MANUAL OF PROCEDURES FOR SURVEILLANCE AND CONTROL

Leishmaniases

IN THE AMERICAS

Pan American Health Organization

World Health Organization
REGIONAL OFFICE FOR THE Americas

Manual of procedures for leishmaniases surveillance and control in the Americas

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FOREWORD

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The P**an American Health Organization/World Health Organization (PAHO/WHO)** presents the **Manual of procedures for leishmaniases surveillance and control in the Americas**. This manual represents an instrument to support leishmaniases management and services in the countries of the Region.

This publication is the result of a joint effort by PAHO/WHO with experts on the topic and representatives from the Ministries of Health of endemic countries. It aims to expand knowledge regarding the disease and construct a working tool for use by health professionals that have to deal with the disease. It also aims to support National Leishmaniasis Control Programs and Surveillance Areas in their respective processes to structure health services and, optimize and guide actions to fight leishmaniases.

Finally, this publication calls attention to the need to incorporate available evidence and local knowledge into National Programs and surveillance services. The goal is to support disease surveillance and control in each country by considering peculiarities related to local species of parasites, vectors, and reservoirs and the epidemiological and clinical characteristics of the disease.

This Manual seeks to contribute to strengthening leishmaniases surveillance and control actions in the Region, a commitment assumed by Member States at the World Health Assembly and by the PAHO Directing Council through *Resolution 55.R9* in 2016.

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INTRODUCTION

INTRODUCTION

Leishmaniases— zoonotic and vector-borne diseases —are a public health problem in the Americas. Their complex biological cycle includes different species of parasites, as well as reservoirs and vectors, that cause a set of clinical syndromes in infected humans that can compromise the skin, mucous membranes, and viscera. In the specific case of the Americas, there is a large number of leishmaniases cases, and they are broadly distributed. Furthermore, the principal risk factors, which result from local social, economic, and environmental processes, greatly increase the number of population members at risk of infection.

Member countries of the World Health Assembly committed to strengthening leishmaniases surveillance and control actions through *Resolution WHA60.13* in 2007. In the Americas, this commitment was signed and strengthened by PAHO Member States through the adoption of *Resolutions CD49.R19* in 2009 and *CD55.R9* in 2016.

In 2010, the WHO worked with the Expert Committee on Leishmaniases to update the guidance for leishmaniases surveillance and control at the global level. This information was published in the WHO Technical Report Series (TRS) No 949: Control of the Leishmaniases.

Given the specific characteristics of the disease, regional and local evidence that shows different transmission scenarios and patterns, and major differences across countries in the organization of their health services, PAHO/WHO – through the Regional Leishmaniases Program – confirmed the need to establish criteria, standardize basic procedures, and provide specific definitions to strengthen the actions carried out to fight this disease in the Americas. These actions should include specific guidelines on how to carry out laboratory diagnostic techniques, treatment indications, surveillance and control of human cases of leishmaniases and vectors, and, when necessary, of reservoirs.

Furthermore, the surveillance and control actions presented here are proposed for the different epidemiological scenarios of cutaneous and visceral leishmaniasis and areas with and without transmission. The proposal considers the specific characteristics of the environment, the pattern of transmission, and human cases, vectors and reservoirs, to prioritize actions and optimize the resources invested in prevention and control and to adapt each intervention to the local epidemiological context.

LEISHMANIASES EPIDEMIOLOGY

1. LEISHMANIASES EPIDEMIOLOGY

1.1 Definition

In the Americas, leishmaniases are zoonotic diseases that cause a set of clinical syndromes in humans, which can compromise the skin, mucous membranes, and viscera. They are caused by different species of protozoans of the genus *Leishmania* and are transmitted to animals and humans through insects from the family Psychodidae.

1.2 Etiologic agent

The parasite is a protozoan belonging to the family Trypanosomatidae. The genus *Leishmania* includes about 22 species that are pathogenic to humans. These species are grouped into the subgenera *Leishmania* and *Viannia* (Figure 1). In the New World, 15 species of *Leishmania* have been identified. These species have different tropisms: visceral, cutaneous, and mucosal (Table 1). The parasite is digenetic, which means that it has two forms or states during its life cycle: the promastigote form (Figure 2), which measures between 20 and 30 µm, is extracellular and elongated, and has a flagellum that allows mobility in the intestine of the insect vectors; and the amastigote form (Figure 3), which measures between 2 and 5 µm, is rounded and intracellular, lacks flagellum, and multiplies in the cells of the phagocytic mononuclear system, mainly macrophages. Both forms of the parasite divide through binary fission and also have a single modified mitochondrion known as a kinetoplast.

In the Americas, the promastigote form is transmitted to susceptible mammals, including humans, through the bite of insect vectors of the gender *Lutzomyia*.

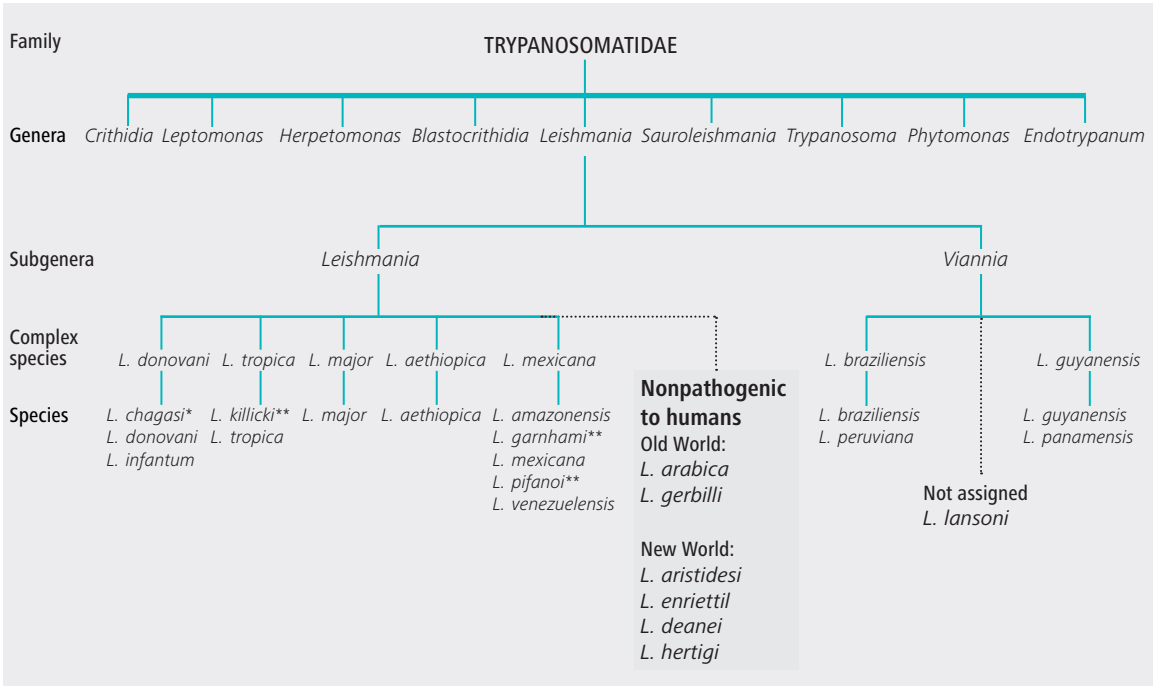

FIGURE 1 - Taxonomy of the genus *Leishmania.*

Source: WHO TRS 949, 2010 - (*) *L. chagasi* in the New World is the same species as *L. infantum* (**) Species status under discussion

TABLE 1 - Types of *Leishmania* identified in humans and tropisms in the Americas.

Subgenera	L. (Leishmania)	L. (Leishmania)	L. (Viannia)	L. (Viannia)
New World	*L. infantum**	*L. infantum* *L. mexicana* *L. pifanoi* ** *L. venezuelensis* *L. garnhami* ** *L. amazonensis*	*L. braziliensis* *L. guyanensis* *L. panamensis* *L. shawi* *L. naiffi* *L. lainsoni* *L. lindenbergi* *L. peruviana* *L. colombiensis* ***	*L. braziliensis* *L. panamensis* *L. guyanensis*
Tropism	**Visceral**	**Cutaneous**	**Cutaneous**	**Mucosal**

Source: Adapted from WHO TRS 949, 2010
(*) *L. infantum* is the same species as *L. chagasi* in the New World
(**) Species status under discussion
(***) Taxonomic position under discussion

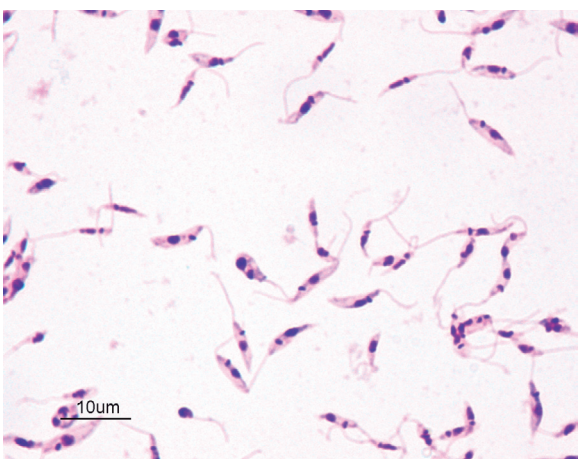

FIGURE 2 - *Leishmania* - Promastigote form.
Source: CLIOC - IOC - Fiocruz, Brazil.

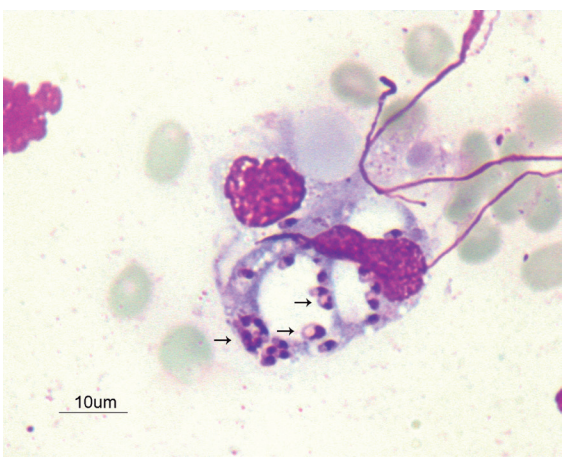

FIGURE 3 - *Leishmania* - Amastigote form.
Source: Laboratory of Leishmaniases Research, IOC, Fiocruz, Brazil.

1.3 Vector

Phlebotomines are small hematophagous diptera of the family Psychodidae. They are of great importance to public health due to their role as vectors of parasites of the gender *Leishmania* and of bacteria of the gender *Bartonella*. They are characterized by veined wings and the presence of dense hair on their wings and thorax. Members of the subfamily Phlebotominae predominate in tropical and subtropical regions. The group comprises 6 genera, but in the Americas, *Lutzomyia* is the most important genus (Figure 4).

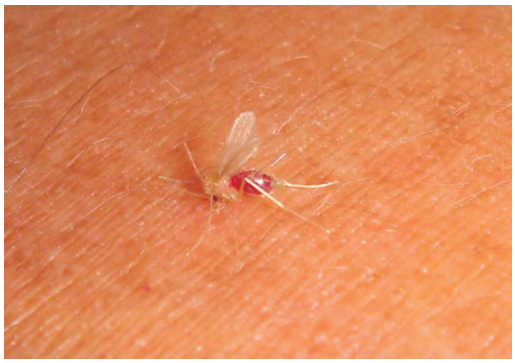

FIGURE 4 - *Lutzomyia* - engorged female (amplified photo)
Source: Vilela, M - IOC-Fiocruz, Brazil

The biology of each species of phlebotomines is unique and complex. The differences between species are remarkable, especially with regard to factors related to the period and local development of immature stages. Aspects related to reproduction, feeding, dispersion, and behavior, which directly influence leishmaniases epidemiology, should be studied by species since they can vary considerably.

Phlebotomines are insects with a complete metamorphosis. Hence, they traverse the stages of: egg, larva, pupa, and adult, each with varying duration depending on the species. Adults measure less than 5 mm long and have: protracted feet; wide, lanceolate wings, with no veins crossing beyond their base; and a protruding thorax. Their bodies are covered with long, fine hair, giving them a hirsute or furry appearance.

These insects are distributed across broad areas of the globe. While species distributed in tropical areas can achieve their complete life cycle throughout the year, species living in subtropical regions can only do so during warm months. These insects' habitats range from moist jungle to very arid regions. Their flight is short, silent and in small leaps.

The species of the genus *Lutzomyia* have mainly crepuscular, nocturnal activity, although they can also be active during daytime.

Phlebotomines are recognized by various common names in different regions (Table 2).

TABLE 2 - Common names of *Lutzomyia* in Latin America.

REGIONS	COMMON NAMES OF *LUTZOMYIA* IN THE AMERICAS
Central Americ	"aliblanco", "carachais", "chiclera", "chiroso", "chitras", "manta", "mosca" "palomilla", "papalotillas", "pringador", "toritos".
South America	"angoleta", "asa branca", "birigui", "blanca", "capotillo", "carachais", "chamapari", "chitra", "manta", "mosquito palha", "palomilla", "plumilla", "pringador", "quechicho", "roco roco", "tatuquira", "tarrayitas", "torito", "ya te vi".

1.4 Reservoirs

Reservoirs are vertebrate animals that maintain the parasite in nature, allowing vectors to get infected from them and enabling the transmission cycle to persist. There is usually a principal reservoir for every species of *Leishmania* in every given focus, but other mammals in the same zone can also become infected, thus becoming secondary or accidental hosts. Domestic and sylvatic mammals — marsupials, carnivores, rodents, edentates, and primates — infected by *Leishmania* may or may not show visible signs of infection. There are both domestic and wild reservoirs, but for some species of the parasite in the Old World, humans are the main reservoir. This is the case for visceral leishmaniasis — caused by *L. (L) donovani* — and cutaneous leishmaniasis — caused by *L. (L) tropica* — both from the Old World.

In the New World Americas, leishmaniases are mainly zoonoses. Identified reservoirs include marsupials (*Didelphis spp.*), sloths (*Choloepus spp. and Bradypus spp.*), the silky anteater (*Tamandua tetradactyla*), the crab-eating fox (*Cerdocyon thous*), and rodents (*Rattus spp., Proechimys spp., Nectomys spp., Oryzomys spp., etc.*). The most important domestic reservoir of *L. (L) infantum* is the dog (Figures 5 to 8).

The interaction between reservoirs and parasites is complex, multifactorial, circumstantial, and dynamic. Thus, it constitutes a biological unit that can vary with changes in the environment. Hence, only animals that guarantee both the circulation and maintenance of the different species of *Leishmania* in nature are considered reservoirs of *Leishmania spp*. The sole finding of an animal infected with *Leishmania* is not sufficient evidence to incriminate it as a reservoir.

FIGURE 5 - Opossum (*Didelphis albiventris)*, one of the species of the genus *Didelphis* frequently found infected with *Leishmania spp.*

Source: Ana Maria Jansen

FIGURE 6 - Caviomorph rodent (*Thrichomys pachyurus)*, species considered to be a potential reservoir of *Leishmania spp.*

Source: LABTRIP Collection

FIGURE 7 - Crab-eating or forest fox (cachorro do mato in Portuguese) (*Cerdocyon thous)*, wild canid species most frequently infected with *Leishmania infantum.*

Source: Fabiana Lopes Rocha

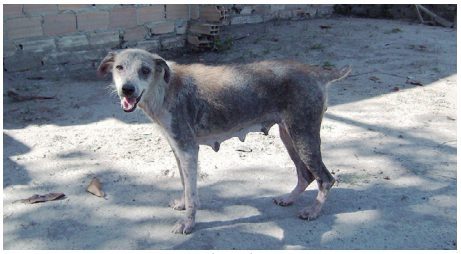

FIGURE 8 - Domestic dog (*Canis lupus familiaris*) with skin disorders suggestive of infection with *Leishmania infantum.*

Source: André Luiz Rodrigues Roque

1.5 Life cycle of *Leishmania* spp.

The image below portrays the life cycle of *Leishmania sp*. in the Americas.

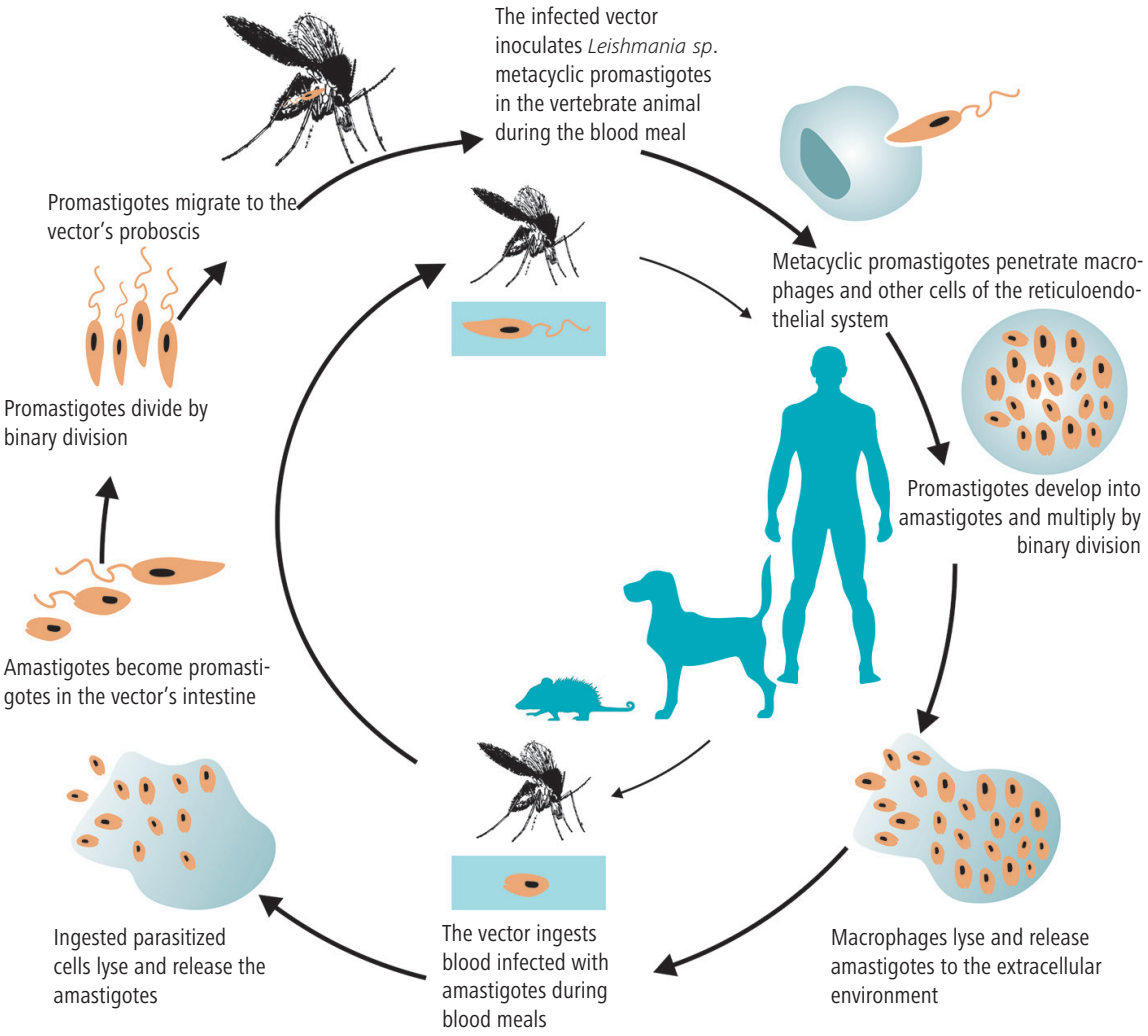

FIGURE 9 - Life cycle of *Leishmania sp*. in the Americas.

Source: Adapted from the Goiás Federal University.

1.6 Distribution of leishmaniases in the Americas

TABLE 3 - Distribution of *Leishmania* species, clinical forms, vectors, and confirmed or suspected reservoirs of disease transmission in countries in the Americas.

Country or territory	*Leishmania* spp.	Clinical form	Vector (confirmed or suspected)	Animal reservoir (confirmed or suspected)
Argentina	*L. guyanensis*	LC	Unknown	Unknown
	L. amazonensis	LC	Unknown	Unknown
	L. braziliensis	LC, LM	*Lu. whitmani* *Lu. neivai* *Lu. migonei*	Dog
	L. infantum	LV	*Lu. longipalpis*	Dog
Belize	*L. braziliensis*	LC	*Lu. ovallesi*	Unknown
	L. mexicana	LC	*Lu. olmeca olmeca*	*Heteromys* spp., *Nyctomys* spp., *Ototylomys* spp., *Sigmodon* spp., *Oryzomys* spp.
Bolivia	*L. braziliensis*	LC, LM	*Lu. nuneztovari anglesi* *Lu. carrerai carrerai* *Lu. llanosmartinsi* *Lu. shawi* *Lu. ayrozai* *Lu. yucumensis*	Unknown
	L. amazonensis	LC, LCD	*Lu. flaviscutellata*	*Oryzomys* spp.
	L. infantum	LV	*Lu. longipalpis*	Dog
	L. guyanensis	LC	*Lu. shawi*	*Choloepus* spp., *Didelphis* spp., *Tamandua* spp.
	L. lainsoni	LC	*Lu. nuneztovari anglesi*	*Agouti paca*
Brazil	*L. guyanensis*	LC	*Lu. umbratilis* *Lu. anduzei* *Lu. whitmani*	*Choloepus* spp. *Tamandua* spp. *Didelphis* spp., *Proechimys* spp. *Proechimys* spp.
	L. amazonensis	LC	*Lu. flaviscutellata* *Lu. longipalpis*	*Oryzomys* spp. *Wiedomys* spp.
	L. braziliensis	LC, LM	*Lu. whitmani* *Lu. intermedia* *Lu. wellcomei* *Lu. complexa* *Lu. neivai* *Lu. edwardsi* *Lu. migonei*	Dog, *Rattus rattus*, *Akodon arviculoides* *Bolomys* spp. *Nectomis* spp. *Thrichomys* spp.
	L. infantum	LV	*Lu. longipalpis* *Lu. cruzi* *Lu. almerio* *Lu. salesi*	Dog, *Lycalopex vetulus*, *Cedocyon thous* *Didelphis albiventris.*
	L. lainsoni	LC	*Lu. ubiquitalis*	*Agouti paca*
	L. shawi	LC	*Lu. whitmani*	*Cebus apella, Chiropotes satanus,* *Nasua nasua* *Bradypus tridactylus* *Choloepus didactylus*
	L. naiffi	LC	*Lu. squamiventris* *Lu. paraensis* *Lu. amazonensis* *Lu. ayrozai*	*Dasypus novemcinctus*
	L. lindenbergi	LC	Desconocido	Unknown
Colombia	*L. braziliensis*	LC, LM	*Lu. spinicrassa* *Lu. colombiana* *Lu. pia* *Lu. towsendi*	Dog, *Akodon* spp., *Micoureus demerarae, Melanomys caliginosus,* *Rattus rattus, Didelphis marsupialis.*
	L. panamensis	LC, LM	*Lu. trapidoi* *Lu. gomezi* *Lu. panamensis* *Lu. yuilli*	Dog *Choloepus hoffmanni* *Metachirus nudicaudatus,* *Didelphis marsupialis, Coendou* spp.

Country or territory	*Leishmania* spp.	Clinical form	Vector (confirmed or suspected)	Animal reservoir (confirmed or suspected)
Colombia (continuation)	*L. guyanensis*	LC, LM	*Lu. umbratilis* *Lu. longiflocosa*	Unknown
	L. colombiensis	LC	*Lu. hartmanni*	Unknown
	L. amazonensis	LC, LCD	*Lu. flaviscutellata*	Unknown
	L. mexicana	LC	*Lu. columbiana*	*Didelphis marsupialis*
	L. infantum	LV	*Lu. longipalpis* *Lu. evansi*	Dog *Didelphis marsupialis*
Costa Rica	*L. panamensis*	LC, LM	*Lu. ylephiletor* *Lu. trapidoi*	*Bradypus griseus, Choloepus hoffmanni, Heteromys desmarestianus*
	L. mexicana	LC, LM,	*Lu. olmeca olmeca,* *Lu. olmeca bicolor*	Unknown
	L. braziliensis	LCD	*Lu. youngi*	Unknown
	L. garnhami	LC, LM	*Lu. youngi*	Unknown
	L. infantum	LC LV	*Lu. longipalpis* *Lu. evansi*	Dog *Didelphis marsupialis*
Dominican Republic	*L. mexicana**	LCD	Unknown	Unknown
Ecuador	*L. braziliensis*	LC, LM	Unknown	Unknown
	L. panamensis	LC	*Lu. trapidoi* *Lu. hartmanni* *Lu. gomezi*	*Potus flavus, Tamandua tetradactyla, Sciurus vulgaris Choloepus didactylus*
	L. guyanensis	LC	Unknown	Unknown
	L. amazonensis	LC, LCD	*Lu. flaviscutellata*	*Sciurus* spp.
	L. mexicana	LC, LCD	*Lu. ayacuchensis*	Unknown
El Salvador	*L. infantum*	LV, LC	*Lu. longipalpis* *Lu. evansi**	*Dog*
United States of America	*L. mexicana*	LC, LCD	*Lu. anthophora* *Lu. diabolica*	*Neotoma* spp.
	L. infantum	Unknown	Unknown	Dog
French Guyana	*L. guyanensis*	LC	*Lu. umbratilis*	*Choleopus didactylus* *Proechimys* spp. *Didelphis marsupialis*
	L. braziliensis	LC, LM	*Lu. wellcomei* *Lu. intermedia*	Unknown
	L. amazonensis	LC	*Lu flaviscutellata*	*Proechimys* spp.
	L. naiffi	LC	Unknown	Unknown
	L. lainsoni	LC	Unknown	Unknown
Guatemala	*L. infantum*	LV	*Lu. longipalpis* *Lu. evansi***	Dog
	L. panamensis	LC. LM	*Lu. ylephiletor* *Lu. panamensis* *Lu. trapidoi*	Unknown
	L. braziliensis	LC, LM	*Lu. ovallesi* *Lu. panamensis* *Lu. ylephiletor*	*Rattus rattus*
	L. mexicana	LC, LCD	*Lu. olmeca olmeca*	Unknown
Guyana	*L. guyanensis*	LC	*Lu. umbratilis* *Lu. anduzei*	Unknown
Honduras	*L. infantum*	LV, LC	*Lu. longipalpis*	Dog
	L. panamensis	LC. LM	*Lu. ylephiletor* *Lu. panamensis* *Lu. trapidoi*	Unknown
	L. braziliensis	LC, LM	*Lu. ovallesi* *Lu. panamensis* *Lu. ylephiletor*	Unknown

Source: Adapted from WHO TRS 949, 2010. Legend: CL = cutaneous leishmaniasis; ML = mucosal leishmaniasis; DCL = diffuse cutaneous leishmaniasis; VL = visceral leishmaniasis

* Characterization performed at the Laboratory on Leishmaniasis Research "Laboratório de Pesquisa em Leishmaniose," IOC, FIOCRUZ, Brazil

** Identification and taxonomy performed by the Entomology Service of the Ministry of Health of Guatemala

Country or territory	*Leishmania* spp.	Clinical form	Vector (confirmed or suspected)	Animal reservoir (confirmed or suspected)
México	*L. braziliensis*	LC, LM	*Lu. ovallesi* *Lu. cruciata*	Unknown
	L. mexicana	LC, LM, LCD	*Lu. olmeca olmeca* *Lu. cruciata,* *Lu. shannoni*	*Heteromys* spp. *Nyctomys* spp. *Ototylomys* spp. *Sigmodon* spp. *Peromyscus* spp.
	L. infantum	LV	*Lu. longipalpis* *Lu. evansi*	Dog
Nicaragua	*L. infantum*	LV, LC	*Lu. longipalpis* *Lu. evansi*	Dog
	L. panamensis	LC	*Lu. trapidoi* *Lu. ylephiletor* *Lu. cruciata* *Lu. panamensis*	Unknown
	L. braziliensis	LC, LM	*Lu. panamensis*	Unknown
Panamá	*L. panamensis*	LC, LM	*Lu. trapidoi* *Lu. ylephiletor* *Lu. sanguinaria* *Lu. panamensis* *Lu. gomezi*	*Choloepus hoffmanni*
	L. braziliensis	LC	*Lu. panamensis*	Unknown
	L. colombiensis	LC	Unknown	*Choloepus hoffmanni*
Paraguay	*L. braziliensis*	LC, LM	*Lu. whitmani,* *Lu. migonei,* *Lu. intermedia*	Unknown
	L. infantum	LV	*Lu. longipalpis*	Dog
Perú	*L. peruviana*	LC, LM	*Lu. peruensis* *Lu. verrucarum* *Lu. ayacuchensis*	Dog *Didelphis albiventris* *Phyllotis andinum* *Akodon* spp.
	L. lainsoni	LC	*Lu. ubiquitalis*	Unknown
	L. amazonensis	LC	Unknown	Unknown
	L. guyanensis	LC, LM	Unknown	Unknown
	L. braziliensis	LC, LM, LCD	*Lu. tejadai* *Lu. pescei*	Unknown
Surinam	*L. guyanensis*	LC	*Lu. umbratilis* *Lu. anduzei*	Unknown
	L. amazonensis	LC	*Lu. flaviscutellata*	Unknown
	L. lainsoni	LC	Unknown	Unknown
Venezuela	*L. braziliensis*	LC, LM	*Lu. ovallesi* *Lu. trinidanensis* *Lu. spinicrassa* *Lu. panamensis*	Unknown
	L. colombiensis	LC	*Lu. panamensis* *Lu. gomezi*	Unknown
	L. venezuelensis	LC, LCD	*Lu. olmeca bicolor*	Unknown
	L. amazonensis	LC, LCD	*Lu. flaviscutellata* *Lu. reducta*	Unknown
	L. pifanoi	LCD	*Lu. flaviscutellata*	Unknown
	L. garnhami	LC	*Lu. youngi*	Unknown
	L. infantum	LV	*Lu. longipalpis* *Lu. evansi* *Lu. pseudolongipalpis*	Dog
	L. guyanensis	LC	Unknown	Unknown
Uruguay	*L. infantum*	LV	*Lu. longipalpis*	Dog

Source: Adapted from WHO TRS 949, 2010. Legend: CL = cutaneous leishmaniasis; ML = mucosal leishmaniasis; DCL = diffuse cutaneous leishmaniasis; VL = visceral leishmaniasis

* Characterization performed at the Laboratory on Leishmaniasis Research "Laboratório de Pesquisa em Leishmaniose," IOC, FIOCRUZ, Brazil

** Identification and taxonomy performed by the Entomology Service of the Ministry of Health of Guatemala

1.7 Epidemiological aspects

Leishmaniases are present on the five continents and are endemic in 102 countries or territories. It is estimated that nearly 350 million people live in regions where there is risk of acquiring the infection. 1.3 million new cases of leishmaniases and 20,000 to 30,000 deaths are registered every year. According to the overall analysis of the burden of infectious diseases, leishmaniases in their different clinical forms are responsible for 2.35 million disability-adjusted life years (DALY).

Nearly 90% of the burden of visceral leishmaniasis (VL) in the world is concentrated in Brazil, India, Sudan, South Sudan, Ethiopia and Kenya. 95% of cutaneous leishmaniasis (CL) cases occur in the Americas, the Mediterranean and Central Asia and the Middle East. Three quarters of the new cases of CL in the world occur in only five countries: Afghanistan, Brazil, Iran, Iraq, and Syria. Finally, mucosal leishmaniasis (ML) occurs mainly in the American region, with the highest numbers of this clinical form in Bolivia, Brazil, and Peru.

Leishmaniases cases have been recorded in the Americas from the southern United States to northern Argentina, with the exception of Chile, given that Uruguay reported its first human case of VL in December 2018. Between 2001 and 2017, 940,396 cases of CL and ML were recorded, with an annual average of 55,317 cases. In 2017, 49,959 cases of CL and ML were reported to the PAHO/WHO Leishmaniases Regional Information System (SisLeish). Of these, 41.3% were located in countries in the Andean Region (20,636/49,959), 35.9% in the Southern Cone (17,924/49,959), 20.8% in Central America (10,404/49,959), and the remaining cases in Mexico and the non-Latin Caribbean. The country that registered the highest number of cases is Brazil (17,526), followed by Colombia (7,764) and Peru (6,631). However, the disease is also endemic and of great epidemiological importance in Nicaragua (4,343), Venezuela (2,326), Bolivia (2,283), Costa Rica (2,224), Honduras (1,854), Panama, Ecuador, Mexico, Guatemala, Argentina, and Paraguay (Figure 10). Among the different clinical forms, the mucosal form can cause disfigurement and severe disability, meaning that it deserves special attention. Of all cases of CL and ML notified in 2017 in the region, 3.77% (1,882/49,959) were of the mucosal form, with very high proportions in Paraguay (67.4%), Bolivia (10.12%), and Peru (8.29%) and important rates in Brazil (4.7%) and Argentina (3.92%). These data, however, present cumulative prevalence. In other countries such as Ecuador (1.65%), Colombia (1.3%), Honduras (1.13%), Nicaragua (0.9%), Panama (0.69%), and Venezuela (0.56%), this clinical form is less frequent.

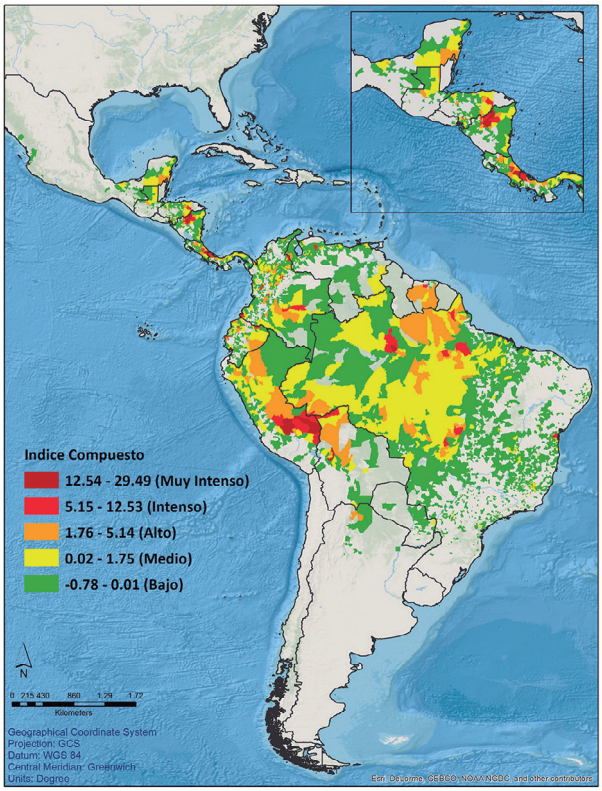

FIGURE 10 - Risk stratification of cutaneous and mucosal leishmaniasis by second subnational administrative level, Americas, 2017.

Source: SisLeish-PAHO/WHO. Data available from countries' leishmaniases programs, accessed on 01 December 2018.

In the same period, from 2001 to 2017, 59,769 cases of VL were recorded, with an annual average of 3,516 cases distributed across 12 countries. In 2017, 4,239 cases were reported to SisLeish, with Brazil (4,114), Venezuela (40), Paraguay (34), and

Colombia (29) contributing to 99.5% of the cases, although cases have also been reported in Argentina (9), Honduras (8), Guatemala (2), El Salvador (2), and Mexico (1) (Figure 11).

Three different transmission cycles have been characterized in the Americas: sylvatic, domestic-rural, and domestic-urban. In the sylvatic cycle, infection in humans occurs when man penetrates the forest or jungle and is bitten by the infected vectors there. In this case, humans are an accidental host that does not intervene in the transmission cycle and sylvatic animals are the reservoirs. In the domestic-rural and domestic-urban cycles, the vectors approach areas around the residence, enter the dwellings, and transmit the infection to household members, with greater incidence in children. Some evidence, still unconfirmed, suggests that humans, animals with synanthropic behavior and domestic animals could participate as reservoirs in the domestic-rural cycle. On the other hand, studies show that the dog is the main reservoir in the transmission of visceral leishmaniasis in urban environments (domestic-urban cycle).

Leishmaniases are limited to specific geographical areas, called disease natural foci, where essential elements for transmission are present, such as vectors, reservoirs and parasites. The presence of the latter in the environment is conditioned, in turn, by multiple factors such as weather, moisture, temperature, vegetation, vector presence and density, etc. Thus, it is important to study them through a broad assessment approach that includes the incidence of social and economic aspects, to achieve a better understanding that makes it possible to develop specific effective control measures. This task poses a great challenge in the Americas because different regional features constitute extensive challenges and difficulties for adequate control of the disease.

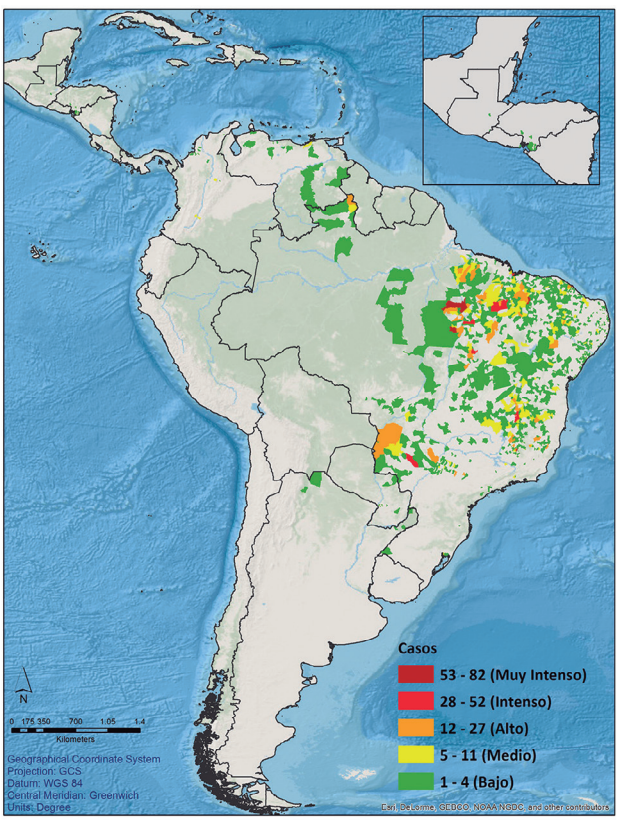

FIGURE 11 - Number of cases of visceral leishmaniasis by second subnational administrative level, Americas, 2017

Source: SisLeish-PAHO/WHO. Data available from countries' leishmaniases programs, accessed on 01 December 2018.

Although important studies in recent decades have allowed improved understanding of this parasitosis, there are still many gaps in the information regarding the elements involved in transmission, risk factors and, above all, the relationship between the parasite and the host. All of these factors determine the pertinent therapeutic response for each case. Additionally, all current efforts are directed to ensuring early diagnosis and adequate, timely treatment of the disease, to prevent deaths from VL, disfigurements or mutilations caused by ML, and high morbidity caused by CL. Furthermore, other potential interventions for the vectors and reservoirs should be evaluated and implemented, considering the specific epidemiological situation of each transmission area.

