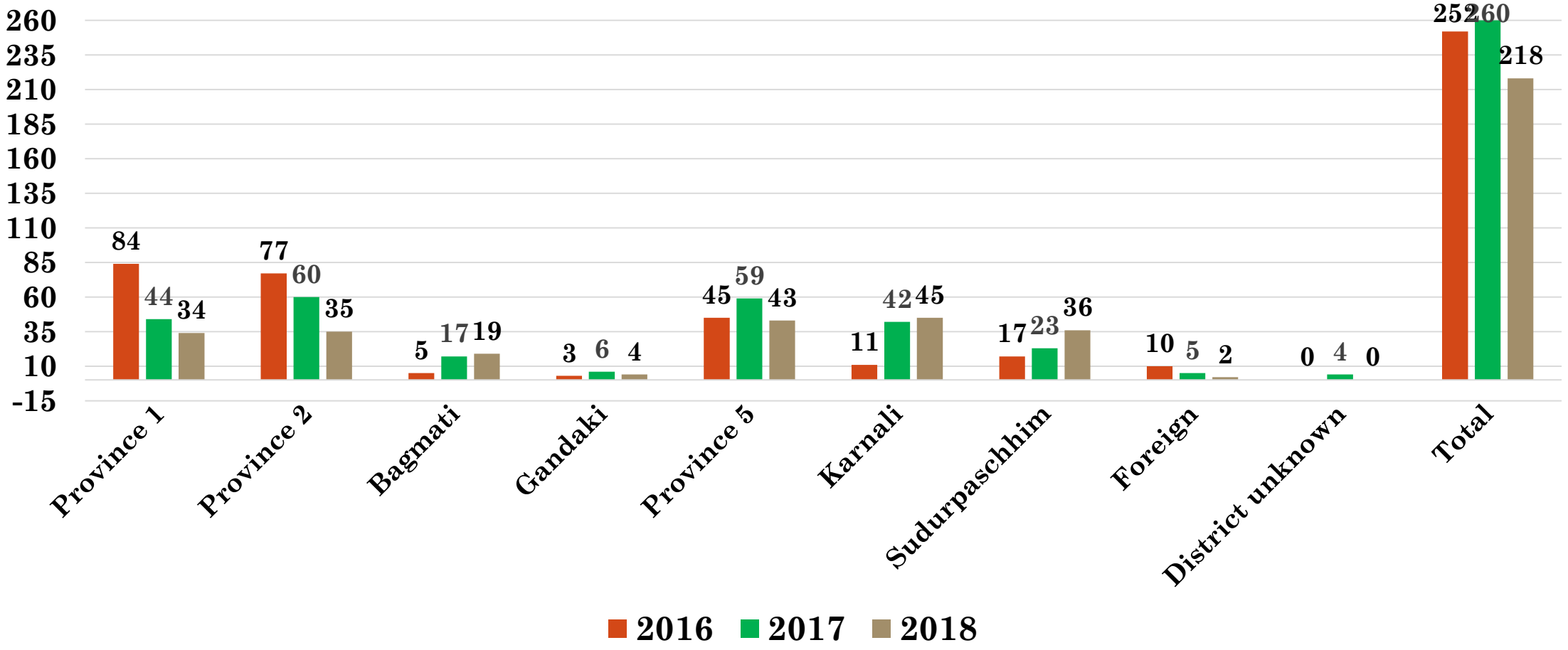


Leishmaniasis situation-2018



All the districts have reported annual incidence of < 1 case/10,000 population in 2018

Province wise VL distribution 2016-2018



District wise distribution –VL 2016 – 2018 (Province 1)

Province - 1	2016	2017	2018
Taplejung	0	0	0
Panchthar	0	0	0
Ilam	2	0	0
Jhapa	12	5	6
Morang	52	16	12
Sunsari	8	9	3
Dhankuta	2	0	1
Terhathum	0	0	0
Sankhuwasabha	0	1	0
Bhojpur	3	7	5
Solukhumbu	0	0	0
Okhaldhunga	1	3	4
Khotang	2	0	3
Udaypur	2	3	0
Total	84	44	34

District wise distribution –VL 2016 – 2018 (Province 2)

Province - 2	2016	2017	2018
Saptari	10	5	2
Siraha	22	14	7
Dhanusha	17	5	3
Mahottari	16	10	6
Sarlahi	10	24	15
Rautahat	0	1	1
Bara	1	1	1
Parsa	1	0	0
Total	77	60	35

District wise distribution –VL 2016 – 2018 (Bagmati)

Province - 3	2016	2017	2018
Sindhuli	1	3	2
Ramechhap	0	1	3
Dolakha	0	0	1
Sindhupalchok	0	0	0
Kabhre	1	0	2
Lalitpur	0	1	0
Bhaktapur	0	2	1
Kathmandu	1	4	1
Nuwakot	0	0	0
Rasuwa	0	0	0
Dhading	1	0	0
Makawanpur	1	6	5
Chitwan	0	0	4
Total	5	17	19

District wise distribution –VL 2016 – 2018 (Gandaki)

Gandaki	2016	2017	2018
Gorkha	0	1	0
Lamjung	0	0	0
Tanahu	0	0	0
Syangja	3	5	3
Kaski	0	0	0
Manang	0	0	0
Mustang	0	0	0
Myagdi	0	0	0
Parbat	0	0	0
Baglung	0	0	0
Nawalpur	0	0	1
Total	3	6	4

District wise distribution –VL 2016 – 2018 (Province 5)

Province - 5	2016	2017	2018
Gulmi	2	0	3
Palpa	13	17	11
Nawalparasi West	1	0	0
Rupandehi	2	1	1
Kapilvastu	1	1	3
Arghakhanchi	2	4	3
Pyuthan	5	11	6
Rolpa	0	1	3
Rukum East	0	0	0
Dang	3	6	3
Banke	8	7	3
Bardiya	8	11	7
Total	45	59	43

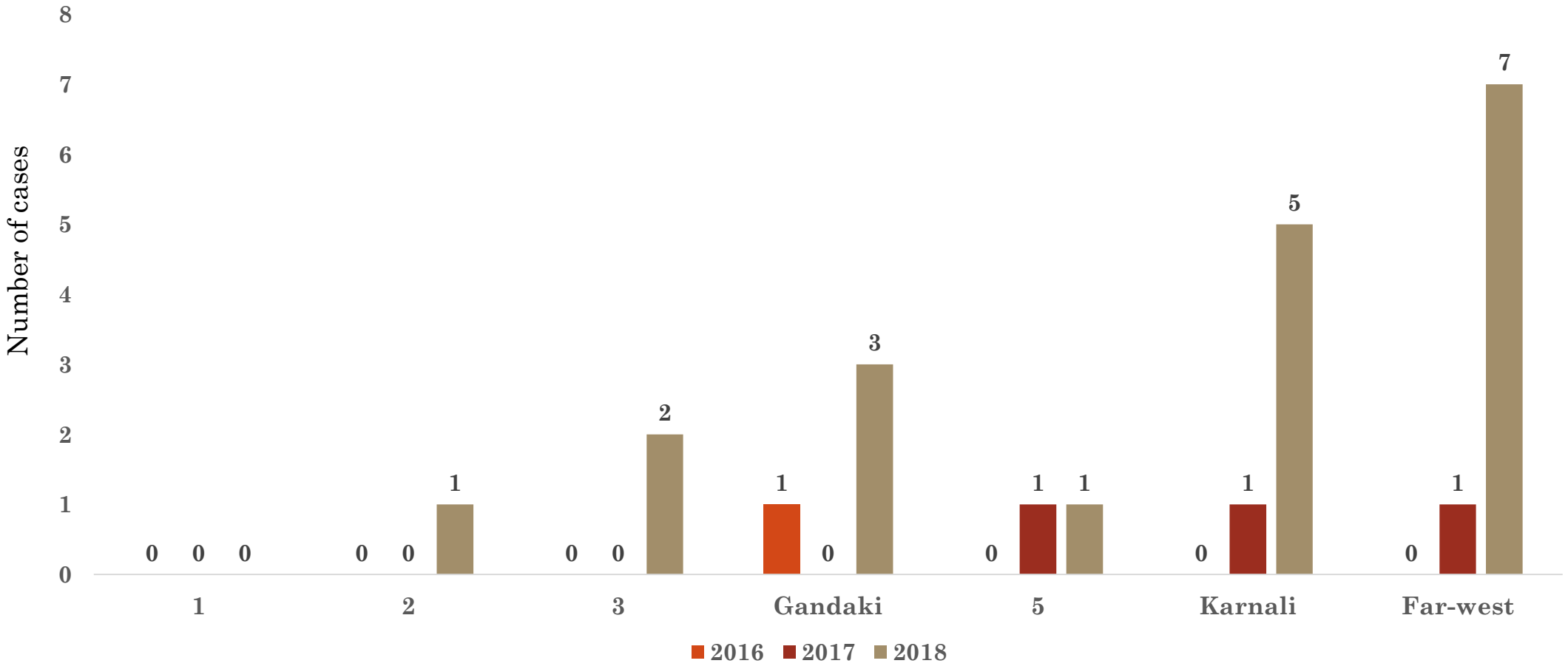
District wise distribution –VL 2016 – 2018 (Karnali)

Karnali	2016	2017	2018
Rukum West	0	1	1
Salyan	3	3	5
Surkhet	4	17	18
Dailekh	0	5	9
Jajarkot	0	1	0
Dolpa	0	6	0
Jumla	0	0	1
Kalikot	0	4	9
Mugu	1	2	0
Humla	3	3	2
Total	11	42	45

District wise distribution –VL 2015 – 2018 (Sudurpashchhim)

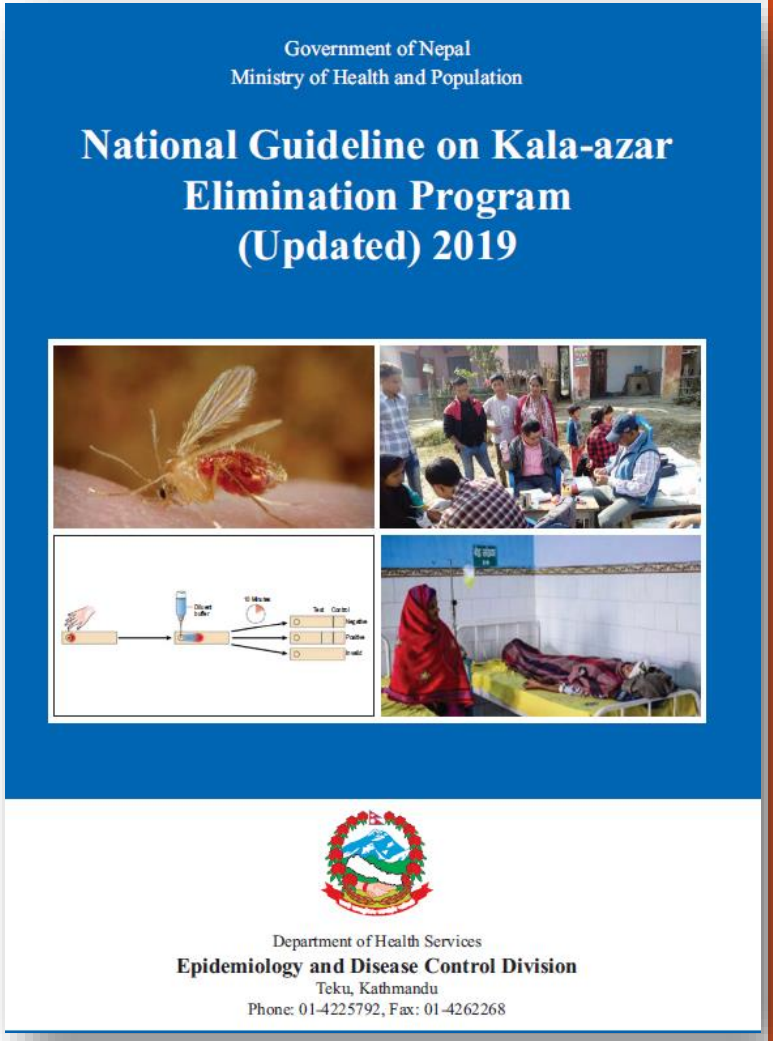
Sudurpashchhim	2016	2017	2018
Bajura	3	9	10
Bajhang	1	0	0
Achham	1	3	7
Doti	2	1	0
Kailali	4	3	3
Kanchanpur	4	2	2
Dadeldhura	0	1	3
Baitadi	1	2	3
Darchula	1	2	8
Total	17	23	36

Province wise CL distribution 2016-2018



National KA Elimination Program

- **2005**-EDCD formulated a **National Plan** for the Elimination of Kala-azar (KA) in Nepal
- **2010**-National Plan revised as National Strategic Guideline on Kala-azar Elimination in Nepal-**rK39** recommended as rapid diagnostic test kit and **Miltefosine** as the first line treatment for KA
- **2014**- National Strategic Guideline updated to introduce **Liposomal Amphotericin B and combination therapy** in the national treatment protocol.
- **2019**- National Guideline on KA elimination revised, **single dose L-AmB**, 10mg/kg as 1st line therapy and surveillance system strengthening (KA Tracker)



National Kala-azar Elimination Program

GOAL

- To contribute to mitigation of poverty in kala azar endemic districts of Nepal by
  - reducing the morbidity and mortality of the disease and
  - assisting in the development of equitable health systems

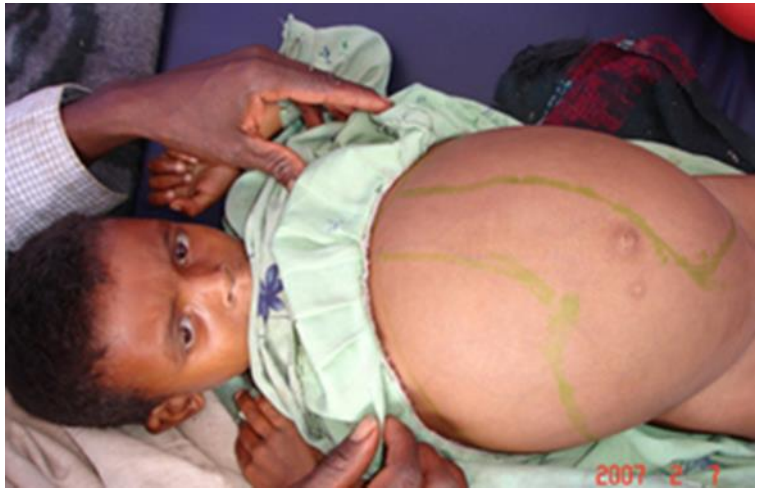
OBJECTIVES

- **Reduce incidence of kala-azar in endemic communities with special emphasis on poor, vulnerable and unreached population**
- **Reduce case fatality rates from primary Kala-azar to ZERO;**
- **Detect and treat PKDL to reduce the parasite reservoir**
- **Prevent and manage kala-azar HIV-TB co-infections**

National Kala-azar Elimination Program

STRATEGIES

1. Early diagnosis and complete treatment
2. Integrated Vector Management
3. Effective disease and vector surveillance
4. Social mobilization and partnership
5. Improve program management
6. Clinical, implementation and operational research



Current interventions

Early Diagnosis and Treatment

- Free diagnosis of KA cases using RDT (rK39) and provision of free treatment with Liposomal Amphotericin B (LAmB), Miltefosine and Paromomycin.
- Provision of NPR 1000 per patient for travel during the whole course of treatment.

Surveillance System Strengthening

- KA surveillance system strengthening along with the introduction of online DHIS2 based reporting

Indoor residual spraying

- IRS activities ongoing in selected districts

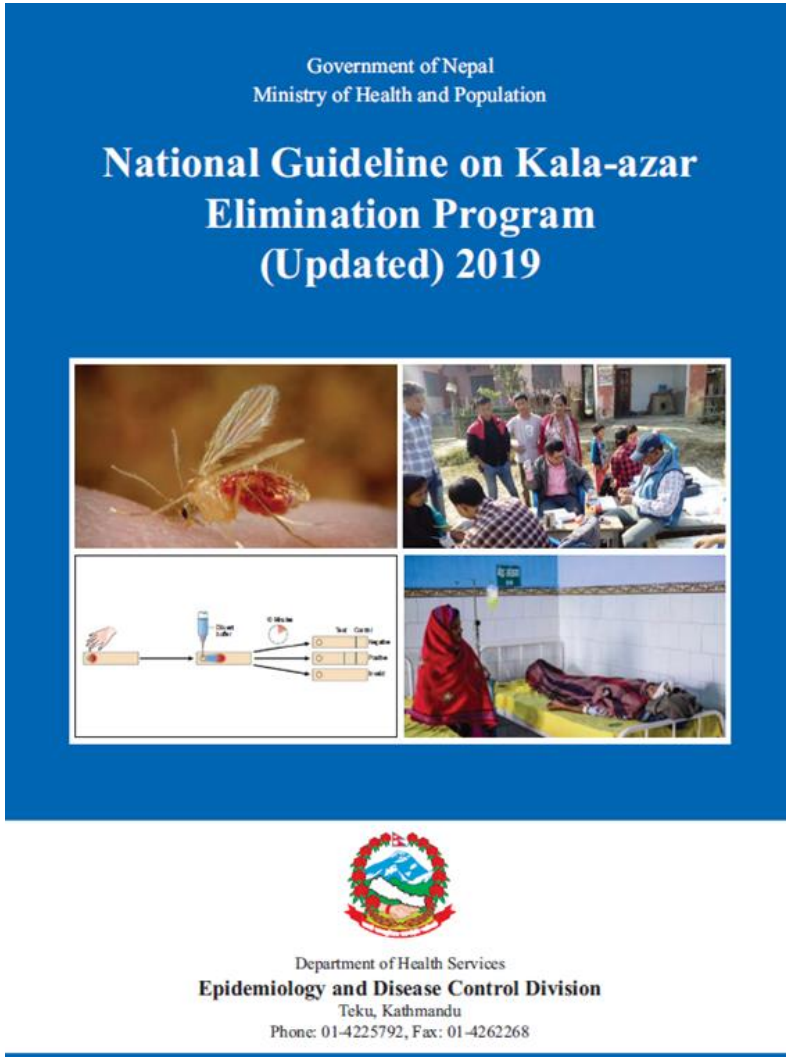
Social mobilization and partnership

IEC/BCC

Training /orientation /symposium

Revised national guideline- 2019

- Recommends Liposomal Amphotericin B as the first line treatment for primary kala azar
- Inclusion of other forms of disease-PKDL, CL, MCL
- Addresses HIV –VL co-infection



Updated surveillance system

- **Updated surveillance system-to expand the existing surveillance system which focuses mainly in the so called endemic districts only.**

- **Surveillance variables and indicators in line with the recent global WHO recommendations.**

Challenges

- Geographical expansion of cases.
- Sustaining the targeted incidence at districts.
- Emergence of CL and MCL.
- Cross border information sharing.
- Lack of HR for effective implementation of integrated vector management and monitoring vector control interventions.

Way forward

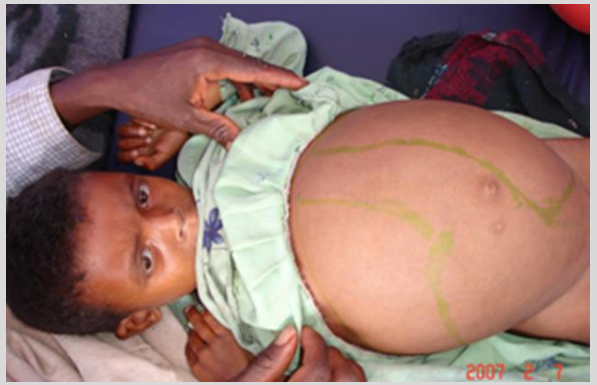
- Classification of endemicity of districts as endemic, endemicity doubtful or non endemic as per the national guideline.
- Intervention intensified as per the new endemicity classification.
- Training on diagnosis, treatment, recording and reporting of kala-azar in line with the revised national guideline 2019.
- Entomological and epidemiological studies.
- Implementation of Integrated vector Management.

Thank you for your patience

Leishmaniasis

Global and Regional Situation

Introduction



- Leishmaniasis -group of diseases caused by protozoan parasites (more than 20 species)
- Transmission- bites of infected **female phlebotomine sand-flies**.
- Manifestation- in 3 main forms:
  - **visceral leishmaniasis** (VL) or kala-azar-most severe form of the disease and **post-kala-azar dermal leishmaniasis** (PKDL)
  - **cutaneous leishmaniasis** (CL)-most common
  - **mucocutaneous leishmaniasis** (MCL)
- Additionally, leishmaniasis can be classified as **anthroponotic** or **zoonotic** depending on whether the natural reservoir of the parasite is human or animal.

Introduction (contd..)

- Mainly affects poor people in Africa, Asia and Latin America and is associated with
  - malnutrition
  - population displacement
  - poor housing
  - weak immune system
  - lack of financial resources

- Estimated 700,000 to 1M new cases occur annually and 26,000 to 65, 000 deaths

Major risk factors

Socioeconomic conditions	• Poverty. • Poor housing & sanitary conditions- may increase sandfly breeding and resting sites, as well as their access to humans. Sandflies are attracted to crowded housing as these provide a good source of blood-meals. • Human behavior (sleeping outside or on the ground) may increase risk.
Malnutrition	• Malnutrition increase the risk that an infection will progress to a full-blown disease.
Population mobility	• Epidemics of both CL and VL are often associated with migration and movement of non-immune people into endemic areas.
Environmental changes	• The incidence of leishmaniasis can be affected by changes in urbanization, and the human incursion into forested areas.

Climate changes	• Leishmaniasis is climate sensitive
	  • Temperature change =strong effect on vector and reservoir hosts, strong effect on developmental cycle of leishmania promastigotes in sandflies
	  • Drought, famine= displacement and migration of people to endemic areas along with poor nutrition

Global Overview

- Out of 200 countries reporting to WHO, 97 countries are endemic for leishmaniasis in 2017
- Out of these 97 countries

Endemicity	No of countries
Endemic for VL and CL	65
Endemic for VL only	10
Endemic for CL only	22
TOTAL	**97**

Global Overview (contd..)

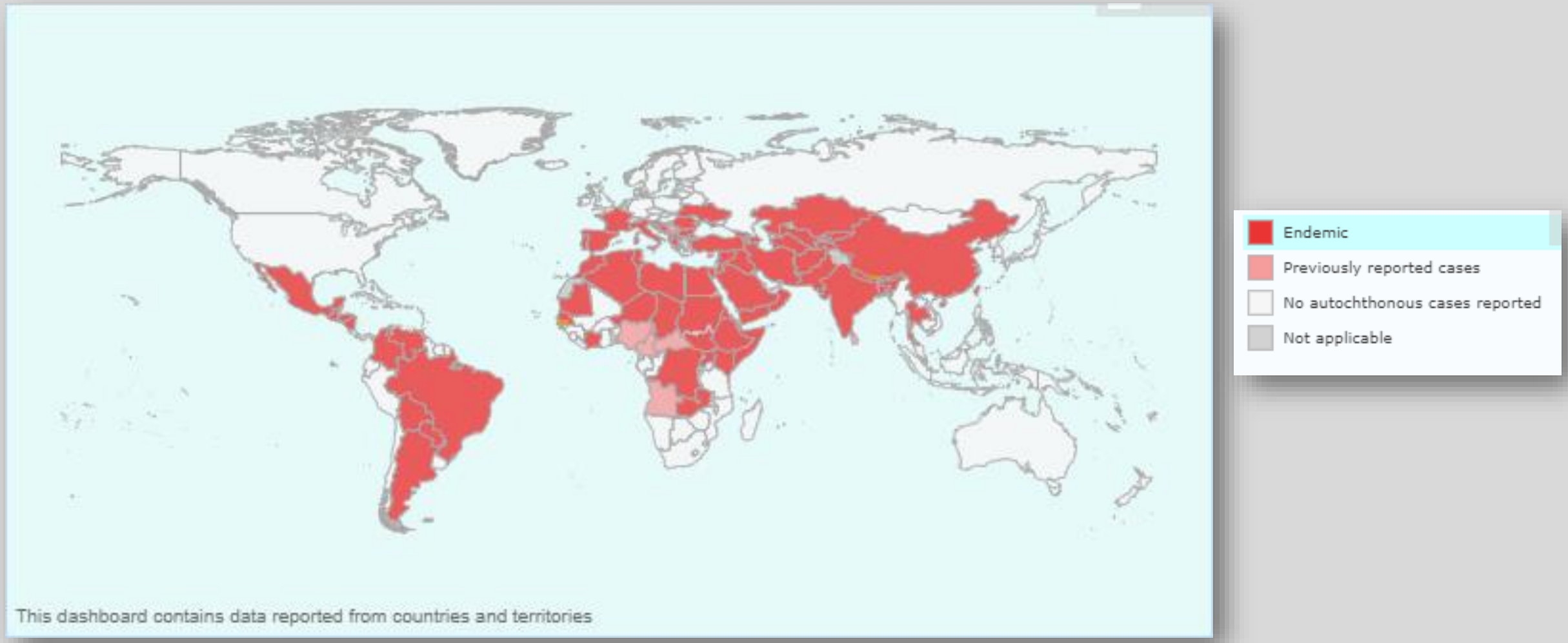
In 2017, over 95% of global VL cases were reported from **10** countries:

- **Brazil**
- **Ethiopia**
- **Kenya**
- **Somalia**
- **South Sudan**
- **Sudan**
- **China**
- **India**
- **Bangladesh**
- **Nepal**

In 2017, over 95% of new CL cases were reported from **7** countries.

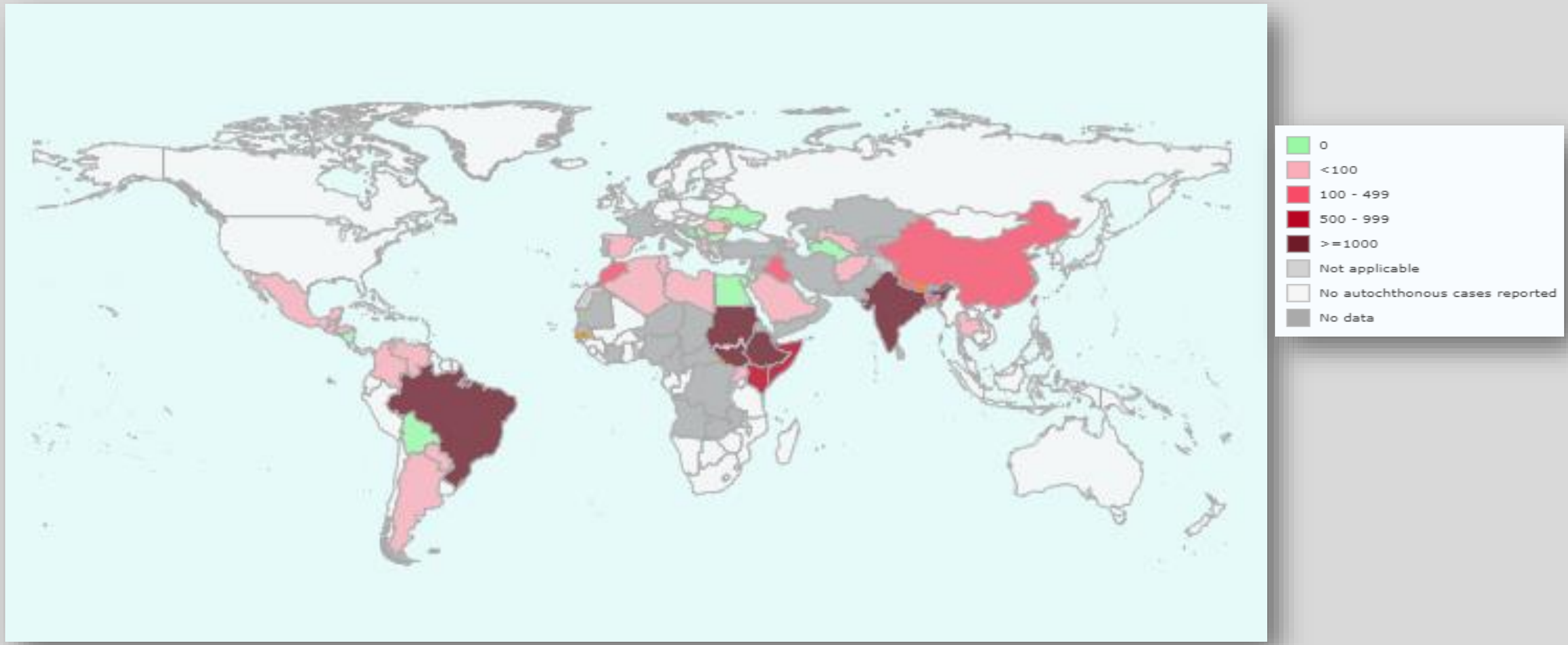
- **Brazil**
- **Colombia**
- **Afghanistan**
- **Algeria**
- **Iraq**
- **Iran**
- **Syrian Arab Republic**