LEISHMANIASES
IMMUNOPATHOGENESIS

The promastigote form is found in the vector, which develops to a metacyclic promastigote in the anterior digestive tract and is transmitted to the vertebrate host through a bite. In the vertebrate host, the promastigote form is phagocytized by skin macrophages, within which, a parasitophore vacuole forms, which in turn fuses with lysosomes to generate what is known as a phagolysosome. Within the phagolysosome, promastigotes turn into amastigotes, which in turn multiply, are released and invade other macrophages that have been attracted to the infection site. When an insect vector bites a human or reservoir again, it ingests cells infected with amastigotes. In the vector's intestine, the cells are disintegrated and release the amastigotes, which rapidly transform into promastigotes once again (Figure 12).

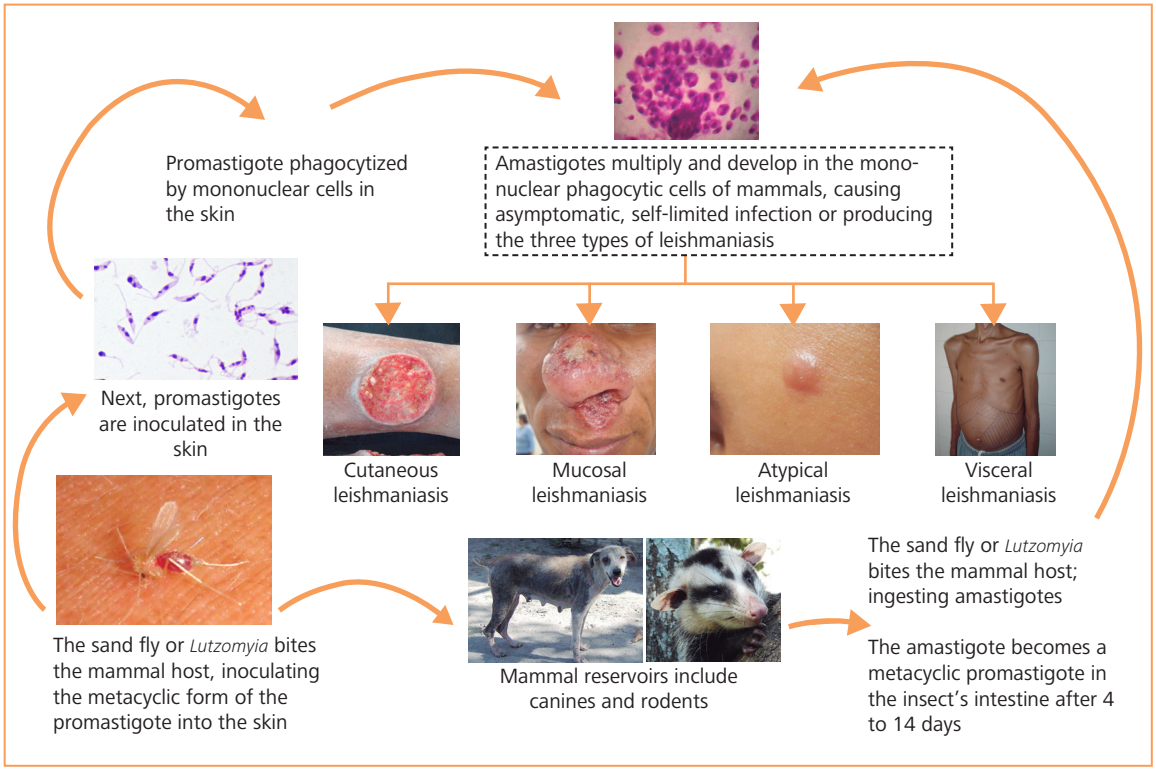

FIGURE 12 - Life cycle of the *Leishmania sp.*, with clinical manifestations in the Americas.

Establishment of the infection, development of evident clinical disease, and resolution of the infection will depend on the inoculum, the route of inoculation, and other factors inherent in the host and vector.

Infection starts when the insect bites a mammal host for a bloodmeal. When the vector is infected, it maintains a large quantity of promastigotes in the esophageal valve, making feeding difficult. Hence, to free the valve, it bites several times and in different areas of the skin. Each bite inoculates promastigotes, which is why several lesions can appear simultaneously in the same patient. With each bite, the insect regurgitates saliva, thus inoculating

10 to 200 promastigotes in the dermis. Immediately afterwards, the same promastigotes, attempting to escape lysis by the activated complement, interact with neutrophils and macrophages present in the dermis. Neutrophils phagocytize the promastigotes. Then, through a process known as receptor-mediated phagocytosis, the promastigotes actively penetrate the macrophage to form the phagosome that fuses with the lysosome and becomes the phagolysosome. Promastigotes turn into amastigotes in the phagolysosome, where they survive and multiply profusely, until they lyse the infected macrophage. Released amastigotes penetrate adjacent macrophages and also spread through lymphatic and blood vessels to infiltrate macrophages of distant sites such as lymph nodes, the liver, the spleen, and bone marrow. Depending on the type of *Leishmania* involved, infection can manifest as a skin, mucosal, mucocutaneous, or visceral lesion.

During the development of CL or ML, the presence of the parasite in tissue can cause a chronic granulomatous inflammatory reaction. This attracts a large number of specific and nonspecific cells, predominantly phagocytes (neutrophils and macrophages). Macrophage activation can promote the release of inflammatory factors like interferon gamma (IFN-ɣ).

The process described above favors the extravasation of cells and fluids from the blood vessels to the infection site, facilitating accumulation of the infection in the tissue and causing edema and local induration (Figure 13). During the development of the lesion, a macule initially appears at the bite location, which then evolves into a papule. The lesion continues to grow and develops into a nodule. The nodule is produced by the dermic mass that contains vacuolated macrophages with abundant *Leishmania* parasites and a lymphocytic infiltrate. Nodules grow in size and necrosis occurs at the center of the granulomatous reaction, induced by the immune response. This results in an ulcer. Initially, the ulcers observed are crusty and rounded, with raised and painless margins.

Finally, after the parasite is eliminated, either because of an effective immune response or due to specific treatment against *Leishmania*, healing of the lesion begins with the production of collagen and metalloproteases from the extracellular matrix of host cells. This allows tissue regeneration through the migration and proliferation of fibroblasts and keratinocytes toward the affected tissue. Fibroblasts turn into myofibroblasts that favor the contraction of wounds. Then, a process of massive angiogenesis to form new blood vessels and connective tissue, known as granulation tissue, takes place. This process concludes with the transition of the tissue to mature scars.

In VL, after the multiplication of the parasite in the skin, which may or may not cause a transient small lesion, the parasites and infected macrophages reach hematopoietic organs and tissues (liver, spleen and bone marrow). Parasites multiply there and infect local macrophages, thus altering the performance of these organs and tissues. This causes systemic injury and the growth of organs rich in cells of the mononuclear phagocytic system, characterized by hepato- and splenomegaly.

The spectrum of infection and disease observed in the natural foci of *Leishmania* transmission is very wide. Within the group of individuals infected by one of the different species of *Leishmania*, some do not develop clinical signs and remain asymptomatic (subclinical infection), while others develop the disease. For some people, the disease resolves easily and they present few clinical manifestations. Other people develop serious symptoms or skin and mucosal lesions.

It is important to point out that the species of infecting *Leishmania* and the immune response triggered by the host determine the body's different responses and the clinical manifestations. The latter range from benign, self-limited forms of CL to the most serious forms, such as ML, diffuse CL, and VL.

In the Americas, leishmaniases of the subgenera *Viannia (L. (V) braziliensis*, *L. (V) panamensis* and *L. (V) guyanensis)* have the capacity to invade naso-oropharyngeal mucosal membranes and produce symptoms of ML.

Other species, such as *L. (L) amazonensis*, *L. (V) braziliensis*, *L. (L) venezuelensis*, *L. (V) guyanensis*, *L. (L) mexicana* and *L. (L) pifanoi* can cause diffuse CL. In turn, *L. (V) panamensis* and *L. (V) braziliensis* can cause disseminated CL. Finally, *L. (L) infantum* can produce both atypical CL or VL.

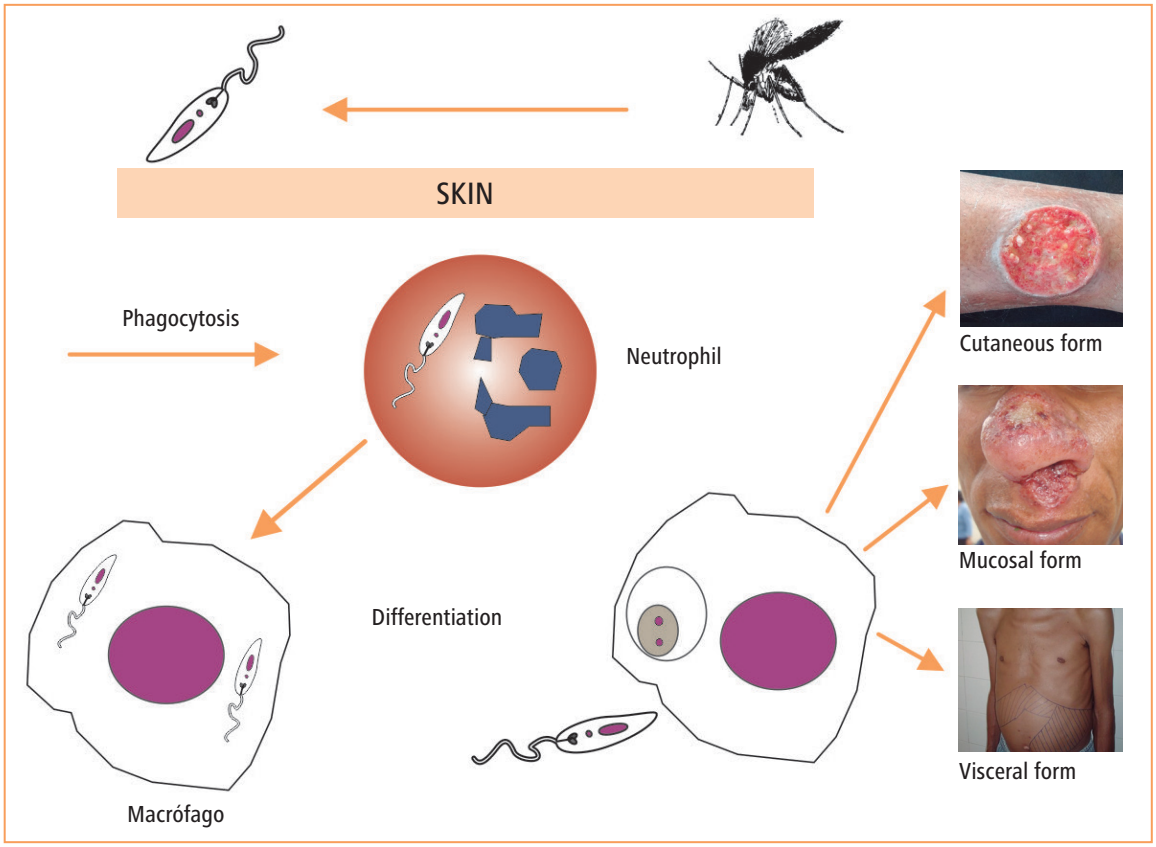

FIGURE 13 - Immune response to infection by *leishmania sp*.
Source: J. Lindoso, NMT-USP Laboratory

CLINICAL MANIFESTATIONS OF LEISHMANIASES

3. CLINICAL MANIFESTATIONS OF LEISHMANIASES

Leishmaniases is characterized by a great clinical polymorphism. It is for this reason that for many authors, leishmaniases does not represent a disease, but a group of diseases. Leishmaniases manifest in different clinical forms: cutaneous, mucosal/mucocutaneous, and visceral.

3.1 Clinical manifestations of leishmaniases and differential diagnosis

3.1.1 Cutaneous leishmaniasis (CL)

When the phlebotomine vector bites the person, it produces a macule of approximately one half of a centimeter in diameter, usually surrounded by a clearer halo, which lasts from 1 to 2 days. This macule is characteristic of the bite itself and does not mean that the insect is infected with the parasite. The incubation period ranges from 2 weeks to 2 months.

An increase in the size of the dermic granuloma is the first sign of cutaneous leishmaniasis (CL). This process consists of a papule evolving to a node that is round and painless, which progressively increases in size and ulcerates. Sometimes, a plaque with epidermal desquamation also forms. Initially, the ulcer is covered by a scab that adheres well to the skin but that bleeds easily when any attempt is made to remove it. When the scab is removed, the typical ulcer can be observed. The ulcer has a clean background, pink color and granulous tissue; is round; has regular, raised margins; is painless; and has an indurated base. At times, ulcers are infected secondarily with other microbial agents, which tends to produce local inflammation with pain and sometimes, purulent secretion. When the disease compromises the auricle, it can cause mutilations to the auricle. This type of injury, produced by *L. (L) mexicana*, was initially described as the "chiclero ulcer" and is very frequent in the Yucatán peninsula, Mexico. It appears that in this geographical region, the flight characteristics of the vector (*Lu. olmeca olmeca*) are such that the vector tends to bite people's ears.

When the first symptom of CL appears, the parasites have already invaded the cords and lymph nodes. Therefore, they may have caused troncular lymphangitis either on its own or accompanied by the appearance of nodes (nodular lymphangitis or sporothricoid syndrome) and regional adenopathies in their pathway. In some cases, regional adenopathies may become evident even before the appearance of the skin lesion. Some types can evolve torridly with central scarring and reactivation on the margins of the lesion. This type is known as leishmaniasis recidiva cutis. It generally presents as reactive to the Montenegro test (intradermal reaction) (Figures 14 to 20).

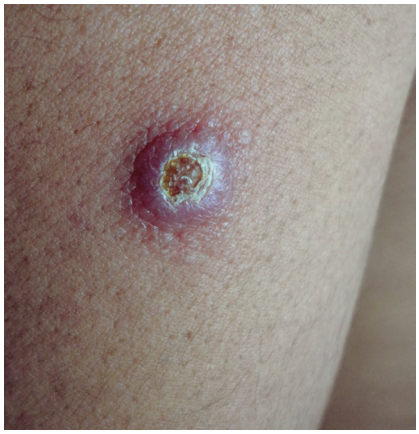

FIGURE 14 - Cutaneous leishmaniasis: sole lesion, ulcerated, small, with infiltrated raised margins.

Source: J. Pereira - Centro Dermatologico, Paraguay.

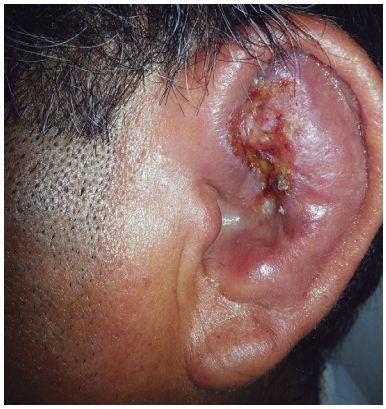

FIGURE 15 - Cutaneous leishmaniasis: ulcerated lesion in the internal auricle of the left ear, approx. 2 cm. in diameter.

Source: JRT Castro - México

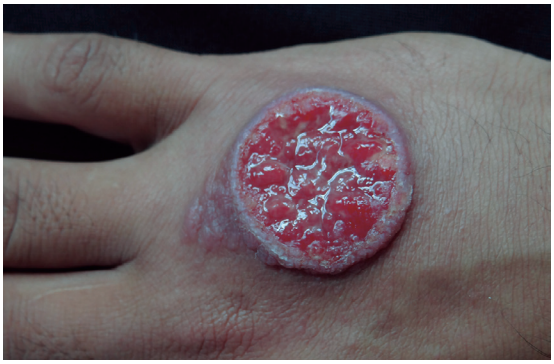

FIGURE 16 - Sole cutaneous leishmaniasis: round ulcer; raised, well-defined, infiltrated margins; and craterform center covered by granulation tissue. The base and area around the ulcer are infiltrated and erythematous.

Source: J. Soto, Jorochito Dermatological Hospital and FUDERMA, Bolivia.

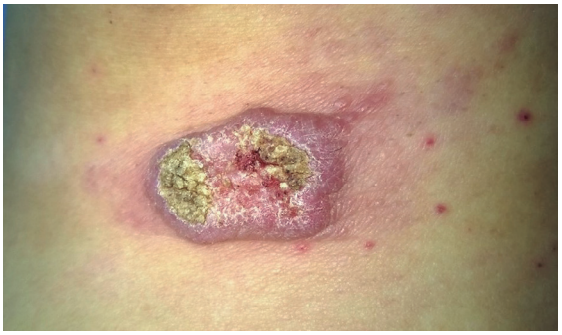

FIGURE 18 - Sole cutaneous leishmaniasis: erythematous violaceous plaque infiltrated with a hyperkeratotic, desquamative center on the posterior region of the thigh.

Source: J. Pereira, Dermatological Center, Paraguay.

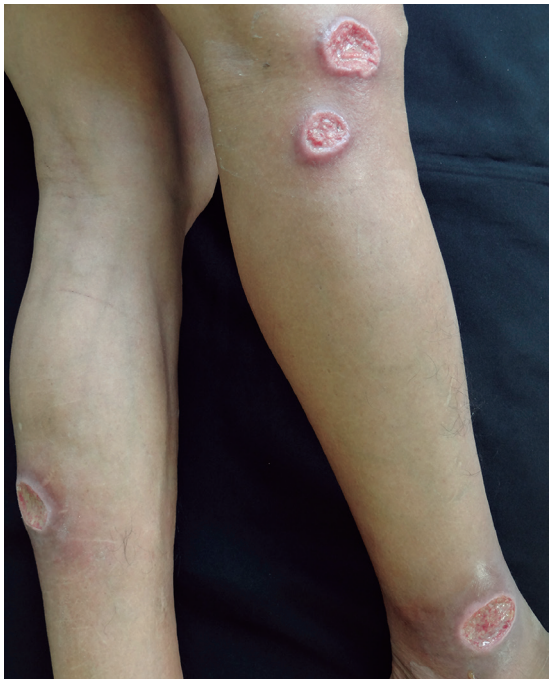

FIGURE 17 - Multiple cutaneous leishmaniases: ulcers of the same evolutionary state, each distant from the others. The most likely scenario is that there were multiple simultaneous bites by different vectors and that each bite had a sufficient parasite burden to cause all of the bites to develop lesions.

Source: J. Soto, Jorochito Dermatological Hospital and FUDERMA, Bolivia.

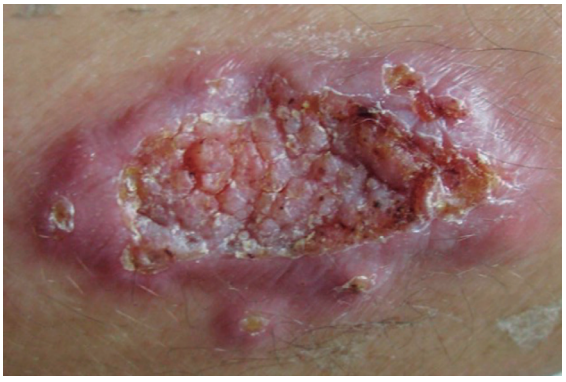

FIGURE 19 - Cutaneous leishmaniasis: ulcerated, not very round, infiltrated, and scabby lesion.

Source: O. Zerpa, Institute of Biomedicine, Central University, Venezuela.

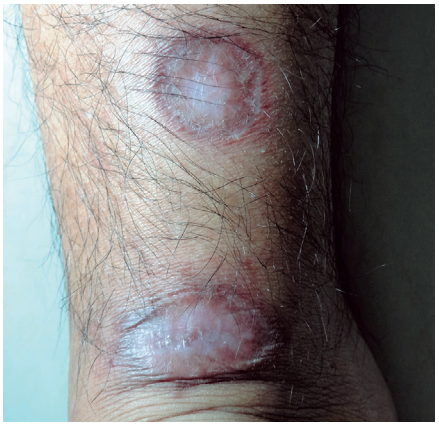

FIGURE 20 - Cutaneous leishmaniasis: scar—round, atrophic lesions; smooth, brilliant skin; without satellites; hypochromic in the center and hyperpigmented in its periphery.

Source: J. Soto, Jorochito Dermatological Hospital and FUDERMA, Bolivia.

In its natural evolution and depending on the etiologic agent, the ulcer can be spontaneously cured after weeks or months. Otherwise, it becomes chronic. When it is cured, the ulcer leaves a characteristic atrophic scar without satellites (Figure 20).

3.1.2 Disseminated cutaneous leishmaniasis

The disseminated form of CL is relatively infrequent. However, in some geographical areas, it can be of great clinical importance due to higher incidence. The species recognized as causes of this clinical type of the disease are *L. (V.) braziliensis*, *L. (L.) panamensis*, *L. (L.) amazonensis*, *L. (V) guyanensis*, and *L. (V) mexicana*.

This clinical presentation is characterized by the onset of multiple papular injuries with an acne-like appearance that affect different segments of the body. The number of lesions can reach several hundred. Disease in these patients starts with one or more lesions with the classic characteristics of granulomatous ulcers with raised margins.

After development of the primary lesions, a more or less acute phenomenon occurs, probably due to the dissemination of the parasite through the blood or lymphatic vessels (metastatic mechanism). This dissemination can be established in a few days, sometimes even in 24 hours, and cause lesions that are distant from the initial lesions (Figures 21 and 22).

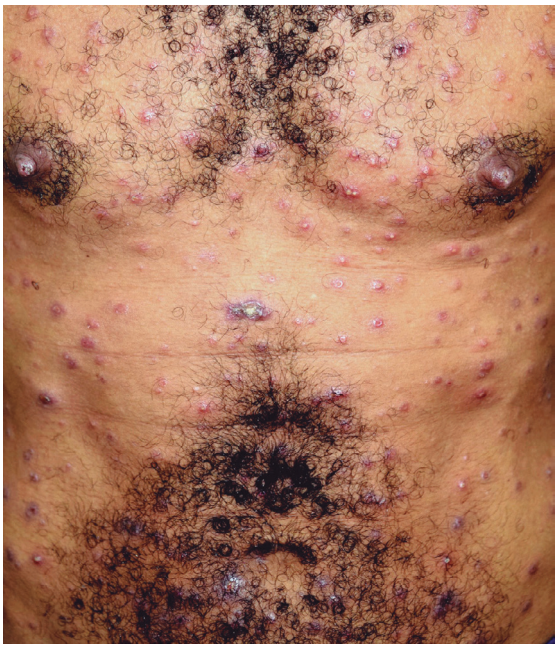

FIGURE 21 - Disseminated cutaneous leishmaniasis: inflammatory papules and acneiform lesions in great quantity, on the trunk.
Source: R. L. Machado, Federal University of Bahia (UFBA), Brazil.

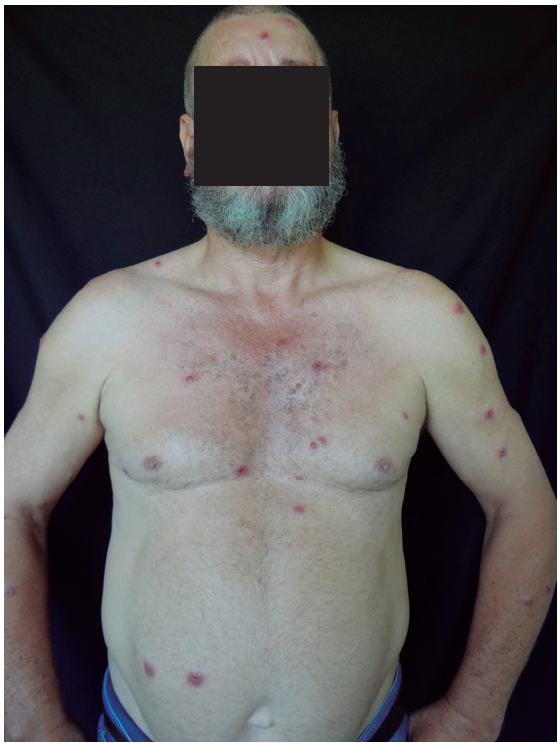

FIGURE 22 - Disseminated cutaneous leishmaniasis: numerous erythematous, edematous, exulcerated, pruriginous papules on the trunk. There are similar lesions in other anatomical regions.
Source: J. Soto, Jorochito Dermatological Hospital and FUDERMA, Bolivia.

3.1.3 Diffuse cutaneous leishmaniasis (DCL)

Diffuse cutaneous leishmaniasis (DCL) is a type of leishmaniasis that has been reported in several countries such as: Brazil, Venezuela, Mexico, the Dominican Republic, Peru and Colombia. It can be produced by *L. (L.) amazonensis*, *L. (L) mexicana*, *L. (L) venezuelensis*, *L. (L.) pifanoi*, and *L. (V.) braziliensis*.

DCL is a serious, anergic form of the disease that prevents the host from adequately responding to the infection due to the direct effect of the parasite or an underlying immunological condition. This is characterized by the presence of abundant parasite-rich lesions. At the beginning, DCL manifests through papules or plaques on one segment of the body's surface, but after a few months, it can extend to other parts of the tegument. The lesions are mainly nodular-type and plaques that resemble lepromatous leprosy (Figure 23). Response to treatment is transitory, with frequent relapses.

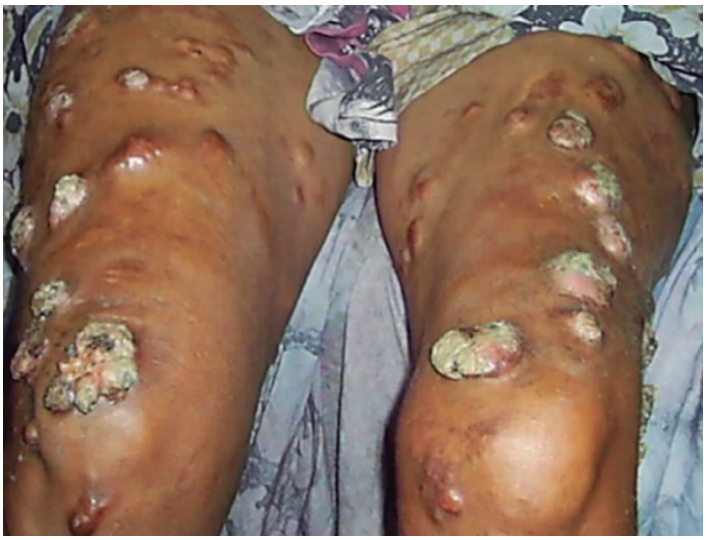

FIGURE 23 - Diffuse cutaneous leishmaniasis: nodular-tumorous lesions located on the thighs and legs.
Lesions with vegetative appearance, associated with exulcerations.
Source: J. M. L. Costa

3.1.4 Atypical cutaneous leishmaniasis (ACL)

In Central America and Venezuela, a form of CL known as atypical cutaneous leishmaniasis (ACL) has been described. ACL manifests with limited non-ulcerated, chronic lesions that are produced by *L. (L.) infantum*. (Figures 24 and 25). ACL has been reported in Nicaragua, Honduras, Costa Rica, El Salvador, and Venezuela.

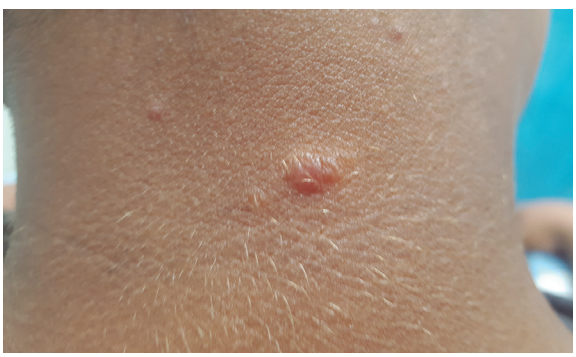

FIGURE 24 - Atypical cutaneous leishmaniasis: sole non-ulcerated lesion.
Source: Regional Program for Leishmaniases, PAHO/WHO/CDE/VT

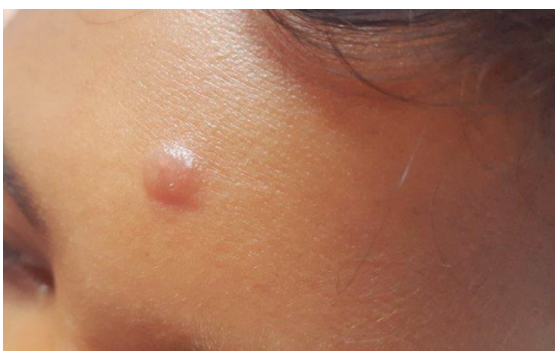

FIGURE 25 - Atypical cutaneous leishmaniasis: sole non-ulcerated lesion.
Source: Regional Program for Leishmaniases, PAHO/WHO/CDE/VT

3.1.5 Mucosal leishmaniasis (ML) / mucocutaneous leishmaniasis (MCL)

On average, mucosal leishmaniasis (ML) or mucocutaneous leishmaniasis (MCL) represents approximately 4% (range 0-16%) of the total leishmaniasis cases reported in the Americas. This varies by country and can reach much higher proportions, as in Paraguay. This clinical type appears to depend on the species involved, genetics, and the host's defense mechanisms. The parasite that is involved most frequently is *L. (V.) braziliensis*, but there are also cases produced by other species such as *L. (V.) panamensis* and *L. (V.) guyanensis*. This type of leishmaniasis represents a complication of a metastasis through a hematogenic or lymphatic route from a distant skin lesion, or more rarely, through the extension to CL mucous membranes in the face or the direct bite of the vector on the mucous membrane.

In general, ML or MCL presents several months or several years after a person heals from CL. Most mucosal injuries appear in the first 2 years after the skin lesion heals, which means that it is very important to look for characteristic CL scars in every patient with clinical suspicion of ML. In some patients, it can simultaneously present with skin lesions while others have no evidence of previous scars or disease history.

The initial, commonly affected site is the mucous membrane of the nasal septum. This causes a sensation of nasal obstruction, pruritus, or pain; sero-hematic scabs; muco-sanguinolent nasal discharge; or hemorrhage. Erythema, edema and infiltration produce increased volume at the tip of the nose and nasal ala and occasionally, extend beyond the nasogenian furrow and up to the cheeks. The lesion can progress until perforating the cartilaginous segment of the nasal septum and can even destroy all of the structures. Therefore, this causes both: severe deformity, making the tip of the nose drooped and thick; and hypertrophy, similar to the nose of the tapir, the denomination given to people with this type of deformities in the Region.

The process can extend to the palate, where it causes infiltrative, proliferative lesions, predominantly in the soft palate and pharynx. As the uvula is infiltrated, it hypertrophies and later, it is amputated (Figures 26 to 32). Between 5% and 15% of patients with ML present dysphonia, which is bitonal in the beginning and aphonic later on due to affectation of the larynx, which can compromise the patients' ability to communicate.

In serious cases, the patient's general state is altered and there is important weight loss. In fatal cases, emaciation, suffocation, or superinfection are observed. Frequently, the terminal phenomenon is pneumonia due to bronchoaspiration.

The mucosal or mucocutaneous forms do not evolve spontaneously toward cure. Instead, they can progress and cause serious destructions and mutilations that affect the patient's quality of life.

Cases that have evolved for several years, with extensive compromise of mucosa or that relapse following treatment, should be considered serious. Follow-up should extend for several years since patients can relapse again.

As post-treatment relapses are frequent, it is important to correctly identify the symptomatology associated with sequelae to avoid administering anti-Leishmania drugs unnecessarily.

The loss of the architecture and function of the nose causes the patient to be unable to humidify or heat the air, which leads to a permanent sensation of over-dryness, irritative cough, pruritus, or pain and scabs. The presence of bacterial superinfections of the paranasal sinuses is also frequent. Some patients present swallowing disorders as a consequence of facial sequelae such as amputation of the uvula or synechias in the soft palate and rhinopharynx.

ML and MCL can occur through different clinical manifestations and degrees of evolution:

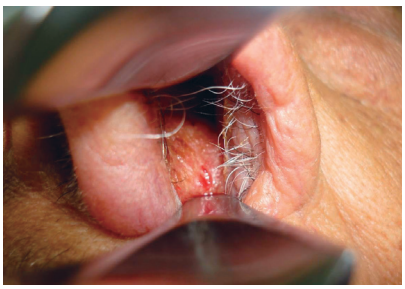

FIGURE 26 - Mucosal leishmaniasis: initial lesion with perforation of the nasal septum.
Source: A. Llanos Cuentas, Cayetano Heredia Peruvian University.

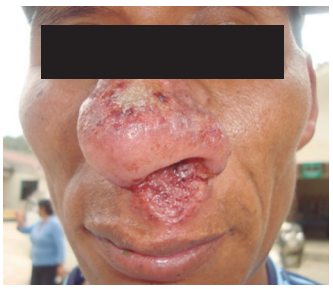

FIGURE 27 - Mucocutaneous leishmaniasis: the inflammatory process has extended through contiguity to the neighboring skin of the nasal ala, upper lip and cheek.
Source: J. Soto, FUNDERMA, Bolivia.

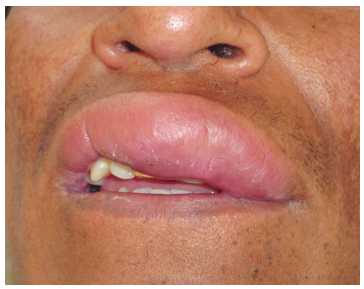

FIGURE 28 - Mucosal leishmaniasis: upper lip edema with small excoriations.
Source: A. N.S. Maia-Elkhoury, PAHO/WHO, Brazil; C.R. Soler, Emílio Ribas Institute, Brazil.

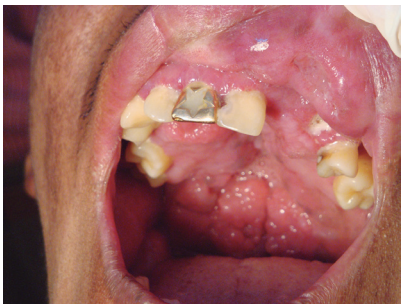

FIGURE 29 - Mucosal leishmaniasis: granulomatous lesion with edema and infiltration in the gingival region and hard palate.
Source: A. N.S. Maia-Elkhoury, PAHO/WHO, Brazil; C.R. Soler, Emílio Ribas Institute, Brazil.

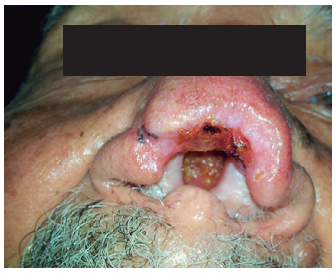

FIGURE 30 - Mucosal leishmaniasis: sequela, loss of architecture due to disappearance of the columella and part of the nasal septum, which causes severe deterioration in nasal function.
Source: J. Soto, FUNDERMA, Bolivia.

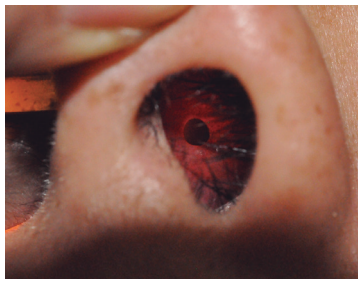

FIGURE 31 - Mucosal leishmaniasis: sequela - absence of columella and total perforation of the nasal septum.
Source: C.R. Soler, Emílio Ribas Institute, Brazil.

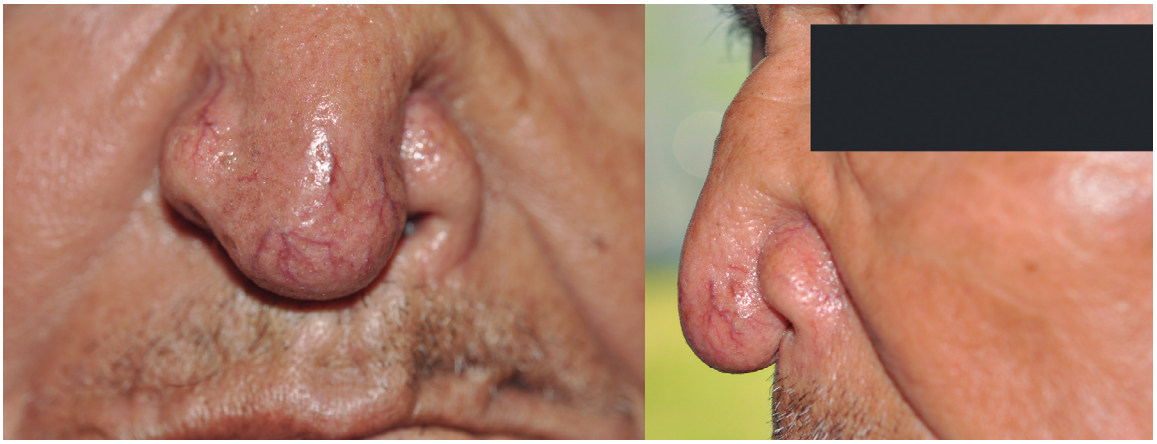

FIGURE 32 - Mucosal leishmaniasis: nasal involvement. Destruction of the nasal septum, frontal and lateral views.
Source: A. Llanos Cuentas, Cayetano Heredia Peruvian University.

3.2 Differential diagnosis of cutaneous, mucosal and mucocutaneous leishmaniasis

3.2.1 Cutaneous leishmaniasis

Pyodermitis, sporotrichosis, chromomycosis, basal cell carcinoma, spinocellular carcinoma, cutaneous tuberculosis, varicose ulcers, traumatic ulcers, psoriasis, cutaneous infections due to mycobacteria that are not pulmonary tuberculosis, cutaneous lymphomas, lobomycosis, foreign body granuloma, discoid lupus erythematous, keratoacanthoma, vasculitis.

3.2.2 Mucosal /mucocutaneous leishmaniasis

- Nasal area: lesions, bacterial infections, syphilis, cocaine use, chromium poisoning, medio-facial malignant granuloma, paracoccidiodomycosis, histoplasmosis, nasal polyps, rhinosporidiosis, leprosy, spinocellular and basal cell carcinoma.
- Palate and larynx area: carcinomas, paracoccidiodomycosis, histoplasmosis, tuberculosis.

3.2.3 Coinfection with cutaneous and mucosal leishmaniasis/HIV

CL and ML can progress differently if the person also has HIV infection, since the immunosuppression caused by HIV facilitates the progression of leishmaniasis. Although there is no exclusive clinical profile associated with coinfection, more serious or unusual clinical manifestations have been observed. As a result, in endemic areas, patients with unusual presentations should be evaluated to determine whether they have HIV infection.

Conditions that can suggest opportunistic patterns of CL and ML are:

- Appearance of any skin lesion in a patient without recent exposure (during the last year) to a geographic area with leishmaniasis transmission.
- Appearance of disseminated CL, with or without affectation of the concomitant mucosa.
- ML with compromise outside the nasal cavity.
- ML or CL with visceral involvement.
- Diffuse CL (DCL).
- Isolation in skin or mucosal material of viscerotropic species of *Leishmania*: *L. (L) infantum* or other species that have not been described as possible causes of skin and mucosal lesions.
- Late relapse (more than six months after the clinical cure).
- Onset of skin lesions after the diagnosis of active ML.
- Absence of clinical cure three months after finalizing adequate treatment.
- CL and ML lesions in patients with HIV coinfection.

3.3 Visceral leishmaniasis

3.3.1 Clinical manifestations

The most serious clinical form of leishmaniasis is the visceral one. Once the infected parasites and macrophages invade hematopoietic organs and tissues (liver, spleen, bone marrow, lymph nodes, etc.) and multiply there, they infect local macrophages and cause the symptoms and signs of VL. The incubation period for VL is usually between 2 weeks and 2 months. VL mainly affects children under 5 years old and can be associated with nutritional aspects and other immunosuppression conditions such as HIV/AIDS. If adequate, timely treatment is not provided, VL can cause the patient's death.

Infection by *L. (L.) infantum* can be asymptomatic since it does not present clinical signs and symptoms. Epidemiological studies show that the majority of infected individuals are asymptomatic; thus, they should not be reported to the surveillance system and they should not receive treatment. Furthermore, infected people can manifest as mild, moderate or serious clinical cases.

The initial period of the disease can easily be confused with different infectious processes. The most frequent signs and symptoms are: fever, which can be constant or irregular; discrete splenomegaly, which manifests in most patients; hepatomegaly, which may or may not be present; lymphadenopathies, which are frequently widespread with firm, movable nodes that do not hurt when palpated; mucocutaneous pallor caused by severe anemia; and finally, weight loss, which occurs slowly and progressively (Figures 33 and 34).

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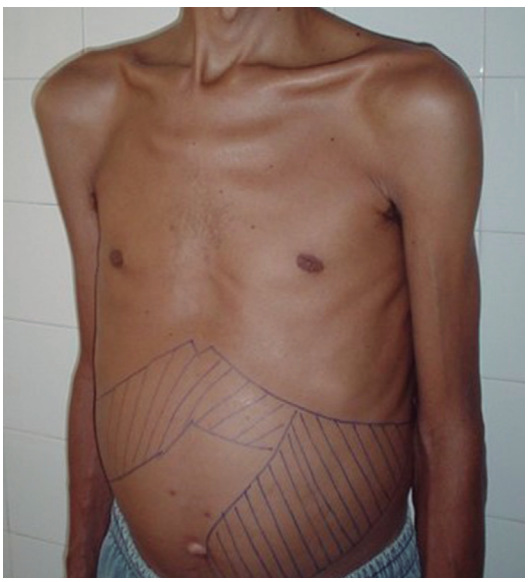

FIGURE 33 - Visceral leishmaniasis: patient with weight loss and presence of hepatosplenomegaly.

Source: D. L. Costa, Piauí Federal University, Brazil.

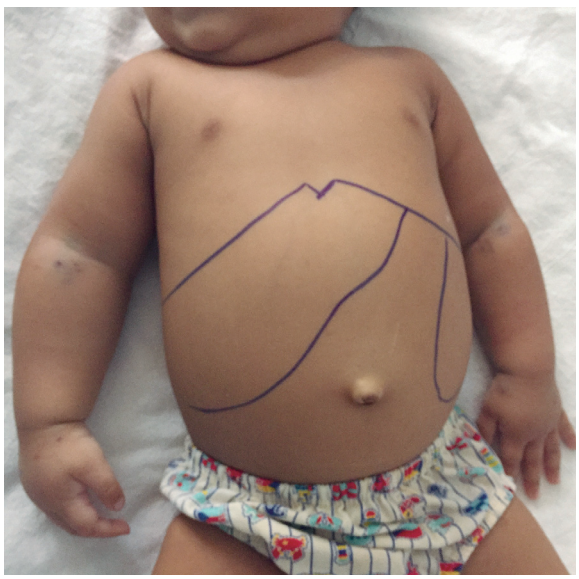

FIGURE 34 - Visceral leishmaniasis: presence of hepatomegaly and splenomegaly.

Source: D. L. Costa, Piauí Federal University, Brazil.

In the evolutionary period, the following is observed: persistent fever associated with progressive weight loss; decline in overall well-being; anorexia; more intense mucocutaneous pallor; increased abdominal volume; and increased hepatosplenomegaly. Other secondary signs and symptoms can progress quickly and include: respiratory disorders that present in patients due to their advanced immunosuppressive state, making them susceptible to intercurrent bacterial or viral infections; nonspecific gastrointestinal symptoms, such as diarrhea, which can manifest as dysenteric syndromes and can be associated with recurrent infections by amoebas such as *Shigella* or *Salmonella*.

Bleeding can become serious and jeopardize the patient's life. Pathogenesis is primarily due to: decreased platelets in the blood; infection of bone marrow by parasites; and the sequestration of blood platelets in the enlarged spleen. Therefore, anorexia is a frequent symptom of the disease.

The least frequent manifestations are jaundice, edema in advanced cases of the disease, and neurological alterations described as a burning sensation in the feet and cerebellar ataxia.

The presence of leukopenia, hypoalbuminemia, thrombocytopenia, and hypergammaglobulinemia makes patients more susceptible to bleeding and opportunistic infections, which can aggravate the disease and cause death.

In this phase, signs and symptoms of disease severity can evolve, elevating case-fatality. This is why clinical examination with great care is emphasized, primarily palpation of the liver and spleen since they are of utmost importance for diagnostic suspicion.

3.3.2 Differential diagnosis of visceral leishmaniasis

For differential diagnosis of VL, every prolonged febrile syndrome with hepatomegaly or splenomegaly should be considered. Phenomena to consider for differential diagnosis include: tropical splenomegaly syndrome (hyper-reactive malarious splenomegaly); tuberculosis with involvement of the spleen; visceral syphilis with hepatosplenomegaly; acute or reemerging American trypanosomiasis (Chagas disease); brucellosis; salmonellosis; septicemia; bacterial endocarditis; systemic histoplasmosis; typhoid fever; lymphomas; leukemias and other neoplasms; hemolytic anemias; hemoglobinopathies; sarcoidosis; and deposit diseases such as Gaucher's disease; among others.

3.3.3 Coinfection with visceral leishmaniasis/HIV

In people with HIV, infection with *L. (L) infantum* is more prevalent in male adults. It presents as an opportunistic disease given that, as CD4 lymphocytes decrease, the parasite spreads through the entire organism, facilitating the isolation of the blood, healthy skin, bronchial aspirate, etc.

Evaluation of the clinical manifestations of VL in this group of patients indicates that there is no clinical profile associated with coinfection. When the clinical triad is present, atypical manifestations can occur more frequently, such as pleural compromise, compromise of the gastrointestinal tract, and increased frequency of relapses. As a consequence, there is also greater risk of death. Early diagnosis is very important. Therefore, patients with VL should always be offered HIV testing. For patients with HIV/AIDS who have cytopenia and splenomegaly with or without fever, or hepatomegaly and cytopenia with or without fever, it is suggested that they be evaluated and investigated for the eventual presence of VL.

LABORATORY DIAGNOSIS OF LEISHMANIASES TECHNICAL PROCEDURES FOR SAMPLE COLLECTION

4. LABORATORY DIAGNOSIS OF LEISHMANIASES TECHNICAL PROCEDURES FOR SAMPLE COLLECTION

The clinical leishmaniases spectrum is very broad and can be confused with other diseases. Therefore, early diagnosis of leishmaniases is very important. Early diagnosis makes it possible to administer specific treatment in a timely manner, in turn controlling the natural history of the disease, relieving signs and symptoms, reducing mortality due to VL, and improving patients' quality of life. The latter is particularly true for patients with CL, ML or MCL, who experience more social stigma because of the physical and psychological sequelae of the disease.

Laboratory diagnosis is needed to confirm clinical suspicion and epidemiological findings of leishmaniases. A laboratory procedure is vital for confirming the case and safely formulating specific treatment, which will be addressed in the next chapter.

Diagnostic tools vary depending on the different clinical forms of the disease. Therefore, leishmaniases diagnosis should be made through visual detection of the parasite. However, it is not always possible to see or isolate the parasite, which means that the diagnosis should also be clinical, meaning complemented by specific immunological tests (indirect methods).

Currently, the principal tools available for leishmaniases diagnosis are based on the detection of amastigotes in samples from skin lesions, mucous membranes, tissues, or lymph nodes. In addition, for diagnosis of the mucosal and visceral forms, the detection of anti-*Leishmania* antibodies in serum or whole blood has been developed. The purpose of this chapter is to present sample collection techniques for leishmaniases diagnosis that are standardized in the Region.

Figure 35 presents a summary of the clinical manifestations of the disease and their respective indications for sample collection for laboratory diagnosis.

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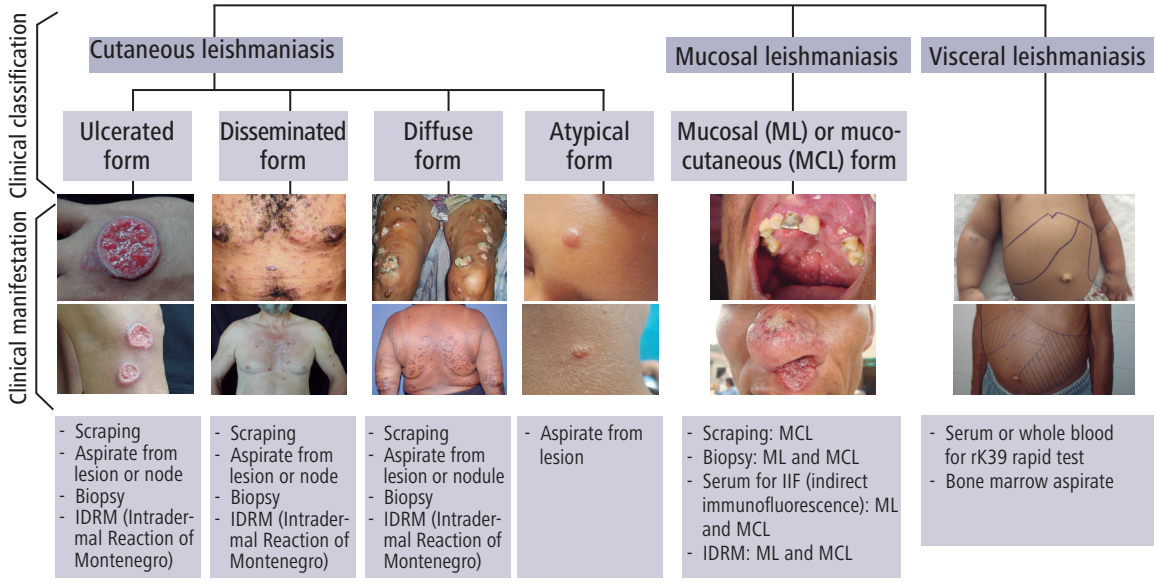


FIGURE 35 - Clinical classification and sample collection for leishmaniases diagnosis in the Americas, PAHO/WHO, 2018.

4.1 Laboratory diagnosis of cutaneous, mucosal, and mucocutaneous leishmaniasis

Direct methods are those that permit the visual detection of the parasite in the patient's sample. Samples can be collected using the following techniques: scraping, biopsy, and aspirates from lesions and lymph nodes (Annexes 1 to 3).

The diagnostic methods are: parasitological or direct examination, culture, histopathological analysis, and polymerase chain reaction (PCR) (Annex 4).

The sensitivity of parasitological diagnosis or direct examination varies based on the experience of the analyst, the techniques used to collect and process the sample, the location of the lesion, the species or strains available from the parasite, the lesion evolution time, and the use of previous treatments (empirical or conventional). For example, studies have shown the following sensitivity for direct examination of the sample material: 90.4% when obtained from the active margin of the lesion and 78.3% when obtained from the base of the ulcer.

Given that isolation, culture and molecular diagnosis of *Leishmania* require greater laboratory infrastructure and more experienced technical personnel, their use in routine health services is limited. Furthermore, it is important for these methods to be available for all cases through reference laboratories.

Indirect methods are based on the detection of anti-*Leishmania* antibodies, mainly IgG type, in the organism. This is done through serological tests or evaluation of the cellular response through a delayed hypersensitivity skin test that is better known as the Montenegro or Leishmanin test. However, this test is not commercially available in the Region (Annex 5).

Serological diagnosis is of limited use for CL, due to its low sensitivity and variable specificity. However, it can be very useful as diagnostic support for ML. The most widely used indirect methods in the public network are indirect immunofluorescence test (IIF) and the enzyme-linked immunosorbent assay (ELISA), whose specificity depends on the antigen used.

Antibody-based tests can be used as diagnostic support when there are clinical manifestations that are compatible with the definition of a suspected case of ML or MCL. However, treatment is not prescribed for patients who have a positive serological result, unless they also present clinical manifestations of the disease.

To strengthen the laboratory diagnosis of leishmaniasis, it is necessary for countries' Ministries of Health to establish and organize a laboratory network for leishmaniases diagnosis that considers epidemiological and clinical issues and the health services network.

It is suggested that countries define the laboratory diagnosis flow chart, according to the complexity of the levels of care.

4.1.1 Direct methods

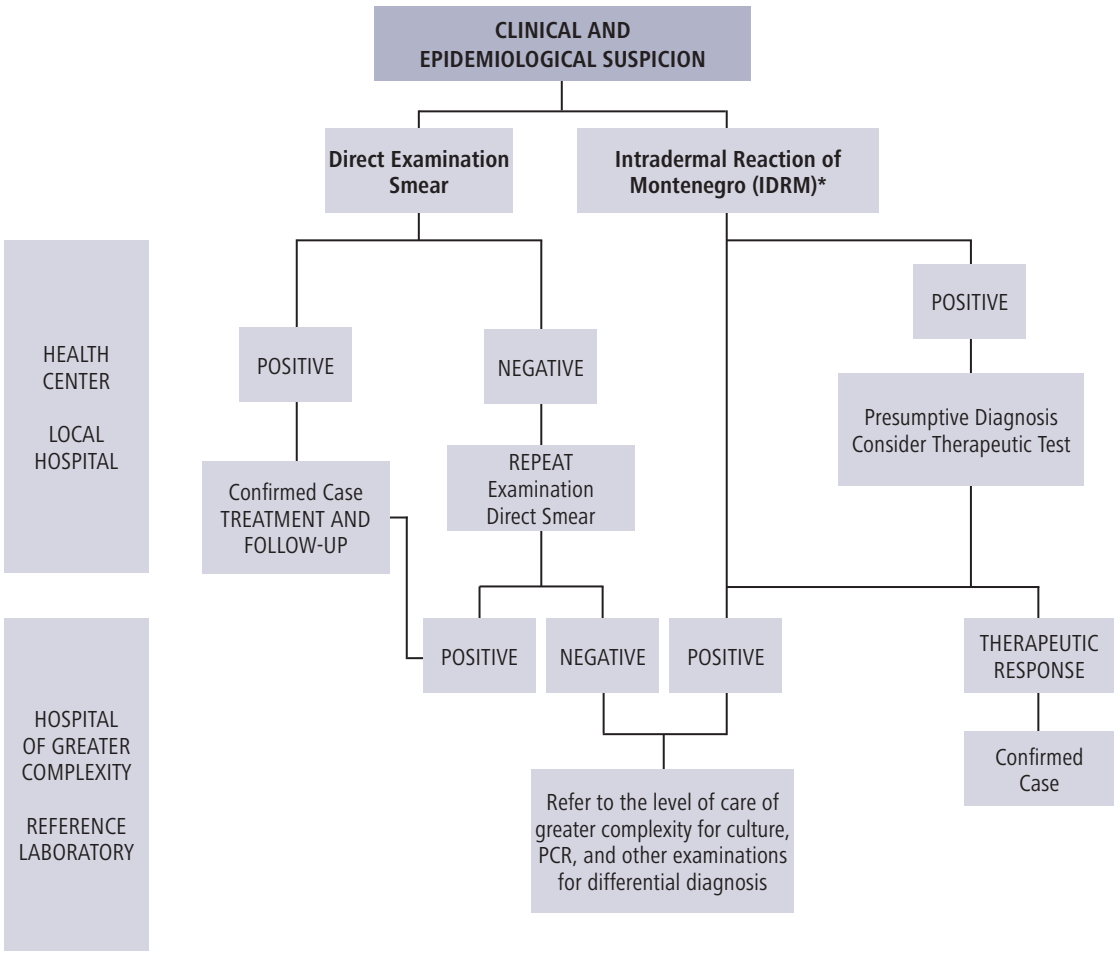
METHOD	DESCRIPTION
Direct parasitological	This refers to the detection of amastigotes in material obtained through scrapes, biopsies, or aspirates from lesions or lymph nodes. This is a very easy, low-cost and rapid procedure to implement. The details for the procedures to collect and process the sample and carry out the diagnosis are presented in Annexes 1, 2 and 3.
Culture	This refers to the visualization of promastigotes either through culture of the material obtained from aspirates from skin lesions or lymph nodes or from biopsies of skin lesions or mucous membranes. The details for the procedures to collect and process the sample and carry out the diagnosis are presented in Annexes 2 and 3.
Histopathological analysis	This refers to the direct observation of amastigotes in biopsy tissue. This is a method that is of great importance for the differential diagnosis of skin lesions that manifest with unusual presentations or that are caused by other etiologies. However, this is a test with low sensitivity. This is probably due to the distortion that parasites experience during the process of fixation and staining and the high level of difficulty in recognizing parasites in histopathological cuts. The details for the procedures to collect and process the sample and carry out the diagnosis are presented in Annex 3.
Polymerase chain reaction (PCR)	This refers to the amplification and detection of genetic material from the parasite (DNA or RNA). The material used for PCR can be a scraping, swab or aspirate from a skin lesion or lymph node or a small fragment of the biopsy. PCR consists of amplifying and detecting a specific region of the parasite's DNA or RNA. To achieve this, DNA/RNA should be extracted from the sample and added to a mixture that contains the essential reagents for amplification of the target sequence. Then, the product can be visualized: in an agarose gel, through hybridization with specific probes (conventional PCR); or in real-time, through fluorescence detection (qPCR). In the Region, PAHO/WHO and the Laboratory on Leishmaniases Research at the Oswaldo Cruz Institute (IOC) established a free reference service, where all countries can send samples for species identification and genetic sequencing of *Leishmania*, in accordance with the criteria and procedures established in Annex 4.

4.1.2 Indirect methods

METHOD	DESCRIPTION
Indirect immunofluorescence test (IIF)	This refers to the detection of specific antibodies for *Leishmania sp*. in the patient's serum using fluorescence. Since this method is not used for CL, it is not routinely available in health services. This method is indicated for ML or MCL.
Enzyme-linked immunosorbent assay (ELISA)	This refers to the screening test for specific antibodies against *Leishmania sp*. in the patient's serum or plasma, through the use of an enzyme-linked reaction. This method is not used frequently since it is not routinely available in public health services.
Montenegro or Leishmanin test	This refers to the delayed hypersensitivity test that evaluates the patient's exposure to *Leishmania*. It is usually applied in the patient's left forearm. It is mainly used as a support tool for the diagnosis of mucosal forms and in epidemiological studies to evaluate whether the person had previous contact with the parasite. Although it is a highly sensitive and specific test, it does not allow for differentiation between previous or current infection. In diffuse CL, the reaction is always negative. The details for the procedures to conserve the antigen and apply and read the test are presented in Annex 5. However, this test is not commercially available in the Region.

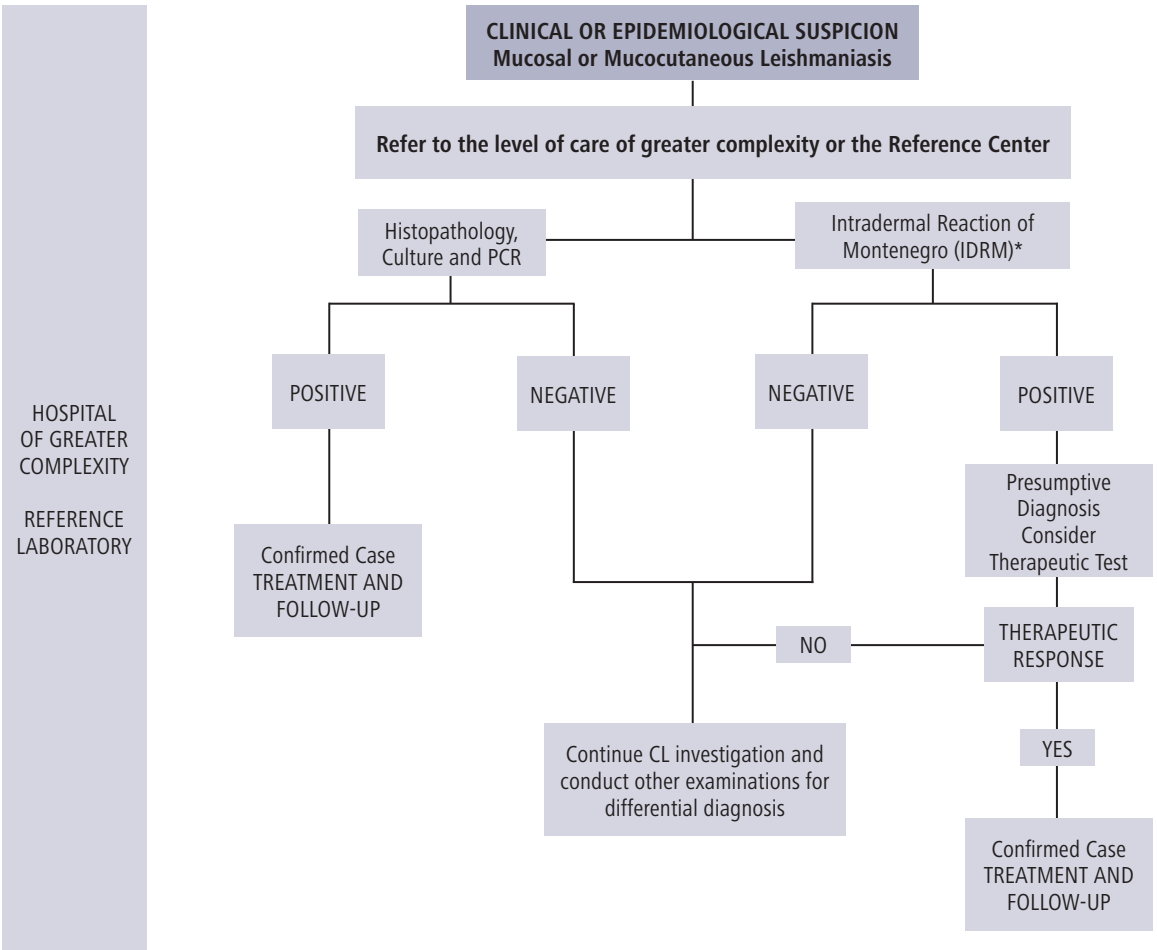
Flow charts 1 and 2 describe the diagnostic steps according to the complexity of the levels of care. The diagnostic methods used at the intermediate and high complexity levels will depend on their availability in each health service.

FLOW CHART 1 - Diagnosis of cutaneous leishmaniasis by level of care.



* The IDRM is not commercially available in the region.

FLOW CHART 2 - Diagnosis of mucosal leishmaniasis by level of care.



* The IDRM is not commercially available in the region.

Table 4 presents the summary for sample collection for the parasitological diagnosis of CL, ML and MCL in the first level of care.

TABLE 4 - Summary of sample collection for the parasitological diagnosis of leishmaniases according to the clinical form and level of care.

CLINICAL FORM	ORIENTATION
Cutaneous leishmaniasis (CL) with ulcerated lesions	Parasitological diagnosis of the skin lesion based on Flow chart 1.
Cutaneous leishmaniasis (CL) without ulcerated injuries	Evaluate the possibility of sample collection at the local level and if this is not possible, refer to second or third level of care. In areas where atypical cutaneous leishmaniasis caused by *Leishmania infantum* occur, the guidance described in the country's national guidelines should be followed.
Mucosal leishmaniasis (ML) without cutaneous compromise	Refer to second or third level of care.
Mucosal leishmaniasis (ML) with cutaneous compromise	Parasitological diagnosis of the skin lesion based on Flow chart 2. If it is not possible to do the examination and, depending on the need for evaluation of MCL, refer to second or third level of care.

4.2 Laboratory diagnosis of visceral leishmaniasis (VL)

The timely diagnosis and adequate treatment of VL are of vital importance since they reduce disease-related mortality and complications. The presumptive diagnosis of VL is based on the clinical and epidemiological aspects of the disease, starting with an adequate case history and a thorough physical examination. When there is a suspected case characterized by a prolonged febrile syndrome of longer than one week with or without hepatosplenomegaly, accompanied by pallor, examinations should be conducted to detect anti-*Leishmania* specific antibodies (rK39) or visualize the parasite in the patient's samples.

Factors to consider when selecting diagnostic methods are the invasiveness of the method, the medical expertise required to take and read the sample, and all of the aspects involved in assuring the quality of a good diagnosis. The diagnosis of VL in the first level of care is through serology using rapid immunochromatographic tests, such as those based on recombinant antigen rK39, that are validated in the region. However, at the primary level of care, it is not always possible to carry out the diagnosis of all cases. As a result, despite the fact that efforts to improve the performance of available tests are needed, it is also the physician's responsibility to provide suspected cases of VL that do not have a diagnosis with timely referrals to levels of care of greater complexity.

The frequency of reported VL-HIV coinfection in the Region of the Americas is growing and is currently at approximately 8% of all new cases of VL (SisLeish, PAHO/WHO, 2017). As a result, it is important to carry out diagnostic tests for HIV in confirmed VL cases.

It is important to consider that conventional histopathological and serological tests for VL and HIV are commonly analyzed in the second level of care, while immunohistochemical analyses, culture, PCR, and development/evaluation of innovative tests correspond to reference laboratories or research centers.

4.2.1 Direct methods

METHOD	DESCRIPTION
Parasitological diagnosis or direct examination	The parasitological confirmation of VL is based on the visualization of amastigotes through microscopic examination of tissue aspirates. Despite its high specificity, the sensitivity of the different methods is variable, with greater sensitivity for spleen aspirates (93-99%) than for bone marrow (53-86%) or lymph node (53-65%) aspirates. However, the spleen aspirate poses a high risk for the patient due to possible complications (e.g. splenic rupture and uncontrolled bleeding). Thus, this type of sample requires very strict precautions, such as operator technical expertise and know-how, adequate health service installations, and support for the realization of a blood transfusion and surgery in the event of an emergency. The details for the procedures to collect and process the sample and carry out the parasitological diagnosis of VL are presented in Annex 6.
Culture	The culture of organ aspirates also increases the sensitivity of the diagnosis. The definition and description of the marrow puncture method are presented in Annex 6.
Polymerase chain reaction (PCR)	The material used for PCR can be a bone marrow aspirate or peripheral blood. The detection of parasite using PCR in bone marrow aspirates or blood is more sensitive than microscopic examination. However, its use is currently limited to reference services and research centers. The description of the marrow puncture method and PCR are presented in Annexes 4 and 6.

4.2.2. Indirect methods

METHOD	DESCRIPTION
Serological diagnosis	Serological tests based on IIF and ELISA have demonstrated high diagnostic precision in most studies, but their use in the field is limited. In immunosuppressed patients, their results on these tests can be non-reactive. There are currently rapid serological tests, which were developed specifically for use in the field, that have demonstrated sensitivity and specificity in the majority of endemic areas. These tests, based on rK39, are easy to carry out, fast, and low-cost. As a result, their use for the timely diagnosis of VL is particularly relevant for the primary care level. The description of this test is available in Annex 7. The challenge of having access to rapid, simple, low-cost tests is documented in the WHO report "Visceral Leishmaniasis Rapid Diagnostic Test Performance" (WHO/TDR Diagnostics Evaluation series Nº 4, 2011, available at https://www.who.int/tdr/publications/documents/vl-rdt-evaluation.pdf).

Antibody-based tests should always be used when there are clinical manifestations compatible with the definition of a suspected case of VL. As a result, the application of serological diagnostic tests for cohabiting partners is not recommended without clinical evidence of VL.

The Montenegro reaction test is not used for the diagnosis of VL since it is not always reactive during the active phase of the disease. Generally, it is reactive after treatment is completed (after 3 to 6 months).

The details and procedures to collect, process, conserve, and transport samples for the parasitological diagnosis of VL are presented in Annex 6.

The details for serological tests based on rK39 are presented in Annex 7.

Table 5 presents the diagnosis of VL according to the three levels of care.

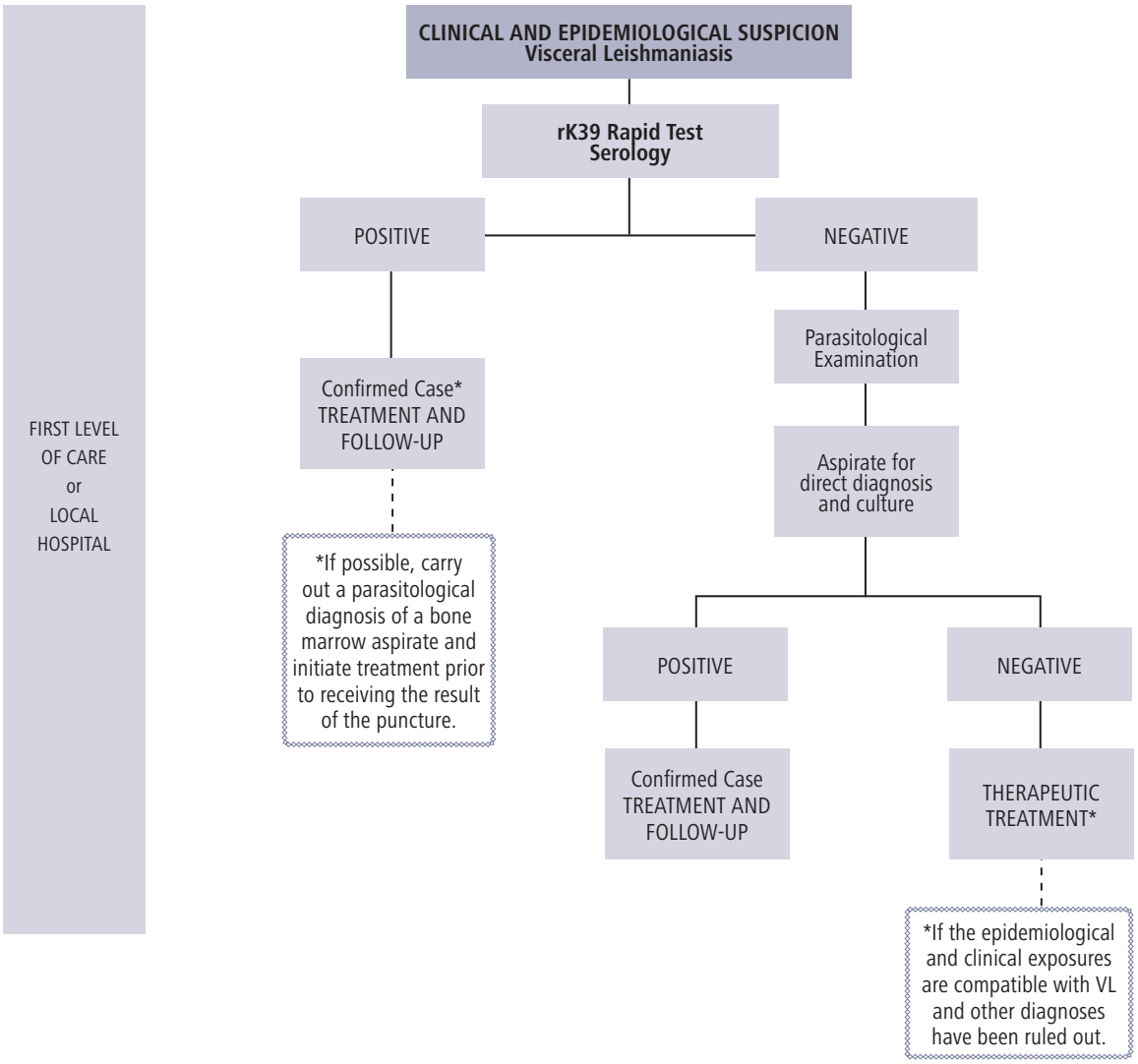
TABLE 5 - DIAGNOSIS OF VISCERAL LEISHMANIASIS IN THE THREE LEVELS OF CARE.

LEVEL OF CARE	DIAGNOSTIC TECHNIQUES
Level I	Serological: rK39 antigen, detected in immunochromatographic test.
Level II	Serological: rK39 antigen, detected in immunochromatographic test. Parasitological: Direct examination of bone marrow, lymph node or spleen* aspirate.
Level III	Serological: rK39 antigen detected in immunochromatographic or other serological test, when available (for example, IIF or ELISA). Parasitological: Direct examination of bone marrow, lymph node or spleen* aspirate, culture or PCR.

Source: WHO TRS 949, 2010; Maia et al, 2012.

(*) The spleen aspirate should be avoided since it can cause hemorrhagic complications. When this type of aspirate is carried out, conditions for treating complications should be available.

FLOW CHART 3 - Diagnosis of visceral leishmaniasis at the primary level.



4.2.3 Laboratory diagnosis when *Leishmania*/HIV coinfection is present

In individuals with *Leishmania*/HIV/AIDS coinfection, the parasite burden is greater. Hence, parasites can be found in unusual places, especially in patients that are critically immunosuppressed. As a result, the microscopic examination, culture result, blood PCR (whole blood or leukocyte layer), and evaluation of bone marrow aspirates are more sensitive than in immunocompetent patients. On some occasions, the parasite can be found in biopsies of the skin, gastrointestinal tract, or lungs.

The sensitivity of serological tests is lower in coinfected patients. In VLendemic areas, and also considering the epidemiological characteristics of HIV, HIV testing is recommended. Coinfected patients also have a greater risk of presenting serious atypical visceral manifestations of CL and ML (visceralization of dermotropic species).

TREATMENT

5. TREATMENT

In the Region of the Americas, CL tends to be more severe and have a long evolution period. Some patients infected by *L. amazonensis* and *L. mexicana* can develop the diffuse cutaneous form, which is difficult to cure with available treatments. Furthermore, *L. braziliensis*, *L. panamensis* and *L. guyanensis* species can evolve with compromise of mucous membranes due to metastasis, even in patients that have received or are undergoing systemic or local treatment. For local therapies in the Americas, there is still limited evidence to support their widespread use in public health. Despite this, the utilization of local therapies is recommended in certain special situations and when the attending health professional considers that the benefits outweigh the risks for the patient.

Treatment options for leishmaniasis in the Americas should be incorporated while considering certain aspects such as clinical manifestations, the number and location of the lesions, the species of *Leishmania*, the geographic location, the patient's general state, the availability of drugs, among others.

Control programs should incorporate new therapeutic regimens with broad use in public health only after considering 1) the quality of the tests, as determined in available local studies (evidence), 2) the balance of the benefits versus the risks and burdens, 3) costs and preferences, 4) use of resources, and 5) the organization of the services, with the capacity to provide follow-up for the detection of long-term complications.

Recommendations for the treatment of cutaneous, mucosal and visceral leishmaniasis in the Americas were prepared according to the GRADE methodology, based on the WHO Handbook for Guideline Development that was published in 2010 and updated in 2012.

Those recommendations, including the criteria for local treatment, the level of care, and a summary of the evidence, are available in detail in Spanish at: https://www.paho.org/hq/dmdocuments/2013/PAHO-Guia-Leishmaniasis-Americas-2013-Spa.pdf.

5.1 Recommendations for leishmaniasis treatment

The upcoming sections present a summary of the recommendations for the treatment of leishmaniasis in the Americas, according to the quality of the evidence and the strength of the recommendation. Furthermore, the patient's previous or current clinical condition should be considered when choosing the best therapeutic option.

5.1.1 Cutaneous leishmaniasis (CL)

- The use of pentavalent antimonials is recommended for the treatment of CL (high quality evidence and strong recommendation).
- For CL produced by *L. guyanensis* and *L. panamensis*, the use of miltefosine is recommended (high quality evidence and strong recommendation).
- For CL produced by *L. mexicana* and *L. panamensis*, the alternative use of ketoconazole is recommended (low quality evidence and weak recommendation).
- The use of pentamidine isethionate (low quality evidence), or ketoconazole (low quality evidence), or miltefosine (moderate quality evidence), or liposomal amphotericin B (very low quality evidence), or amphotericin B deoxycholate (very low quality evidence) is recommended in the case of treatment failure after other drug options have already been used or in special situations (weak recommendation).

- The use of thermotherapy (moderate quality evidence) or intralesional antimonials (very low-quality evidence) is recommended, when the application of systemic treatments is not indicated or local treatment of CL is needed, according to established criteria (weak recommendation).

Tables 6 to 8 present suggested treatment regimens and options.

TABLE 6 - Local treatments for cutaneous leishmaniasis, by quality of evidence and strength of recommendation.

Intervention (by quality of evidence)	Route of administration	Regimen	Quality of evidence	Strength of recommendation
Thermotherapy	Application of local heat with an electromagnetic device that generates high frequency waves	Following local anesthesia, an electrode at 50°C is applied for periods of 30 seconds, until covering the entire surface of the lesion, for 1 to 3 sessions, with an interval of 1 week between sessions.	**Moderate**	**Weak** Restricted for ongoing indications in the table of treatment options. It is necessary to conduct random trials in different areas and with different species, and to expand the number of applications and follow-up time when lesions are produced by *L. braziliensis*.
Intralesional antimonial	Intradermal injection	1 to 5 infiltrations of 1 to 5 ml per session (depending on the size of the lesion. The quantity used is the amount necessary to cover the lesion) every 3 to 7 days.	**Very Low**	**Weak** Use restricted to groups with contraindications to systemic treatments, as indicated in the table of treatment options. It is necessary to conduct random trials in different areas and with different species, and to expand the number of applications and follow-up time when lesions are produced by *L. braziliensis* (Blum, 2012).

Source: Leishmaniasis en las Américas: Recomendaciones para el tratamiento. https://www.paho.org/hq/dmdocuments/2013/PAHO-Guia-Leishmaniasis-Americas-2013-Spa.pdf

TABLE 7 - Systemic treatments for cutaneous leishmaniasis, by quality of evidence and strength of recommendation.