Endemicity status-VL (2017)



Endemicity status-CL (2017)

No of VL cases reported- 2017



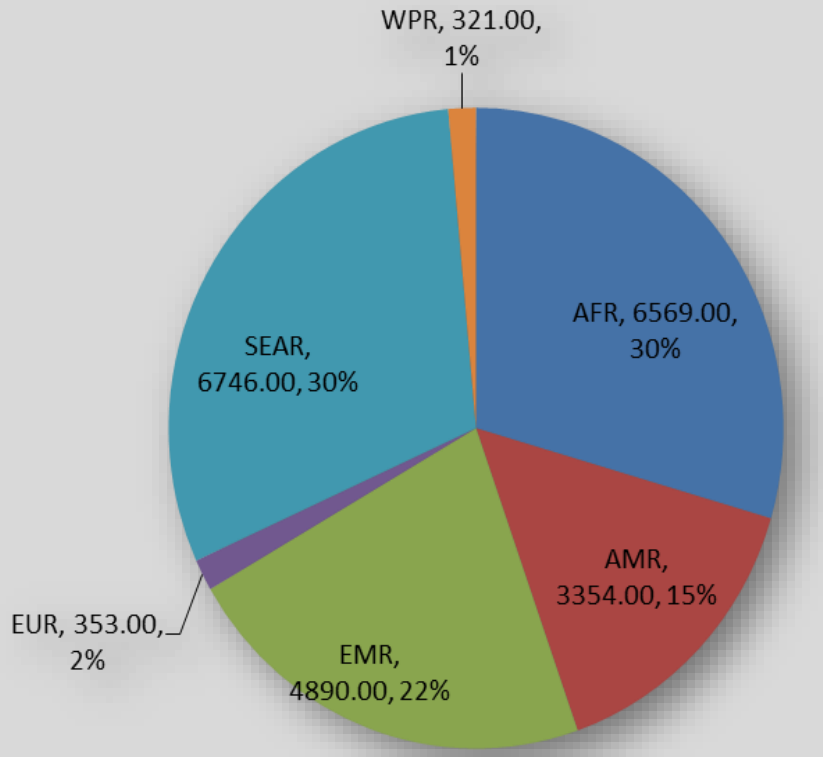
World Health Organization
Nepal

No of CL cases reported- 2017

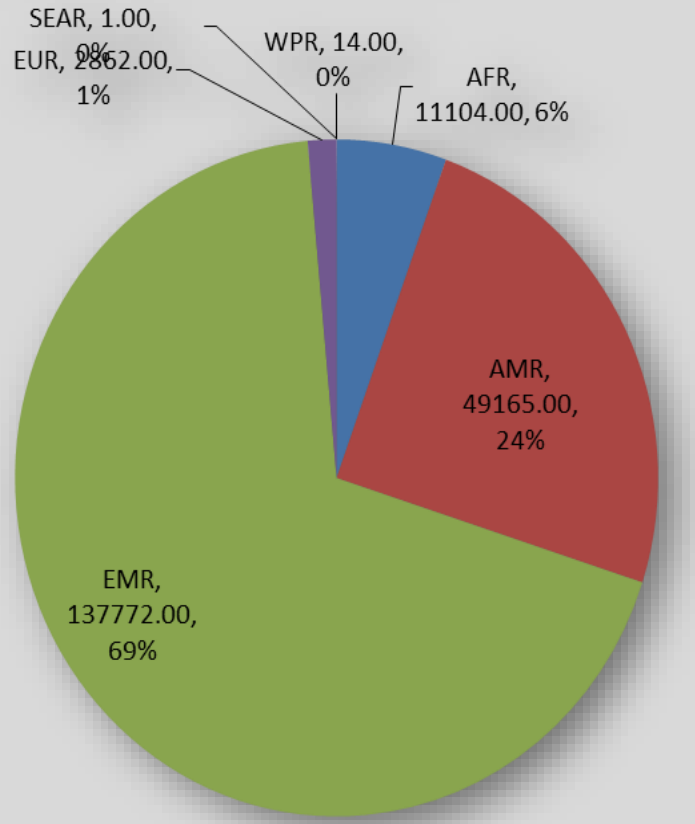
World Health Organization
Nepal

REGIONAL DISTRIBUTION- VL and CL (2016)

New VL autochthonous cases, 2016

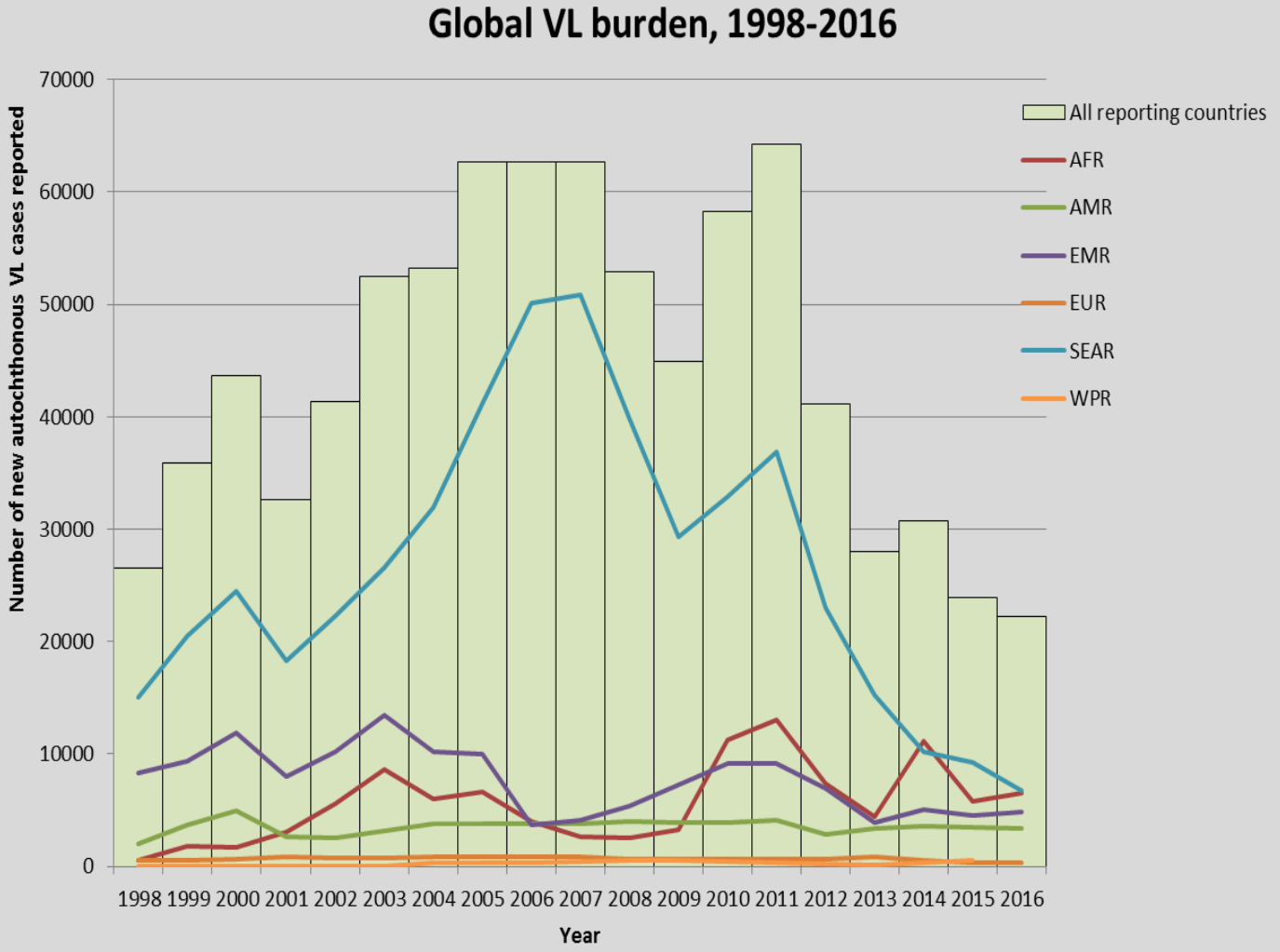


New CL autochthonous cases, 2016



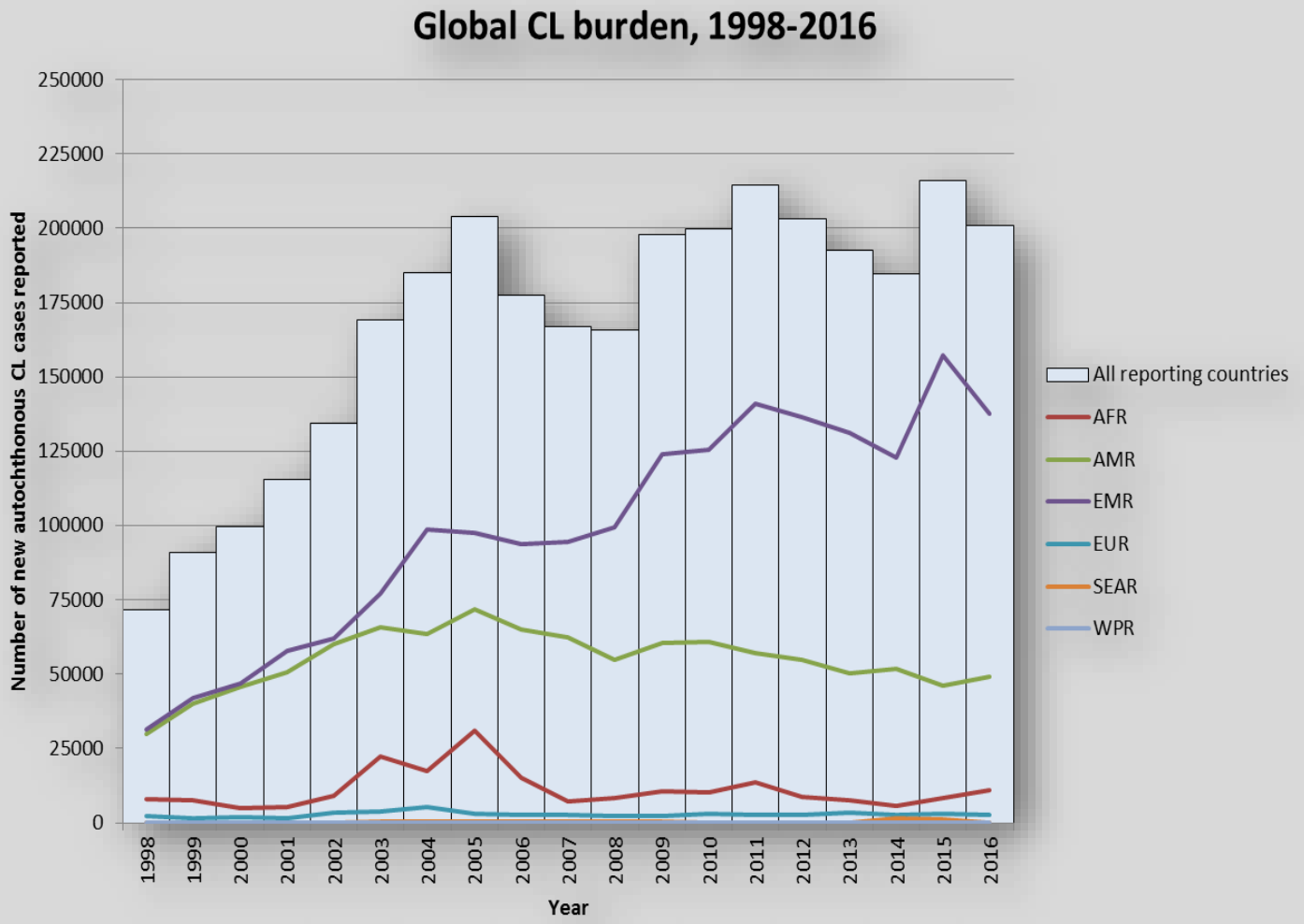
World Health Organization
Nepal

GLOBAL TREND- VL (1998-2016)



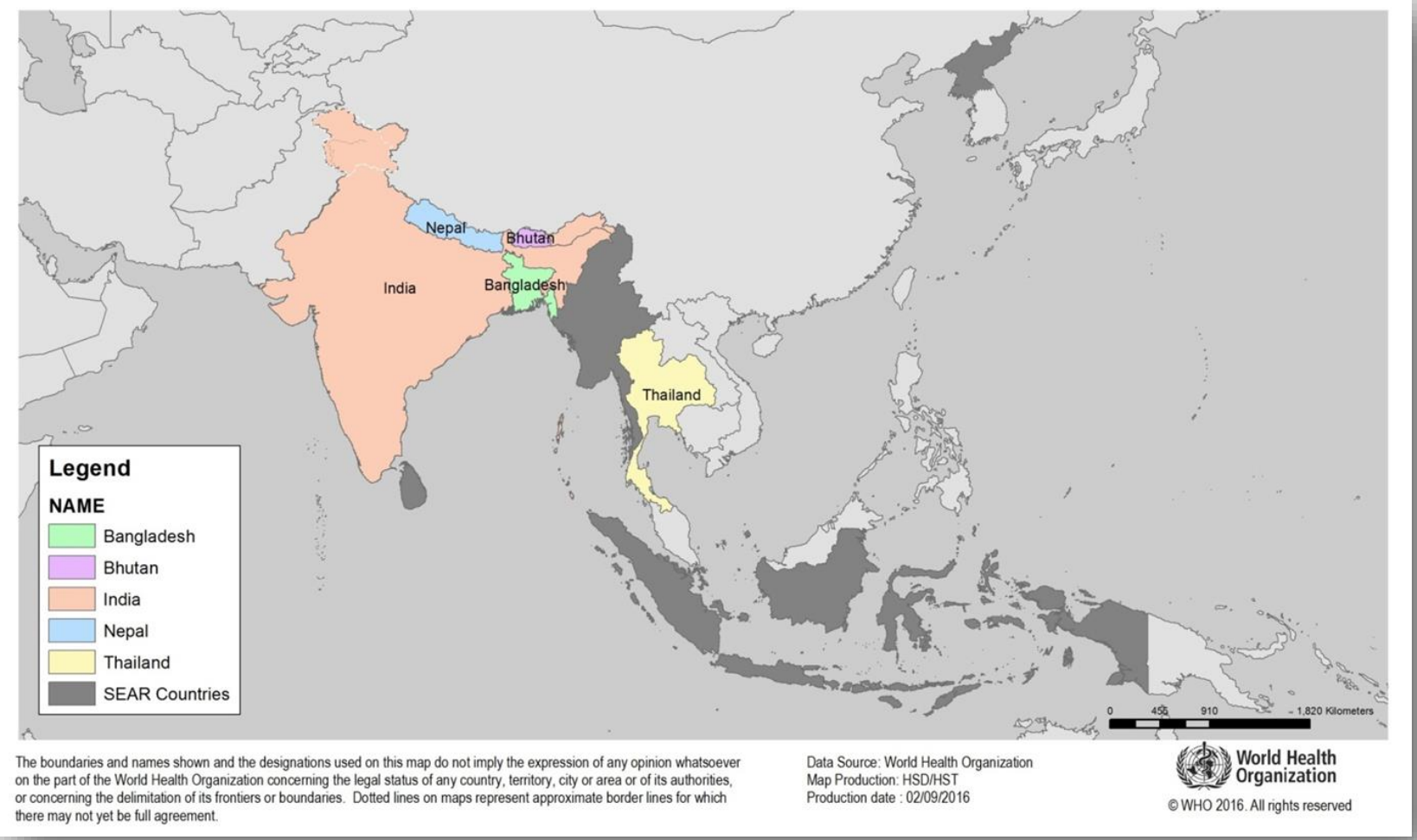
World Health Organization
Nepal

GLOBAL TREND- CL (1998-2016)