Intervention (by quality of evidence)	Route of administration	Regimen	Quality of evidence	Strength of recommendation
Pentavalent antimonials	Intravenous or intramuscular	10 to 20 mg Sb^{+5} / kg/day of pentavalent antimonials in a single daily dose for 20 days. The indication for the doses (10, 15 or 20 mg Sb^{+5}) should be in accordance with local evidence. Maximum dose of 3 ampoules/day to reduce adverse effects.	**High**	**Strong**
Miltefosine	Oral	1.5 to 2.5 mg/kg/ day, with maximum dose of 150 mg/day for 28 days. Dividing doses and taking them after meals are suggested to reduce adverse gastrointestinal effects.	**High** For localized skin lesions **Moderate** For localized skin lesions	**Strong** Indicated for *L. guyanensis* y *L. panamensis* **Weak** For all other species of *Leishmania*. Randomized trials with different species in different areas are recommended.
Pentamidine isethionate	Intramuscular	3 to 4 mg/kg/day in 3 to 4 doses on alternate days.	**Low**	**Weak** Better results with *L. guyanensis.* Randomized trials with different species in different areas are recommended..
Ketoconazol	Oral	600 mg/day for 28 days.	**Low**	**Weak** Indicated for *L. panamensis* y *L. mexicana*. Randomized trials with different species in different areas are recommended.
Anfotericina B liposomal	Intravenous	2 to 3 mg/kg/day up to 20 to 40 mg/kg of total dosage.	**Very Low**	**Weak** Alternative in cases with counterindications for the use of amphotericin B deoxycholate; treatment failure with other drug options; or special situations.
Anfotericina B desoxicolato	Intravenous	0.7 to 1 mg/kg/day up to 25 to 30 doses.	**Very Low**	**Weak** Alternative in cases of treatment failure or special situations. Its management requires care due to adverse effects.

Source: Leishmaniasis en las Américas: Recomendaciones para el tratamiento. https://www.paho.org/hq/dmdocuments/2013/PAHO-Guia-Leishmaniasis-Americas-2013-Spa.pdf

5.1.2 Mucosal or mucocutaneous leishmaniasis (ML or MCL)

The use of pentavalent antimonials is recommended for treating ML or MCL (low quality evidence and strong recommendation).

The use of the following is recommended: pentavalent antimonials + oral pentoxifylline (low quality evidence); or liposomal amphotericin B (very low quality evidence); or amphotericin B deoxycholate or pentamidine isethionate (very low quality evidence); or miltefosine (very low quality evidence) in the case of treatment failure after other drug options or special situations (weak recommendation).

Table 8 presents suggested treatment regimens and options.

TABLE 8 - Treatments for mucosal or mucocutaneous leishmaniasis, by quality of evidence and strength of recommendation.

Intervention (by quality of evidence)	Route of administration	Regimen	Quality of evidence	Strength of recommendation
Pentavalent antimonials	Intravenous or intramuscular	20 mg Sb^{+5}/kg/day of pentavalent antimonial in a single daily dose for 30 continuous days.	**Low** **Very Low**	**Strong**
Pentavalent antimonials + Oral pentoxifylline	Intramuscular Sb^{+5} or intravenous oral pentoxifylline	20 mg Sb^{+5}/kg/day for 30 days + 400 mg pentoxifylline every 8 hours for 30 days.	**Low**	**Weak** Evidence from a randomized trial with a limited number of participants. More studies are needed.
Anfotericina B liposomal	Intravenous	2 to 3 mg/kg/day up to a cumulative dose of 3.5g.	**Very Low**	**Weak** Alternative for cases of treatment failure or treatment for special cases.
Anfotericina B desoxicolato	Intravenous	0.7 to 1 mg/kg/day up to 25 to 45 doses.	**Very Low**	**Weak** Alternative for cases of treatment failure or treatment for special cases. Its management requires care due to adverse effects.
Pentamidine isethionate	Intramuscular	3 to 4 mg/kg/day in 7 to 10 doses on alternate days.	**Very Low**	**Weak**
Miltefosina	Oral	1.5 to 2.5 mg/kg/day for 28 days with a maximum dose of 150 mg/day.	**Very Low**	**Weak**

Source: Leishmaniasis en las Américas: Recomendaciones para el tratamiento. https://www.paho.org/hq/dmdocuments/2013/PAHO-Guia-Leishmaniasis-Americas-2013-Spa.pdf

Table 9 presents a summary of the treatment options for CL, ML or MCL.

TABLE 9 - Treatment options for cutaneous and mucosal leishmaniasis in the Americas, by clinical form, treatment indications, and level of care.

	Treatment	
Description	**Therapeutic indications**	**Level of care**
Localized cutaneous leishmaniasis • Sole lesion up to 900 mm^2 (diameter of 3 cm) in any location, except for the head and periarticular regions. Absence of immunosuppression and possibility of carrying out follow-up	**Local treatment (options by quality of evidence):** • Thermotherapy. See restrictions on use in the table with treatment regimens for CL. • Intralesional pentavalent antimonials.	Reference center
	Systemic treatment (options by quality of evidence): *First line* • Pentavalent antimonials • Miltefosine • Pentamidine isethionate (*L. guyanensis* and *L. panamensis*) • Ketoconazole (*L. mexicana* and *L. panamensis*) *Second line* • Anfotericina B	First or second level of care Second level of care or reference center
Localized cutaneous leishmaniasis • Sole lesion of greater than 900 mm^2 in any location • Sole lesion of any size on the head or periarticular regions • Multiple lesions • Sole lesions that were previously treated locally and did not respond or relapsed	**Systemic treatment (options by quality of evidence):** *First line* • Pentavalent antimonials • Miltefosine • Pentamidine isethionate (*L. guyanensis* and *L. panamensis*) • Ketoconazole (*L. mexicana* and *L. panamensis*) *Second line* • Pentamidine isethionate • Amphotericin B • Liposomal amphotericin B	First or second level of care Second level of care or reference center
Disseminated cutaneous leishmaniasis	**Systemic treatment (options by quality of evidence):** *First line* • Pentavalent antimonials *Second line* • Liposomal amphotericin B • PAmphotericin B	Second level of care or reference center
Diffuse cutaneous leishmaniasis	**TSystemic treatment (options by quality of evidence):** • Pentavalent antimonials • Liposomal amphotericin B • Pentamidine isethionate • Amphotericin B deoxycholate	Reference center
Mucosal leishmaniasis	**Systemic treatment (options by quality of evidence):** • Pentavalent antimonials + pentoxifylline • Pentavalent antimonials • Liposomal amphotericin B • Pentamidine isethionate • Amphotericin B deoxycholate	Reference center

Source: Leishmaniasis en las Américas: Recomendaciones para el tratamiento. https://www.paho.org/hq/dmdocuments/2013/PAHO-Guia-Leishmaniasis-Americas-2013-Spa.pdf

5.1.3 Treatment for special cases of cutaneous and mucosal leishmaniasis

For recommendations related to the treatment of special cases, given that there are no clinical trials or observational studies, experts considered existing clinical experience, case reports, and the risks/benefits of interventions for each of the following situations:

- Pregnant women: thermotherapy is recommended and cases that require systemic treatment should be referred to a reference center. The suggested prescribed drug is liposomal amphotericin B or amphotericin B (weak recommendation). The use of antimonial salts, miltefosine, and pentamidine is contraindicated.
- Lactation stage: the use of intralesional antimonials, or thermotherapy, or amphotericin B or miltefosine is recommended, while guaranteeing contraception (weak recommendation). The contraindication of systemic antimonials is relative.
- Patients with alterations on their electrocardiogram: local or systemic treatments with miltefosine are recommended (weak recommendation). The use of antimonial salts and pentamidine is contraindicated.
- Patients with nephropathies, hepatopathies, heart diseases: Local treatments are recommended for CL (weak recommendation). The use of liposomal amphotericin B is suggested (weak recommendation).
- Comorbidity with tuberculosis: Special care in monitoring adverse events is recommended due to drug interactions, mainly when two treatments are used concomitantly.
- Patients with HIV and other causes of immunosuppression: liposomal amphotericin B or amphotericin B deoxycholate is recommended (weak recommendation).
- Patients over 50 years of age: Carry out a careful clinical evaluation. Consideration of alternatives other than systemic antimonials is recommended given the risk of serious adverse effects.
- Patients with treatment failure: In the case of failure with local treatment, treatment should be repeated, or the patient should be switched to systemic treatment. In the case of failure with systemic treatment, after two treatment regimens, use of a different drug or regimen from the one used initially is recommended.

5.1.4 Visceral leishmaniasis (VL)

Ideally, VL treatment should cure the patient, reduce the risk of relapse, and reduce the possibility of drug-resistant parasite strains. To guarantee the full completion of treatment and detect eventual adverse effects, full supervision of treatment by the health team is recommended. Etiological treatment options for VL are described below, but it is important to ensure comprehensive treatment that includes adequate hydration and nutrition. If necessary, severe anemia should be corrected with blood transfusions and concomitant infections should be treated with the corresponding anti-infective agents, according to health professionals' criteria. Successful specific treatment improves the person's general condition, resolves fever, permits the involution of hepatosplenomegaly, and makes it possible for blood tests to return to normal levels.

An initial cure can be considered by the absence of fever and clinical improvement at the end of treatment. Complete regression of hepatomegaly or splenomegaly can take several months. A good indicator of definitive cure is the absence of clinical relapse at 6 months post-treatment.

The use of liposomal amphotericin B, pentavalent antimonials or amphotericin B deoxycholate is recommended for the treatment of VL (very low-quality evidence and strong recommendation).

The use of liposomal amphotericin B, or pentavalent antimonials or amphotericin B deoxycholate is recommended for the treatment of VL/HIV/AIDS coinfection (very low-quality evidence and strong recommendation).

The effectiveness of secondary prophylaxis after the first successfully treated episode of VL has not been established. A meta-analysis study (not carried out in Latin America) showed that secondary prophylaxis in patients with VL and HIV/AIDS significantly reduces VL relapse rates.

To date, there are no controlled clinical trials that demonstrate the superiority of any of the treatment schemes. Thus, the selection of the scheme should consider the toxicity profile and the interactions with other drugs used by the patient. Secondary prophylaxis is recommended for all patients with a CD4T-lymphocyte count lower than 350 per mm^3.

The use of liposomal amphotericin B, pentavalent antimonials or amphotericin B deoxycholate is recommended as secondary prophylaxis after the first episode of VL (very low-quality evidence and strong recommendation).

The clinical course of VL is complex and requires care and follow-up during treatment. Thus, for special cases, the use of liposomal amphotericin B is recommended (very low-quality evidence and strong recommendation).

The suggested drug regimens are presented in Tables 10, 11 and 12.

TABLE 10 - Treatment for visceral leishmaniasis, by quality of evidence and strength of recommendation.

Intervention	Route of administration	Regimen	Quality of evidence	Strength of re-commendation	Level of care
Liposomal amphotericin B	Intravenous	3-5 mg/kg/day for 3 to 6 days up to total dosage of 20 mg/kg.	**Very Low**	**Strong**	Second level of care or reference center
Pentavalent antimonials	Intravenous or intramuscular	20 mg/Sb^{+5}/kg/day for 28 days.	**Very Low**	**Strong**	First and second level of care and reference center
Amphotericin B deoxycholate	Intravenous	1 mg/kg/day for 14 days up to total dosage of 800 mg.	**Very Low**	**Strong**	Second level of care or reference center

Source: Leishmaniasis en las Américas: Recomendaciones para el tratamiento. https://www.paho.org/hq/dmdocuments/2013/PAHO-Guia-Leishmaniasis-Americas-2013-Spa.pdf

TABLE 11 - Treatment for visceral leishmaniasis and HIV/AIDS coinfection.

Intervention (by order of priority considering drug availability in each country)	Route of administration	Regimen	Level of care
Liposomal amphotericin B	Intravenous	3-5 mg/kg/dose up to total dosage of 20-40 mg/kg	Reference center
Pentavalent antimonials	Intravenous or intramuscular	20 mg/Sb^{+5}/kg/day for 28 days	
Amphotericin B deoxycholate	Intravenous	1 mg/kg/day for 14 days up to total dosage of 800 mg	

Source: Leishmaniasis en las Américas: Recomendaciones para el tratamiento. https://www.paho.org/hq/dmdocuments/2013/PAHO-Guia-Leishmaniasis-Americas-2013-Spa.pdf

TABLE 12 - Recommended regimens for secondary prophylaxis in patients with visceral leishmaniasis and HIV/AIDS coinfection.

Intervention (by order of priority considering drug availability in each country)	Route of administration	Regimen	Quality of evidence	Strength of recommendation	Level of care
Liposomal amphotericin B	Intravenous	3-5 mg/kg/dose every 3 weeks	**Very Low**	**Strong**	Reference center
Pentavalent antimonials	Intravenous or intramuscular	20 mg/Sb^{+5} c/ 2 semanas	**Very Low**	**Strong**	
Anfotericina B desoxicolato	Intravenous	1mg/kg/dose every2 weeks	**Very Low**	**Strong**	

Source: Leishmaniasis en las Américas: Recomendaciones para el tratamiento. https://www.paho.org/hq/dmdocuments/2013/PAHO-Guia-Leishmaniasis-Americas-2013-Spa.pdf

5.1.5 Management of special cases of visceral leishmaniasis (VL)

For management of special cases of VL, drug selection should consider the toxicity profile of the drugs and the risk of death associated with the disease. Liposomal amphotericin B is prescribed in patients who meet at least one of the following criteria:

- Age older than 50 years;
- Age under one;
- Kidney failure;
- Liver insufficiency;
- Cardiac insufficiency;
- Corrected QT interval longer than 450 milliseconds (ms);
- Concomitant use of drugs that alter the QT interval;
- Hypersensitivity to pentavalent antimonials or other drugs used to treat VL;
- HIV infection;
- Comorbidities that compromise immunity;
- Use of immunosuppressive medication;
- Treatment failure with pentavalent antimonials or other drugs used to treat VL;
- Pregnant women.

In light of the impossibility of using liposomal amphotericin B for the situations described above, the therapeutic alternative is amphotericin B deoxycholate.

5.2 Criteria for therapeutic responses and treatment follow-up

After treatment is finalized, patients should be followed to evaluate the therapeutic response and detect an eventual relapse or mucosal compromise in cases of CL. The following orientations are provided to this end:

5.2.1 Cutaneous leishmaniasis (CL)

Patients should receive a clinical evaluation, at a minimum, on completing treatment and 45 and 90 days after completion, until at least 6 months post-treatment. Given that they are visible, accessible, and usually well-delimited, skin lesions are easy to compare. Therefore, on initiating treatment, lesions should be duly measured and registered in each patient's medical record (Annex 8). When this happens, the health professional providing the next check-up will have quantifiable evidence to determine whether there is a response to therapy. During the first half of treatment, it is frequent for lesions to not decrease in size or to even increase in size as a consequence of inflammatory processes associated with therapy. This does not represent failure. At each follow-up visit, there should be a complete clinical examination that includes evaluation of symptoms and signs of eventual mucosal compromise. If the lesion has decreased in size, additional treatment should NOT be administered. The lesion should be completely healed 45 to 90 days after finishing treatment. When the lesion is very large or the patient has comorbidities, healing can be more difficult. If there is no clinical cure

after this period or in the case of reactivation of the lesion at any time, the lesion should be evaluated, and new treatment should be considered.

Clinical criteria for cure of CL at 3 months post-treatment is:

- Scarring with complete reepithelialization and flattening of the lesions' margins;
- Disappearance of the induration of the base;
- Disappearance of lymphangitis or adenitis in the event that it has occurred;
- Absence of new lesions.

5.2.2 Mucosal leishmaniasis (ML)

Patients should receive a clinical evaluation, at a minimum, on completing treatment and at 3, 6, 12, 18, and 24 months. Clinical signs should be evaluated clinically and registered (erythema, edema infiltration, erosion, ulceration, and dysphonia). When this happens, the health professional providing the next check-up will have quantifiable evidence to determine whether there is a response to therapy.

It is necessary to evaluate the therapeutic response 6 months after completion of treatment to define the need for a new therapeutic regimen. Furthermore, complete resolution of clinical signs is expected to define cure, in accordance with the following criteria.

Clinical criteria for cure of ML is:

- The criterion for cure is regression of all clinical signs. This should be confirmed by an ear, nose and throat examination at six months post-treatment;
- Clinical assessment on completing treatment, at 3 and 6 months and then semiannually until 2 years;
- In the absence of a specialist, the physician should be trained to carry out at least rhinoscopy and oroscopy;
- Where conditions are not appropriate, the patient should be referred to the reference service for the evaluation of cure;
- In situations where there is no clinical cure or in the case of reactivation of the lesion, evaluate and provide new treatment.

Treatment failure

Treatment failure is defined as no clinical cure following complete treatment, evaluated at 3 months for CL and up to 6 months for ML. For those cases, follow the recommendations described for special case management.

Relapse and reinfection

For the purposes of epidemiological surveillance, relapse is defined as the reactivation of a previously cured lesion regardless of observation time. Reinfection should be considered when new lesions appear in different anatomical sites and when there is history of new exposure.

5.2.3 Visceral leishmaniasis (VL)

Criteria for therapeutic responses and treatment follow-up for VL

Evaluation of the response to treatment is essentially clinical. The disappearance of fever occurs at the beginning of treatment and reduced hepatosplenomegaly starts in the first week. At the end of treatment, the spleen usually decreases by 40% or more in comparison to the baseline value. Improved hematological parameters (hemoglobin, white blood cells, and blood platelets) appear in the second week. Albumin and globulin normalize during the following weeks. Patient weight gain is evident, with improved appetite and general state.

The patient should be examined at the end of treatment and should continue with monthly follow-up during the first quarter, then every 3 months until 12 months post-treatment and every 6 months until 2 years post-treatment.

Treatment failure

Treatment failure is defined as no clinical remission after receiving two therapeutic schemes with the same drug, provided in a regular manner.

Relapse

This consists of intensification of symptomatology within 12 months after clinical cure.

5.3 Antileishmanial drug costs

The estimated costs of the most commonly used antileishmanial drugs in the Region are available in Annex 9.

INTRODUCTION TO LEISHMANIASES SURVEILLANCE

6. INTRODUCTION TO LEISHMANIASES SURVEILLANCE

Leishmaniases Programs in the Americas have the following objectives established:

6.1 Objectives

General objective

Reduce morbidity due to leishmaniases, case-fatality due to VL, and deformities caused by ML and MCL.

Specific objectives

- Conduct timely, adequate diagnosis and treatment of human cases of leishmaniases.
- Maintain an effective epidemiological surveillance system in time and structure.
- Reduce the vector's contact with susceptible hosts.
- Reduce sources of infection for the vector.
- Promote prevention, health education and social mobilization actions related to the achievement of these objectives.

To accomplish the proposed objectives, Leishmaniases Programs' activities include:

- Health care for human cases.
- Monitoring and follow-up of human cases and registry of mortality, as corresponds.
- Surveillance and evaluation of treatment-associated adverse events.
- Entomological surveillance and vector control.
- Surveillance and management of domestic reservoirs in areas with presence of VL, when the latter is of epidemiological importance in the transmission cycle.
- Promotion of health education and environmental management actions.

6.2 Surveillance and control

Strengthening of public health surveillance is essential to understand, intervene, and disseminate actions that are pertinent to achieving the objectives of the Control Program.

With regard to leishmaniasis, health surveillance actions comprise the collection and subsequent analysis—with regard to its role in the transmission cycle —of data on human cases, vectors, and canine reservoirs. The evidence gathered will be used to make decisions on how to carry out prevention, surveillance, and control of the disease to optimize resources invested in prevention and control and adapt every intervention to the local epidemiological context. The surveillance decision-making process allows, among other things, identification of risk strata, monitoring of trends in the time and dispersion of leishmaniasis transmission, and generation of adequate, timely responses to endemic transmission and epidemic outbreaks. On the other hand, the prevention decision-making process involves the promotion of integrated, intersectoral actions for individual, community, and structural prevention, environmental management, and health education regarding disease transmission. Finally, with regard to control, decision-making involves the eventual implementation of recommended control measures and assessment of their impact.

For epidemiological and operational purposes, it is necessary to identify and classify leishmaniasis scenarios in the Americas. This will allow for recognition of areas with and without transmission and determination of risks to in turn direct actions.

6.3 Classification and identification of leishmaniases epidemiological scenarios in the Americas

The purpose of the classification and identification of leishmaniases epidemiological scenarios is to understand the magnitude and risk of occurrence of the disease in order to prioritize and guide surveillance, prevention and control actions. Therefore, the different transmission cycles and the role of each element in the disease transmission chain should be considered. This information should be used to examine the concepts, definitions and indicators for each form of leishmaniasis. The proposal is presented below in detail.

It is important to mention that the epidemiological analysis and classification for leishmaniases can be done at any administrative level. However, it should ideally be done at the most disaggregated operational level, so that surveillance and control actions are more effective in time-space defined scenarios. Each of the Region's countries has its own administrative political structure, with a hierarchical relationship of definitions and names that differs across countries. For comparative purposes, the denomination of the national and subnational administrative levels and their equivalents in every country is presented in Table 13.

TABLE 13 - List of leishmaniases endemic countries and respective administrative levels, Americas, 2018.

Endemic countries for CL and/or VL	ADMINISTRATIVE LEVELS		
	1st Subnational administrative level	2nd Subnational administrative level	3rd Subnational administrative level
Argentina	Provinces	Departments	Municipalities
Bolivia	Departments	Provinces	Municipalities
Brazil	States	Municipalities	Districts/Localities
Colombia	Departments	Municipalities/Districts	Localities
Costa Rica	Provinces	Cantons	Districts
Ecuador	Provinces	Cantons	Parishes
El Salvador	Departments	Municipalities	Towns
Guatemala	Departments	Municipalities	Localities
Guyana	Regions	Neighborhood councils	-
Honduras	Departments	Municipalities	Villages
Mexico	Departments	Municipalities	Localities
Nicaragua	Departments/Regions	Municipalities	Localities
Panama	Provinces/Regions	Districts	Jurisdictions/Regions
Paraguay	Departments	Districts/Municipalities	Localities
Peru	Departments	Provinces	Districts
Suriname	Districts	Suburbs	Cities
Venezuela	States	Municipalities	Localities
Uruguay	Departments	Municipalities	-

6.3.1 Cutaneous leishmaniasis (CL) / mucosal leishmaniasis (ML)

It is necessary to consider the following definitions, classification, and epidemiological indicators to define risk stratification for surveillance of CL.

6.3.1.1 Definitions

Area	Geographical space with data that can be stratified
Areas with no transmission or silent	Areas without registry of the occurrence of autochthonous human cases of CL. These areas are classified according to the vulnerability and receptivity to the vector.
Vulnerable areas	Areas with no transmission or silent, where a) there are biomes favorable to presence of the vector, b) that are contiguous to areas with transmission or c) that have undergone or are undergoing environmental modifications (deforestation, new settlements, development plans, etc.).
Non-vulnerable areas	Areas with no transmission or silent that do not fulfill the criteria for vulnerability.
Receptive areas	Vulnerable or non-vulnerable areas with recorded presence of the vector.
Non-receptive areas	Vulnerable or non-vulnerable areas where there is no recorded presence of the vector. To characterize an area as non-receptive, there should be a corresponding entomological study.
Areas with transmission	Areas with a historical record of the occurrence of at least one autochthonous human case of CL. These areas are in turn classified according to whether or not they have an outbreak occurrence.
Endemic areas	Areas with historical records of the occurrence of autochthonous human cases of CL, whether continuous or not, in the last ten years.
Outbreak occurrence	Presence of cases of CL in an area with no transmission/silent or an increase in cases relative to the expected number in areas with transmission or endemic areas.
Primary sylvatic environment	Territory with dense vegetation, without previous significant modification in the environment due to human activity.
Intervened sylvatic environment	Territory with dense vegetation, with significant intervention in the environment due to human activity.
Rural environment	Territory with medium to low vegetation density and low population density, which is used for agricultural, agroindustrial, extractive, forestry, or other activities.
Peri-urban environment	Territory with low to medium population density, usually peripheral to cities, but without the high population density of the urban space, and if it is used for rural activities, these are exclusively on a household scale.

6.3.1.2 Indicators for stratification of areas WITH transmission of CL

The following indicators were identified for the classification and risk stratification of CL: number of cases, case incidence, and case density. These indicators were analyzed individually. After assessing the advantages and disadvantages of each indicator for adequately representing the epidemiological scenarios, the proposals were discussed with the countries. The decision was made to establish composite indicators, which have been validated for the risk stratification of CL. A distribution analysis using the natural breaks was the method used for the analysis, categorization in classes, and use of the indicators, to reduce the variance within classes and minimize the variance between classes. Five transmission strata were generated based on the classes: low, moderate, high, intense, and very intense. The different indicators are detailed below in Table 14:

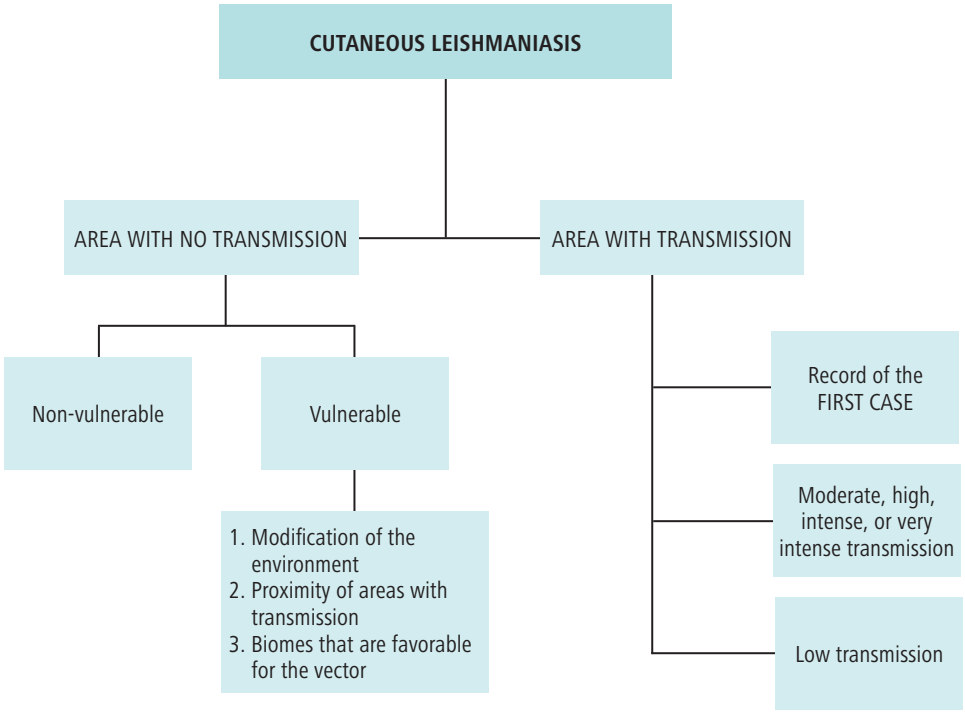
TABLE 14 - Cutaneous leishmaniasis indicators, calculation and use.

INDICATORS	CALCULATION	USE
Cutaneous / mucosal leishmaniasis cases	Num. of total new confirmed cases of cutaneous leishmaniasis reported during the year in the region, subregion, country and 1st and 2nd subnational administrative levels. NOTE: Confirmed cases according to the standardized case definition.	Identify the occurrence, profile, and evolution of cutaneous leishmaniasis cases, their distribution and trends.
Cutaneous / mucosal leishmaniasis incidence rate	Num. of total new cases of cutaneous leishmaniasis that occurred during the year / total population in transmission areas in the region, subregion, country and 1st and 2nd subnational administrative levels x 100,000 inhabitants	Identify the risk of occurrence of cutaneous leishmaniasis and monitor disease trends.
Cutaneous / mucosal leishmaniasis density rate	Num. of total new cases of cutaneous leishmaniasis that occurred during the year / area of transmission in km^2 in the region, subregion, country and subnational administrative levels, or in the specific transmission area	Quantify the occurrence of cutaneous leishmaniasis cases in a limited geographical space.
Cutaneous / mucosal leishmaniasis Annual Composite Indicator (CICl)	After calculating the annual indicators of cases, incidence, and density for the region, country or 1st and 2nd subnational administrative levels, for each indicator we calculate the average and standard deviation and then standardize into one single metric, with the calculation: Standardized case indicator = Num. cases – average cases/standard deviation. Standardized incidence indicator = Incidence – average incidence/ standard deviation. Standardized density indicator = Density – average density/ standard deviation. ICLc= ∑ Standardized case indicator + Standardized incidence indicator + Standardized density indicator. The ICLc for each territorial unit analyzed is categorized by calculating the natural break points, making it possible to generate five transmission risk strata: low, average, high, intense, and very intense.	Identify the areas of occurrence and risk of cutaneous leishmaniasis, integrating the information contained in the case, incidence and density indicators. The indicator categories are used to direct and prioritize surveillance, prevention, and control actions in defined territories.
Cutaneous / mucosal leishmaniasis Triennium Composite Indicator (TCICl)	After calculating the indicators for the last 3 years for cases and incidence for the region, country or 1st and 2nd subnational administrative levels, for each indicator we calculate the average and standard deviation and then standardize into one single metric, with the calculation: Average cases = (Num. cases in year X + Num. cases in year Y + Num. cases year Z) / 3. Average incidence = (Incidence in year X + Incidence in year Y + Incidence in year Z) / 3. Standardized case indicator = Case average – general average for cases / general standard deviation for cases. Standardized incidence indicator = Average incidence – general average for incidence / general standard deviation for incidence. TCICl= ∑ Standardized case indicator + Standardized incidence indicator . The TCICl is categorized by calculating the natural break points for each territorial unit, making it possible to generate five transmission risk strata: low, average, high, intense, and very intense.	Identify the areas of occurrence and risk of cutaneous leishmaniasis integrating the information contained in the average of the last 3 years of cases and incidence indicators. The indicator categories are used to direct and prioritize surveillance, prevention, and control actions in defined territories.

6.3.1.3. Epidemiological classification and surveillance and control actions

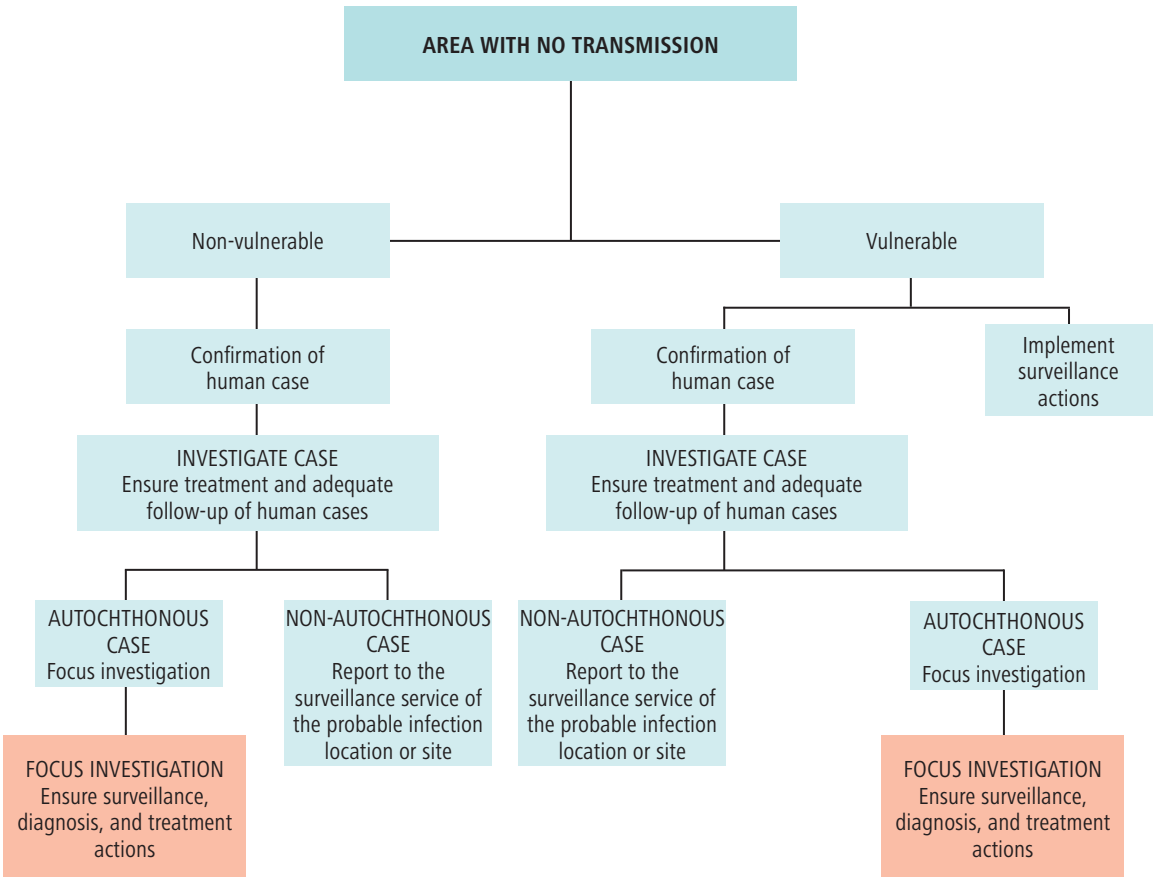
The flow charts below present the different epidemiological classifications of CL for the purpose of guiding, planning and directing surveillance and control actions.

FLOW CHART 4 - Epidemiological classification for the surveillance and control of cutaneous leishmaniasis in the Americas.



NOTE. In areas with transmission of atypical cutaneous leishmaniasis - ACL, where the transmission cycle is the same as for VL and where the importance of dogs as a domestic reservoir has been verified, guidelines for the surveillance and control of VL should be followed.

FLOW CHART 5 - Surveillance and control actions for areas **WITH NO CL TRANSMISSION**, when a suspected case of CL emerges.



Procedures in areas WITH NO CL transmission, when a suspected case of CL emerges

- Confirm diagnosis of CL human case using laboratory criterion.
- Ensure timely treatment and follow-up of confirmed case.
- Investigate the case and assess whether it is autochthonous, based on epidemiological background and entomology.
- If there is confirmation that the case is autochthonous, initiate relevant actions for active case-finding in the area and the corresponding diagnosis and treatment. Carry out other recommended actions based on an outbreak situation with presence of the first case.
- If the case is not autochthonous, report the case's probable site of infection to the corresponding surveillance service.

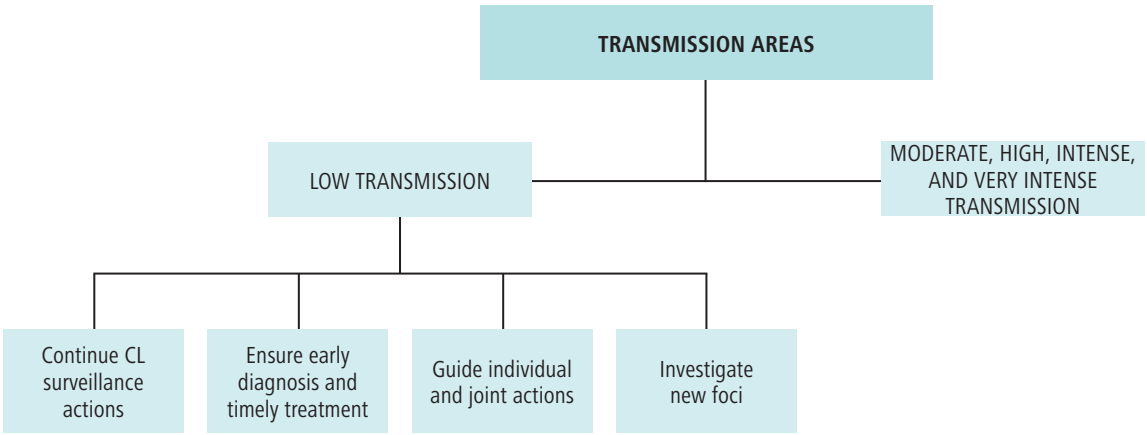
Procedures in areas WITH NO CL transmission, vulnerable

- Conduct entomological studies every 5 years or when there is an environmental modification or any other type of event —such as intense migration from endemic or outbreak areas— that can increase the population's risk of infection.
- In the event that the area is characterized as "receptive vulnerable," implement epidemiological surveillance. When there is an environmental modification or event that can increase the population's risk of infection — extraordinary meteorological phenomena or intense migration from endemic or outbreak areas— intensify surveillance through active case-finding.

Procedures for areas WITH NO CL transmission, non-vulnerable

- No surveillance actions are recommended for non-vulnerable areas. However, whenever there is any environmental modification or event that implies a potential increase in the population's risk, proceed as if it were a vulnerable area.

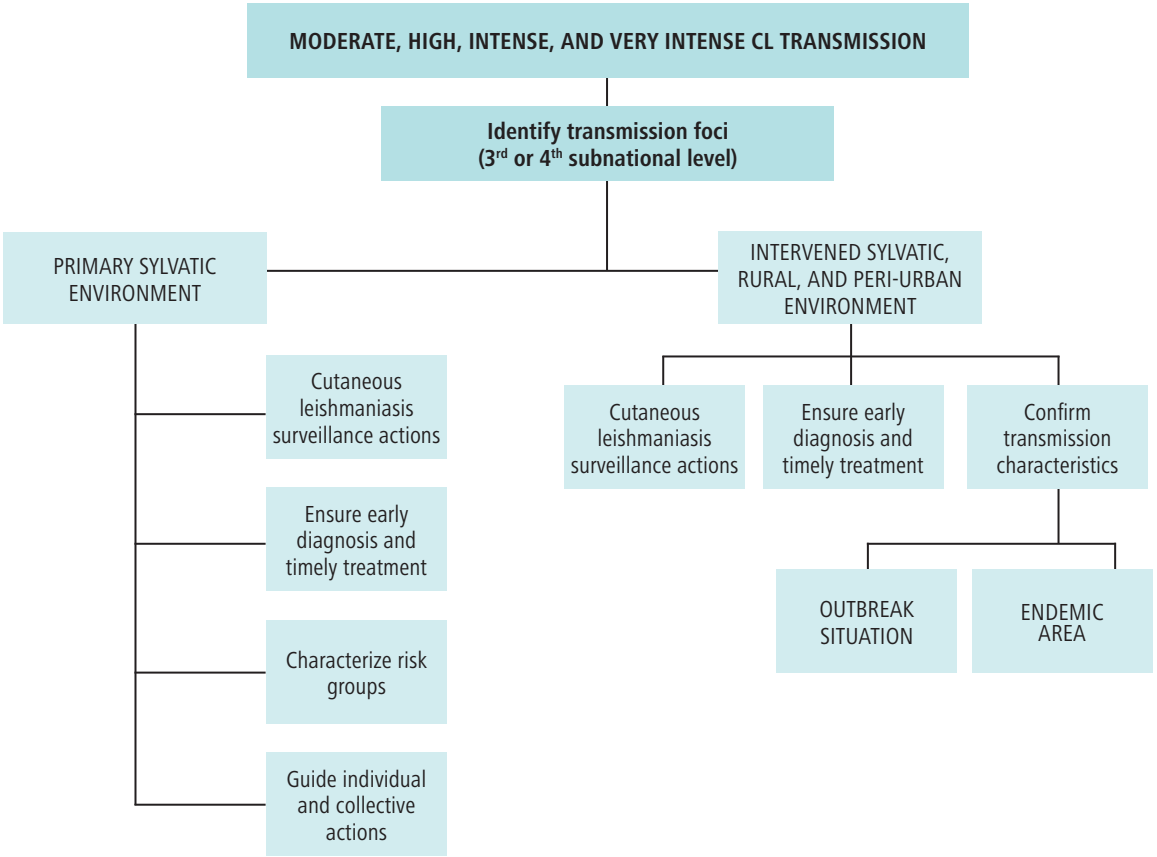
FLOW CHART 6 - Surveillance and control actions for areas **WITH low CL** transmission.



Procedures in areas WITH low CL transmission

- Strengthen and/or continue actions for surveillance of human cases.
- Guarantee access to early diagnosis through the organization and operational performance of the laboratory network, including processes for internal quality control and external assessment.
- Ensure timely treatment and individual follow-up for cases, including health services capacity and timely availability of materials and supplies. Consider reporting and monitoring of adverse events.
- Case reporting and monitoring— Consider guidelines for surveillance, diagnosis, treatment and follow-up.
- Guide individual and collective promotion and prevention actions.
- Investigate new transmission foci, as detailed in the Leishmaniases Research section.
- Monitor surveillance actions and epidemiological status.

FLOW CHART 7 - Surveillance and control actions for areas **WITH moderate, high, intense, and very intense CL transmission** in primary sylvatic, intervened sylvatic, and rural and peri-urban environments.



Procedures in areas WITH moderate, high, intense, and very intense CL transmission

- Surveillance and control actions are the same for all transmission levels. However, categories are intended to guide and prioritize resources. Actions to be conducted are the following:
  - Identification of CL transmission foci (3rd or 4th administrative level, as relevant to each country, for delimitation of the foci).
  - Identification of the different transmission scenarios: primary sylvatic, intervened sylvatic, rural, or peri-urban.

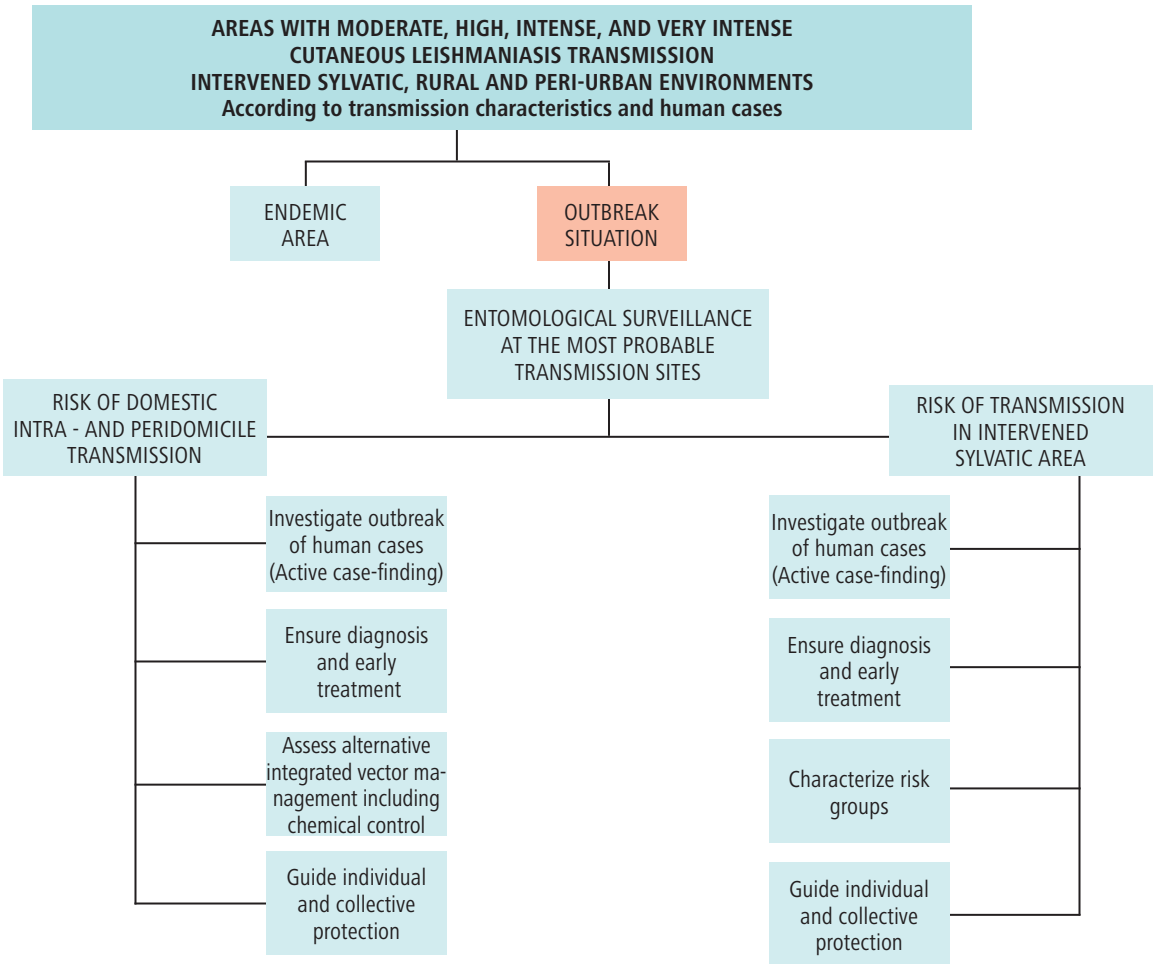
Procedures in areas WITH moderate, high, intense, and very intense CL transmission, in the primary sylvatic environment.

- Guarantee access to early diagnosis through the organization and operational performance of the laboratory network, including processes for internal quality control and external assessment.
- Ensure timely treatment and individual follow-up for cases, including health services capacity and timely availability of materials and supplies.
- Characterize the epidemiological situation and identify risk groups (for example: age, sex, occupation, probable exposure time and place).
- Guide individual and collective promotion and prevention actions according to the identified risk groups.
- Implement and continue surveillance activities for human cases.
- Investigate and characterize new transmission foci.
- In this scenario, implementation of entomological surveillance and vector control activities is not recommended.

Procedures in areas WITH moderate, high, intense, and very intense CL transmission in intervened sylvatic, rural and peri-urban environments

- Implement and continue surveillance activities.
- Guarantee access to early diagnosis through the organization and operational performance of the laboratory network, including processes for internal quality control and external assessment.
- Ensure timely treatment and individual follow-up for cases, including health services capacity and timely availability of materials and supplies.
- Implement individual and collective promotion and prevention actions according to the risk groups.
- Characterize the foci and transmission characteristics:
  - Outbreak situation (refer to Flow chart 8)
  - Endemic area

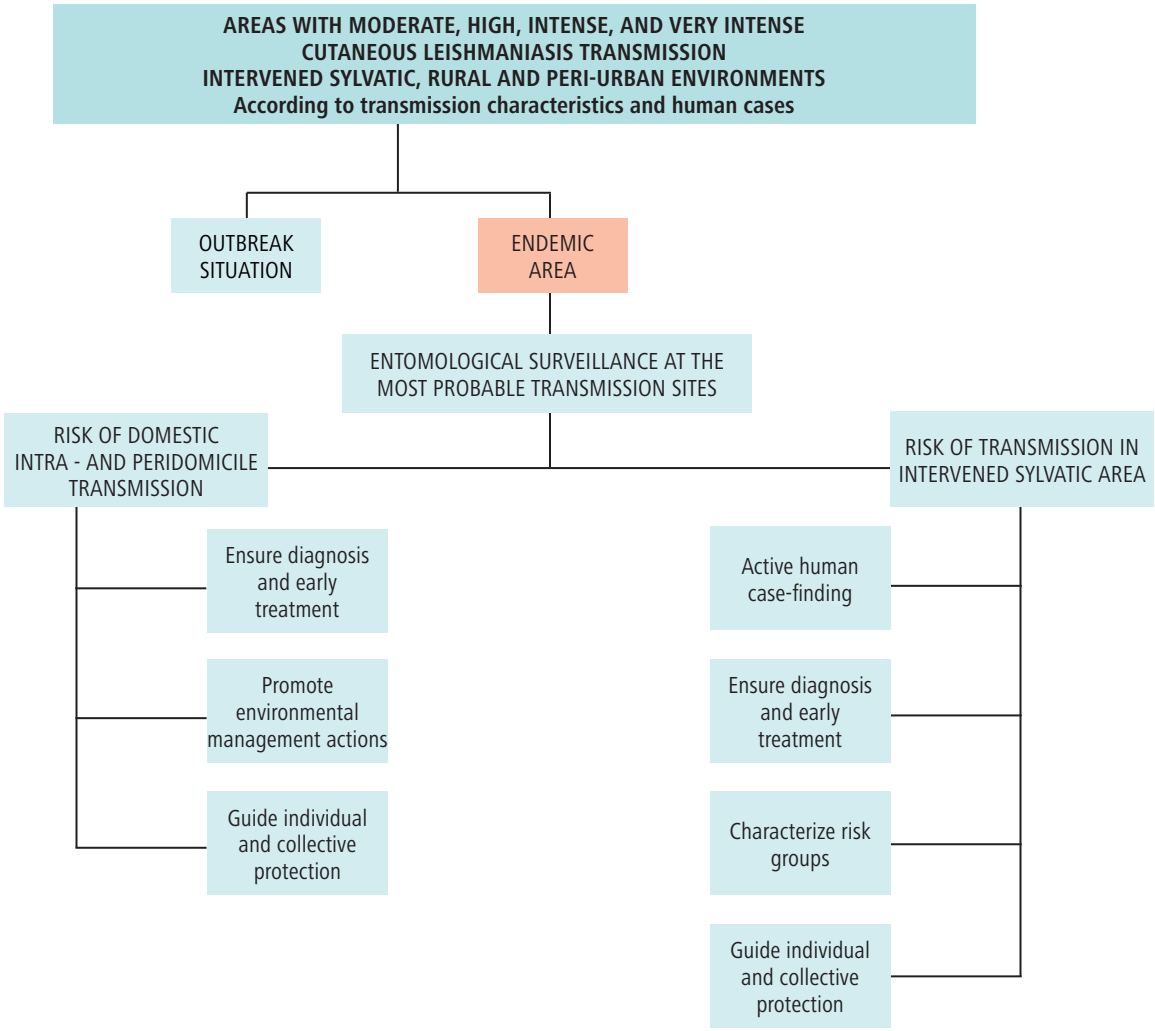
FLOW CHART 8 - Surveillance and control activities for areas WITH moderate, high, intense, and very intense CL transmission in intervened sylvatic, rural, and peri-urban environments during an **outbreak**.



Procedures for areas WITH moderate, high, intense, and very intense CL transmission in intervened sylvatic, rural and peri-urban environments during an outbreak

- Investigate the outbreak to establish whether the most probable transmission sites are in the domestic environment — intra- or peridomicile — or extradomicile — intervened sylvatic environment (refer to Outbreak Investigation).
- In the event that the outbreak is defined as domestic-intradomicile transmission, integrated vector management alternatives, including the feasibility and relevance of vector chemical control, should be assessed and implemented if possible. Actions should be carried out in a limited area and with effectiveness indicators, at least in the short term.
- Guide individual and collective promotion and prevention actions with intersectoral involvement, according to the identified risk groups.
- Implement and continue surveillance actions for human cases.
- In an endemic situation, refer to Flow chart 9..

FLOW CHART 9 - Surveillance and control actions for areas with moderate, high, intense, and very intense CL transmission, in intervened sylvatic, rural and peri-urban environments in an **endemic area**.



Procedures for areas WITH moderate, high, intense, and very intense CL transmission in intervened sylvatic, rural and peri-urban environments in an endemic area

- Conduct epidemiological and entomological studies to establish whether the most probable transmission sites are in the domestic environment — intra - and peridomicile or extradomestic — i ntervened sylvatic environment.
- In the event that an endemic situation with domestic transmission is defined, promote environmental management actions and guide and implement pertinent actions for individual and collective protection.
- In the event that extradomestic transmission is defined, promote and implement individual and collective protective actions and environmental management, according to the site and risk exposure period.
- Continue epidemiological surveillance actions.

6.3.2 Visceral leishmaniasis (VL)

To define risk stratification for surveillance of VL, it is necessary to consider the following definitions, classification, and epidemiological indicators.

6.3.2.1 Definitions

Transmission scenarios	Ecological characterization of the environment where transmission occurs
Concept of area	Geographical space with data that can be stratified.
Areas with no transmission or silent	Areas with no historical record of autochthonous cases of VL in humans or canines. These areas are classified as vulnerable or non-vulnerable.
Vulnerable areas	Areas that fulfill at least one of the following criteria: a) have a favorable condition for vector presence; b) are contiguous to transmission areas, whether a country, department, municipality, or locality; c) have intense migratory transit with transmission areas within the country or with border areas of neighboring countries; or d) share road networks with transmission areas.
Non-vulnerable areas	Areas that do not meet the criteria for vulnerability.
Receptive areas	Vulnerable or non-vulnerable areas with recorded presence of the vector.
Non-receptive areas	Vulnerable or non-vulnerable areas without recorded presence of the vector. To characterize an area as non-receptive, it should have a corresponding entomological study according to the entomological surveillance chapter.
Areas with transmission	Areas in which at least one autochthonous, human, or canine case has occurred. These areas are also classified according to whether or not there is an outbreak.
Endemic areas	Areas with a historical record of the occurrence of autochthonous human or canine VL cases, whether or not they are continuous.
Outbreak	In an area without transmission, when the first human or canine case is present. In an area with canine transmission, when the first human case is present. In an area with transmission, when there is an increase in the number of human cases relative to expected cases.

6.3.2.2 Epidemiological classification of visceral leishmaniasis in the Americas

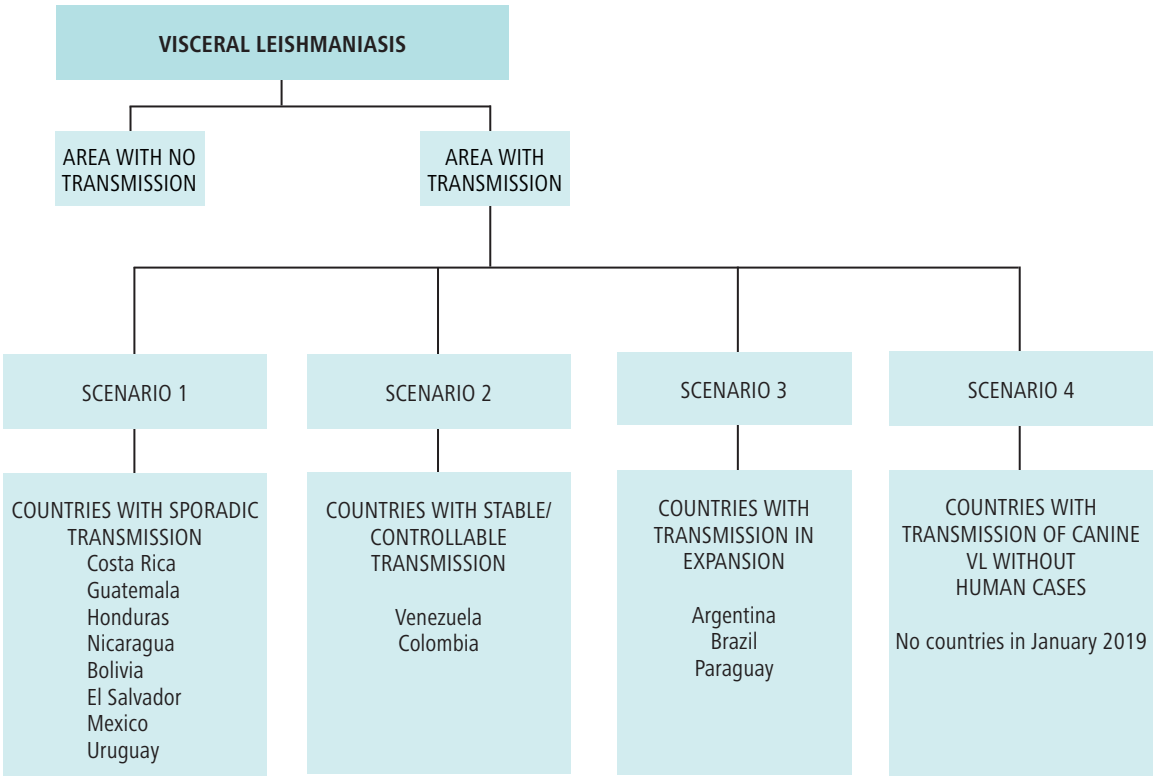
In the Americas, for epidemiological purposes, VL is categorized in scenarios and areas with and without transmission, as shown in Flow charts 10 and 11.

- Areas WITH NO transmission or silent, which can also be:
  - Vulnerable
  - Non-vulnerable

The areas of onset or occurrence of visceral leishmaniasis, according to the scenario or country involved, are classified at the regional level in the following way:

- Areas WITH transmission:
  - Scenario 1: countries with a record of sporadic cases of VL.
  - Scenario 2: countries in which VL transmission is stable or controlled.
  - Scenario 3: countries with a growing number or expanded geographical distribution of cases of VL.
  - Scenario 4: countries with canine transmission of VL, without occurrence of human cases of VL.

FLOW CHART 10 - Classification of visceral leishmaniasis in the Americas, according to the epidemiological scenario and countries corresponding to each scenario.

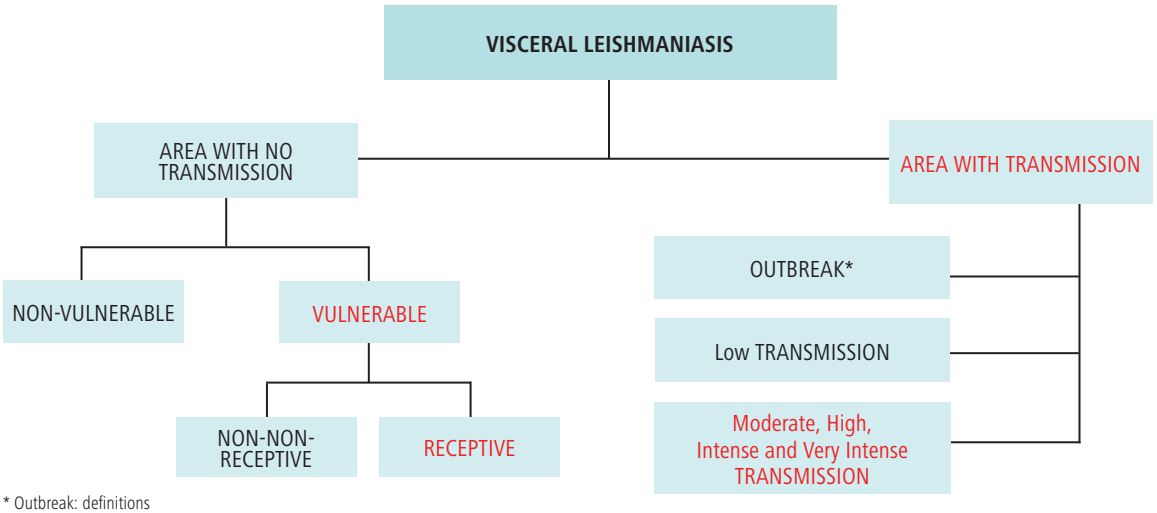


6.3.2.3 Indicators for the stratification of areas with VL transmission in the country and in the 1st and 2nd subnational administrative levels.

TABLE 15 - Visceral leishmaniasis indicators, calculation, and use.

INDICATORS	CALCULATION	USE
Visceral leishmaniasis cases	Num. of total new confirmed cases of visceral leishmaniasis reported during the year in the region, subregion, country and 1st and 2nd subnational administrative levels. NOTE: Confirmed cases according to the standardized case definition.	Identify the occurrence, profile, and evolution of visceral leishmaniasis cases, their distribution and trend.
Visceral leishmaniasis incidence rate	Num. of total new cases of visceral leishmaniasis that occurred during the year / total population in transmission areas in the region, subregion, country and 1st and 2nd subnational administrative levels x 100,000 inhabitants.	Identify the risk of occurrence of visceral leishmaniasis and monitor disease trends.
Visceral leishmaniasis Trienium Composite Indicator (TCIVl)	After calculating the indicators for the last 3 years for cases and incidence of VL for the country or 1st and 2nd subnational administrative levels, for each indicator we calculate the average and standard deviation and then standardize into one single metric, with the calculation: Average cases = (Num. cases in year X + Num. cases in year Y + Num. cases year Z) / 3. Average incidence = (Incidence in year X + Incidence in year Y + Incidence in year Z) / 3. Standardized case indicator = Case average – general average for cases / general standard deviation for cases. Standardized incidence indicator = Average incidence – general average for incidence / general standard deviation for incidence. TCIVl= ∑ Standardized case indicator + Standardized incidence indicator. The TCIVl is categorized by calculating the natural break points for each territorial unit, making it possible to generate five transmission risk strata: low, average, high, intense, and very intense.	Identify the areas of occurrence and risk of visceral leishmaniasis integrating the information contained in the average of the last 3 years of cases and incidence indicators. The indicator categories are used to direct and prioritize surveillance, prevention, and control actions in defined territories.

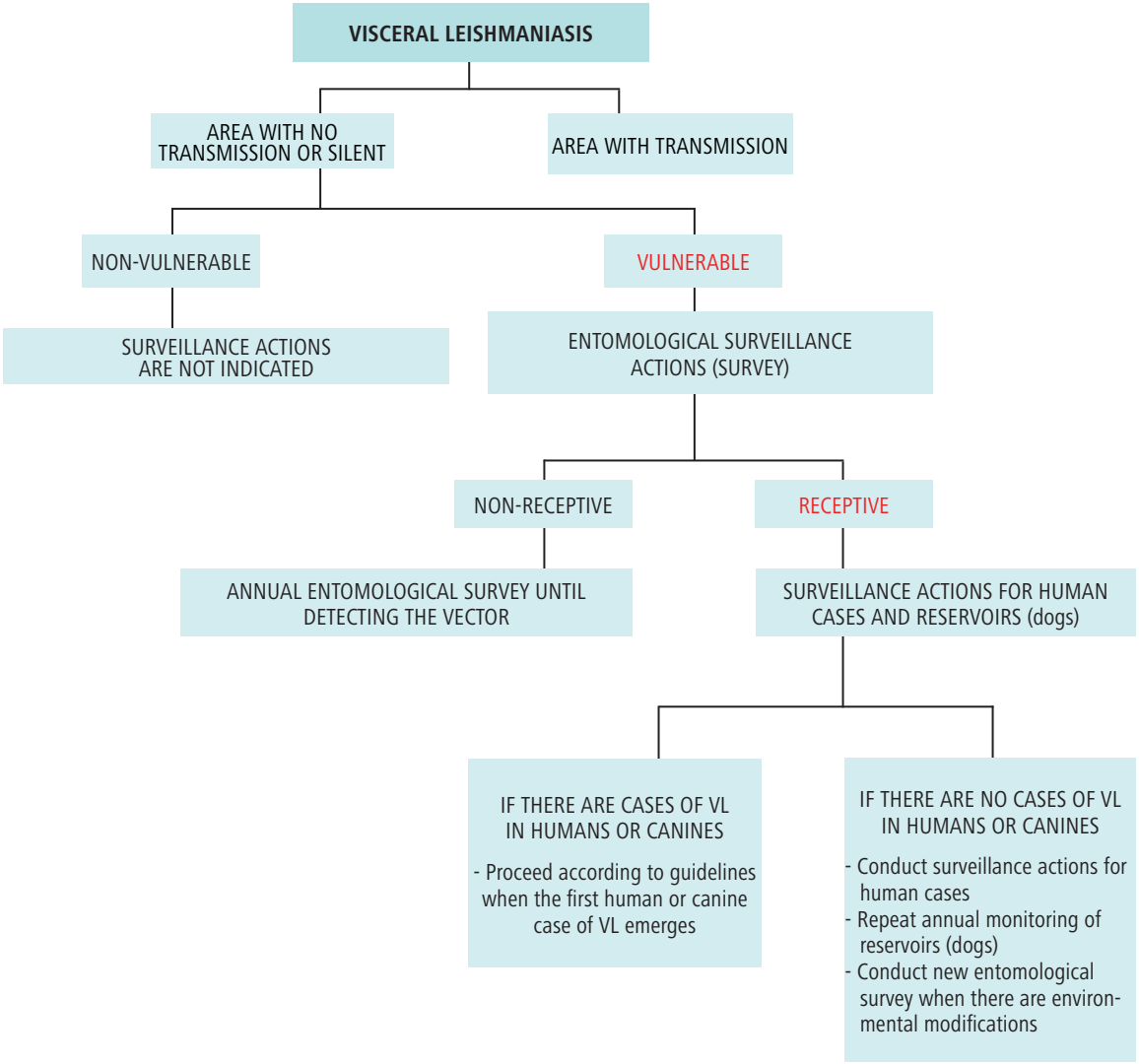
FLOW CHART 11 - Epidemiological classification of visceral leishmaniasis in the Americas.



* Outbreak: definitions
In an area with no transmission, with the presence of the first human or canine case.
In an area with canine transmission, with the presence of the first human case.
In an area with transmission, when there is an increase in the number of human cases relative to the number of expected cases..

6.3.2.4 Surveillance and control activities for areas with no VL transmission or Silent

FLOW CHART 12 - Surveillance and control actions for areas with no **VL transmission or silent.**



Surveillance and control procedures and actions in areas WITH NO VL transmission or Silent

For the classification of vulnerable and non-vulnerable areas, consider the same criteria as for CL and include other important criteria for each country.

NON-vulnerable areas

- Surveillance and control actions are not indicated.

Vulnerable areas

- Trigger entomological surveillance activities using the survey methodology, as described in the Entomological Surveillance chapter.

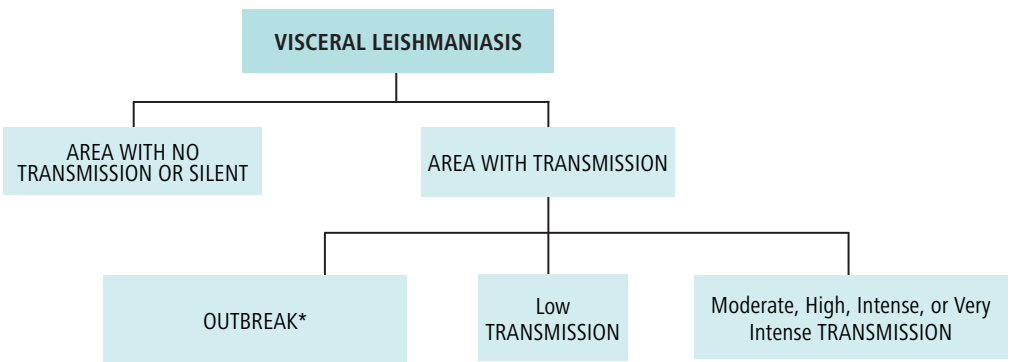
Vulnerable areas with no presence of the vector (non-receptive): When the vector is **NOT** found during the entomological survey.

- Conduct entomological monitoring annually until the vector is detected.

Vulnerable areas with presence of the vector (receptive): When the vector **IS** found during the entomological survey.

- Continue entomological surveillance activities.
- Conduct studies of reservoirs (dogs) and active case-finding in humans according to the methodology described in the corresponding chapter.
- Initiate surveillance of human cases and reservoirs through active case-finding and reservoir surveys in the area around where the vector was found.
- If a suspected case of human or canine VL is reported, initiate surveillance actions.
- If an autochthonous case of human or canine VL is confirmed, proceed according to the guidelines for the first human or canine case of VL, regardless of which one occurs first.
- If there is no confirmation of human or canine cases of VL, continue surveillance of human cases and repeat reservoir monitoring annually. Conduct a new entomological survey if there are environmental modifications.

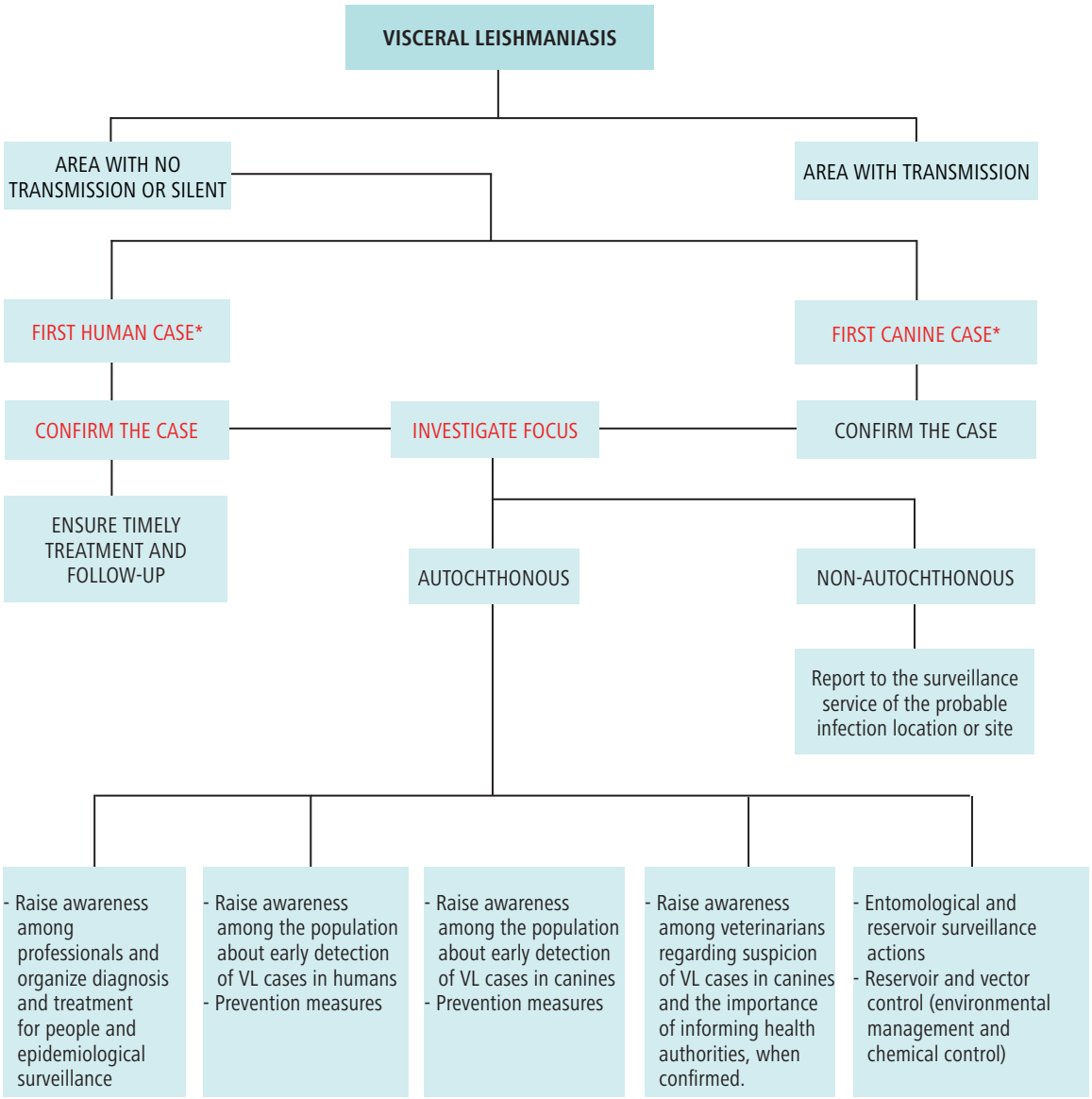
FLOW CHART 13 - Epidemiological classification in areas **WITH** visceral leishmaniasis transmission in the Americas.



***Outbreak: definitions**

In an area with no transmission, with the presence of the first human or canine case.
In an area with canine transmission, with the presence of the first human case.
In an area with transmission, when there is an increase in the number of human cases relative to the number of expected cases.

FLOW CHART 14 - Surveillance and control actions for areas **WITH NO** Transmission, when the first human or canine case of VL is recorded.



NOTE: * Area in transition: This refers to the area WITH NO transmission or silent that is in transition to becoming an area WITH transmission. This confirmation will depend on the focus investigation, when autochthony confirmed.

Surveillance and control actions for areas WITH NO transmission WITH recording of the 1st human or canine case of VL

When there is suspicion of the first human case of VL

- Conduct laboratory diagnosis with immunoserological or immunochromatographic methods that are specific to *Leishmania infantum* or with parasitological or molecular methods. If there is confirmation, ensure timely treatment and individual follow-up of the human case (Annexes 4, 6 and 7). Preserve appropriate material, in adequate conditions, for identification of the species of *Leishmania* (Annex 10).
- If VL is confirmed, investigate the case to assess whether it is autochthonous, based on epidemiological background and the entomological research (150-meter radius or nine-block area).
- If the case is not autochthonous, meaning that the VL vector is not found, report the case's probable infection location to the corresponding surveillance system.
- If there is confirmation of an autochthonous case, meaning that the VL vector is found, initiate active case-finding actions for humans and canines, ensure diagnosis and management of detected human cases, and conduct other actions for an outbreak situation due to the presence of the first case.
- Proceed with the identification of the *Leishmania* species through molecular biology techniques. For characterization of the species, there is support from the "Laboratório de Referência Regional, IOC/Fiocruz" (Regional Reference Laboratory, IOC/Fiocruz). The procedures for sample collection, preservation, and shipment are described in Annexes 4 and 10.
- Raise awareness and organize health services for the implementation of epidemiological surveillance, diagnosis, and treatment actions for people with suspected VL.
- Raise awareness of veterinarians about the early diagnosis of canine VL infection and the importance of reporting canine VL cases to health authorities.
- Raise awareness of local decisionmakers, involved sectoral agents and the community about adequate prevention measures and measures to promote early detection of human and canine cases of VL.
- Initiate vector surveillance, prevention, and control actions — following criteria for integrated vector management (Annex 11) — in the minimum area determined by the entomological research.
- For areas where dogs are important in the VL transmission cycle, initiate surveillance, prevention, and control actions for the domestic reservoir. The details of the proposed actions are described in the following section, Suspicion of the first canine case of VL.

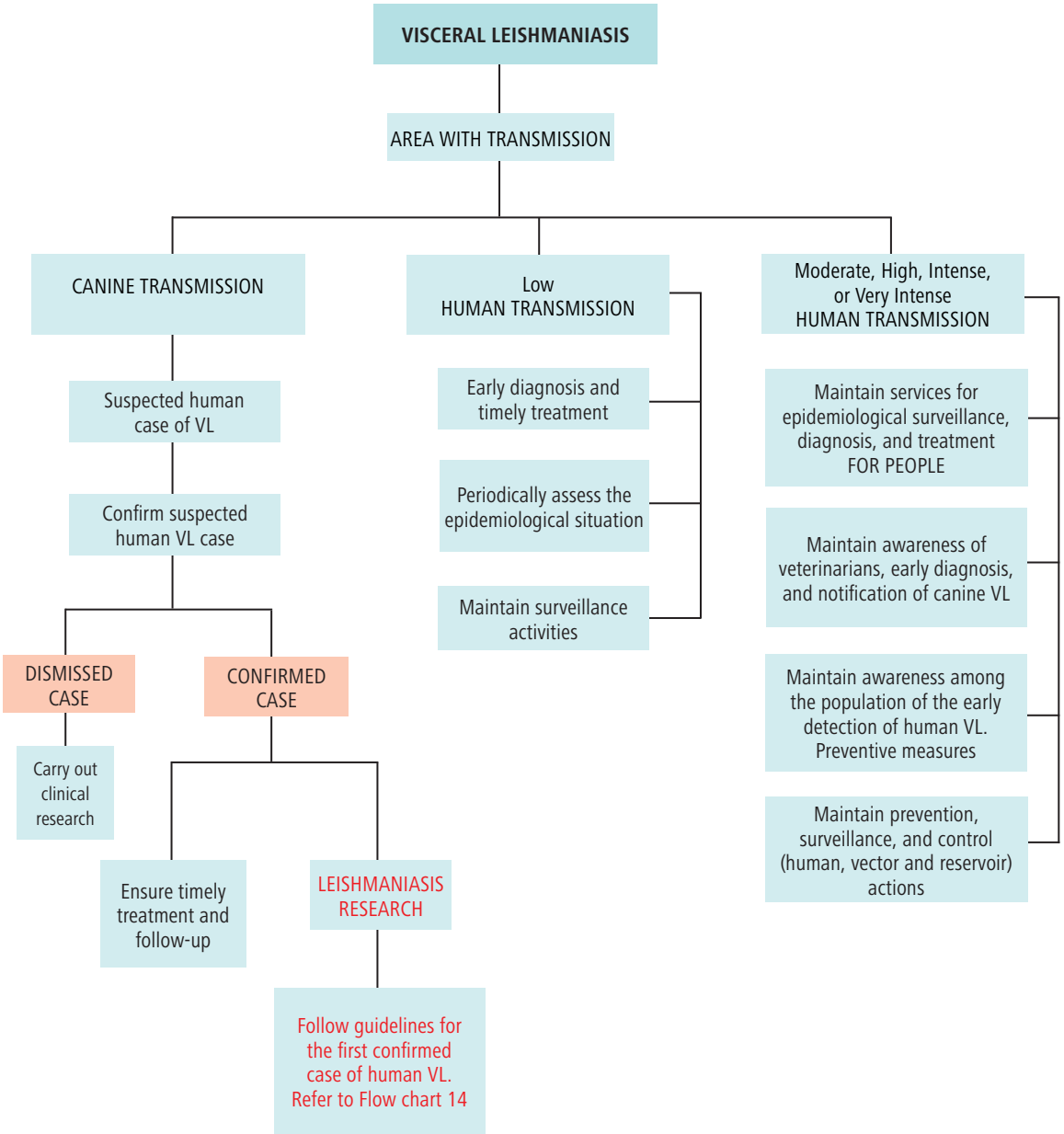
When there is suspicion of the first canine case of VL

- Conduct laboratory diagnosis with immunoserological or immunochromatographic methods that are specific to *Leishmania infantum* or with parasitological or molecular methods. If positive, collect material for species identification.
- If VL is confirmed, investigate the case to assess whether it is autochthonous, based on epidemiological background and the entomological research (150-meter radius or nine-block area).
- If the canine VL case is not autochthonous, report the canine case's probable infection location to the corresponding surveillance system.