World Health Organization
Nepal

Leishmaniasis- South East Asia Region



VL-

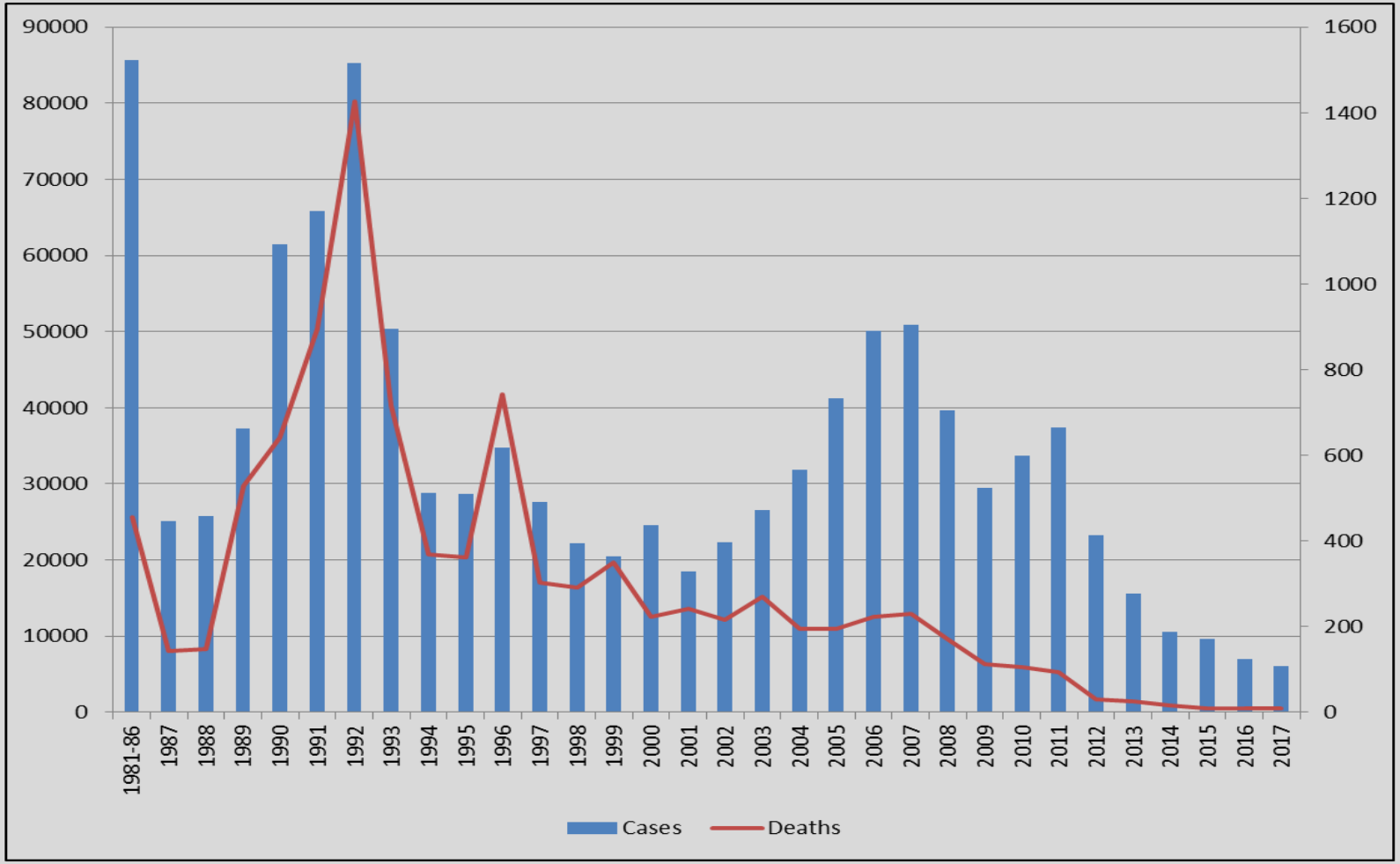
- Bangladesh
- Bhutan
- India
- Nepal
- Sri Lanka
- Thailand

CL-

- India
- Nepal
- Sri Lanka

Trend of VL

South East Asia Region(1981-2017)



World Health Organization
Nepal

The KA elimination initiative- SEAR

Target

Elimination as a public health problem

Defined as

Achieving less than 1 case per 10 000 populations

Implementation unit

Districts in NEP, Upazilla in BAN, Blocks in IND

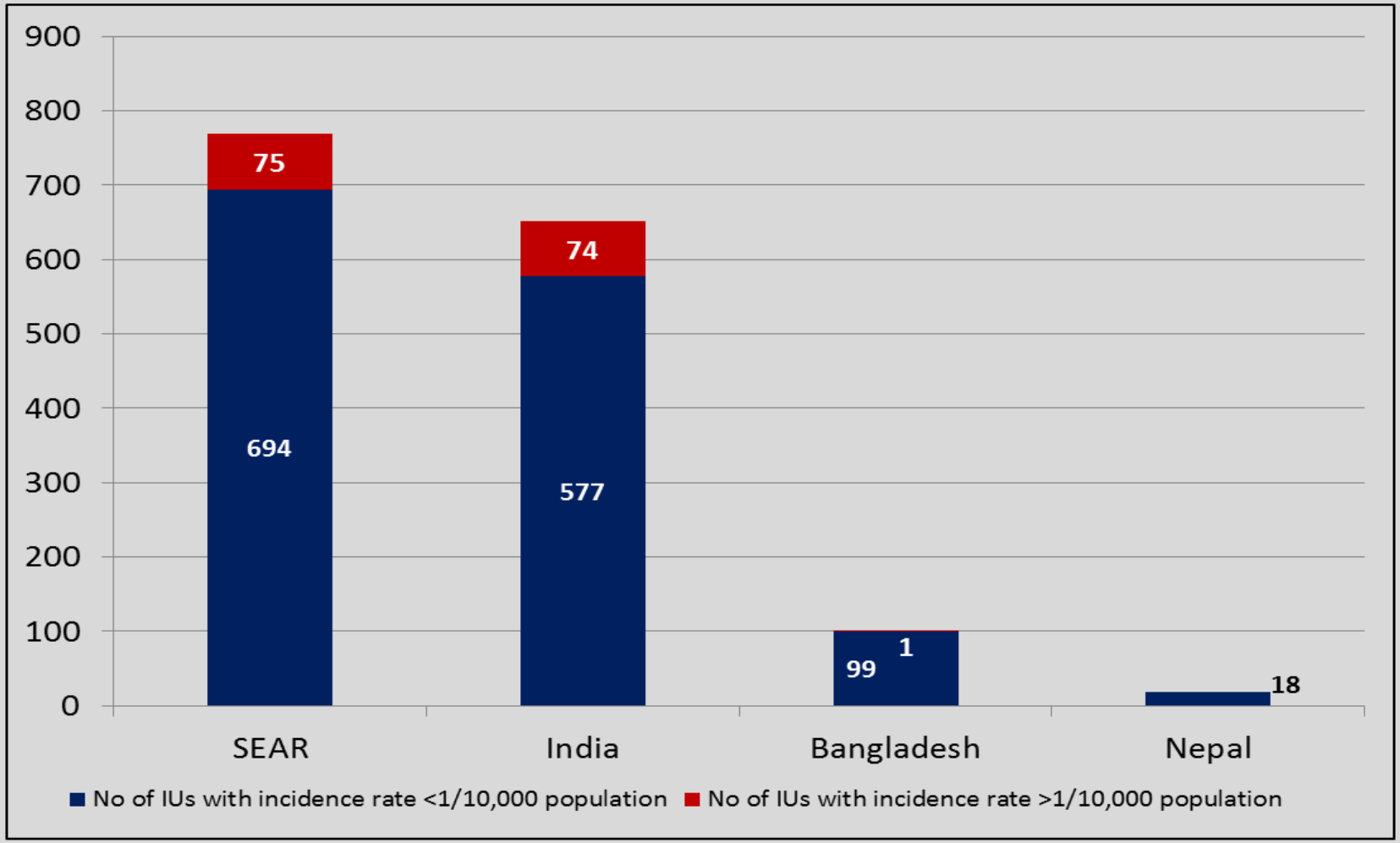
Timeframe

By 2020

Confirmation of achievement

Validation

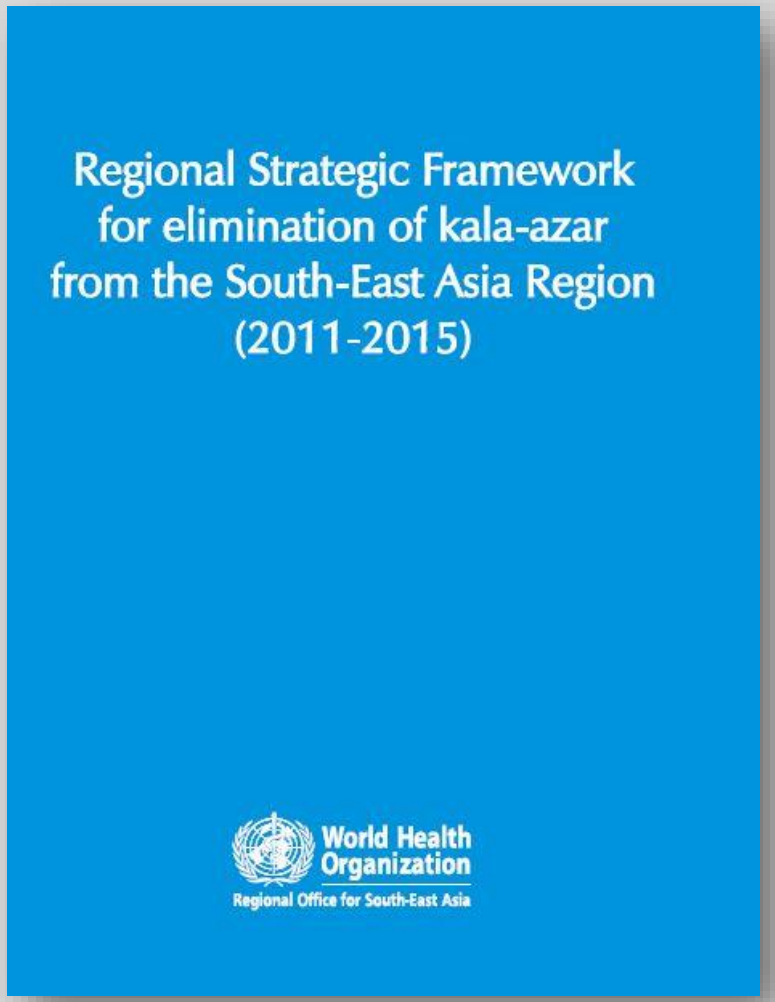
Status of VL elimination in Implementation units in SEA (2017)



World Health Organization
Nepal

Strategies
Kala- azar Elimination

1. Early diagnosis and complete case management
2. Integrated vector management and vector surveillance
3. Effective disease surveillance through passive and active case detection
4. Social Mobilization and building partnerships
5. Implementation and operational research



Challenges & opportunities Leishmaniasis-South East Asia Region

Challenges

- VL reported in new areas
- PKDL and HIV-VL coinfection
- CL increasingly being reported
- Sustaining and consolidating the gains

Opportunities

- VL elimination initiative
- Liposomal Amphotericin received by countries on donation through WHO

Thank you for your kind attention

Introduction & Clinical Features

- Visceral Leishmaniasis (VL)
- Post Kala-azar Dermal Leishmaniasis (PKDL)