- If there is confirmation of an autochthonous VL canine case (determination of VL vector presence), initiate activities of human and canine active case-finding, ensure diagnosis and management of detected human cases, and conduct other activities for an outbreak situation due to the presence of the first case. The details of reservoir surveillance actions are described in the corresponding chapter and Annexes 13 and 14.
- If the vector is not detected initially, continue with entomological surveillance activities, implementing them at least in the known or estimated period of greatest vector abundance.
- For reservoir surveillance activities, delimit the area of the focus investigation. The delimited area will be extended radially around the first confirmed canine case, including at least 100 dogs to be examined (variable radius according to canine density).
  - Active case-finding of symptomatic dogs and serological survey with rapid tests will be conducted in the area, to identify possible positive cases.
  - Part of the positive cases should be confirmed using parasitological methods (Annex 14).
  - Conduct identification of the species of *Leishmania* using molecular techniques for confirmation of canine VL transmission. There is support from the "Laboratório de Referência Regional, IOC/Fiocruz" (Regional Reference Laboratory, IOC/Fiocruz) for species characterization. The procedures for sample collection, conservation, and shipment are described in Annexes 4 and 10.
- Raise awareness and organize health services for the implementation of epidemiological surveillance, diagnosis, and treatment actions for people with suspected VL.
- Raise awareness of veterinarians about the early diagnosis of canine VL infection and the importance of reporting canine VL cases to health authorities. Review case definitions in the Reservoir Surveillance chapter.
- Raise awareness of local decisionmakers, involved sectoral agents and the community about adequate prevention measures and measures to promote early detection of human and canine cases of VL.
- Initiate vector surveillance, prevention, and control actions — according to criteria for integrated vector management — in the minimum area determined by the entomological study.

6.3.2.5 Surveillance and control activities for areas **WITH VL** transmission

FLOW CHART 15 - Surveillance and control activities for areas WITH VL transmission.



Surveillance and control actions in areas WITH canine transmission with suspicion of the first human case of VL:

When there is suspicion of the first human case of VL in areas WITH already established canine transmission

- Conduct laboratory diagnosis with immunoserological or immunochromatographic methods specific to *Leishmania infantum* or with parasitological or molecular methods. If VL is confirmed, ensure timely treatment and individual follow-up of the human case. Preserve appropriate material, in adequate conditions, for identification of the species of *Leishmania*. (Annexes 5 to 8)
- If VL is confirmed, investigate the human case to assess whether it is autochthonous, based on epidemiological background and the entomological research (150-meter radius or nine-block area).
- If there is confirmation of an autochthonous case, meaning that the VL vector is found, initiate active case-finding actions for humans and canines, ensure diagnosis and management of detected human cases, and conduct other actions for an outbreak situation due to the presence of the first case.
- Delimit the occurrence area for the human case and initiate prevention, and surveillance and control actions for human cases, reservoirs, and the vector. (Consider a minimum of 150 meters.)
- Raise awareness and organize health services for the implementation of epidemiological surveillance, diagnosis, and treatment actions for people with suspected VL.
- Raise awareness of veterinarians about the early diagnosis of canine VL infection and the importance of reporting canine VL cases to health authorities.
- Raise awareness of local decisionmakers, involved sectoral agents and the community about adequate prevention measures and measures to promote early detection of human and canine cases of VL.
- Initiate vector surveillance, prevention, and control actions — following criteria for integrated vector management in the minimum area determined by the entomological research.
- Initiate surveillance, prevention, and control activities for the domestic reservoir.

Surveillance and control actions for areas with LOW human VL transmission

- Ensure the early diagnosis, timely treatment, and individual follow-up of human cases.
- Systematically assess the epidemiological situation to implement, if necessary, prevention, surveillance and control measures.
- Permanently and systematically maintain surveillance activities.
  - Periodically monitor and assess the occurrence of human (any time) and canine (annually) cases;
  - Investigate all suspected or confirmed cases of VL;
  - Monitor entomological indicators and intervene with surveillance, prevention and control activities when necessary;
  - Monitor canine indicators and conduct surveillance, prevention, and control activities, when indicated.

In countries with LOW transmission, characterize the role of dogs in the VL transmission cycle and, if necessary, plan reservoir surveillance and control activities.

Surveillance and control actions for areas WITH moderate, high, intense, and very intense human VL transmission:

The procedures for areas with moderate, high, intense and very intense transmission are the same. However, activities should be prioritized according to stratification by higher transmission risk.

- Maintain health service organization to initiate actions for prevention, epidemiological surveillance and diagnosis and treatment of VL in humans.
- Maintain veterinarian awareness for early diagnosis and reporting of canine VL cases (reporting will depend on the country's epidemiological situation and regulations). Implement reservoir surveillance, prevention, and control measures.
- Raise awareness of local decisionmakers, involved sectoral agents and the community about pertinent measures of VL surveillance, prevention, and control.
- Permanently and systematically maintain VL surveillance and control activities.
  - Delimit the transmission area based on the occurrence of human cases, presence of infected dogs, and presence of the vector;
  - For each delimited area, have the number of blocks, dwellings, people and dogs, as well as the characteristics of the environment and transmission patterns, available;
  - Monitor epidemiological, entomological, and canine indicators and include other indicators (social, economic, vulnerability, among others) if possible;
  - Annually plan surveillance and control actions according to the frequency and distribution of cases and vectors and the prevalence in canines.
- Maintain the organization of services and supplies to initiate entomological and reservoir surveillance activities. Conduct reservoir and vector control (following criteria for integrated vector management), when indicated. Consider the need to monitor the impact of the surveillance and control activities conducted.

Details on surveillance activities for human cases, entomological surveillance and vector control, reservoir surveillance and control, and leishmaniasis research can be found in the following chapters.

SURVEILLANCE OF HUMAN CASES AND PREVENTION MEASURES

7. SURVEILLANCE OF HUMAN CASES AND PREVENTION MEASURES

Leishmaniases in their different clinical forms require an individual notification system given that each form requires different prevention, surveillance, and control actions. Additionally, these actions depend on the data obtained and the investigation conducted for each case. In turn, prevention activities should be adapted to transmission cycles and patterns.

7.1 Case definitions

Cutaneous leishmaniasis (CL)

- Suspected case: This refers to a person who has traveled to CL endemic areas or lives in one of them and has characteristic skin lesions — blot, papule, node or ulcer.
- Laboratory-confirmed case: This refers to a suspected case that has been confirmed using parasitological diagnosis — smear, culture or PCR.
- Case confirmed by clinical and epidemiological criteria: This refers to a suspected case that, although the person received a negative or inconclusive result when evaluated using a laboratory diagnosis method, responds favorably to treatment with specific anti-CL drugs.

Mucosal or mucocutaneous leishmaniasis (ML and MCL)

- Suspected case: This refers to the person who has traveled to ML/MCL endemic areas at least once or lives in one and has mucosal lesions.
- Laboratory-confirmed case: This refers to a suspected case that has been confirmed either using parasitological diagnosis (direct or culture), PCR testing, or serological diagnosis.

Visceral leishmaniasis (VL)

- Suspected case: This refers to a person who has traveled to VL endemic areas or lives in one of them and presents nonspecific fever for over a week, in addition to signs of splenomegaly and/or hepatomegaly.
- Confirmed case: This refers to a suspected case that has been confirmed using parasitological — direct, culture or PCR — or serological diagnosis.
- Case confirmed by clinical and epidemiological criteria: This refers to a suspected case without laboratory confirmation of the diagnosis, but that responds favorably to treatment with specific drugs.

***Leishmania*/HIV coinfection**: This refers to a patient with a diagnosis of HIV infection that presents some clinical form of leishmaniasis (CL, ML/MCL and VL) confirmed using serological and/or parasitological diagnosis.

7.2 Strategies for leishmaniases case-finding

Passive case-finding (PCF): This refers to when the patient with signs and/or symptoms of CL, ML/MCL or VL seeks medical care in public or private health facilities. The professional who handles the case should report it to the surveillance system according to pre-established criteria. It is important that the necessary forms for the case report be available and that an information flow is defined so that data arrive to the surveillance system on a timely basis.

Active case-finding (ACF): This refers to when a healthcare professional or community promoter carries out case-finding in a population where there are people with signs and symptoms compatible with leishmaniases. For CL, active case-finding is indicated in outbreak situations, when there is suspicion of peri- or intradomiciliary transmission, or when people live in risk areas from which it is difficult to access health services where they can obtain adequate diagnosis and treatment. Active case-finding is indicated in areas with VL transmission when there is information on a human case or in areas without transmission when the first autochthonous canine or human case of VL is confirmed.

7.3 Investigation of leishmaniases cases

The objectives of a leishmaniases case investigation are:

Cutaneous leishmaniasis

- Identify the epidemiological, biological, and environmental characteristics associated with the presence of this clinical form, considering the influence that socioeconomic activities may have on these characteristics. Identify whether the patient comes from or has traveled to endemic areas. If not, determine whether there are characteristics of a new transmission focus.
- Assess the need to conduct active case-finding.
- Weigh the need to carry out entomological surveillance activities.
- Guide individual and collective prevention measures and, when required, environmental management or chemical control measures.

Visceral leishmaniasis

- Identify whether the case is autochthonous or imported and adopt the pertinent measures in each case.
- Confirm whether it is an endemic area or a new transmission focus.
- Identify the epidemiological, biological, and environmental characteristics associated with the emergence of the case, vector, reservoir, and transmission pattern.
- Assess the need to conduct active case-finding.
- Guide the pertinent prevention, surveillance and control actions, according to the epidemiological situation and the classification of the area.

7.4 Surveillance and minimum recommended variables for data collection and analysis

Although each country of the Region has established its own surveillance system — which defines whether surveillance is individual or aggregate, whether notification includes suspected or confirmed cases, which forms to use, what variables to consider, how data flows, what type of analysis to perform, which interventions are pertinent, and how information is disseminated — the following minimum guidelines can be defined for leishmaniases monitoring and evaluation.

Cutaneous, mucosal or mucocutaneous leishmaniasis

Individual case reporting (when the case has already been confirmed using laboratory methods or in special situations confirmed by clinical epidemiological criteria).

- Identification data: age, sex, current residence, health care facility, probable transmission place, travel history.
- Clinical, diagnosis, laboratory, and treatment data: dates for onset of signs/symptoms, diagnosis, notification time, and start of treatment. Type of entry (new case or relapse), clinical form (CL, ML/MCL), number of lesions, lesion size and location, laboratory diagnosis method (parasitological, serological), type of treatment (local or systemic), drug used, treatment evolution (cure, treatment failures, death), HIV diagnosis and, for women, gynecological aspects (pregnancy).

Outbreaks: there is consensus that CL outbreaks in international border areas are reported as alerts in the SisLeish.

Visceral leishmaniasis

Individual case reporting (when there is clinical suspicion)

- Identification data: age, sex, current residence, probable transmission place, travel history.
- Clinical, diagnosis, laboratory, and treatment data: dates for onset of signs/symptoms, diagnosis, notification, and start of treatment. Type of entry (new case or relapse), laboratory diagnosis method (parasitological, serological), drug used and evolution (cure, treatment failures, complications, death), HIV status and, for women, gynecological aspects (pregnancy).

NOTE: Human and canine cases of VL and presence of VL vectors in international border areas should be reported as alerts in the SisLeish.

7.5 Epidemiological and operational indicators

TABLE 16. Epidemiological and operational indicators.

INDICATORS	CALCULATION	USE
Cutaneous/mucosal and visceral leishmaniasis cases	Total num. of new confirmed cases of leishmaniases reported in the year in the region, subregion, country and 1st and 2nd subnational administrative levels. NOTE: Confirmed cases according to the PAHO/WHO standardized case definition	Identify the occurrence, profile, and evolution of cutaneous/mucosal and visceral leishmaniasis cases and their distribution and trend.
Cutaneous/mucosal and visceral leishmaniasis incidence rate	Total num. of new leishmaniases cases that occurred during the year / total population in transmission areas in the region, subregion, country and 1st and 2nd subnational administrative levels x 100,000 inhabitants	Identify the risk of occurrence of cutaneous/mucosal and visceral leishmaniasis cases and monitor disease trends.
Cutaneous/mucosal and visceral leishmaniasis case density	Total num. of new cases of cutaneous/mucosal and visceral leishmaniasis that occurred during the year / transmission area in km^2 in the region, subregion, country and 1st and 2nd subnational administrative levels or the specific transmission area.	Quantify the occurrence of cutaneous/mucosal and visceral leishmaniasis cases in a limited geographical space.
Proportion of cutaneous/mucosal and visceral leishmaniasis cases by sex	Total num. of new cases of cutaneous/mucosal and visceral leishmaniasis that occurred during the year by sex / total num. of cutaneous/mucosal and visceral leishmaniasis cases in the region, subregion, country and 1st and 2nd subnational administrative levels x 100	Identify the occurrence of cutaneous/mucosal and visceral leishmaniasis cases by sex and monitor risk groups.
Proportion of cutaneous/mucosal and visceral leishmaniasis cases by age group	Total num. of new cases of cutaneous/mucosal and visceral leishmaniasis that occurred during the year by age group / total num. of cutaneous/mucosal and visceral leishmaniasis cases in the region, subregion, country and 1st and 2nd subnational administrative levels x 100	Quantify the occurrence of leishmaniases cases by age group, identify and monitor risk groups.
Proportion of cutaneous/mucosal and visceral leishmaniasis cases by clinical form	Total num. of new cases of cutaneous/mucosal and visceral leishmaniasis that occurred during the year by clinical form / total cutaneous/mucosal and visceral leishmaniasis cases in the region, subregion, country and 1st and 2nd subnational administrative levels x 100	Identify the incidence and distribution of leishmaniases cases by clinical form.
Proportion of cutaneous/mucosal and visceral leishmaniasis cases by confirmation criterion	Total num. of new cases of cutaneous/mucosal and visceral leishmaniasis that occurred during the year by confirmation criterion / total cutaneous/mucosal and visceral leishmaniasis cases in the region, subregion, country and 1st and 2nd subnational administrative levels x 100	Identify the occurrence of leishmaniases cases by confirmation criterion and monitoring of the laboratory diagnosis.
Proportion of cutaneous/mucosal and visceral leishmaniasis cases by case evolution	Total num. of new cases of cutaneous/mucosal and visceral leishmaniasis that occurred during the year by evolution / total cutaneous/mucosal and visceral leishmaniasis cases in the region, subregion, country and 1st and 2nd subnational administrative levels x 100	Identify the occurrence of leishmaniases cases by case evolution and monitor deaths due to cutaneous/mucosal and visceral leishmaniasis.
Case-fatality rate	Total num. of deaths from visceral leishmaniasis that occurred during the year / total visceral leishmaniasis cases in the region, subregion, country and 1st and 2nd subnational administrative levels x 100	Identify the severity of visceral leishmaniasis and monitor and identify risk groups.

7.6 Data reporting and flow from countries to PAHO/WHO

According to the discussions and definitions developed in 2012 and 2013 by the Regional Leishmaniases Program, PAHO/WHO representatives and countries' National Leishmaniases Programs and Epidemiological Surveillance Services, it was established that:

- Each country will report human leishmaniases data to PAHO/WHO once a year, on 30 April of the subsequent year, through the Leishmaniases Regional Information System - SisLeish.
- To update population data, the country leader should complete a form in SisLeish once a year, by 30 March. The data entered should be population data from the 2nd subnational administrative level for the previous year (these data make it possible to maintain indicator estimates).
- Data reported to SisLeish should include previously established variables that should be disaggregated to the 2nd subnational administrative level. In the case of countries that do not have a 2nd administrative level in their political administration, an exception will be made to report cases disaggregated to the 1st level (as is the case for Guyana and Uruguay).
- The epidemiological and operational indicators were standardized so that the denominators established to calculate incidence and density will be the population and area in km^2 for the 2nd subnational administrative level with transmission.
- Data and indicators are available in the SisLeish aggregated by region, subregion, country, and subnational administrative levels (1st and 2nd). Analysis of these indicators can be conducted at the country, department or regional level.
- For international border areas, VL is reported in SisLeish — VL border alert — when a vector or a case of VL in dogs or in humans is identified. The alert immediately sends the case report to all bordering countries.
- CL outbreak reports in international border areas should be reported in SisLeish — CL outbreak border alert. The alert immediately sends the case report to all bordering countries.
- When there is confirmation of the existence of a new circulating species of *Leishmania* or a suspected or incriminated vector that is not described in the system, the country should send a report or publication to the Regional Leishmaniases Program. The report, which should follow the guidelines regarding the specific type of event as described in the section on SisLeish, will be assessed by a group of experts who will decide whether data on the corresponding species should be included in SisLeish.

7.7 Preventive Measures

Some preventive individual or collective protection measures should be promoted to avoid the transmission risks of cutaneous or visceral leishmaniasis. Measures depend on the environment and transmission pattern (primary sylvatic environment, intervened sylvatic environment, rural environment, peri-urban environment, and urban environment).

- Use of repellents when exposed to environments where vectors are usually found.
- Avoid exposure during hours of vector activity (from dusk to dawn) in environments where vectors are usually found.
- Use fine small mesh mosquito screens or nets with or without impregnation with insecticide and screens on doors and windows..

- Manage the environment by cleaning patios and land, to change the environmental conditions that favor the establishment of breeding sites for immature forms of the vector.
- Keep organic waste in adequate, separate sites to avoid potential vector breeding sites.
- Prune trees to increase illumination and reduce shaded land to avoid a favorable environment (temperature and moisture) for vector development.
- Keep animal shelters separate and always very clean.
- In areas of potential CL transmission, maintenance of a safety zone of 400 to 500 meters between dwellings and the woods is suggested.
- In areas of urban VL transmission, always keep plazas, streets and barren lots clean and reduce the time between garbage collection periods.

ENTOMOLOGICAL SURVEILLANCE, PREVENTION, AND VECTOR CONTROL

8. ENTOMOLOGICAL SURVEILLANCE, PREVENTION, AND VECTOR CONTROL

The goal of Leishmaniases Programs' entomological surveillance is to gather quantitative and qualitative information on transmitting vectors to support disease prevention, surveillance and control actions.

This chapter presents the objectives, entomological methodologies, and standardized indicators for CL and VL in the Region, as well as the activities according to the type of environment and risk stratification. Data analysis is important for decision-making; hence, it is necessary to record data for entomological collections using a specific format. A model is suggested in Annex 12 for countries that need a form for entomological data registration.

8.1 Cutaneous leishmaniasis (CL)

Entomological surveillance objectives and enabling activities

OBJECTIVES	ACTIVITIES/ ENTOMOLOGICAL METHODS
Confirm autochthony	Focus investigation
Estimate the most probable transmission sites	Entomological survey[1]
Identify existing vector species	Focus investigation and/or entomological survey
Guide collective and individual prevention and control actions	Focus investigation and/or entomological survey[1]

Entomological surveillance and control guidelines in areas with CL transmission

1) Primary sylvatic environment: it is not pertinent to establish entomological surveillance and vector control programmatic actions in this environment.

2) Intervened sylvatic, rural, or peri-urban environment:

   a) CL cases or outbreaks in areas with no transmission history:
      - Activity: focus investigation.
      - Indication for chemical control[2]: when the focus investigation confirms autochthony, conduct a chemical blockade of the focus and provide prevention and environmental management recommendations that have been validated for CL.

   b) CL outbreaks in areas of low transmission:
      - Activity: focus investigation.
      - Indication for chemical control: when the focus investigation demonstrates that there is domiciliary/ peri-domiciliary transmission, conduct a chemical blockade of the area where CL cases are concentrated and provide prevention and environmental management recommendations. Information on vector control is described in Annex 11.

NOTE 1: Methodologies for focus investigation, entomological survey, and monitoring are described in section 8.1.1.
NOTE 2: Information on vector control is described in Annex 11.

c) Moderate, high, intense, or very intense endemic transmission

- Activity: entomological survey
- Indication for chemical control:
  - When indices for transmission cases in children and/or women are higher than the country average and the entomological survey indicates that there is high vector abundance in peri-domiciliary areas far from primary / secondary vegetation. In the presence of atypical CL, discriminate the analysis groups when calculating transmission indices.
  - When the periodic entomological survey signals an increase in vector abundance in peri-domiciliary areas far from primary/secondary vegetation.

Chemical control and environmental management should be carried out according to the validated methodology for the species of CL vectors present in the focus, according to the site and moment of exposure risk. When validated methods are lacking, conduct action-oriented research to validate the methods.

NOTE: the implementation of seasonal vector monitoring studies stratified by transmission indicators and risk environments is recommended as complementary actions to better understand the bio-ecology of the vector and effectively guide surveillance and control actions. These tasks can be conducted by program experts or coordinated with research groups.

8.1.1 Entomological methods

8.1.1.1 CL focus investigation

Objectives

EPIDEMIOLOGICAL STATUS	OBJECTIVE
Presentation of first case in areas with no previous CL transmission	Identify the presence of the cutaneous leishmaniasis vector species at the probable infection location/s.
In outbreak areas	Guide chemical control measures, if feasible.

Methodologies (minimum activity proposed):

a) CDC light traps: traps are set in the case's home and probable transmission locations (determined through case history or inference about the socio-environmental risk), at a minimum. Traps are set within the residence (intra domicile), in the peri-domiciliary area, and in the extra domicile area (on the border with vegetation). The traps should be activated before dusk and remain functional for 12 hours, when they should be turned off. This process should be repeated for three nights during which environmental conditions are favorable (suitable temperature, little rain and moisture) for collection. If there is sufficient operational capacity, the placement of additional traps in four sites close to the case's residence is recommended, again, where favorable environmental conditions for vector presence exist. Considering available resources, it is possible to set traps simultaneously or consecutively, which means that upon closing a collection site, another one can be initiated.

b) Manual collection with motorized or manual suction tube: captures take place in the case's residence and probable transmission locations, at a minimum. Captures should be conducted both in the residence and the peri-domiciliary area of each location, for at least one night, when climate conditions are favorable for vector presence, and in a period between dusk and 22:00 or 23:00. Captures should last at least 30 minutes per person at the residency and 30 minutes per person in the peri-domiciliary area. If there is sufficient operational capacity, repetition of the procedure in the same locations where the CDC light traps were set is recommended. In the case of motorized suction, control the suction force so that it is adequate for sand flies.

c) Shannon traps: if there is operational capacity, complementing the captures using CDC light traps with captures using Shannon traps is recommended. The trap is set in the case's residence and in probable transmission locations. In each location, a trap is set either in the peri-domiciliary or extradomiciliary area (on the border with vegetation), in environments with favorable conditions for vector presence. Collection should be carried out from dusk to 22:00 or 23:00 and for at least one night with favorable climate conditions.

NOTE: temperature, relative humidity and precipitation should be recorded during collection days using the standardized form from the entomology service (Annex 12). These data should be analyzed according to existing knowledge of the vector species present in the area to determine when vector collection should be repeated or how collection may contribute to knowledge of the species in the area.

If the focus investigation results are NEGATIVE, the investigation should be repeated monthly, depending on climate conditions and according to operational capacity. If results continue to be negative (including the season of the year and climate conditions with greater theoretical abundance of the vector) for 6 months, the focus investigation is considered negative, but the locality remains designated as an area that requires periodic entomological surveillance. The result will be POSITIVE when at least one specie, considered of medical importance, is found using one or more of the collection methods. Confirmation of transmission in the domestic environment requires a more complex entomological study design, which can be carried out by program experts or coordinated with research groups.

NOTE: The focus entomological and epidemiological investigation should be integrated to define locations, activities, and probable moment of exposure to the vector-infection.

8.1.1.2 CL Entomological survey

Objective

EPIDEMIOLOGICAL STATUS	OBJECTIVE
In areas with moderate, high, intense, or very intense transmission.	• Identify the spatial distribution of vector abundance. • Guide prevention and chemical control activities. • Evaluate the impact of chemical control (periodic survey - monitoring).

Methodology

In areas without a previous survey, the guidance is to carry out the initial survey and repeat it whenever there is a situation of increased cases or an environmental modification.

a) CDC light traps: traps are set in at least 10 locations where recent cases reside and in probable transmission sites (established through case history or inference about the socio-environmental risk). Set two traps in every location, one in the residence (intra domicile) and another in the peri-domiciliary area (preferably in animal shelters). If there is primary or secondary vegetation, traps are added in the extra domicile area (on the border with vegetation). The traps should be set before dusk and remain functional for 12 hours. They should be set for three consecutive nights when there are favorable climate conditions.

NOTE: temperature, relative humidity and precipitation should be recorded during collection days using the standardized form from the entomology service (Annex 12). These data should be analyzed according to existing knowledge of the vector species present in the area to determine when vector collection should be repeated or how collection may contribute to knowledge of the species in the area.

8.1.1.3 CL Entomological Monitoring

Objectives

EPIDEMIOLOGICAL STATUS	OBJECTIVE
In areas with moderate, high, intense, or very intense transmission.	• Identify the bio-ecology and annual distribution of vector abundance. • Guide prevention and chemical control actions.

Methodology

Place CDC light traps and Shannon traps in at least 10 locations where recent cases reside and in probable transmission sites (established through case history or inference about the socio-environmental risk). Set two traps in every location, one in the residence (intra domicile) and another in the peri-domiciliary area (preferably in animal shelters). If there is primary or secondary vegetation, traps are added in the extra domicile area (on the border with vegetation). The traps should be set before dusk and remain functional for 12 hours. They should be set monthly for three consecutive nights when there are favorable climate conditions. This process should be repeated over a period of two years, preferably during the same week or lunar phase of every month.

To guide the operational objectives, according to logistical availability, initial stratification of the area based on transmission indicators and homogeneous biomes-landscapes is recommended. Then, proceed with the assessment and monitoring in each sector, to prioritize interventions according to risk probability.

NOTE 1: temperature, relative humidity and precipitation should be recorded during collection days using the standardized form from the entomology service (Annex 12). These data should be analyzed according to existing knowledge of the vector species present in the area to determine when vector collection should be repeated or how collection may contribute to knowledge of the species in the area.

NOTE 2: this methodology can be coordinated with research groups through specific entomological studies.

8.2 Visceral leishmaniasis (VL)

Objectives of entomological monitoring of VL

- Determine the presence of vectors and the risk of local transmission.
- Define the transmission risk in cases' residences in order to conduct the focus intervention.
- Specify vector abundance in space and time to guide canine survey and transmission control activities.

8.2.1 Methodologies

The methodologies are the same as those described for cutaneous leishmaniasis:

- Focus investigation
- Entomological survey
- Entomological monitoring

8.2.2 Guidelines for control

In all situations, prevention and environmental management recommendations will be made to reduce the reproductive success of vectors and minimize vector contact with humans or reservoirs. Furthermore, it is necessary to intensify guidance on responsible pet ownership and reproduction. Complementary chemical control is only indicated for specific situations that are detailed for each epidemiological status. Information concerning vector control is described in Annex 11.

1) Rural environment:

In outbreak situations:

Human or canine cases of VL in areas WITH NO transmission history

- Activity: focus investigation. Follow guidelines already described for CL.
- Indication for chemical control: if the focus investigation confirms that the first cases are autochthonous, conduct chemical blockade in the focus investigation area or locality, as justified by the number and density of the residences. A methodology that has been validated for VL vectors should be used or, if lacking, impact-action research should be used to validate the intervention.

Increase in human VL cases in areas WITH known transmission:

- Activity: entomological survey. Follow guidance already described for CL.
- Indication for chemical control: if there is a concentration of cases and presence of vectors (according to the survey), chemical blockade should be implemented in the locality, as justified by the dispersion of the cases and the density of the residences. Ongoing evaluation of the impact of chemical control, through periodic surveys and incidence studies of domestic reservoirs, is suggested.

2) Peri-urban/urban environment:

a) In OUTBREAK situations:

First case of human or canine VL in areas WITH NO transmission history

- Activity: focus investigation. Follow guidance already described for CL.
- Indication for chemical control: if the focus investigation confirms that the first cases are autochthonous, conduct chemical blockade in the focus investigation area. A methodology that has been validated for VL vectors should be used or, if lacking, impact-action research should be used to validate the intervention.

First case of human VL in areas WITH previously established canine transmission

- Activity: entomological survey. Follow guidance already described for CL.
- Indication for chemical control: the relevance of a chemical intervention will be evaluated.

Interventions should be conducted in circumscribed spaces whose size can guarantee the quality and operational feasibility of the intervention in a short time. Ongoing evaluation of the impact of chemical control, through periodic surveys and incidence studies of domestic reservoirs, is suggested.

Increase in human cases of VL relative to the number of expected cases

- Activity: entomological survey. Follow guidance already described for CL.
- Indication for chemical control: the relevance of conducting a chemical intervention when there is a high concentration of incident human cases and an increase in vector abundance will be evaluated. The intervention should be conducted in a circumscribed space whose size can guarantee the quality and operational feasibility of the intervention in a short time. Ongoing evaluation of the impact of chemical control, through periodic surveys and incidence studies of domestic reservoirs, is suggested.

b) Areas with LOW transmission

- Indication for chemical control: specific entomological surveillance or control actions are NOT recommended beyond the recommendations of prevention and environmental management.

c) Areas with MODERATE, HIGH, INTENSE, OR VERY INTENSE transmission

- Activity: According to the intensity of transmission and the operating capacity, implementation of monitoring or seasonal or annual survey is recommended.
- Indication for chemical control: considering the lack of evidence about the impact of chemical control and given the low effectiveness and/or residual effect of insecticides that are currently in use, the following is suggested: intensification of prevention and environmental management actions, the actions indicated in the chapter on reservoir surveillance and control, and actions related to responsible pet ownership and reproduction. When chemical control is implemented, carry out both pre- and post-surveys using an impact research design and serological surveys at least once a year.

NOTE: the implementation of seasonal vector monitoring studies stratified by transmission indicators and risk environments is recommended as complementary actions to better understand the bio-ecology of the vector and effectively guide surveillance and control actions. These tasks can be conducted by program experts or coordinated with research groups.

8.3 Entomological Indicators

a) Averages for CDC light traps, by collection point

- Objective
  - Estimate and compare the average vector abundance by capture site and environment (intra-domiciliary area, peri-domiciliary area, extra-domiciliary area).
- Usefulness
  - Guide prevention and control actions according to the absolute and relative vector abundance within environments, taken as an indicator of probable transmission location.
  - Evaluate the impact of control actions.

$$\text{Intra-domiciliary area} = \frac{\Sigma \text{ Num. of specimens captured in the home, by species}}{\text{Num. of days worked (monthly average)}}$$

$$\text{Peri-domiciliary area} = \frac{\Sigma \text{ Num. of specimens captured in peri-domiciliary area, by species}}{\text{Num. of days worked (monthly average)}}$$

$$\text{Border of vegetation} = \frac{\Sigma \text{ Num. of specimens captured on the border vegetation, by species}}{\text{Num. of days worked (monthly average)}}$$

Σ = **Summation**

b) Monthly average for Shannon traps, by species and trapping site

- Objective
  - Estimate the average abundance of anthropophilic vector species in the peri-domiciliary area.
- Usefulness
  - Guide prevention and control activities according to the absolute and relative vector abundance with CDC light traps and Shannon traps in the same site, taken as an indicator of probable transmission location.
  - Evaluate the impact of control actions.

$$\text{Peri-domiciliary area} = \frac{\Sigma \text{ Num. of specimens captured in the trap, by species}}{\text{Num. of collectors/capture day}}$$

Σ = **Summation**

c) Monthly average for manual capture, by species and by site

- Objective
  - Increase the probability of finding anthropophilic vector species in the domestic (endophilic and/or endophagic) and peri-domestic environments with the CDC light trap, to complement focus investigation.

- Usefulness:
  - Contribute to the characterization of autochthonous transmission.
  - Guide prevention and control actions in the probable transmission location.

$$\text{Intra-domiciliary area} = \frac{\Sigma \text{ Num. of specimens captured in the home, by species}}{\text{Num. of collectors}}$$

$$\text{Peri-domiciliary area} = \frac{\Sigma \text{ Num. of specimens captured in the peri-domicile, by species}}{\text{Num. of collectors}}$$

Σ = **Summation**

N.º of collectors: with standardized capture effectiveness (time/person)

NOTE: indicators by site can be used to average the abundance by locality or by higher aggregation level, to compare infestation intensity.

8.4 Preventive measures

Vector preventive measures are described in the chapters: Surveillance of human cases and Surveillance and control of domestic reservoirs.

SURVEILLANCE, PREVENTION, AND CONTROL OF DOMESTIC RESERVOIRS

9. SURVEILLANCE, PREVENTION, AND CONTROL OF DOMESTIC RESERVOIRS

In the Americas, leishmaniases are zoonotic diseases whose reservoirs are wild or synanthropic animals or domestic animals, depending on the species of *Leishmania* involved. In general, there is a main reservoir for each specific type of *Leishmania* in each focus. However, the same area can have other infected mammals, which then constitute incidental reservoirs that can form a "community" and participate in maintaining the transmission chain. On the other hand, the mere presence of infection in a species of mammals, even if it is in numerous individuals, does not necessarily indicate that the species is a reservoir. Other pre-established criteria need to be met for a species to be considered a reservoir.

CUTANEOUS LEISHMANIASIS (CL)

Different wildlife, synanthropic and in some situations domestic animals have been confirmed as possible reservoirs of CL for this Region. However, **no surveillance and control actions have been indicated** for them.

VISCERAL LEISHMANIASIS (VL)

For *Leishmania infantum*, the parasite species that causes VL in the Americas, the dog is the main urban reservoir. Additionally, it has been demonstrated that asymptomatic dogs that have been infected naturally can be very infective for sand flies. Hence, dogs play an important role in transmission maintenance, since nearly 50% of all infected dogs are asymptomatic or pre-symptomatic carriers.

The sum of several phenomena related to the urbanization of VL in some countries of the Region, — Brazil, Paraguay and Argentina — such as vector dispersion and adaptation to the urban environment and an increase in the dog population in these areas, has made the importance and role of dogs in the VL transmission chain emerge as more evident and fundamental. In addition to the increased number of dogs and humans exposed to VL in areas with high population density, other factors such as mobility and frequent environmental changes induced by occupations and migration to urban areas also favor the territorial expansion of the disease and hinder the development of systematic, integrated disease control actions. Thus, the implementation of such actions constitutes a true challenge for public health institutions, which are the parties directly responsible for making decisions and implementing tasks to meet this obligation.

In the Technical Report Series 949 (2010), the WHO emphasizes the importance of dogs as reservoirs of *Leishmania infantum* in the Americas and the complexity of controlling this canine reservoir as part of VL control. Despite the complexity of the diagnosis and epidemiology, the document points out that when working toward public health-related objectives, canine euthanasia is justified as a control method and that this method should adapt to each local situation. The document also considers that the theoretical ideal would be to eliminate all symptomatic or seropositive dogs in epidemiological scenarios where dogs are important for the transmission cycle. However, screening and mass euthanasia of seropositive dogs has not demonstrated uniform effectiveness in control programs (for example, in Brazil), due to operational problems, diagnostic sensitivity, rejection by the population, replacement of dogs in scenarios that maintain the exposure level (see below), and the need to maintain the area under continuous surveillance and with systematic control.

To date, endemic countries have had several experiences with the implementation of surveillance and control actions for canine VL in the Americas. In each case, they have been adapted to the different epidemiological transmission scenarios, which can be sporadic, controllable/stable, or in expansion. It has been noted that in countries with sporadic transmission, where the transmission pattern is predominantly rural, reservoir surveillance and control actions have not been necessary. On the other hand, in countries in which VL is urban and is expanding geographically or controllable, reduction of the prevalence of infected dogs has been the greatest challenge for control programs, even when carried out with the first canine or human cases, when the intervention is more likely to be implemented and be effective, due to its dimensions and impact.

Of the activities currently conducted by National Leishmaniases Programs, those targeted at the domestic reservoir infected by *Leishmania infantum* have been and continue to be the most questioned by different stakeholders. This is partly because scientific uncertainties persist about the effectiveness of such activities for reducing human disease incidence, but it is also due to the negative social impact of the practice of euthanasia in dogs.

In 2014, with the goal of building a baseline for decision-making on this problem, the Ministry of Health of Brazil — a country that reports 96.6% of the VL cases in the Region — commissioned a group of researchers to carry out an exhaustive literature review on the subject. The review's objective was to evaluate available evidence on laboratory diagnostic tools for canine VL and control measures for infected reservoirs and vectors that are applicable to the Region.

Subsequently, with PAHO/WHO support, another group of specialists met to work on the task of evaluating the evidence and effectiveness of the implementation of reservoir control actions. This group had the foundation of both the results of the aforementioned review and an unpublished assessment of service and study results. The conclusions showed that:

- There is insufficient evidence to reach conclusions about the effectiveness or ineffectiveness of the canine VL surveillance and control strategy on the incidence of human VL, whether interventions are targeted at the vector or combined with actions targeted at the reservoir.
- There is no evidence to validate the effectiveness of national risk stratification to guide activities, which started in 2004. However, services' experiences in Brazil, with risk stratification to define action and the theoretical foundations of communicable disease surveillance and control suggest that the approach was effective for prioritizing control actions in municipalities with intense and moderate transmission. However, given that control actions are not proposed for municipalities without transmission or with sporadic transmission when this stratification criterion is applied, the needs of these municipalities were not considered in the conclusions.
- Despite some theoretical evidence and observational studies that prove that control measures for reservoirs and vectors are effective, there are no controlled experimental studies that demonstrate the effectiveness and impact of such measures on transmission.
- Currently proposed control actions have not been able to be implemented with strict fidelity to all of their technical requirements, due to logistical, operational, and managerial difficulties such as, among others: the lack of necessary human resources, vehicles and supplies; the low coverage and sustainability of the actions themselves; the limitations of diagnostic techniques; community resistance to accepting control actions; and the difficulties represented by the common community practice of immediately replacing the euthanized dog with new dogs that are susceptible to infection, maintaining the risk environment in the affected areas. Furthermore, it is recognized that there are difficulties with data collection and analysis at the local level. Another

aspect that has hindered the effectiveness of actions is the widespread notion that VL control is the exclusive responsibility of the health sector. This demonstrates that in addition to promoting the executive areas of control, the following should be facilitated: the integration of other public sectors such as those responsible for the environment, sanitation, infrastructure, and urban planning; and efforts to ensure that the entire community assumes the commitment to disease control.

- For countries with greatest number of cases, it is essential to establish a local stratification model for medium - and large-sized municipalities, through representative indicators that consider local conditions and operational feasibility at the municipal level. For example, these could include intraurban strata according to canine prevalence, number of human cases and incidence, vector density, and environmental and social characteristics.

Based on the points addressed and summarized above, the group of expert meeting participants concluded that surveillance and control actions for reservoirs and vectors should be maintained according to current standards, until new evidence emerges.

CANINE VISCERAL LEISHMANIASIS (CVL)

CVL is a systemic disease in which the clinical manifestations depend on the immunological response of the infected animal. The clinical expression can range from an apparently healthy animal to severe disorders that can be fatal.

Infection starts in the site of the vector bite, where the parasites are found. Subsequently, the parasites migrate toward the viscera and finally, the parasites are distributed through the dermis.