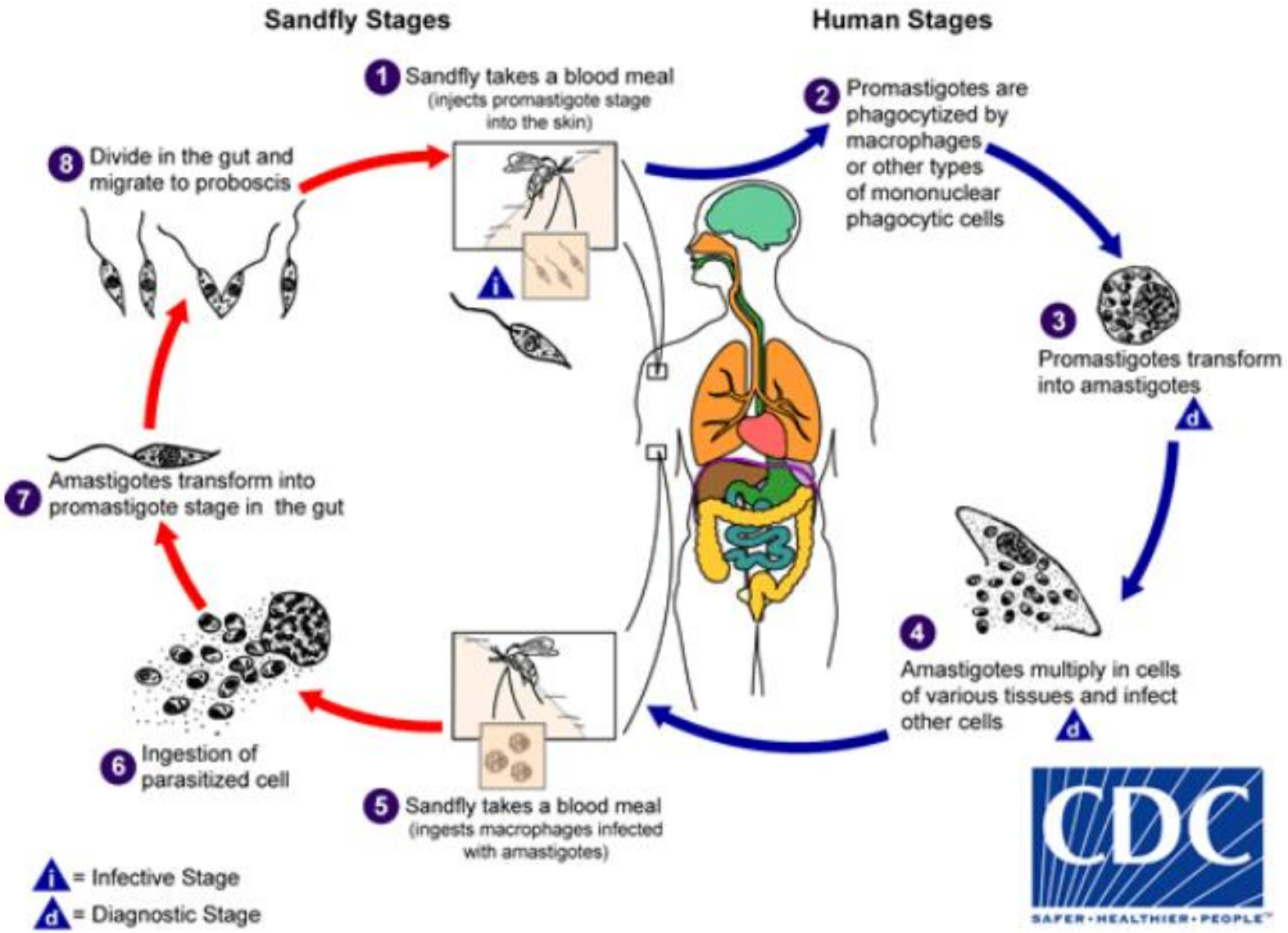
Introduction- Leishmaniasis

- A neglected tropical disease found in **tropical and subtropical** areas.
- There are three main forms of leishmaniasis:
  - **Cutaneous** (most common)
  - **Visceral or kala-azar** (most severe form)
  - **Mucocutaneous**
- **Caused by**: Protozoan parasites-from more than 20 **Leishmania** species.

Introduction- Leishmaniasis

- **Transmission**: Bites of infected female phlebotomine **sandfly**.
- Sand fly- generally most active during twilight, evening, and night-time hours (from dusk to dawn)
- Transmission can be: Anthroponotic or zoonotic
- In Nepal, known transmission is **anthroponotic** (human—sandfly—human cycle)
- In anthroponotic transmission, effective treatment of individual patients can help control the spread of the parasite

Life Cycle:

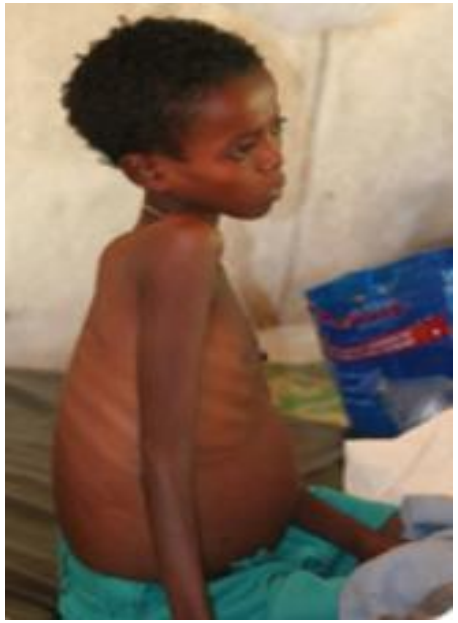

Sandfly Stages
Human Stages
1 Sandfly takes a blood meal (injects promastigote stage into the skin)
2 Promastigotes are phagocytized by macrophages or other types of mononuclear phagocytic cells
3 Promastigotes transform into amastigotes
4 Amastigotes multiply in cells of various tissues and infect other cells
5 Sandfly takes a blood meal (ingests macrophages infected with amastigotes)
6 Ingestion of parasitized cell
7 Amastigotes transform into promastigote stage in the gut
8 Divide in the gut and migrate to proboscis
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™

Clinical Features-VL (Kala-azar)

- Mainly affects **under privileged rural communities**.
- Majority of cases found in **children and young adults** in Nepal.
- Incubation period→ **2-6 months.**
- Most people infected by the parasite do not develop any symptom at all in their life.
- Should be suspected in a patient from an **endemic area** who presents with **prolonged irregular fever, splenomegaly and weight loss** as its main manifestations.

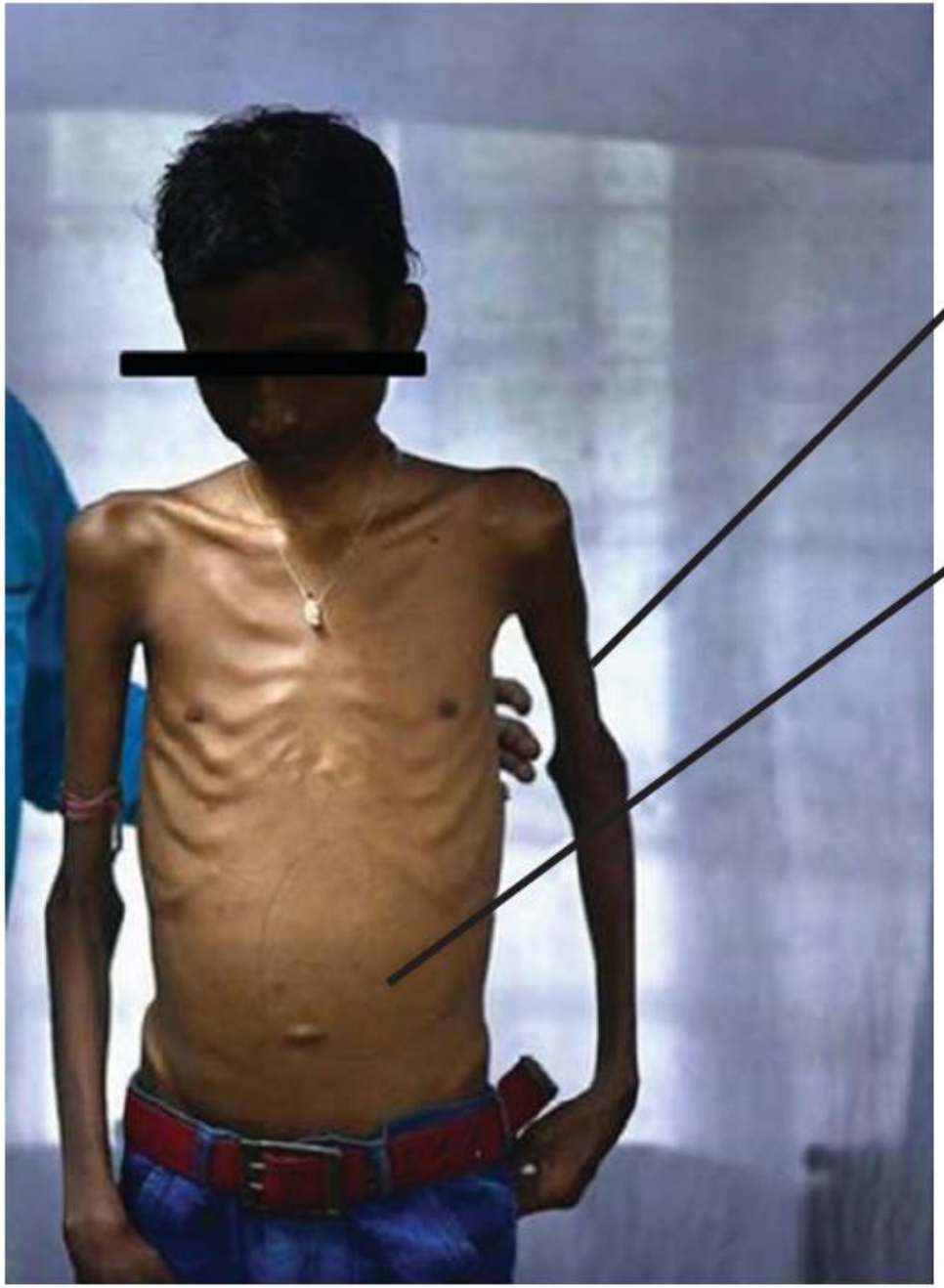
Clinical Features-VL (Kala-azar)..

SYMPTOMS	
Fever	High grade, irregular, prolonged duration (>2 weeks)
Weight loss	In the form of wasting, low body mass index (BMI)
Abdominal swelling	Due to splenomegaly, hepatomegaly
Loss of appetite	Seen invariably in al patients
Epistaxis	



Clinical Features-VL (Kala-azar)..

SIGNS	
Pallor	Usually moderate to severe pallor Tachycardia and signs of heart failure in severe anemic cases
Emaciation	
Splenomegaly	Most specific sign, non tender with smooth surface
Hepatomegaly	Less common than splenomegaly
Lymphadenopathy	Mainly in teenage population
Icterus	Usually seen in severe cases
Petechiae	
Edema, Ascites	



Wasting with decreased middle upper arm circumference (MUAC)

Distended abdomen with massive splenomegaly mark

(PC: Dr. V. Kattel)

Clinical Features-VL (Kala-azar)..

LAB FINDINGS	PARAMETER
Hemoglobin	Low
WBC count	Low
Platelet count	Low
Albumin	Low
Gamma globulin	High
Transaminases	High
Bilirubin	High

Risk of disease

- Young age
- Malnutrition
- Immunosuppressive conditions
- Poverty

Differential Diagnosis-Kala-azar

If symptoms > 2-4 weeks	
Tuberculosis	Usually not massive spleen as VL
Brucellosis	Usually associated with bone and joint symptoms and signs
Malnutrition	Usually not massive spleen as VL
AIDS	Usually not massive spleen as VL
Chronic hepatitis	Usually features of liver failure
Liver cirrhosis	Usually features of portal hypertension
Lymphomas and Leukemias	Usually rK39 negative

Differential Diagnosis-Kala-azar..

If symptoms of 2 weeks	
Malaria	Usually clinically very sick, hemodynamically unstable and antigen detection for malaria is highly sensitive and specific followed by very good response to standard malaria treatment
Typhoid fever	Usually clinically very sick, hemodynamically unstable and not massive spleen as VL and rK39 negative
Leptospirosis	Usually clinically very sick, hemodynamically unstable and not massive spleen as VL and rK39 negative

Case Definitions (Kala-azar)

PROBABLE VL (Kala-azar)	CONFIRMED VL (Kala-azar)
A person living in or having travelled to kala-azar endemic areas showing clinical signs and symptoms of kala-azar (mainly irregular fever lasting more than two weeks and splenomegaly and/or weight loss), after ruling out malaria in endemic areas.	**Laboratory confirmed** A probable VL case with laboratory confirmation either serologically (RDT, DAT, ELISA, IFAT) and /or parasitological (smear, culture) and/or positive by PCR or related techniques
	Clinically confirmed A probable VL case that has not been confirmed by any laboratory test (i.e. test not done or negative) but is assessed by a clinician to be a confirmed VL case based on clinical grounds.

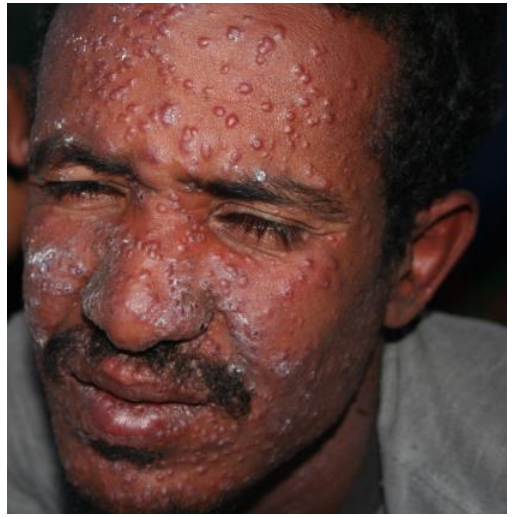
Post Kala-azar Dermal Leishmaniasis (PKDL)

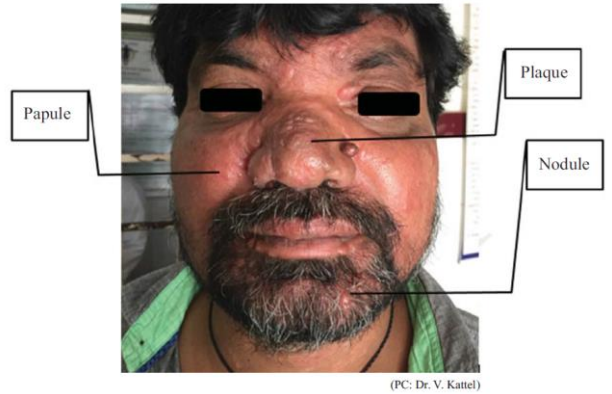
Introduction-PKDL

- Post kala-azar dermal leishmaniasis (PKDL) is a **complication of kala-azar** in areas where Leishmania donovani is endemic.
- It is common in East Africa and Indian subcontinent.
- Usually appears 6 months to 1 or more years after apparent cure of the disease but may occur earlier or even concurrently with visceral leishmaniasis.
- Considered to have an important role in **maintaining and contributing to transmission of the disease** particularly in interepidemic periods of VL, acting as a reservoir for parasites.

Clinical Features-PKDL

- Most patients on the Indian subcontinent have **mixed presentations** comprising **macules, papules, plaques or nodules** mainly on or around the chin and mouth or face.
- The lesions are
  - **non ulcerative**
  - **non itchy**
  - **non anesthetic**
  - Prolonged duration, persistent and progressive
- Macules can progress over weeks to months into papule and plaque then progress to nodules.





Clinical Features-PKDL..