The classical symptoms of CVL are skin lesions, mainly furfuraceous desquamation and eczema, which are more common in the nasal region and ear. There are also often small shallow ulcers on the ears, muzzle, tail, joints, and dull coat. In the most advanced cases, the following is observed more frequently: onychogryphosis (atypical nail growth), splenomegaly, lymphadenopathy, alopecia, dermatitis, skin ulcers, keratoconjunctivitis, coryza, apathy, diarrhea, intestinal hemorrhage, feet edema, vomiting, and hyperkeratosis. In the final phase of infection, paresis in the posterior limbs, cachexia, starvation and death occur. Furthermore, infected dogs can remain without clinical symptoms for a long period of time.

Classification according to clinical symptoms:

- Asymptomatic dogs: absence of clinical symptoms suggestive of infection by *Leishmania*.
- Oligosymptomatic dogs: presence of lymphoid adenopathy, minimal hair loss, and dull coat.
- Symptomatic dogs: all or at least three concurrent symptoms of disease. The most common are skin alterations (alopecia, eczema, ulcers, hyperkeratosis), onychogryphosis, weight loss, keratoconjunctivitis, and paralysis of posterior limbs.

9.1 Diagnostic methods for canine visceral leishmaniasis (CVL)

Currently, the diagnosis of CVL continues to be a problem for public health services, since there is a wide range of signs and clinical symptoms of CVL that can be caused by other pathologies and there is no diagnosis test that is 100% specific and sensitive.

For the use of CVL diagnostic tests and the design of activities and actions based on these tests in public health services, programs should consider the transmission area's pattern and distribution, human and financial resources, operational limitations, and limitations when interpreting the method used.

For surveillance and stratification of CVL at the population level, health services use immunological tests given their ease of use and acceptable precision and that these tests can be applied simultaneously to a large number of animals in a short amount of time. The limitation is that while symptomatic dogs generally produce higher levels of antibodies and can be easily detected, the sensitivity of antibody detection is usually weaker for initial infections or asymptomatic animals. There can also be problems with specificity depending on the antigens used, especially cross-reactivity with other *Leishmania* or *Trypanosoma* species.

Available immunochromatography rapid tests, which are specific for the *L. donovani* complex (that includes *L. infantum*), use recombinant protein K39 as the antigen. These are qualitative tests that detect antibodies in canine serum, plasma or blood (the use in all or some of these is indicated by the manufacturer). They are the most common routinely used immunological tests among health services in the Region.

The Kalazar Detect (Kalazar DetectTM Rapid Test, Canine, InBios International) is a test that detects antibodies in canine serum. It was validated in two countries: Brazil, with a sensitivity of 83% and a specificity of 100% (Lemos et al., 2008); and Argentina, with a sensitivity of 90% and a specificity of 99%, using a controlled sera panel (Solomon et al., in press).

The Dual Path Platform rapid test (TR-DPP®-Bio-Manguinhos) was also validated in two countries: Brazil, with a sensitivity of 83% and a specificity of 73% (Peixoto et al., 2014); and Argentina, with a sensitivity of 93% and a specificity of 98%, using a controlled sera panel (Solomon et al., in press). This test can be applied with sera, plasma or whole blood samples, making it easier to use in the field.

Several other immunochromatographic tests are available in the Americas, but they have not been validated on a large scale. Furthermore, other serological tests that can be used are: indirect immunofluorescence (IIF), with a sensitivity of 88% and a specificity of 63%; the immunoenzymatic test (ELISA), with a sensitivity of 89% and a specificity of 87%; and the direct agglutination test (DAT), with a sensitivity of 91% and a specificity of 100% (Peixoto et al., 2015; Oliveira et al., 2016). It should be considered that the values of all immunological diagnostic tools can vary with transmission intensity, the geographical scenario, and the reference standard (gold standard) used.

Parasitological diagnosis is a method based on demonstrating the presence of the parasite in biological material obtained from liver, lymph node, spleen, and bone marrow biopsies and from skin biopsies or smears. These procedures are invasive, meaning that there are risks for the animals, and are impracticable methods for widespread use by public health programs, where a large number of animals should be tested in a short period of time. The use of this method by public health services is indicated only for case confirmation and identification of the *Leishmania* species, when the first autochthonous canine case of CVL appears in an area previously considered as with no VL transmission.

9.2 Canine surveillance for visceral leishmaniasis

The different epidemiological scenarios in the Region should be considered for canine surveillance, as already described in chapter 6. It is important to understand the relative weight of dogs in the VL transmission cycle in areas that have cases of human VL.

In South America, there is a high prevalence of dogs infected by *L. infantum* and their function as a domestic reservoir of VL is well-characterized in countries with scenarios of transmission that is stable/controlled or in expansion. However, surveillance and control actions should be applied in accordance with risk stratification at the local level. Stratification should rely on the greatest possible amount of available information, such as: intraurban strata according to canine prevalence; number of human cases and incidence; vector density; and environmental and social characteristics.

Furthermore, in countries with sporadic VL transmission scenarios, like in Central America, it is necessary to understand the importance of dogs in the VL transmission cycle. This will allow decision-makers to make the best decisions and direct appropriate surveillance and control actions. For atypical cutaneous leishmaniasis (ACL), it is necessary to know if the dog acts as a domestic reservoir seeing that VL and ACL occur in the same transmission cycle.

9.2.1 Case definition for CVL

- Suspicious canine: animals from VL endemic areas or where a VL outbreak is occurring, with one or more clinical manifestations compatible with the disease (irregular fever, apathy, weight loss, furfuraceous desquamation, skin ulcers that are usually on the muzzle, ears and limbs, conjunctivitis, paresis of posterior limbs, bloody feces, and exaggerated growth of nails).
- Confirmed case:
  - Laboratory criterion: suspicious canine with clinical manifestations compatible with CVL and that has a positive serological test and/or a positive parasitological examination.
  - Clinical criterion and epidemiological link: suspicious canine with clinical manifestations compatible with CVL and epidemiological nexus without laboratory test confirmation.
  - Asymptomatic infected canine: every canine without clinical symptoms, but with positive serology and/or a positive parasitological examination for VL and that lives in a confirmed VL transmission area or is from a VL endemic area.

9.2.2 Surveillance activities for CVL

Surveillance activities for CVL are targeted at areas WITH transmission and areas WITH NO transmission that are vulnerable and receptive to VL.

- Areas WITH NO transmission that are vulnerable and receptive to VL: Follow the guidelines described for surveillance and control actions in detail when suspicion of the first human or canine VL case emerges (Flow charts 12 and 14).
- Areas WITH low, moderate, high, intense and very intense VL transmission; and only canine transmission: Follow the guidelines described for surveillance and control actions in detail (Flow chart 15).

9.2.3 Surveillance of domestic reservoirs for visceral leishmaniasis

9.2.3.1 Serological surveys

Selected methodologies are proposed below to conduct serological surveys in dogs for the purposes of surveillance and control.

Serological surveys comprise the collection of biological samples from dogs in a population or area of interest for VL surveillance and control, to confirm the presence of dogs infected with *Leishmania infantum*. The purpose is to be able to direct measures, prioritize areas of public health interest, and identify VL prevalence in a specific area. Considering their objective and other factors such as information and available resources, surveys can be classified as:

a) Census survey

In the census survey, samples are collected from all of the dogs in the population or reference area. The purpose of the census survey is to identify infected dogs so that it is possible to direct control actions and identify the prevalence of canine infection in a given area or population. This type of survey is indicated in the following epidemiological situations:

- In rural areas of municipalities with transmission of human or canine VL.
- In urban areas of municipalities with presence of VL cases, where the canine population is less than 500 dogs.
- In urban areas of municipalities with presence of VL cases (moderate, high, intense and very intense transmission) and a dog population greater than 500 animals, if the action area is delimited to a sector/neighborhood or area where human cases occur and the vector is present. The periodicity can be annual or semiannual, according to transmission intensity, supply availability, and operational capacity.

b) Sample survey

In the case of a sample, specimens are collected from a selected group of dogs in the population or reference area. Samples are used when it is not possible to collect samples from all of the dogs in a required population or area for logistical or resource reasons. According to the epidemiological situation, the sampling process can have two essential objectives (which will determine its design): one can be to estimate the prevalence of canine infection in known transmission areas to prioritize control actions; and another can be to detect the presence (and/or distribution) of canine infection in receptive and vulnerable areas with no transmission.

In a simple sampling process, the purpose is to achieve a representative sample of the population from which the sample will be extracted. Random methodologies (drawings) are used to avoid biases when selecting the dogs to be tested. However, these sampling strategies, since they depend on the population and prevalence, tend to be extensive and therefore expensive.

Another strategy consists of directed or risk-based sampling. The situations that pose a greater risk receive a higher resource priority, since they favor an increased cost-benefit ratio performance. Thus, these sampling strategies focus on those components of the population or area where there is greater probability of finding the infection. To carry out risk-based sampling, it is necessary to have reliable, exhaustive, complete, and up-to-date information about the population, the area, vector distribution, infection risk factors, etc. This type of sampling is indicated particularly in the following situations:

- In areas with no VL transmission that are classified as vulnerable and receptive or with the occurrence of the first human or canine case, and according to the guidelines specified for that epidemiological situation.
- In urban areas with VL transmission, to identify the distribution and prevalence of the infection and direct surveillance and control actions based on this information. It is necessary to delimit the sampling area. Periodicity can be annual or semiannual in accordance with transmission intensity, supply availability, and operational capacity.

9.2.3.2 Methodologies for sample surveys

Methodology 1: This methodology is used by the National Leishmaniases Program of the Ministry of the Health of Brazil in municipalities or areas that are delimited according to their size and vector distribution.

For each area, the sample is calculated by considering the expected CVL prevalence and the number of dogs, as shown in Table 17. For localities with known prevalence estimates, the value in the table should be used as a parameter. When the estimated prevalence is unknown, use of a prevalence of 2% is recommended.

In each area, a given number of blocks is drawn until reaching the number of dogs that represents the sample. To calculate the number of blocks, the following averages should be considered: each block has 20 dwellings; each dwelling has 4 people; and the ratio of the number of dogs to the number of people is 1:5. As a result, it is estimated that each block has an average of 16 dogs. Thus, the number of assigned blocks could be determined through the formula:

$$Q = N \times 2 / \hat{A}$$

Where:

Q is the estimated number of blocks to be sampled;

N is the estimated number of dogs in the sample/sector;

Â is the average number of dogs/block, which in this case is estimated to be 16.

TABLE 17 - Sample size (num. of dogs) according to the estimated dog population in the area and the expected disease prevalence in dogs, at a significance level of 5%.

ESTIMATED POPULATION BY SECTOR	EXPECTED / OBSERVED PREVALENCE (≤0.05), $\alpha = 0.05$						
	≤1.0	1.1- 2.0	2.1 - 3.0	3.1 - 4.0	4.1 - 5.0	5.1 - 9.9	≥ 10.0
500 - 599	356	300	240	212	184	137	108
600 - 699	430	334	272	228	196	144	112
700 - 799	479	363	291	242	206	149	115
800 - 899	524	388	306	252	214	153	118
900 - 999	565	410	320	262	220	157	120
±1000	603	430	332	269	226	159	121

Source: Secretariat of Health Surveillance, Ministry of Health, Brazil.

Any available method can be used to select the blocks. For a better spatial distribution of the sample in the area, systematically working with 50% of the dwellings that exist in each block is suggested. This way, sampling starts in the northernmost corner of each block, from the margin of the house that is selected (first or second), continuing alternately in a clock-wise direction, until the block has been covered in its entirety.

Methodology 2: This methodology is used in Argentina for the simultaneous survey of vectors and canine visceral leishmaniasis (Argentine Leishmaniasis Research Network (REDILA) - National Institute of Tropical Medicine)

The total area of the city is divided into a grid formed by 400 x 400-meter quadrants. This area was defined based on the spatial autocorrelation of the abundance registered for *Lutzomyia longipalpis* in the region. Depending on city size and operational capacity, sampling is conducted in all of the quadrants or in a sub-sample by strata. Sampling is usually conducted in all of the grid cells in localities that have up to 100 quadrants. However, in cases of specific interest, sampling has been conducted in all of the grid cells in localities that have up to 400 quadrants.

In the quadrants to sample, select a house that is considered a "critical site" since it presents the environmental criteria with greater probability of vector presence and/or history of human VL cases or canine density. The distance between selected critical sites from contiguous cells should be greater or equal to 150 meters. In each selected critical site: vector sampling is conducted as explained in the corresponding survey section; and a serological sample is drawn from at least five dogs whose sleeping area is near the light traps, whether they are dogs from the same house or from several neighboring dwellings, until completing the number, regardless of their clinical condition.

Canine sampling may not be simultaneous to vector sampling, just as analysis of vector abundance and estimated canine prevalence is not simultaneous. The number of dogs sampled to characterize the point prevalence in the critical site is based on the estimated attraction area for the light trap and the average number of dogs per household. For stratified sampling, in the event of resource or operational limitations, the strata are delimited after making the 400 x 400-meter quadrant grid, which are uniform areas according to landscape criteria. The landscape approach presumes the presence of one or more environmental factors that affect vector distribution and human and dog demography and that these factors can be expressed in a map (for example, vegetation cover density and urban heterogeneity). Between 25 to 40 quadrants are randomly selected in each stratum, with a higher number of quadrants assigned to those strata that have greater environmental variability (more heterogeneous areas). In each selected quadrant, selection and sampling of the critical site is conducted, as described in the previous paragraph.

This method is used to define and associate the entomological risk and the distribution of canine VL. With regard to the random selection, canine prevalence obtained using this method tends to be biased toward higher values since part of the sampling criterion is sites with greater probability of vector presence. Nevertheless, it allows a delimitation adjusted by autocorrelation of risk areas to prioritize actions. Furthermore, in sites with recently established transmission, it has greater sensitivity for detection of vectors and canine cases.

9.2.4 Indicators for surveillance and control of domestic reservoirs

1. Expected prevalence in dogs:

$$\frac{\text{Num. of VL-positive dogs in an area}}{\text{Total dog population in an area}} \times 100$$

USE: this indicator evaluates the estimated proportion of animals infected in a population in a specific area and time. This indicator should be evaluated jointly with the other vulnerability indicators to complement the definition of priority areas. It can also be used to evaluate the impact of control measures.

NOTE: it should be calculated based on data generated from a census or sample survey.

2. Proportion of dogs examined in census surveys:

$$\frac{\text{Num. of infected dogs screened for VL diagnosis by area}}{\text{Num. of dogs that exist in the area}} \times 100$$

USE: this indicator evaluates the coverage of the serological census survey.

NOTE: it should be calculated based on data generated from a serological census survey.

3. Proportion of dogs that is positive for CVL:

$$\frac{\text{Num. of infected dogs}}{\text{Num. of examined dogs}} \times 100$$

USE: this indicator evaluates canine positivity for VL in an area where a serological survey was conducted.

NOTE: it should be calculated based on data generated from a census or sample survey.

4. Proportion of infected dogs subjected to euthanasia:

$$\frac{\text{Num. of infected dogs subjected to euthanasia}}{\text{Num. of infected dogs}} \times 100$$

USE: this indicator seeks to evaluate the coverage of the control measure (euthanasia) in reservoirs in the locality.

NOTE: it should be calculated based on data generated from a serological census or sample survey.

9.3 Individual prevention measures for visceral leishmaniasis in dogs

The following prevention measures are recommended for owners.

- Contact between animals and vectors should be reduced as much as possible, mainly at the time of greater vector presence, which goes from dusk until dawn.
- Use of repellents with residual effect on dogs.
  - In Brazil, a significant reduction (50%) in CVL prevalence was demonstrated with the use of collars impregnated with deltamethrin 4% ScaliborTM in areas where 50% of the canine population used the collar, in comparison with the area with no intervention (control group) (unpublished observations, Werneck 2016). The same method also reduced *L. longipalpis* in the peri- and intra-domiciliary area (Albuquerque et al., 2018). For public health use, cost-effectiveness studies should be carried out, to subsequently evaluate specific strategies and indications while considering the availability of financial resources and logistics associated with the activity.
- Vaccine: there are currently two vaccines to prevent CVL in the global market, one in Europe and another in Brazil, with variable levels of protection. However, studies to verify the effectiveness of vaccine use on reducing the human incidence of VL are still lacking. This means that their use has been restricted for the individual protection of dogs but has not been validated as a strategy for human disease control.

9.4 Clarification about the treatment of canine visceral leishmaniasis

The success rate of treatment of dogs with VL is lower than the rate observed in humans treated with similar drugs. Dogs have frequent relapses and, in many cases, drug administration is needed for life. In countries in the European Mediterranean, the individual treatment of infected dogs is practiced widely, with the main purpose of extending the animal's life by reducing the parasitic burden and diminishing clinical signs, even when the treatment does not have the capacity to promote parasitological cure. As a result, animals treated in this way continue to be sources of infection for the vector over a longer period than if they had been sacrificed or had died due to disease progression.

Theoretically, treatment of CVL could be effective for the control of human VL. However, according to a systematic review on the treatment of CVL conducted by the Ministry of Health of Brazil (unpublished data), current scientific evidence is insufficient to draw conclusions about the efficacy of any of the drugs or the immunotherapy of the different therapeutic regimens, due to studies' methodological limitations. On the other hand, there is scientific evidence showing that treatment can reduce the infectiousness of treated dogs sufficiently so as to significantly impact transmission to the human population. Authors recommend that new controlled studies with appropriate methodologies be carried out, for the purpose of clarifying gaps related to the potential efficacy of treatment of CVL for reducing transmission of the parasite to humans.

In this context, treatment of dogs is **not recommended** as a control strategy for canine or human VL, until there is rigorous scientific evidence of its effectiveness. However, even if the impact of treatment for dogs on reductions in cases of human VL were verified, a systematic program of mass treatment of dogs with VL may not be practical from a financial and operational standpoint.

Furthermore, due to the absence of an effective parasitological cure and the recurrence of relapse in dogs, treatment should be repeated periodically. However, this would increase the risk of parasite populations that are resis-

tant to the drugs currently used for human treatment. Thus, avoidance of treatment of these animals with drugs used for human treatment is recommended.

In Southern Cone countries, public and private veterinary services often discuss this subject. For example, in March 2015, using the aforementioned systematic review as a foundation, the Ministry of Health of Brazil held a forum to discuss the effectiveness of treatments for CVL on the incidence of human VL. At this meeting, a group of experts, researchers, and representatives of several societies of human and veterinary health professionals found that, with the current evidence, it is not possible to recommend the use of CVL treatment in Brazil as part of the VL Program's monitoring and control efforts. However, the same group also pointed out that the treatment of dogs with products that are not used to treat human VL is admissible as an individual measure, whenever:

- There are established treatment protocols that have the best scientific evidence about the treatment's efficacy for clinical cure and reduced parasitic burden;
- Treatment is associated with protective measures for the dog, such as insecticide-impregnated collars and topical repellents, during pharmacological treatment to minimize risk of the transmission to the human population;
- Criteria are established for follow-up of the dog in treatment for the purpose of reducing the risk of infection for the human population;
- The responsibilities for follow-up and compliance with treatment protocols are defined;
- There is an established flow of information about the animals under treatment among the organisms in charge of animal and public health;
- When there are animals that do not respond well to treatment or whose owners do not comply with treatment, treating veterinarians should inform the official health services and submit the animal for euthanasia.

Another example took place in Argentina in August 2015 when public health agents and professionals from the federation of private practice veterinarians met to reach a consensus on the management of CVL and the creation of an intersectoral commission for periodic evaluation of new evidence (in progress). Considering that vector-borne transmission risk only affects a limited area of the territory but that CVL is potentially distributed across the country due to the movement and trafficking of dogs, activities were differentiated according to vector risk scenarios. The consensus proceedings were consistent with what is presented in the previous paragraph for Brazil. The meeting also resulted in the commitment of signing a document for animals treated, between the local program representative, the attending veterinarian, and the animal's owner. Furthermore, it included castration of infected animals to avoid vertical and horizontal transmission of the infection.

LEISHMANIASES RESEARCH

10. LEISHMANIASES RESEARCH

The leishmaniases research for surveillance and/or control are indicated in the following situations:

- Areas with registry of autochthonous cases, but absence of sufficient epidemiological and entomological information to generate surveillance and control programmatic actions.
- In outbreak situations, the research will be defined separately for CL and VL.

10.1 Cutaneous leishmaniasis (CL)

10.1.1 Areas with registry of cases, but absence of epidemiological and entomological information

- Epidemiological investigation of current and historical cases: carry out analysis of secondary data available in the health system. Carry out interviews with any recent cases to determine perceptions of transmission sites and moments. Interview stakeholders, including cases, to develop hypotheses about transmission.
- Entomological investigation: carry out entomological surveys in risk sites where there is a favorable environment for the vector and epidemiological history. If there are any recent cases, include additional studies in the domestic environment. The trapping methodology and scheme will depend on the environments and intensity of transmission. The suggested approach is to repeat trapping efforts in each environment evaluated during successive days and to repeat, if necessary, during the season with the highest expected vector abundance. The trap placement methodology according to the epidemiological situation was already described in the section on entomological surveillance and control.
- Active case-finding: carry out case-finding in environments considered to be high exposure and with identified risk groups (for example, occupational risk).
- Evaluation of the sensitivity and specificity of the notification system: evaluation of sensitivity using the capture-recapture methodology, carried out collaboratively with research groups, is recommended.
- Analysis and return of results: present and discuss results with local health agents and managers. Epidemiological and entomological aspects should be detailed, with an emphasis on the species of sand flies identified, probable vectors, the distribution of sand fly and vector abundance in time and space, the transmission pattern, and other biological and social risk factors. If necessary, adequate guidance should be provided regarding the development of training activities on surveillance and the need to strengthen the technical capacity of professionals and the service.
- Guidance: prevention, surveillance, and control activities for human cases and the vector should follow previously-established indications.
- Identification of the parasite: if feasible, sample collection and identification of the circulating parasite species is recommended. This recommendation is of greater importance when: the circulating parasite is unknown in the region; in situations in which there are confirmed clinical manifestations that are different from the habitual ones; and in cases with treatment failure and frequent relapses. According to PAHO, the Regional Reference Laboratory for the identification and genetic sequencing of Leishmania is the Laboratory on Leishmaniases Research, IOC/FIOCRUZ. For more information on CLIOC, go to: http://clioc.fiocruz.br/.

- Specific reservoir and entomological studies: studies whose objective is to learn about reservoirs and vectors in detail — for example, absence of known vectors, eating habits, schedule pattern, rate of infection, etc. — should be coordinated with research groups. The feasibility of research will depend on the environments involved and is higher in the case of peridomestic transmission (domestic and synanthropic animals).

10.1.2 Outbreak situation: in areas with no transmission, with the first cases of CL, or in areas with transmission, but with increased cases (relative to the expected number)

- Epidemiological investigation of current cases: carry out collection of demographic and clinical data such as place, date, probable infection sites, date of symptom onset, diagnosis date, diagnostic methods, treatment scheme and response. Carry out an interview about the risk factors (trips, visits to area(s) with transmission, occupation, etc.) and disease itinerary. With regard to the probable infection period, pay special attention to occasional visits to endemic areas, which are not usually perceived as "trips" by the interviewee. Compile data (delimited in time and space) about the natural events and anthropic actions that could be related to the outbreak (for example, climatic or hydrologic change, migration, deforestation, increase in risky practices, etc.).
- Active case-finding: conduct surveys in health centers and review medical records with compatible symptomatology. Through interviews and using the "snowball" method: locate other cases; contact and speak with stakeholders and members of identified risk groups (for example, occupational risks); determine environments considered to be high exposure (for example, settlements at the edge of the primary vegetation); and refer people with suspected clinical manifestations to the health center.
- Entomological investigation: carry out this type of investigation in risk sites if there is an environment that is favorable for the vector and epidemiological history, including recent domestic environment conditions of the case(s) and recent modifications in the environment, include sites that the community perceives to be risk sites. The trapping methodology and scheme will depend on the environments and the intensity of transmission. The recommendation is to repeat trapping efforts in each environment evaluated during successive days and to repeat, if necessary, during the season with the highest expected vector abundance. The trap placement methodology according to the epidemiological situation was already described in the section on entomological surveillance and control.
- Analysis and return of results: present and discuss results with local health agents and managers, generating hypotheses about causes and the frequency of transmission in time and space. The epidemiological and entomological aspects should be detailed, with an emphasis on the species of sand flies identified, probable vectors, the distribution of sand fly and vector abundance, the pattern of transmission, and other biological and social risk factors.
- Guidance: prevention, surveillance, and control actions for human cases and the vector should follow previously-established indications, which should be discussed, adapted and detailed according to the results found. In the case of environmental modification or occupational risk, preventive and mitigation guidance should be provided for the institutions responsible for risk events.
- Identification of the parasite: sample collection and identification of the parasite species circulating in the outbreak is recommended until it is identified (not as a measure of individual diagnosis). This recommendation is of greater importance when: the circulating parasite is unknown in the region; in situations in which there are confirmed clinical manifestations that are different from the habitual ones; and in cases with treatment failure and frequent relapses. According to PAHO, the Regional Reference Laboratory for the identification and genetic

sequencing of *Leishmania* is the Laboratory on Leishmaniasis Research, IOC/FIOCRUZ. For more information on CLIOC, go to: http://clioc.fiocruz.br/.

- Specific reservoir and entomological studies: studies whose objective is to learn about reservoirs and vectors in detail — for example, absence of known vectors, eating habits, schedule pattern, rate of infection, etc.— should be coordinated with research groups. The feasibility of research will depend on the environments involved and is higher in the case of peridomestic transmission (domestic and synanthropic animals).

NOTE: in situations in which the presence of ML or an increased number of cases of ML is verified or in areas with transmission of CL in which there is no history of this clinical form or this causal magnitude, specific epidemiological research should be undertaken. If necessary, conduct a leishmaniasis research with socio-environmental characterization of the risk and identification of the parasite strain.

10.2 Visceral leishmaniasis

10.2.1 Areas with registry of cases, but absence of epidemiological and entomological information

- Epidemiological investigation of current and historical cases: carry out analysis of secondary morbidity and mortality data available in the health system at the national and local levels. Carry out interviews with any recent cases, including a patient itinerary. Interview stakeholders to develop hypotheses about the initial transmission. In border areas, there should be contact between countries and sharing of available data on cases served on both sides of the border. This will facilitate the joint review of medical records from hospitals or health centers in neighboring areas.
- Entomological investigation: carry out in risk sites if there is an environment that is favorable to the vector and epidemiological history (areas with concentration of cases). The trapping methodology and scheme will depend on the environments and have as a goal to understand the distribution of vector abundance in time and space. The trap placement methodology according to the epidemiological situation was already described in the section on entomological surveillance and control.
- Active case-finding: carry out active case-finding in environments considered to be high exposure and domestic areas identified as risky.
- Prevalence study in dogs: its purpose is to confirm and determine infection due to *Leishmania infantum* in reservoirs and to describe the distribution of the abundance of cases in environments considered to be high exposure and domestic areas identified as risky. If human cases are concentrated, implementation of a census survey of dogs in the area is recommended, which should be geographically delimited for the baseline analysis and follow-up of cases. If human cases are scattered across the territory, there should be a selection of samples and a survey that considers the distribution and aggregation of human cases (refer to information on canine surveillance and Annexes 13 and 14).
- Evaluation of the sensitivity, specificity, and relevance of the notification system: evaluation of sensitivity using the capture-recapture methodology, carried out collaboratively with research groups, is recommended.
- Analysis and return of results: return results to those involved, discuss and analyze results with local health workers, generate recommendations, raise awareness of human and animal health professionals and the community. Include the subjects of responsible pet ownership and reproduction, management of canine populations in the community and at the municipal level, and regulation of breeding sites, shelters, and centers for the commercialization and display of dogs.

- Guidance: prevention, surveillance, and control actions for human cases, the vector and the reservoir should follow previously-established indications
- Identification of the parasite: both in the case when non-species-specific diagnostic tests are used and in the case when there is confirmation of transmission for the first time, sample collection and identification of the circulating parasite species is recommended, if feasible, in accordance with the indications already described.
- Entomological and reservoir studies: studies whose objective is to identify more detailed aspects of sylvatic reservoirs and vectors — for example, absence of known vectors, eating habits, schedule pattern, rate of infection, etc. — should be coordinated with research groups.

10.2.2 Outbreak situation: First reported case of canine VL in an area with no transmission or without previous registry of visceral leishmaniasis

- Confirmation of case of canine VL: confirmation of the laboratory diagnosis of the case should be carried out through species-specific tests.
- Clinical-epidemiological and entomological investigation of the first confirmed canine case of VL, for definition of autochthony.
  - Clinical-epidemiological investigation: compile information on date of appearance of symptoms, origin of dog, trips, visits to area with transmission, breeding history, daily movement habits, cohabitating dogs, probability of vertical transmission, etc.
  - Focus investigation: this should be carried out in the domestic environment and in other possible infection sites for the canine case. The trapping methodology and scheme will be carried out according to the methodology for confirmation of autochthony described in the section on entomological surveillance and control.
- Epidemiological research: carry out analysis of secondary morbidity and mortality data available in the health system at the national and local levels. Interview animal health agents and private sector professionals in search of a history of canine cases with compatible clinical signs. In border areas, request and share available data on human or canine cases.
- Entomological investigation to evaluate risk of local transmission in risk sites: entomological history and/or favorable environment for the vector and epidemiological history of the canine case.
  - If presence of the vector was confirmed in previous studies of autochthony, the entomological sampling methodology will be a entomological survey.
  - If autochthony could not be confirmed, the focus investigation should be expanded based on transmission hypotheses.
- Active case-finding for infection in dogs:
  - If the canine case was not autochthonous and there is no presence of the vector: carry out active case-finding for the infection in cohabitating dogs and in dogs at risk of horizontal (breeding history) and vertical (offsprings) transmission.
  - If the canine case is autochthonous: carry out census active case-finding for canine infection, moving radially from the canine case until including at least 100 dogs.

- If active case-finding of canine infection confirms local transmission, previously established guidelines on prevention, surveillance, and control of human cases, the vector, and the reservoir should be carried out.
- If active case-finding of canine infection does not confirm local transmission, previously established surveillance guidelines should continue.

- Analysis and return of results: return, discuss, and analyze results with local health workers. Generate recommendations, raise awareness of human and animal health professionals and the community. Include the subjects of responsible pet ownership and reproduction, management of canine populations in the community and at the municipal level, and regulation of breeding sites, shelters, and centers for the commercialization and display of dogs.
- Guidance: if local transmission is confirmed, the actions described for prevention, surveillance, and control for human cases, the vector and the reservoir should continue.
- Identification of the parasite: when non-species-specific diagnostic tests are used, sample collection and identification of the circulating parasite species is recommended, if feasible, in accordance with the indications already described.
- Entomological and reservoir studies: studies whose objective is to identify more detailed aspects of sylvatic reservoirs and vectors — for example, absence of known vectors, eating habits, schedule pattern, rate of infection, etc. — should be coordinated with research groups.

10.2.3 Outbreak situation: first reported case of human VL in an area with no transmission of human or canine VL

- Confirmation of case of human VL: confirmation of the laboratory diagnosis of the case should be carried out through species-specific tests.
- Historical epidemiological research and research on the first recorded case: analysis of secondary morbidity and mortality data available in the health system at the national and local levels. Interview the case and key actors to generate transmission hypotheses. Interview animal health agents and private sector professionals in search of a history of canine cases with compatible clinical signs. In border areas, available data on cases that received care should be shared among neighboring countries. There should be joint review of medical records from hospitals or health centers in neighboring areas.
- Entomological investigation: this is carried out in the domestic environment and in other possible infection sites for the case or critical environments (sites with accumulation of dogs). The trapping methodology and scheme will be carried out according to the methodology for evaluation of autochthony and what is described in the section on entomological surveillance.
- Investigation of canine infection: if the epidemiological and entomological history indicates the possibility of autochthonous cases, census active case-finding for canine infection should be carried out using a radial strategy that starts from the residence of the human case and proceeds until at least 100 dogs are included. Include the subjects of responsible pet ownership and reproduction, management of canine populations in the community and at the municipal level, and regulation of breeding sites, shelters, and centers for the commercialization and display of dogs.

- Guidance: if local transmission is confirmed, the actions described for prevention, surveillance, and control for human cases, the vector and the domestic reservoir should continue.
- Analysis and return of results: return results to stakeholders, discuss and analyze results with local health agents, generate recommendations, raise awareness of human and animal health professionals and the community.
- Identification of the parasite: when using non-species-specific diagnostic tests, sample collection and identification of the circulating parasite species is recommended, in accordance with the indications already described.
- Entomological and reservoir studies: studies whose objective is to identify more detailed aspects of sylvatic reservoirs and vectors — for example, absence of known vectors, eating habits, schedule pattern, rate of infection, etc. — should be coordinated with research groups.

10.2.4 Outbreak situation: in areas with transmission, increase in cases relative to the expected number

In the case of outbreaks of VL in areas with confirmed transmission, follow the recommendations already described in the section on epidemiological classification and surveillance and control actions for areas with transmission. Observations should be made about whether there are epidemiological, entomological and reservoir aspects that are particular to the outbreak, and general recommendations should be adapted accordingly.

ANNEXES

ANNEX 1. DIRECT SMEAR

Procedures for detailed techniques to collect, process, conserve, and transport the sample for the parasitological diagnosis of cutaneous, mucosal, and/or mucocutaneous leishmaniasis

I. Purpose

Obtain an ideal sample from suspected cutaneous leishmaniasis lesions. Stain and use optical microscopy to visualize the parasite of the genus *Leishmania* in its amastigote form and prepare the corresponding report.

II. Biosafety

- Use the biosafety elements necessary to collect the sample, such as: gloves, facemask, glasses, and gown, to follow adequate biosafety practices.
- Apply universal precautions when handling sharp materials (scalpel and glass slides).
- Consider every blood sample as potentially infectious.

In the case of material spills, clean and disinfect splatters of samples or reagents using 70% alcohol or a disinfectant such as sodium hypochlorite 0.5% solution.

III. Materials, reagents, and protection elements

For sample collection

- Adequate location to collect samples (clean, ventilated, illuminated, and with privacy).
- Laboratory gowns.
- Disposable gloves.
- Paper towels for cleaning the work area.
- Safety disposal container for discarding sharps.
- Container with red bag.
- Black lead #2 pencil or indelible marker.
- Disposable scalpel blade #15.
- Handle for scalpel #3.
- New and defatted microscope slides.
- Microporous adhesive tape.
- Wooden tongue depressor for application of antibiotic cream.
- Form to register direct examination or patient file.
- Pencil.

Material for cleaning the lesions:

- Sterile gauze, antiseptic alcohol, sterile saline solution, and other solutions available in the service.

NOTE: if scalpel blade cannot be used, it can be replaced with another element when collecting the sample, provided that the quality of the specimen and asepsis are guaranteed.

For fixation of the sample:

- Methanol absolute.
- Pasteur pipette or dropper.
- Support for drying slides.

Material for conservation and shipment:

- Slide holder or absorbent paper.
- Form to register direct examination or patient file.

IV. Description of activities

A. Sample collection

1. Record the patient's data on the registry form.
2. Clean the work area with 70% alcohol and/or disinfectant.
3. Prepare the needed materials before initiating the procedure.
4. Wash hands and put on gloves.
5. Explain the following to the patient: the procedure, its limitations, and the time needed to provide the results report. Clarify patient doubts.
6. Label one of the extremes of the two microscope slides with the patient's medical record number or code and the date when the sample is taken.
7. Select the lesion with the shortest evolution time and look for the most indurated margins, which indicate that the lesion is active. Depending on the case, it may be necessary to collect samples from more than one lesion. Ideally, the lesion should be free from bacterial superinfection (when there is superinfection, the recommendation is to inform the physician to consider the need for prescription antibiotics and to make another appointment for the patient when antibiotic treatment is complete).
8. Clean the lesion with disinfectant solution using circular movements from the center toward the periphery of the ulcer.
9. In the event that the lesion has crust, humidify the lesion with saline solution and remove the crust. Then, clean the lesion again.
10. Select the area for the incision around the most active margin using the index fingers and thumb. Apply pressure for 20 seconds to achieve good hemostasis. Without stopping the pressure, use the scalpel blade to make a parallel superficial incision at the margin of the lesion that is 5 mm long by 3 mm deep.
11. Introduce the scalpel blade by guiding the edge toward the external part of the lesion and scrape the interior of the incision. The material obtained from the scraping should have a lumpy or granular aspect,

which indicates the presence of cells with limited quantity of blood.

12. Distribute the material obtained on a microscope slide by placing the scalpel blade parallel to the edge of the slide and slowly and smoothly spread the material obtained from the inside to the outside using a circular motion.
13. If the material obtained is abundant, make several imprints from the same scraping. If the material is limited, extract additional sample with the same scalpel blade, either from the same or a different incision.
14. Prepare a total of three slides per patient with three imprints per slide. Each imprint should have a diameter of approximately 8 to 10 mm.
15. Discard the scalpel blade in the safety disposal container.
16. Apply antibiotic cream to the lesion with a tongue depressor and cover it with sterile gauze and microporous adhesive tape.
17. Allow the slides to dry in a horizontal position on the worktable, at room temperature, for 15 to 20 minutes. In warm and humid climates, take the necessary precautions to avoid the harmful effect of external contamination agents such as fungi and spores or direct contact with sunlight.
18. Fix the slides by covering the entire slide with methanol absolute. Place them in a vertical position to drain excess methanol and allow them to dry for 15 to 20 minutes.
19. For shipment of the slides to the laboratory, they should be placed in slide holders or wrapped in absorbent paper. Then, they should be deposited into a secondary container, to then store them in a tertiary or exterior holder, so that they are contained in a triple packaging system.

NOTE: If there is no local laboratory that can carry out the diagnosis, samples should be sent in the shortest time possible following collection.

B. Staining

1. Place the slides with the sample face down on the concave support that contains the stain and dye by immersion to avoid the formation of precipitates.
2. Use the stain according to manufacturer recommendations, especially with regard to the time and standardized concentration for each lot. In general, they can be stained with any Romanowsky stain (Wright, Field, Giemsa, or Quick Panoptic).
3. Wash the slides with tap water (pH 6.5 to 7), making sure that the water does not fall directly on the sample.
4. Allow the slides to dry at room temperature for 10 to 15 minutes and incline them on the staining rack to remove excess water with absorbent paper.

C. Reading and evaluation

1. Place the slide on the platina of the microscope and focus with a 10X eyepiece - 10X objective (100X magnification) to locate the sample. Add one drop of immersion oil to the sample and focus with a 10X eyepiece - 100X objective (1000X magnification).
2. Evaluate the sample and classify it according to the following characteristics of the size and staining:

Optimal sample: cells with correct morphology are observed: abundant leukocytes and limited erythrocytes and adequate staining of the leukocytes (intense blue-violet nucleus, clear blue cytoplasm, and pale pink red blood cells).

Inadequate sample: the sample lacks a granular aspect, is limited in quantity, contains abundant red blood cells or bacteria, and has unsatisfactory staining.