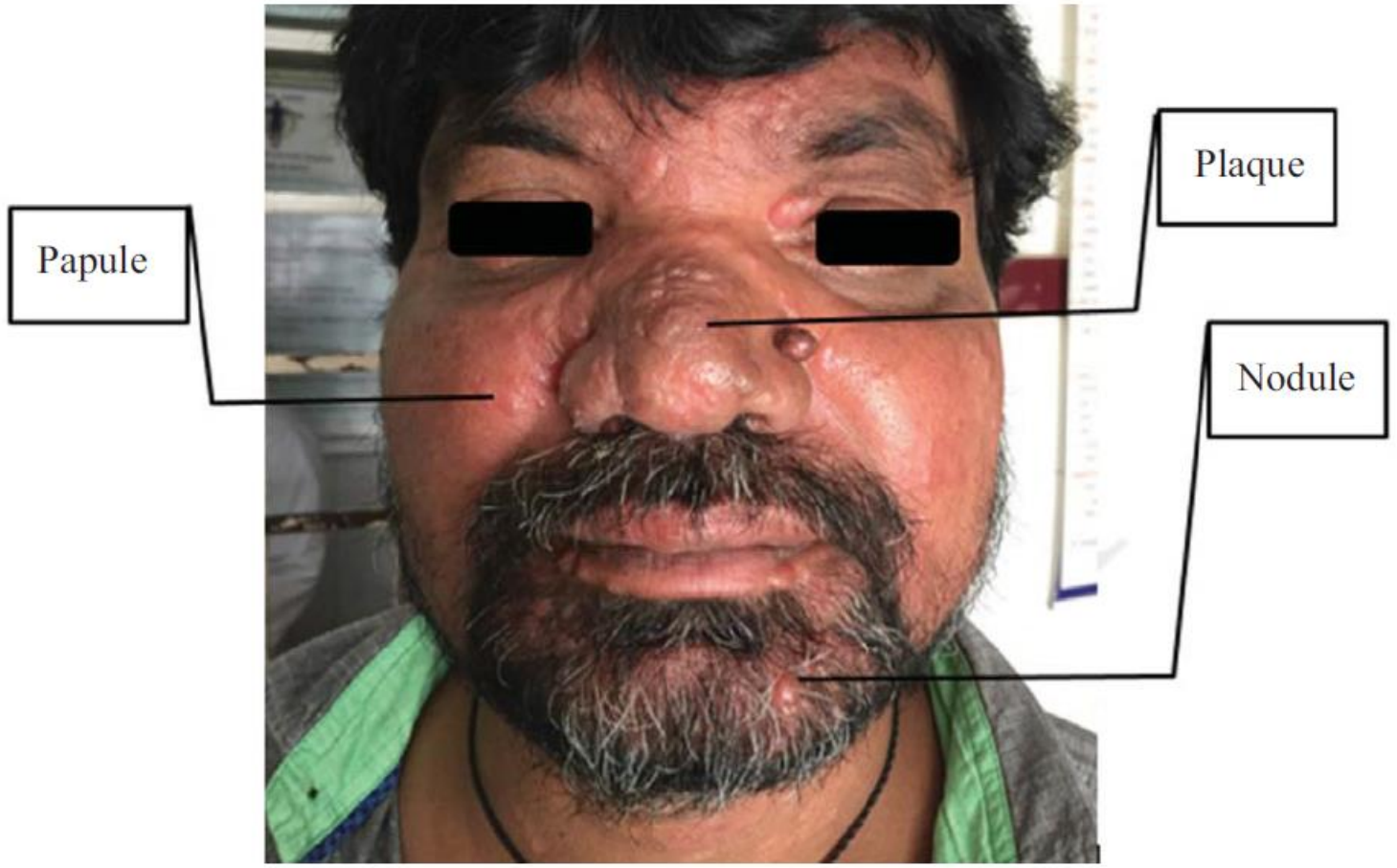
PKDL can present in different forms

- **Monomorphic-** macular and nodular
- **Polymorphic or mixed-** both macules and indurated lesions such as papules are present
- **Rare presentations-** erythrodermic

There is no standard system for grading the severity of PKDL on the Indian subcontinent. The severity may be described as:

- **Mild-** very few lesions, usually on the face
- **Moderate-** lesions easily visible and generalized
- **Severe-** dense coverage with lesions and little normal skin remains

Clinical Features-PKDL..



(PC: Dr. V. Kattel)

Severe and polymorphic lesion

Differential Diagnosis-PKDL

▪ Leprosy	▪ Nutritional deficiencies
▪ Pityriasis alba	▪ Milaria rubra
▪ Neurofibromatosis	▪ Acne
▪ Secondary syphilis	▪ Lupus vulgaris
▪ Chronic arsenic poisoning	▪ Discoid lupus erythematosus
▪ Pityriasis versicolor	

Case Definitions- PKDL

PROBABLE PKDL	CONFIRMED PKDL
A patient living in or having travelled to visceral leishmaniasis (kala-azar) endemic areas presenting with a typically symmetrical multiple hypopigmented macules, papules, plaques, or nodules without loss of sensation.	A probable PKDL case with Leishmania infection confirmed parasitologically, by PCR or a slit-skin smear or biopsy.

PKDL can occur in patients with previous or concomitant kala-azar. In some cases, it occurs without the history of VL. Serological test such as rK39 rapid diagnostic test positivity acts as a strong evidence when other diseases (for example, leprosy) are considered in the differential diagnosis, or if a history of VL is uncertain.

Diagnosis

Visceral Leishmaniasis (VL)

PKDL

Diagnosis-Kala-azar

- Combination of
  - **Clinical signs** with
  - **Parasitological** or **serological** tests.
- **Parasitological**- Definitive diagnosis of VL is by
  - Culture or
  - Microscopic confirmation –
- **Serological**
  - Rapid diagnostic test –rK39

Laboratory Diagnosis-Kala-azar..

Serological- Rapid diagnostic test (rK39)

- Simple test- can be used at all levels of the health care services.
- Does not require highly skilled lab staff and results can be read easily and within 30 minutes.
- The test is based on the **detection of antibodies in blood**.
- Due to the persistence of antibodies over long periods, they cannot be used to differentiate between current and past infection.
- Can be false negative in immunocompromised status like HIV/Kala-azar co infection, pts on immunosuppressant therapy and severe acute malnutrition.

Laboratory Diagnosis-Kala-azar..

rK39 test- Before you start

- Read manufacturer's instructions.
- Serum preferred over whole blood.
- Check for expiry date.
- The strip should be taken out from the pouch only at the time of performing the test.
- If the strip has not been used within one hour of taking out from the pouch, it should be discarded.

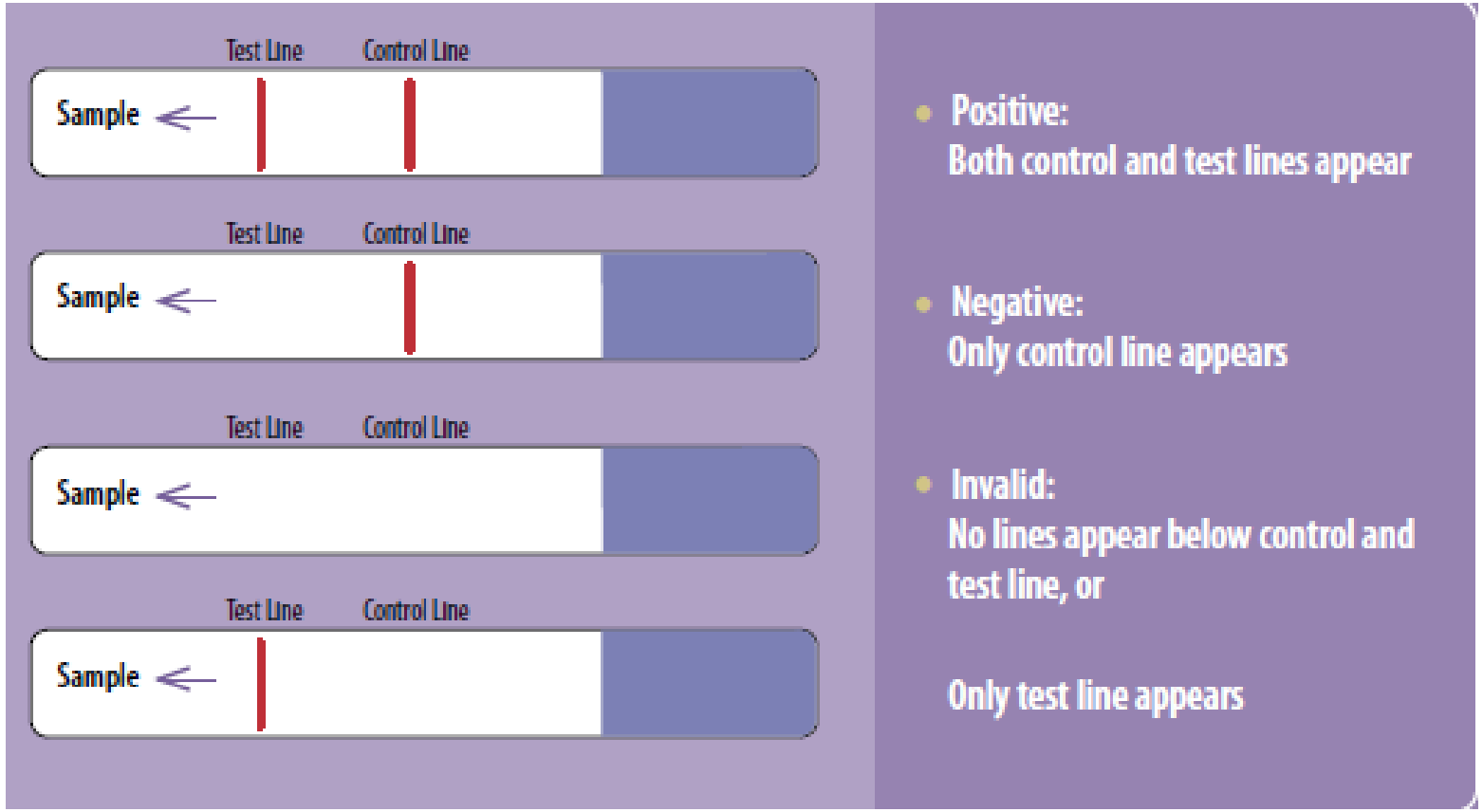
Laboratory Diagnosis-Kala-azar..

rK39 test- Test procedure

- Remove the test strip from the pouch.
- With a new lancet, prick the finger tip of the patient suspected to be suffering from kala-azar.
- Let the blood come out on its own. Do not use pressure or squeezing for obtaining blood.
- Place one drop of blood or serum 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.
- Add 2-3 drops of buffer solution provided with the kit to the pad.
- Read the results in10 minutes.
- Use universal precautions for infection prevention.

Laboratory Diagnosis-Kala-azar..

Serological- Rapid diagnostic test (rK39)-Test reading



Laboratory Diagnosis-Kala-azar..

Advantages and disadvantages of rK39

Advantages	Disadvantages
■ Can be used in field settings ■ Enables individual patients be tested at the bedside/field e.g. camps ■ Tests are individually packed and easy to store and transport ■ Simple to perform with minimal training ■ Results are reproducible ■ Does not require a laboratory set up ■ Test can be performed using finger prick whole blood, serum or plasma. ■ Kits can be transported and stored at ambient temperature (up to 30°C). ■ Results are easy to read and interpret and are available within 10 – 30 minutes.	■ Remains positive after the successful treatment of VL patients (past infection) ■ Cannot distinguish between active cases (current infection)-NEW CASES and RELASPES cases ■ The rK39 antibodies can also be present in healthy persons from endemic areas who were exposed to Leishmania but have not developed clinical disease, therefore, interpretation must always be done in combination with clinical case definition ■ In patients with advanced HIV infection a negative result cannot rule out the diagnosis of VL

Laboratory Diagnosis-Kala-azar..

Scenario when rK39 is not definitive

- Patients under current VL treatment or treated.
- PKDL with past history of VL. However, in PKDL cases treated for past VL, positive rK39 makes such patients as probable PKDL.
- Is false negative in HIV-VL co-infected persons with CD4 less than 200 or those with other immune disorders or severely malnourished.

Laboratory Diagnosis-Kala-azar..

rK39 test- Storage of kits

- The test strips and the buffer should be
  - stored safely at **room temperature between 20-30°C**.
  - not be frozen since freezing deteriorates the quality of the reagent.
- Not advised to store large quantities of ‘rK39’ test kits in the peripheral locations since it is difficult to maintain appropriate temperature.
- Stored at central locations in the districts where the temperature can be properly maintained as required in the specifications.

Laboratory Diagnosis-Kala-azar..

Parasitological- Microscopic confirmation

- Definitive diagnosis- microscopic confirmation of the amastigote form of the parasite in tissue aspirates from spleen, bone marrow or lymph nodes.
- **INDICATIONS**
  - rK39 negative but suspicion of kala-azar high in normal individual
  - rK39 negative but suspicion of kala-azar is high in cases like PLHIV with low CD4 count, severe malnutrition and severe immune suppression.
  - Patient treated for VL in the past presenting again with symptoms suggestive of VL (high suspicion of relapse).
  - In settings where studies are done for monitoring of drug resistance.

Laboratory Diagnosis-Kala-azar..

Comparative features of different clinical specimen for microscopic confirmation of kala-azar

Features	Splenic	Bone marrow	Lymph node
Sensitivity	■ 95% ■ Sensitivity highest among the three methods ■ Considered reference standard	■ 53-86%	■ Low (52-58%)
Availability	■ Not recommended for field settings ■ Only in district hospitals or higher (referral) centre ■ Where surgical services are available ■ Where blood transfusion services are available ■ Where nursing surveillance is present	■ Not recommended for field settings ■ District hospitals or higher (referral) centre	■ proper tissue preparation is needed
Procedure	■ Expertise required for the procedure	■ Painful	■ Need experience
Risk	■ Risk of fatal bleeding (0.1%)	■ Sterilization is required	

Laboratory Diagnosis-Kala-azar..

Parasitological- Microscopic confirmation

Important points for considerations

- Demonstration of parasites in aspirates is proof of VL.
- Sensitivity of the test depends on the expertise and quality of reagents.
- Identification of amastigotes under microscope requires experience and skill. At least 1000 microscopic fields for amastigotes using x100 oil immersion lens should be examined.
- Inability to find amastigotes in aspirates cannot be a reason to exclude VL in a patient having a strong suspicion of the disease.

Laboratory Diagnosis-Kala-azar..

Parasitological- Culture

- Needs special media-NNN media.
- Time consuming process and not feasible for field settings.

Polymerase chain reaction (PCR)

- Higher sensitivity than parasitological examination, however not available in all referral hospitals

Laboratory Diagnosis-Kala-azar..

Other recommended laboratory tests-to monitor the side effects of drugs and progress of treatment, the following laboratory tests are recommended.

- **CBC**
- **Prothrombin time**
- **Renal function tests**
- **Liver function tests**
- **Pregnancy tests**
- **Malaria parasite**
- **HIV-should be checked in all VL cases**
- **Urine dipstick test for protein**

Note: Since TB is endemic in Nepal and VL-TB coinfections have been reported, it is recommended to screen all kala-azar cases for TB as per the national protocol

Laboratory Diagnosis-VL Relapse

VL Relapse

- Kala-azar patient who was successfully treated in the past but has presented again with clinical manifestations of kala-azar with parasitological confirmation at any point after cure. In many cases relapses usually occur **within 6 months** after treatment.
- Diagnosis of relapse should be based on parasitological diagnosis. rK39 cannot differentiate between recent (new) and past infection (relapse) because it can still be positive for months to years after a case is successfully treated.

Laboratory Diagnosis-VL Relapse

VL Relapse

- Sometimes especially in children during or post treatment there may be worsening of symptoms in the form of high grade fever, pancytopenia, mild to moderate icterus, progression of hepatosplenomegaly, generalized lymphadenopathy and rashes.
- This is rare case scenario however serum ferritin, triglyceride level and fibrinogen level may help to differentiate hemophagocytic syndrome a close differential of relapse. Bone marrow biopsy will reveal hemo-phagocytosis. Thus, role of microscopy plays vital in such scenario.

Laboratory Diagnosis-PKDL

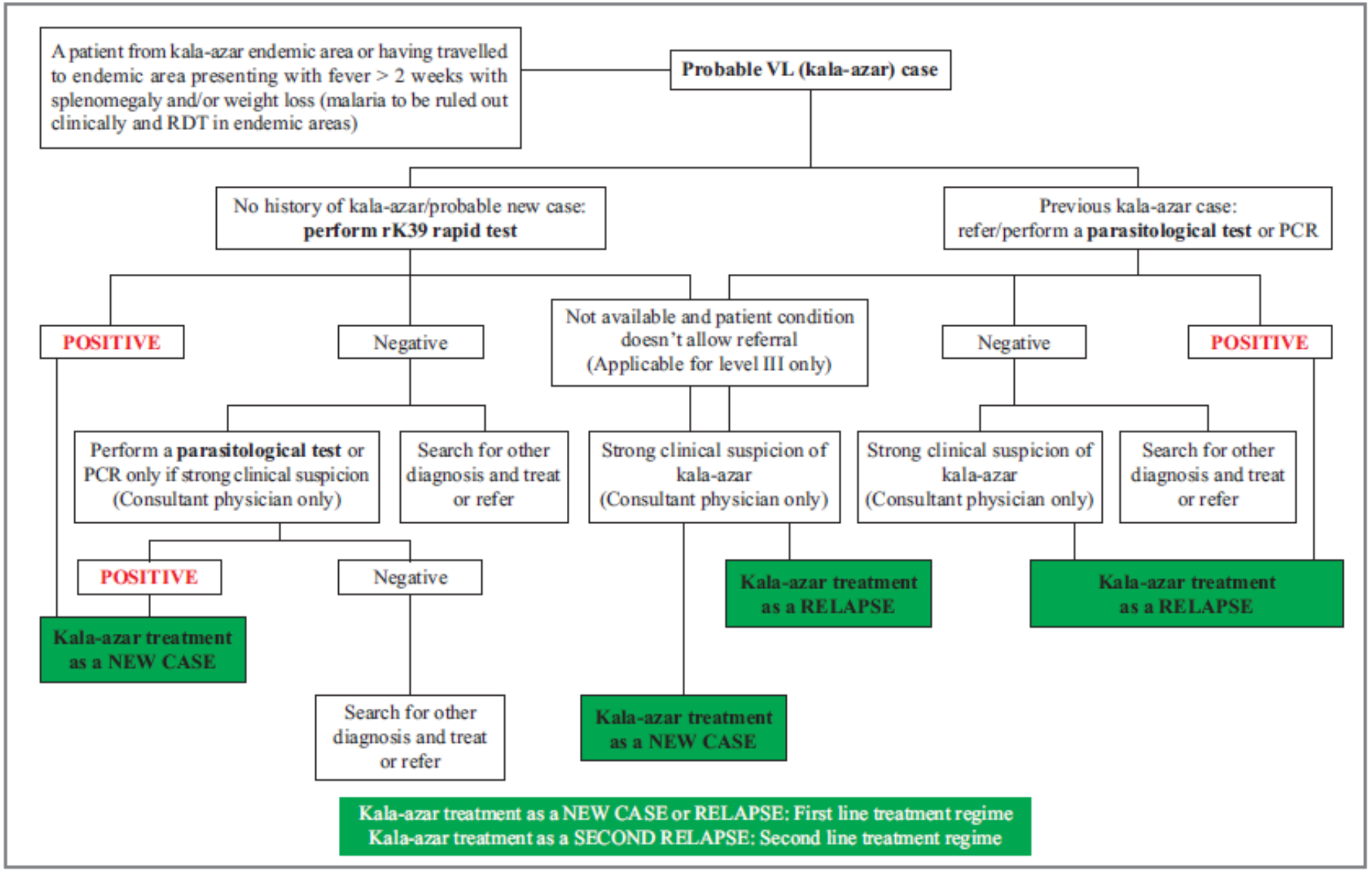
Parasitological

- Confirmation of diagnosis is usually done by skin slit smear (SSS) microscopy or histopathology.
- Sensitivity of SSS microscopy is, 40-60% from patients with nodular lesions. The advantage of microscopy is the acknowledged high specificity, which leads to low numbers of patients unnecessarily treated with anti-leishmanial drugs.

PCR

- Higher sensitivity than microscopy however it is not available due to cost factor in resource limited set up.

Figure 3: **Kala-azar Diagnostic Algorithm**



Treatment

Visceral Leishmaniasis (VL)

PKDL

Treatment- Kala-azar

MAIN OBJECTIVES OF TREATMENT

- Clinical cure of the patient
- Minimize drug toxicities and side effects, if any
- Prevent and/or identify and treat complications
- Support patient's nutritional and hydration status
- Manage other medical conditions
- Reduce the risk of relapse and PKDL
- Report to national kala-azar elimination program.

Treatment- Kala-azar

POOR PROGNOSTIC FACTOR OF VL TREATMENT

- Jaundice
- Severe wasting
- Severe anemia
- HIV co-infections
- Extreme of ages
- Pregnancy
- Hemodynamically unstable
- Hemophagocytic Syndrome
- Comorbidities
- Biochemical markers (Neopterin)

Treatment- Kala-azar

- **Supportive management**
  - Nutritional support
  - Treatment of inter-current infections
  - Treatment of anaemia

- **Drug treatment**
  - Primary kala-azar
  - Relapse kala-azar

Treatment- Kala-azar

Supportive management

Nutritional support

- Most of the kala-azar patients are malnourished and require adequate nutrition and vitamin supplements.
- In some patients therapeutic feeding may also be needed.

Treatment- Kala-azar

Supportive management..

Treatment of inter-current infections

- Intercurrent infections are very common in kala-azar patients. Many patients die due to secondary bacterial infections.

- Intercurrent infections should be treated
  - **Pneumonia**-appropriate antibiotics
  - **Skin/mouth infections**-maintain skin and oral hygiene, appropriate antibiotics, drugs
  - **Other parasitic infections/infections-Malaria, TB** with appropriate drug
  - **Dysentery**

Treatment- Kala-azar

Supportive management..

Anaemia

- Many patients suffer from anaemia and may occasionally require blood transfusion to correct severe anaemia or bleeding due to thrombocytopenia.

Treatment- Kala-azar

Drug treatment

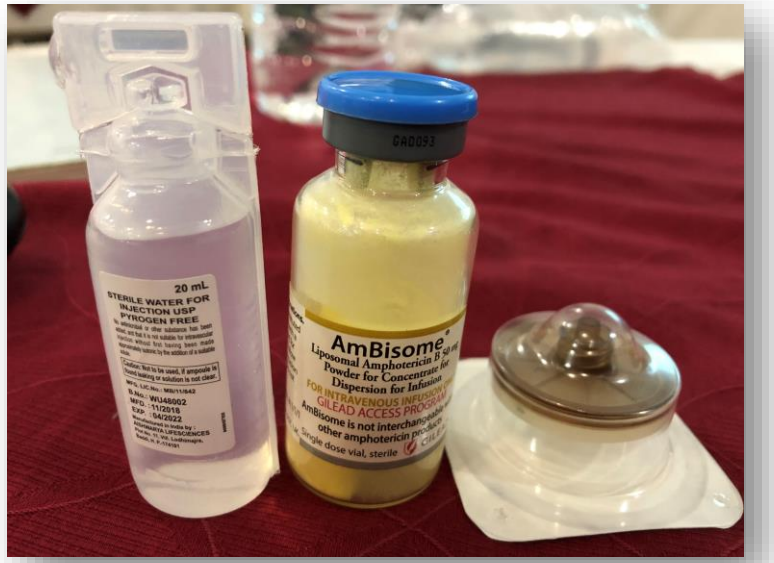
Primary Kala-azar

		Drugs
1st line	Monotherapy	Liposomal Amphotericin B
2nd line	Combined regimen	Liposomal Amphotericin B+ Miltefosine or Liposomal Amphotericin B + Paromomycin or Miltefosine+ Paromomycin
3rd line	Monotherapy	Amphotericin B deoxycholate
4th line	Monotherapy	Miltefosine or Paromomycin

Treatment- Kala-azar

Primary Kala-azar- **1st line regimen**

- **Liposomal Amphotericin B**
  - 10 mg/kg single dose over 2 hrs.
  - Single dose ensures 100% compliance.
  - Safe in pregnant women, severely ill patients, children less than 2 yrs. old, old aged and HIV co-infected patients.



Treatment- Kala-azar

Primary Kala-azar- 1st line regimen

- **Liposomal Amphotericin B..**
  - Successful therapy-
    - Improves general condition
    - Resolves fever in most cases by end of week
    - Regression of splenomegaly (complete regression may take several months, most cases have complete regression by 6 months)
    - Recovery of blood count towards normal
    - Indicator of definitive cure-absence of clinical relapse at 6 months.

Treatment- Kala-azar

Primary Kala-azar- 1st line regimen

- **Liposomal Amphotericin B..**

THINGS TO REMEMBER FOR L-AmB TREATMENT

- Do not use underweight dose
- Give test dose before starting L-AmB infusion
- Do not freeze
- Always prepare in Dextrose solutions
- L-AmB is not compatible with saline and other fluids
- Prevent foam formation while constituting drug

Treatment- Kala-azar

Primary Kala-azar- 1st line regimen

- **L-AmB: Adverse Events-** In order of frequency of occurrence
  - Infusion related fever and rigor, chills-common
  - Nausea/vomiting-common
  - Headache/backache-common
  - Chest pain
  - Hypokalemia
  - Dyspnea
  - Bronchospasm
  - Tachycardia, Hypotension
  - Nephrotoxicity and hepatobiliary disorders.
- Life threatening adverse drug reaction- very rare.
- Infusion related reactions- infusion can be slowed down and/or physician may give medicines to prevent or treat infusion related reactions, such as diphenhydramine (antihistamine), paracetamol and or hydrocortisone to reduce immune system response.

Treatment- Kala-azar

Primary Kala-azar- 1st line regimen

- **L-AmB: Indication for stopping L-AmB treatment**
  - Patients who develop hypersensitivity reactions- cessation of L-AmB and switching to an alternative treatment.
  - If a severe anaphylactic reaction- the infusion should be immediately discontinued, and the patient should not receive any further infusions.

- **L-AmB: Storage conditions**
  - Before use, medicine should be stored at 2-25°c and should not be frozen.
  - It should also be protected from exposure to light. Once reconstituted, the product must be used immediately.

Treatment- Kala-azar

Primary Kala-azar- 2nd line (combination drugs)

- Use of combination regimen
  - reduces dose requirement of individuals drugs and its consequences without compromising the cure rate.
  - reduces the probability of selection of drug-resistant parasites, thereby prolonging the effective life of the available medicines.
- Combination regimen will be used in patients where the first line treatment is not indicated or not available.

Treatment- Kala-azar

Primary Kala-azar- **2nd line regimen (combination)**

1. Liposomal Amphotericin B + Miltefosine

- **L-AmB**: 5mg/kg single infusion and
- **Miltefosine**: 50 mg BID in adult or 2.5 mg/kg/day-7 days

Treatment- Kala-azar

Primary Kala-azar- **2nd line regimen (combination)..**

2. Liposomal Amphotericin B + Paromomycin

- **L-AmB**: 5mg/kg single infusion and
- **Paromomycin**: 11 mg/kg base-10 days

Treatment- Kala-azar

Primary Kala-azar- **2nd line regimen (combination)..**

3. Miltefosine+ Paromomycin

- **Miltefosine**: 50 mg BID in adult or 2.5 mg/kg/day-7 days
- **Paromomycin**: 11 mg/kg base-10 days

Treatment- Kala-azar

Primary Kala-azar- 3rd line regimen

Amphotericin B deoxycholate

- **0.75-1.0 mg/kg per day-** IV infusion in 5 % dextrose over 4 hours for 14 days.
- If poor response to treatment drug needs to be continued for 21-28 days.
- Due to its side effects it has been replaced by safer liposomal formulations, however, still a rescue medicine in non responsive pts to antileshmanial medicines.
- Patient must be admitted in higher/special referral centers for administering Amphotericin B as it requires for monitoring renal parameters.

Treatment- Kala-azar

Primary Kala-azar- 3rd line regimen

Amphotericin B deoxycholate

- Generally recommended in the following conditions:

  - **When 1st and 2nd line regimen are not available.**
  - **Kala-azar treatment failure- unresponsive to first and second line regimen or cases of relapse**
  - **Kala-azar patients whose first and second line therapy is discontinued due to severe side effects.**

Treatment- Kala-azar

Primary Kala-azar

Amphotericin B deoxycholate

- **Side effects:**

Renal impairment	Hypokalaemia and hypomagnesaemia
Headache	Nausea
Vomiting	Chills
Fever	Malaise
Muscle and joint pain	Diarrhoea, gastrointestinal cramps
Hypertension/hypotension	Cardiac arrhythmias including ventricular fibrillation
Skin rashes	Anaphylactoid reactions
Blurred vision	Vertigo
Hearing loss	Tinnitus
Liver disorders,	Peripheral neuropathy
Convulsions	Thrombophlebitis at the injection site and anaemia.

Treatment- Kala-azar

Primary Kala-azar- 4th line regimen

1. Miltefosine

- Only to be considered when 1st, 2nd and 3rd line treatment are not available.
- Only available oral anti kala-azar drug, available in 10 mg and 50 mg capsule.
- Miltefosine monotherapy has observed a higher relapse rates in Nepal, so used as a part of combination therapy. If used as monotherapy should be given for 28 days.

Recommended doses of Miltefosine according to body weight

Drug category	Morning	Evening	Duration
More than 12 years of age and more than 50 kg body weight): 150mg/day	100 mg	50 mg	28 days
More than 12 years of age and more than 25-50 Kg body weight) at a dose of 100mg/day	50 mg	50 mg	28 days
≥12 years of age and less than 25 Kg body weight) at a dose of 50mg /day.	50 mg	0	28 days
Children aged (2-11 years age) at (2.5mg/kg body weight 10mg formulation in divided doses)			28 days

Note:

- **Miltefosine should be taken after meal**
- **If any 28 days course drug is missed can be completed by 35 days without exceeding the maximum recommended dose**

Miltefosine

Contraindication	Method of verification
Pregnancy	History of last menstrual period (LMP) and Pregnancy test
MWRA not using contraceptives	History
Lactating mother	History
Less than 2 years	History
Severe illness, bed bound	History and Physical examination
Malnutrition	< 10 percentile weight for age
Severe anemia (Hb% < 5 gm)	Level of Hb
Patients with known kidney disease	Edema, decreased urine output, Proteinuria
Patients with known liver disease	Jaundice
Chronic alcoholism	History

Miltefosine
Side effects and its management

Side effect	Management
Vomiting, Diarrhea and Abdominal pain	Usually during 1st week of treatment-mild, short duration and reversible. Advise ORS. If severe refer to higher center
Liver and renal side effects	Puffiness of face, decreased urine output, jaundice may be liver or kidney related side effects. These symptoms should be monitored.
	If fever persists in spite of taking Miltefosine for two weeks, then the patient may have other infections along with kala-azar. Such patients should be referred for further investigation and treatment.

Treatment- Kala-azar

Primary Kala-azar- **4th line regimen**

1. Miltefosine..

- **Indications for stopping Miltefosine treatment**
  - Pregnancy during treatment
  - Jaundice
  - Puffiness of face
  - Decreased urine output
  - Breathlessness
  - Severe vomiting/diarrhea

Treatment- Kala-azar

Primary Kala-azar- 4th line regimen

2. Paromomycin

- **15 mg/kg/day** IM- 21 days
- Should not be administered intravenously
- Patient must remain well hydrated
- If patient has severe vomiting and diarrhea, do not give injections
- Not recommended in pregnancy
- Should be avoided in patients with severe anaemia with hemoglobin <5g/dl
- Contraindicated in patients with renal insufficiency

Treatment- Kala-azar

Drug treatment

Relapse Kala-azar

- **Relapse:** a patient who experiences recurrence of VL symptoms with parasitological confirmation at any time point after initial cure. VL relapses are usually observed within 6 months of completion therapy.
- The treatment of choice is **higher doses of L-AmB** or **Amphotericin B deoxycholate** or **combination regime of two drugs.**
- Liposomal amphotericin B at higher cumulative doses **up to 30mg/kg**

Kala-azar with HIV co-infection

- Kala-azar is an AIDS-defining condition and a valid entry point for starting ART, irrespective of CD4+ count. The baseline CD4+ count is lower in Leishmania-HIV co- infected patients, as kala-azar itself causes a reduction in CD4+ cells.
- The impact of ART-: a reduction of incidence, higher survival rates, a reduction in relapse rate and possible immune reconstitution inflammatory syndrome.
- HIV patients are more likely to develop kala-azar(due to reactivation of a dormant infection or clinical manifestation after primary infection). Patients have high parasite loads. Kala-azar negatively affects the response to ART and is difficult to cure in coinfected patients, especially those with CD4+ counts < 200 cells/mm^3, who typically relapse·

Kala-azar with HIV co-infection..

- **Prognosis** of coinfected patients is characterized by
  - high mortality rate during the first episode
  - increased antileshmanial drug toxicity (predominantly with antimonial)
  - poor long-term clinical response, parasitological cure and a high relapse rate over a lifetime.
- **Risk factors** for relapse are:
  - no antiretroviral treatment
  - low CD4+ cell count
  - previous kala-azar episode
  - failure to achieve clinical or parasitological cure during the first episode
  - no secondary prophylaxis.

Kala-azar with HIV co-infection..

- Visceral leishmaniasis in a HIV patient is an AIDS defining illness
- All VL patients should be offered Provider Initiated Testing and Counselling for HIV screening
- All HIV patients should be clinically assessed for signs and symptoms of visceral leishmaniasis
- In VL-HIV con-infected patients, ART should be started without delay

Kala-azar with HIV co-infection..

Important issues..

- **Higher dose regimen**

- L-AmB at a dose of 5mg/kg/day (D1, 2, 3, 5, 9, 13, 17, 21) total up to 40mg/kg body weight.

Kala-azar with HIV co-infection..

Important issues..

➢Relapse in HIV-VL co-infected

- Relapse is inevitable with VL-HIV co-infection, so it has recommended starting ART as early as possible in such scenario.
- Relapse has to be treated with combination therapy- **L-AmB +Paromomycin**
  - **L-AmB-** 5mg/kg/day; total 30-40mg/kg/ on (D1,2,3,5,9,13,17,21)
  - **Paromomycin-** 15mg/kg/day for 21 days).

Kala-azar with HIV co-infection..

Important issues..

- **Immune Reconstitution Inflammatory Syndrome (IRIS)**

- Usually it is recommended to have a gap of 2 weeks between VL treatment and ART if CD4 count is very low due to IRIS.
- However, in a tertiary care centers both treatments can be initiated simultaneously, and IRIS can be managed if it occurred.
- Symptomatic therapy and/or steroid are recommended in mild to moderate IRIS. In life threatening IRIS it is better to hold ART.

Kala-azar with HIV co-infection..

Important issues..

- **Other conditions**

- L-AmB has been demonstrated safe in kala-azar with Diabetes mellitus, Hypertension, and stable heart disease patients.
- L-AmB is also safe for elevated liver enzymes.

Treatment PKDL

- PKDL- usually do not have any signs of kala-azar like fever/splenomegaly/anemia.
- Although 85-90% of them appear after the cure of kala-azar, 10-15% of cases of PKDL occur without the preceding history of kala-azar.
- Following complete treatment of PKDL case, all skin lesions tend to disappear.
- PKDL cases need to be actively looked for as they act as a potential reservoir of kala-azar and contribute to continued transmission.
- It is advised to refer all the PKDL cases to level III health institution or special referral center for treatment.

Treatment PKDL

- WHO recommended dose for PKDL treatment in Bangladesh, India and Nepal

Drug	Dose	Schedule
Miltefosine*	Daily doses of 100 mg for patients weighing > 25 kg and 50 mg for those <25 kg.	Daily for 12 weeks
Amphotericin B	1 mg/kg IV infusion	60–80 doses over 4-5 months with (20 doses on followed by 20 days interval)

*As the safety of courses of Miltefosine longer than 4 weeks has not been evaluated, all patients should be closely monitored for any unexpected side effects. (All patients should be admitted for first 4-7days).

Disease Surveillance

Objectives
Kala-azar and PKDL surveillance

- The main objectives of surveillance in the elimination context are:
  - To monitor incidence trends over time and progress towards elimination
  - To determine the distribution and potential extension of KA
  - To identify and treat PKDL cases in order to decrease leishmaniasis reservoir
  - To detect outbreaks in order to respond in a timely manner
  - To evaluate elimination activities
  - To identify and prioritize at-risk population
  - To monitor vectors distribution and density in country

Case Definitions (Kala-azar)

PROBABLE VL (Kala-azar)	CONFIRMED VL (Kala-azar)
A person living in or having travelled to kala-azar endemic areas showing clinical signs and symptoms of kala-azar (mainly irregular fever lasting more than two weeks and splenomegaly and/or weight loss), after ruling out malaria in endemic areas.	**Laboratory confirmed** A probable VL case with laboratory confirmation either serologically (RDT, DAT, ELISA, IFAT) and /or parasitological (smear, culture) and/or positive by PCR or related techniques
	Clinically confirmed A probable VL case that has not been confirmed by any laboratory test (i.e. test not done or negative) but is assessed by a clinician to be a confirmed VL case based on clinical grounds.

Case Definitions- PKDL

PROBABLE PKDL	CONFIRMED PKDL
A patient living in or having travelled to visceral leishmaniasis (kala-azar) endemic areas presenting with a typically symmetrical multiple hypopigmented macules, papules, plaques, or nodules without loss of sensation.	A probable PKDL case with Leishmania infection confirmed parasitologically, by PCR or a slit-skin smear or biopsy.

PKDL can occur in patients with previous or concomitant kala-azar. In some cases, it occurs without the history of VL. Serological test such as rK39 rapid diagnostic test positivity acts as a strong evidence when other diseases re considered in the differential diagnosis, or if a history of VL is uncertain.

Characteristic of Leishmaniasis surveillance

- **Permanent**: even when elimination as a public health problem is reached, the surveillance efforts should be sustained in order to prevent an increase/ resurgence of leishmaniasis
- **Continuous**: surveillance should be sustained throughout the year. Particular efforts should be made during the peak season.
- **Exhaustive**: the KA surveillance should aim at capturing all KA cases in order to ensure the reliability of the figures used to calculate KA incidence at district level.

Case detection strategy

- Case detection- a **core function** of the surveillance system.
- Case detection can be
  - **Active-** when the program reaches out to the community to actively screen and find the cases or
  - **Passive-** when patients seek care at health facilities at their own initiative

Case detection strategy

Passive Case Detection

- Timely, regular and accurate reporting of the leishmaniasis cases who seek diagnosis and treatment from a health facility: It covers both endemic and non-endemic areas
- Includes all level of health institution from public sector, but an effort should be made to cover private medical services/NGOs.
- Does not require additional efforts and resources as it is currently part of the existing health system.

Case detection strategy

Active Case Detection

- Health staff reaches out to the community and systematically screens the population to find cases of leishmaniasis.
- A recent study conducted in Nepal, Bangladesh, and India suggests that the chance of case detection by active case detection (ACD) is significantly higher than PCD.
- Helps to reduce disease transmission by shortening the infectious period of cases and earlier diagnosis and treatment improves treatment outcomes.

Case detection strategy

Active Case Detection

- Currently four approaches of active case detection have been validated in KA and PKDL case detection

  - **Blanket approach**
  - **Camp approach**
  - **Index case based approach**
  - **Incentive based approach**

Case detection strategy

Active Case Detection- Blanket approach

- House to house visit by trained health workers for case detection
- Gold standard
- Due to high costs, recommended mainly during outbreaks
- Can be conducted in an integrated manner with other public health programs.

Case detection strategy

Active Case Detection- Camp approach

- Done by organizing health camps for screening of KA/PKDL cases by mobile teams of medical officers, nurses, lab technicians, health workers.
- The community is pre-informed about the visit of the team, its purpose; and the time/date/place of the activities.
- A sensitive tool for the detection of new kala-azar/PKDL cases
- Conducting the camp approach twice in a year is sufficient to capture a substantial number of new KA/PKDL cases in a given area.

Case detection strategy

Active Case Detection- **Index case based approach**

- Search of new KA/PKDL cases among the household members through house-to-house visits around the house (radius of 100 meters or 50 households) of a recently diagnosed (usually in the previous 6 months) KA case.
- The index approach is the **preferred method** for ACD for endemic and non-endemic areas and in those areas where households are scattered.

Case detection strategy

Active Case Detection- Incentive based approach

- Search for new KA/PKDL cases is done through FCHV who receive an incentive for each newly detected, if it is confirmed.
- Can be a useful method, particularly in low KA endemic areas or in combination with the above-mentioned methods.
- However, this method needs meticulous supervision and monitoring to prevent misuse of funds.

Data Collection and Reporting

- Kala-azar register
- EWARS (DHIS-2 based)
- HMIS

Treatment
Completion and Outcome

Treatment completion

- Assessment of whether the **full-course** of treatment has been received by the patient, as per the national protocol.
- Should be assessed and recorded at the **end of the treatment course** or at the time the patient stops the treatment.
- In case of single dose Liposomal amphotericin B regimen, initial assessment after treatment completion done at 15 days.

Treatment completion..

- **Treatment completed:** The patient has completed the full-course of the treatment as per the national protocol, and the clinician's prescription. Length of treatment depends on drug regimen.
- **Treatment stopped for medical reasons**: the treatment was stopped by decision of the clinician (e.g. patient suffering from side effects, treatment failure) or after the death.
- **Default**: 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.

Treatment Outcome

- Treatment outcomes for leishmaniasis patients have to be assessed twice
- **Initial assessment:** At the end of treatment, or 15 days after treatment starts for short- course regimen

and

- **Final assessment:** Six months after the last drug was taken

Treatment Outcome..

Initial assessment

- **Initial cure:** a full course of drugs has been completed AND the patient has clinically improved. Clinical criteria for initial cure defined as "no fever + regression of splenomegaly + 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 15 days 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.

Treatment Outcome..

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 KA symptoms with parasitological confirmation at any time point after initial cure.
- **Loss to follow-up:** patient does not present for assessment at six months.
- **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

Treatment Outcome..

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 SAE
- Death due to non-medical condition (accident)
- Death due to unknown cause

Criteria for cure

- The cure of kala-azar is confirmed by **absence of parasite from splenic and bone marrow smears.** Such provision is available in specialized institutions only.
- For program purpose a case completing treatment is considered clinically cured when there are no sign and symptoms of kala- azar.
- Complete clinical criteria of cure of kala-azar are as follows:
  - The full course of treatment has been taken.
  - Fever is absent.
  - Regression of spleen has occurred. Return of normal appetite is reported. Increase in body weight has been reported.
  - Improvement in anemia and a rise in hemoglobin have been demonstrated.

Thank you for your patience