1. Review at least 100-200 fields of the smear in a sequential way and stop at sites where there is an abundant leukocyte reaction to search for intra- or extracellular amastigotes.
2. A result is positive when at least one intra- or extracellular amastigote is clearly observed with all of its characteristics.
    a. Nucleus: dark blue-violet color.
    b. Kinetoplast: intense violet color.
    c. Cytoplasm: light blue color.
    d. Defined cellular membrane.
3. A result is negative when, after reviewing all of the fields on all of the slides, no amastigotes are observed.

D. Cleaning and care of equipment

1. Once the reading is complete, clean the 100X objective of the microscope with rice paper.
2. Store the slides in a slide holder box for quality control, complying with each service's directions.
3. Register the result on the form established for parasitological reports or direct examination.
4. Enter the result in the laboratory information system database.
5. Deliver the printed, signed results.

V. Report of results

- Positive: amastigotes of *Leishmania sp*. are observed in the sample examined.
- Negative: amastigotes of *Leishmania sp*. are not observed in the sample examined.

NOTE: Every positive result should be reported immediately to the surveillance service or the local leishmaniases program.

VI. Recommendations for sample collection and processing

- When the ulcer is covered by a crust, try to remove it very gently at the same time that the ulcer is being cleaned since this helps with faster scarring. In cases in which the crust is firmly adhered, it should be humidified with gauzes impregnated in saline solution for a few minutes and then carefully removed while trying to prevent traumas for the patient.
- Recall and apply all biosafety standards during the procedure, discard contaminated material such as gauzes or cotton in red bags, and discard the scalpel blade in the safety disposal container.
- The patient should be advised to not use empirical treatments to attempt to heal or scar over the injury.
- In the laboratory, confirm the validity and adequate storage of reagents and other materials.

- If the smear is considered inadequate, it should still be evaluated. If there is no doubt that it is positive, it should be reported as such. If it is negative, it should be reported as an inadequate sample, and collection of a new sample is suggested.
- A negative smear does not rule out disease. There are some circumstances in which the result of the direct smear can be negative even though the disease is present. Some of these circumstances are: bacterial super-infection, abundance of red blood cells, presence of chronic lesions with an evolution longer than six months, presence of scarring lesions, inadequate sample collection, poor preparation of the smear or staining, and technician inexperience.
- Although the kinetoplast is generally easily observed through microscopy, sometimes it may not be evident due to the position in which the amastigote remains when making the smear. It should be considered that it is necessary to clearly identify at least one parasite to avoid interpretation errors.

VII. Quality control

The national laboratory should carry out internal quality control in its entire network and participate in the Regional External Assessment Program of Performance (PEED).

In the Region, PEED is the responsibility of the Parasitology Laboratory of the National Institute of Health of Colombia (INS).

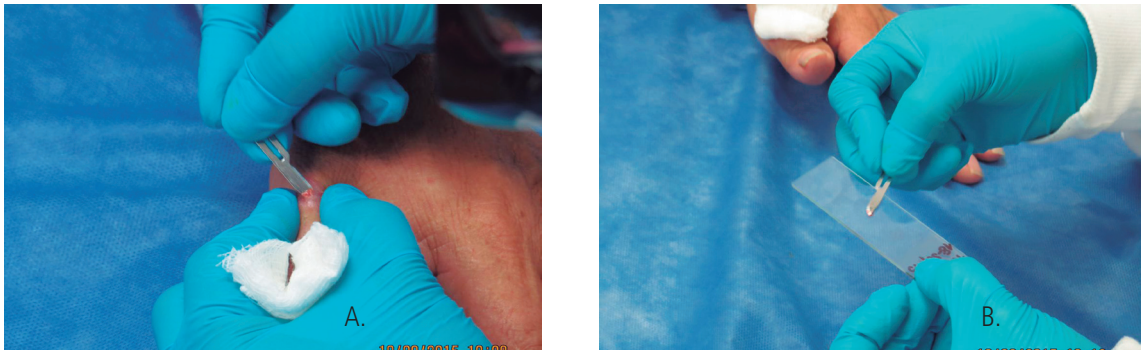


FIGURE 36 - Incision carried out with the scalpel blade on the margin of the lesion
Source: International Center for Medical Training and Research, CIDEIM, Colombia

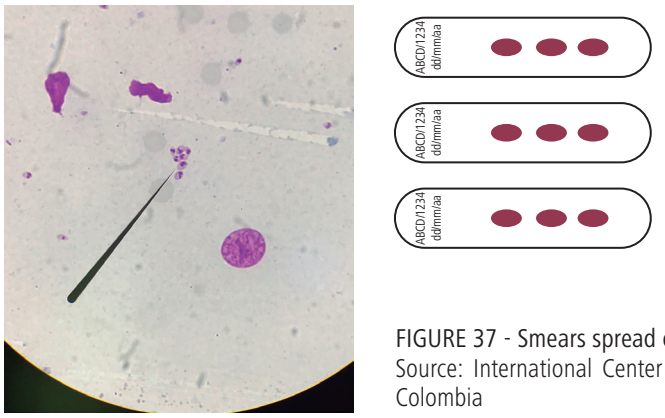


FIGURE 37 - Smears spread on the microscope slide in a circular manner.
Source: International Center for Medical Training and Research, CIDEIM, Colombia

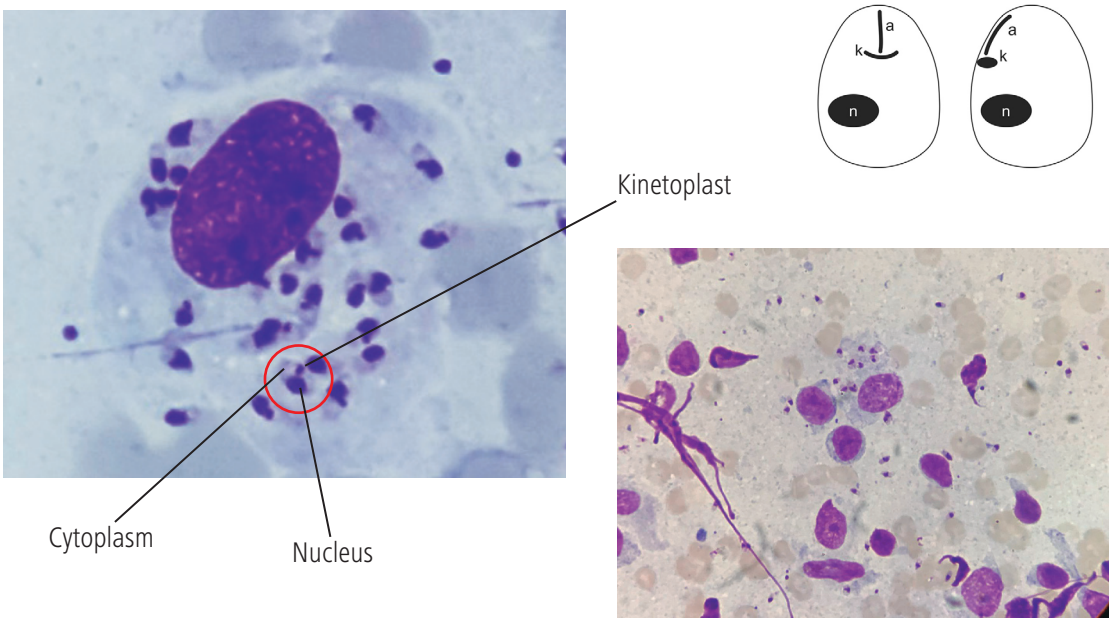


FIGURE 38 - Visualization of all structures of the amastigote: axoneme (a) is not observed through microscopy, nucleus (n), kinetoplast (k), and cytoplasm (c). Visualization of the amastigote and its structures.

Sources: J. Pereira, Dermatological Center, Paraguay; International Center for Medical Training and Research, CIDEIM, Colombia

ANNEX 2. COLLECTION OF LESION ASPIRATE

Procedures for detailed techniques to collect, process, conserve, and transport the sample for the parasitological diagnosis of cutaneous, mucosal, and/or mucocutaneous leishmaniasis

I. Purpose

Obtain an ideal sample from the lesions, through aspirates, which will be used to carry out a culture, direct examination or PCR.

II. Biosafety

- Use the biosafety elements necessary to collect the sample, such as: gloves, facemask, glasses, and gown. Follow required safety practices during the procedure.
- Consider every biological sample as potentially infectious.
- Clean and disinfect splatters from samples or reagents using 70% alcohol and spills from biological material with a disinfectant such as sodium hypochlorite 0.5% solution.

III. Materials, reagents, and protection elements

- Disposable syringes of 1 ml of insulin with 27 gauge 3/8 inch needles.
- Disposable gloves.
- Laboratory gown.
- Rack for culture medium tubes.
- Alcohol or Bunsen burner.
- Indelible marker.
- 70% ethanol to clean the work area.
- Paper towels.
- Sterile wooden tongue depressor or applicator.
- Antibiotic cream.
- Safety disposal container for discarding sharps.
- Container with red bags.
- Lesion cleaning material: sterile gauzes, antiseptic alcohol, sterile saline solution, and other solutions available in the service.
- Phosphate buffered saline (PBS) with antibiotic.
- Culture medium tubes.

IV. Description of activities

A. Sample collection

1. Record the patient's data on the registry form.
2. Clean the work area with 70% alcohol and/or disinfectant.
3. Prepare the needed materials before initiating the procedure.
4. Wash hands and put on gloves.
5. Explain the following to the patient: the procedure, its limitations, and the time needed to provide the results report. Clarify patient doubts.
6. Select the lesion with the shortest evolution time and look for the most indurated margins, which indicate that the lesion is active. Depending on the case, it may be necessary to collect samples from more than one lesion. Ideally, the lesion should be free from bacterial superinfection (when there is superinfection, the recommendation is to inform the physician to consider the need for prescription antibiotics and to make another appointment for the patient when the antibiotic treatment is complete).
7. Clean the lesion with disinfectant solution using circular movements from the center toward the periphery of the ulcer.
8. In the event that the lesion has crust, humidify the lesion with saline solution and remove the crust. Then, clean the lesion again.
9. Have a mixture of PBS with antibiotic, at a total concentration of 100 U/ml of penicillin and 100 µg/ml of streptomycin or 100 µg/ml of gentamicin, available.
10. Prepare 3 or 4 syringes of insulin and add 0.1 ml of the previous mixture to each one.
11. Once the PBS solution and antibiotic are in the syringe, introduce the needle at 3 to 4 mm from the external margin of the lesion so that a 45o angle is formed. Then, aspirate to obtain tissue liquid, carrying out rotational motions to favor the movement of the material through the syringe. If the material cannot be obtained in this way, aspirate gently with the plunger to obtain the tissue liquid. Aspiration of blood should be avoided to the greatest degree possible since this hinders the visual detection of the parasite. Cover the needle with its cover using the single hand technique. The PBS and antibiotic mixture should never be injected on the margin of the lesion.

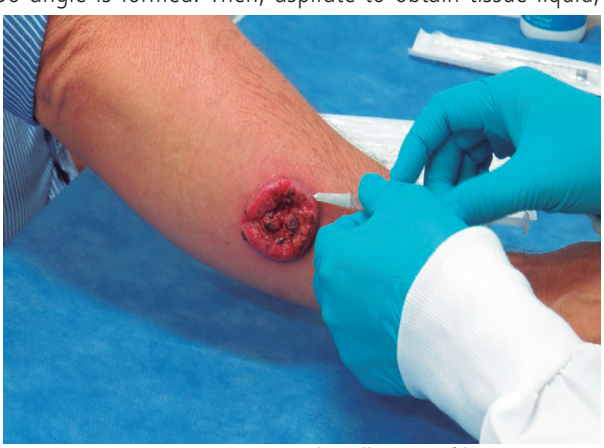


FIGURE 39 - Sample collection of lesion aspirate for culture of *Leishmania sp*

Source: International Center for Medical Training and Research, CIDEIM, Colombia

12. Repeat the same procedure with each remaining syringe, each time at different places on the margin of the lesion.
13. On completing sample collection, apply pressure to the lesion with a sterile gauze until bleeding is controlled. Then, apply antibiotic cream and cover the lesion with sterile gauze and microporous adhesive tape.
14. Thank the patient and recommend that the patient remove the gauze 24 hours after the sample was taken. Indicate the importance of informing the physician about any complications so that the patient can return to the health service if this happens.
15. Inform the patient when the result will be provided, according to the diagnostic method used.

16. For shipment of the aspirate samples to the reference laboratory, the syringes should be deposited in a secondary plastic container and then in a third holder to ensure their refrigeration at 4 to 8 °C. This fulfills the triple packaging system.
17. The time between sample collection and processing cannot be longer than 24 hours.

NOTE: For sample collection from lymph nodes, the same aforementioned procedures should be followed.

B. Laboratory procedure

1. Culture
    a) Once the aspirate sample collection procedure is complete, ensure that there is a sterile area near the Bunsen burner or sterilization source that will be used.
    b) Mark the culture medium tubes in two phases (e.g. NNN) with the patient's information (medical record number and identification code) and the sample collection date.
    c) Turn on the burner.
    d) Uncover the culture medium tubes one by one, as close as possible to the burner.
    e) Flame the mouth of the culture medium tube and immediately deposit all of the aspirate material from one of the syringes, displacing the plunger 10 times. Flame the mouth of the tube again and cover.
    f) Repeat the previous procedure to deposit the content of each syringe in a different culture medium tube.
    g) To ship the seeded cultures to the reference laboratory, they should be deposited in a secondary rigid plastic container, and then in a third holder, to fulfill their transport in a triple packaging system.
    h) Refrigerate the tubes using ice packs placed between the second and third package, so that they stay at a temperature of 10 °C to 20 °C.
    i) Send to the reference centers.
    j) At the reference centers:
        a) Place the inoculated tubes in an incubator at a temperature of 24 °C to 26 °C.
        b) Observe daily using an inverted microscope and register any event or change. Carry out blinded reviews, if necessary, or subculturing every 8 to15 days.

V. Report of results

- Positive: promastigotes of *Leishmania sp*. are observed in the sample examined.
- Negative: promastigotes of *Leishmania sp*. are not observed in the sample examined.

NOTE: Every positive result should be reported immediately to the surveillance service or the local leishmaniases program.

VI. Recommendations concerning the procedure

It is important that adequate storage be guaranteed and that the validity of all biological elements be confirmed before taking the sample.

ANNEX 3. BIOPSY COLLECTION FROM SKIN OR MUCOUS MEMBRANE USING PUNCH BIOPSY

Procedures for detailed techniques to collect, process, conserve, and transport the sample for the parasitological diagnosis of cutaneous, mucosal, and/or mucocutaneous leishmaniasis

I. Introduction

Biopsy is defined as the acquisition of tissues or other materials from the live organism, for their microscopic examination for diagnostic and research purposes. The skin biopsy is the most appropriate diagnostic procedure for dermatological diseases, since it provides very useful information for defining the patient's diagnosis.

A punch skin biopsy is a basic technique for obtaining specimens with complete thickness from skin or mucous membranes.

II. Purpose

Standardize the technique for obtaining a skin or mucous membrane biopsy using punch biopsy, to optimize results and minimize the risks and complications of this procedure.

III. Materials, reagents, and protection elements

- 4 mm punch biopsies.
- Sterile gauzes.
- Disinfectant solution.
- Insulin syringe.
- Local anesthetic.
- Iris straight scissors.
- Small material scissors.
- Tweezers with small clamp.
- Needle holder.
- Monofilament non-absorbable sutures, 4-0.
- Surgical field.
- Antibiotic cream.
- Microporous adhesive tape.
- 10% buffered formal saline.
- Sterilized clean gloves.
- Vials or containers for biopsy collection.

IV. Description of activities

A. Collection of skin biopsy

1. Record the patient's data on the registry form.
2. Clean the work area with 70% alcohol and/or disinfectant.
3. Prepare the needed materials before initiating the procedure.
4. Explain the following to the patient: the procedure, its limitations, and the time needed to provide the results report. Clarify patient doubts.
5. Carry out clinical hand washing using aseptic and antiseptic techniques.
6. Use sterile gloves and apply other necessary biosafety measures.
7. Label the vial that will be used with a black pencil or marker.
8. Select the lesion with the shortest evolution time and look for the most indurated margins, which indicate that the lesion is active.
9. For ulcer-type lesions, which are characteristic of cutaneous leishmaniasis, a biopsy with the following proportions is suggested: 1/3 from healthy skin and 2/3 from the margin of the lesion.
10. Clean the sample area with disinfectant solution.
11. Set up the surgical field.
12. Carry out subcutaneous infiltration according to medical criteria to apply local anesthetic in the area where the biopsy will be taken, considering the anatomical site and size of the lesion.
13. Obtain the biopsy, sustaining the punch biopsy vertically on the skin while exerting downward pressure, making it rotate using the thumb and index fingers of the dominant hand. Remove the punch biopsy on reaching subcutaneous fat or the plastic limit of the instrument.
14. Lift the skin sample obtained using the needle used for the infiltration anesthesia. Tweezers can be used as long as precaution is taken to not pressure the specimen. While holding the scissors in the dominant hand, the specimen should be cut to liberate it from the subcutaneous tissues. The cut should be made below the dermis.
15. Prepare a vial that contains 10% buffered formal saline for histopathology, 70% alcohol for PCR, or sterile saline solution for PCR, PCR culture or other culture. The volume of the solution should be 10 times greater than that of the specimen obtained.
16. Introduce the specimen into the container with the conservation solution in accordance with the diagnostic technique.
17. Material destined for molecular biology should be kept frozen until it is processed. Biopsies for culture should be refrigerated for no more than 24 hours. Biopsies in formal solution for histopathology can be conserved at room temperature until they are processed.
18. Close the wound, carrying out hemostasis with clean gauzes. If necessary and according to medical criteria, close the wound with one or two discontinuous suture points.
19. Apply scar or antibiotic cream, cover with gauze, and set with microporous adhesive tape.

B. Collection of mucous membrane biopsy

Collection of a mucous membrane biopsy requires the intervention of a specialized physician (otorhinolaryngologist) since it is necessary to have special medical equipment such as the rhinoscope. However, the rest of the materials and procedures are the same as those previously described.

V. Report of results

- Positive: amastigotes of *Leishmania sp*. are observed in the sample examined.
- Suggestive: inflammatory changes and chronic granulomatosis, which suggest infection, are observed but the diagnosis is not confirmed given the absence of the parasite in the sample examined.
- Negative: neither amastigotes of *Leishmania sp*. nor changes compatible with a chronic granulomatous reaction are observed in the sample examined.

NOTE: Every positive result should be reported immediately to the surveillance service or the local leishmaniases program.

VI. Recommendations concerning the procedure

It is important that adequate storage be guaranteed and that the validity of all biological elements be confirmed before taking the sample.

Send the sample for processing and reading by the pathologist as soon as possible.

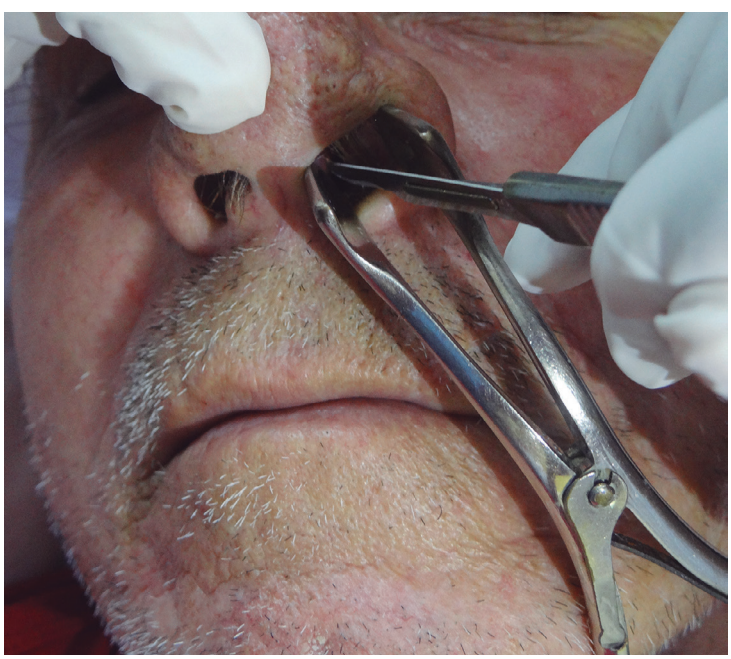


FIGURE 40 - Nasal mucous membrane biopsy
Source: J. Soto, Funderma, Bolivia.

ANNEX 4. POLYMERASE CHAIN REACTION (PCR)

Guidance and procedures for PCR

I. Introduction

Diagnosis using molecular biology (PCR or qPCR) requires standardization and validation at the regional level. This is a proposal that PAHO currently leads.

II. Indications for use of PCR

PCR is indicated for patients who have clinical or epidemiological suspicion that is compatible with leishmaniasis accompanied by a negative parasitological diagnosis (PCR is only recommended once the conventional diagnosis algorithm has been exhausted).

In situations in which the implementation of PCR is required, the local reference laboratory should be contacted, evaluate the logistical feasibility of sample collection, conservation, and shipment following the required recommendations presented in Table 18:

TABLE 18 - Type of sample, mode of conservation, and shipment of material for application of PCR in reference laboratories.

Type of sample	Material	Immediate conservation temperature and maximum time between sample collection and sample reception in the reference center	Long-term conservation	Observation
Aspirate	Syringe with aspirate	Refrigeration 4 °C to 8 °C 48 hours	EIn lysis buffer -20 °C to -80 °C	Avoid freezing or thawing due to degradation of nucleic acids.
Scraping	Sterile Eppendorf tube with scraping	Refrigeration 4 °C to 8 °C 48 hours	In lysis buffer -20 °C to -80 °C	Avoid freezing or thawing due to degradation of nucleic acids.
Swab	Swab with sample	Refrigeration 4 °C to 8 °C 72 hours	- 20°C a -80°C	Avoid freezing or thawing due to degradation of nucleic acids.
Biopsy	Container with biopsy in conservation solution	OCT: -20 °C to -80 °C, until processing	< -80°C	Avoid freezing or thawing due to degradation of nucleic acids.
		70% alcohol, until processing	< -80°C	
		Paraffin at room temperature, until processing	Room temperature	

Source: PAHO/WHO, IOC/Fiocruz 2018.

ANNEX 5. LEISHMANIN TEST (TEST OF MONTENEGRO)

Procedures for application, reading, and interpretation of the test

I. Introduction

The leishmanin test (test of Montenegro) is a test of delayed-type cutaneous hypersensitivity to homologous or heterologous antigens of *Leishmania* promastigotes. The Montenegro skin test (intradermal reaction of Montenegro or IDRM) is very useful for epidemiological studies of leishmaniases. Above all, it serves as support when diagnosing CL and for diagnosing of ML and MCL when the reaction is more intense. However, it is not useful for the diagnosis of VL since during the active phase of VL, patients are always non-reactive to the test and, in the majority of patients, become reactive only until after finalizing treatment (3 to 6 months after completing treatment). The IDRM does not have the capacity to distinguish between current and previous infections. Thus, in areas of endemic transmission, it is a guiding, but not a diagnostic, tool.

Leishmanin (antigen): is an antigen obtained from heat-inactivated promastigotes of the dermotropic *Leishmania* species. To conserve its activity, it should be stored in refrigeration at a temperature of 4 °C to 8 °C. Given that it is an injectable based on homogenates of undefined composition that has variance between lots, local production is not advised until there is an antigen that has been standardized through quality control of production and has regular availability and uniform sensitivity. Currently, this test is not commercially available in the Americas, limiting its use by public health programs.

II. Purpose

Standardize the application and reading of the leishmanin test to optimize results and minimize the risks and complications of the procedure.

III. Materials and reagents

- Tuberculin syringe.
- Needle Num. 26.
- Cotton.
- Alcohol.
- Ballpoint pen.
- Millimetric ruler.
- Leishmanin (antigen).

IV. Description of activities

A. Application

1. Aspirate 0.1 ml of leishmanin with syringe.
2. Clean the superior third of the flexor face of the left forearm with cotton impregnated with alcohol.
3. Introduce only the tip of the needle intradermally, bevel-side up. Inoculate the leishmanin slowly, until ob-

serving the formation of a small papule with an "orange skin" aspect. Marking the application area on the skin with a ballpoint pen is suggested to facilitate follow-up.

4. Recommend to the patient to not scratch or apply alcohol or other substances to the application site.

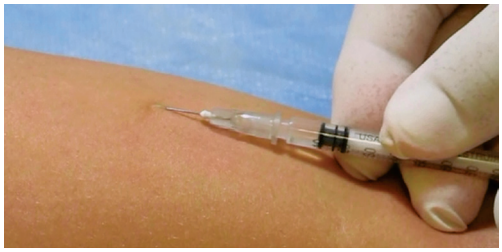


FIGURE 41- Application of the antigen
Source: Regional Leishmaniases Program, PAHO/WHO

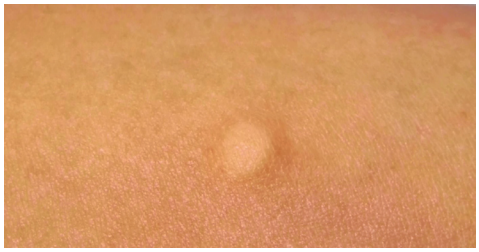


FIGURE 42 - Formation of "orange skin" papule
Source: Regional Leishmaniases Program, PAHO/WHO

V. Reading

1. Within 48 to 72 hours after application, the induration area should be delimited in the following way: with a ballpoint pen placed in a position that is perpendicular to the plane of the skin, trace a line sliding the tip of the pen from the periphery (approximately 4 cm from the induration area) to the center of the induration area until there is resistance. This will result in the drawing of a straight line that indicates where the circumference of the induration area begins. This procedure should be repeated in the other three quadrants of the induration area (each time, 90 degrees from the last line that was drawn), so that the circumference of the induration area is indicated at four different points through four lines that form a cross, corresponding to the four quadrants of the area.
2. Based on the defined limits, measure the diameter of the induration with a millimetric rule or a king foot caliper.

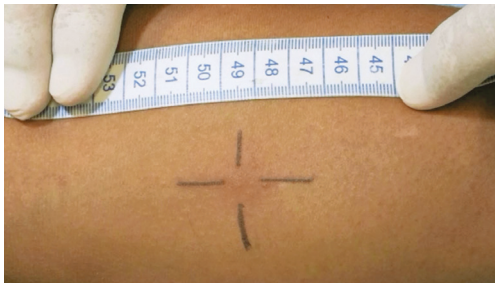


FIGURE 43 - Reading the leishmanin test
Source: Regional Leishmaniases Program, PAHO/WHO

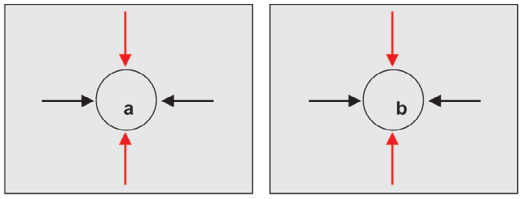


FIGURE 44 - Scheme for reading the leishmanin test
Source: Regional Leishmaniases Program, PAHO/WHO

VI. Report of results

- Positive: when one of the two diameters of the induration is equal to or greater than 5 mm.
- Negative: when none of the two diameters of the induration are equal to or greater than 5 mm.

The result is reported as positive or negative and the diameter of the two readings is reported (e.g. positive: 5 mm x 3 mm; negative: 4 mm x 3 mm)

NOTE: In some cases, mainly in patients with mucosal lesions, hyper-reactivity to the antigen can present as ulceration of the application area. In these cases, a medical evaluation is recommended for timely detection of superinfection of the ulceration.

ANNEX 6. BONE MARROW PUNCTURE

Sampling for visceral leishmaniasis

I. Purpose

Establish the necessary activities for carrying out the diagnosis of VL through bone marrow aspirate for later parasitological analysis or direct examination, either histopathological, culture or PCR.

II. Responsibility

Physician

III. Principle of the method

Parasites present in bone marrow (*Leishmania sp*. amastigotes) can be observed microscopically using a 100X objective (1000X increase). This observation confirms the disease.

Additionally, the parasites present in bone marrow can be collected and cultivated in specific media for amplification and identification of their species.

IV. Materials, reagents, and protection elements

- Antiseptic alcohol.
- Saline solution.
- Iodized solution.
- Sterile gauzes.
- Clean gloves.
- Laboratory gown.
- Sterile field.
- Protective glasses or mask.
- Facemask.
- Marrow puncture needle.
- 10 cc syringes.
- 1" and 1½" needles (subcutaneous and intramuscular).
- Local anesthetic.
- Safety disposal container for discarding sharps.
- New and defatted slides.
- Romanowsky / May Grünwald-Giemsa stain.
- Container for sample transportation.

- Indelible marker.
- Novy-MacNeal-Nicolle (NNN) culture medium.
- Binocular microscope with 10X eyepiece and 100X objective.
- Computer.

V. Description of activities

A. Explain the following to the patient: the purpose of the examination, the steps for the procedure, the risks of the procedure, and diagnostic alternatives. It is important to ask the patient about any history with risk of hemorrhage. Once the procedure has been explained and all of the patient's questions have been resolved, request that the patient sign the informed consent or assent.

B. Position the patient in the decubitus dorsal position and locate the area to carry out the puncture. In this instance, there are three options:

1. Puncture of ileac crest:
    a) This is recommended for adults and children of any age and it is satisfactory even in lactating babies.
    b) It is preferable to carry out the puncture in the posterior ileac crest and, only in the case that this is not possible, in the anterior ileac crest.
    c) The puncture of the ileac crest is not recommendable in obese patients or in patients with any type of immobility.
    d) While pressing on the skin with the thumb placed under the ileac crest and the index finger placed above the crest, penetrate the epidermis with the needle at 90° to the skin, and introduce it firmly.
    e) When the needle is firmly introduced in the bone, withdraw the guide, connect the syringe, and aspirate one to two drops of medullary material. The provider can tell that the needle is well-positioned once negative pressure is felt, that is, once it induces pain and discomfort at the puncture site.
    f) Advantages: it is less painful and safer than a sternal puncture.
    g) Risks: it is possible to exceed the internal bone table and affect the intestine, although the probability of this risk is very low.

2. Sternal puncture
    a) This is recommended for obese patients or patients with immobility.
    b) This is not recommended for children under 2.
    c) A needle with depth protection is used.
    d) This is applied in the sternum at the height of the first, second, or third intercostal space.
    e) While the pinky finger is placed on the clavicle and the thumb and index finger in the intercostal spaces, penetrate the epidermis with the needle at 90° and introduce it into the bone firmly and delicately.
    f) When the needle is firmly positioned in the bone, withdraw the guide, connect the syringe, and aspirate one to two drops of medullary material. The provider can tell that the needle is well-positioned once negative pressure is felt, that is, once it induces pain and discomfort at the puncture site.
    g) Advantages: it is easy to implement and with this method, the thin bone table can be penetrated easily.

h) Risks: it is possible to exceed the internal bone and affect the large veins (this risk is lower in the manubrium (sternal) puncture because the esophagus is posterior to the puncture site).

3. Tibial puncture

a) This is recommended for children under two months in light of the impossibility of making the puncture to the ileac crest.

b) This should be done on the medial, oblate surface of the proximal diaphysis (upper 1/3), one to two centimeters below the tibial tuberosity.

c) While using the thumb and index finger to press on the skin, penetrate the epidermis with the needle at 10° to the vertical plane — not in the caudo-cranial direction — and proceed to introduce the needle into the bone firmly and delicately.

d) When the needle is firmly positioned in the bone, remove the guide, connect the syringe, and aspirate one to two drops of medullary material. The provider can tell that the needle is well-positioned once negative pressure is felt, that is, once it induces pain and discomfort at the puncture site.

e) Risks: it is possible to have rare complications such as osteomyelitis, hematomas, subcutaneous abscess, and bone fracture.

C. C. Once the puncture area has been located, prepare the patient for the test.

D. Using sterilized gloves and a facemask, disinfect the puncture area with a gauze soaked in antiseptic and recall this rule of cleaning: always clean from the center toward the periphery of the area and never return to the center with a previously-used gauze. Once the puncture area is dry, cover with a sterile cloth and organize all of the material that will be needed for the aspirate.

E. Anesthetize the area where the bone marrow extraction will be done with a dose of 0.5 ml to 1.0 ml of xylocaine 1%, starting with the superficial tissues and finishing with the infiltration of the periosteum.

F. Once the site has been identified and anesthetized, perform the extraction of the marrow aspirate. Part of the aspirate can be used to make a culture in NNN media.

G. Use the material obtained to make several bone marrow smears on the slides.

H. If necessary, once the aspirate is finished, proceed with the extraction of the biopsy.

I. To carry out a biopsy: insert another type of needle in the same area and remove a small sample of the bone.

J. Collect the biopsy sample and rotate it between two holders for later staining and analysis.

K. Introduce the aspirated material into a container with a 10% formal solution.

L. Apply pressure to the puncture area for a period of time and apply a dressing that compresses the entire affected area.

M. Once in the laboratory, mark the slides with a graphite or diamond pencil to be able to identify all of the samples collected.

N. Stain with the May Grünwald-Giemsa method and finally, observe microscopically.

VI. Recommendations concerning the procedure

Universal precautions for the manipulation of contaminated fluids and biological material should be considered.

Special temperature or humidity conditions are not required when carrying out this test.

The bone marrow biopsy is considered to be a safe procedure with minimal risks. It is rare that complications arise. However, in some cases, it is possible for the patient to experience discomfort at the biopsy site for 1 or 2 days. In exceptional cases, even infection or hemorrhage can occur.

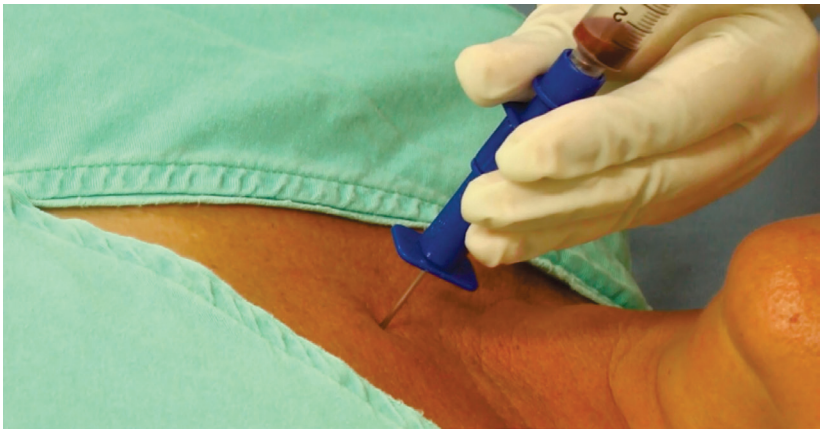


FIGURE 45 - Bone marrow puncture. Sternum
Source: Regional Leishmaniases Program, PAHO/WHO

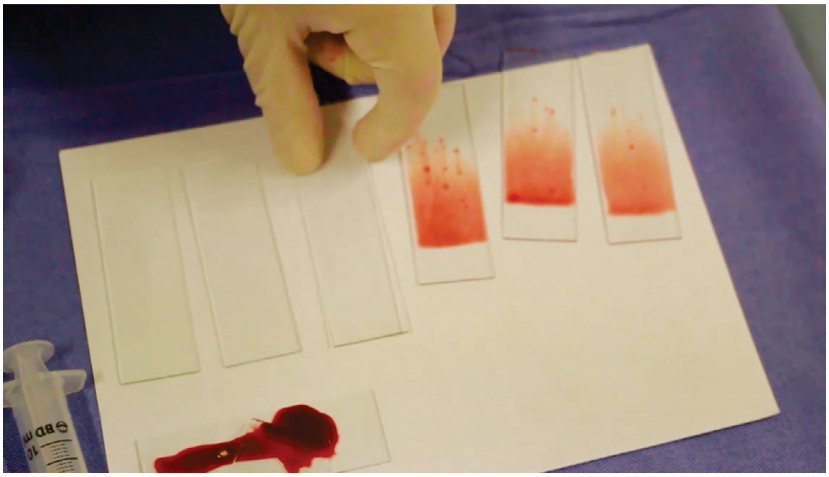


FIGURE 46 - Smear spread on the microscope slide
Source: Regional Leishmaniases Program, PAHO/WHO

ANNEX 7. RAPID IMMUNOCHROMATOGRAPHIC TEST

Procedures for the detection of antibodies to recombinant protein K39

I. Introduction

A rapid method for the diagnosis of VL is currently available. This method is based on the identification of antibodies to recombinant protein K39 in serum or whole blood samples, as specified by the supplier. Protein K39 is an epitope conserved in amastigotes of the *Leishmania* species that is responsible for visceral infection. The sensitivity and specificity of the test is very close to 95% and it is considered as being comparable to parasitological tests. Therefore, it may replace parasitological diagnosis as the universal standard for making decisions about how to treat VL when this decision is being made in peripheral health centers in endemic areas.

II. Purpose

Establish the necessary activities to carry out the diagnosis of VL through the rK39 immunochromatographic test.

III. Responsibility

Bacteriologist or microbiologist.

IV. Biosafety

Universal precautions for the manipulation of contaminated fluids and biological material should be considered. Special temperature or humidity conditions are not required for carrying out this test.

V. Principle of the method

The rK39 reactive strip is a rapid immunochromatographic test that consists of the qualitative detection of antibodies against *L. donovani - L. infantum* in human serum or blood, depending on the producer. The test is useful for the presumptive diagnosis of VL.

During the test, the serum sample reacts to colloidal conjugated protein A, which at the same time is conjugated to a stain that is embedded in the reactive strip. The serum -conjugate mixture migrates by capillarity through the membrane chromatography up to the area where the rK39 antigen is embedded. If the serum sample contains antibodies to the rK39 antigen, which is present on the strip, the antibodies will react to the antigen and a red line will appear at the reaction site. The presence of the red line indicates that the result is positive, while the absence of the red line indicates that the result is negative.

The chromatography strip contains a second region that is embedded with an anti-protein A antibody that is obtained from chickens. Thus, regardless of whether or not there are antibodies in the patient's serum sample, when the mixture migrates up to this second region, a red line will always appear. The presence of a second red line serves as a control that the sample is sufficient and that the strip and reagents are functioning adequately.

VI. Materials, reagents, and protection elements

a) Materials

- rK39 reactive strip.
- Chronometer.

b) Reagents

- Serum, depending on the producer.
- Blood, depending on the producer.

c) Protection elements

- Laboratory gown.
- Gloves.

VII. Description of the activities

Follow the manufacturer's orientations for use.

1. Remove the strip from the packaging,
2. Deposit one drop of blood or serum on the absorbent pad on the lower part of the strip.
3. Submerge the strip's absorbent pad in the well with buffer solution.

Read the results in the time determined by the manufacturer.

VIII. Report of results

Positive: when two red lines appear on the strip, both in the area for the control and in the area for the sample (control and patient sample). The red color can vary in intensity, depending on the amount of antibodies present.

Negative: when the red line appears only in the area corresponding to the control.

Invalid: when no line appears in the area for the control.

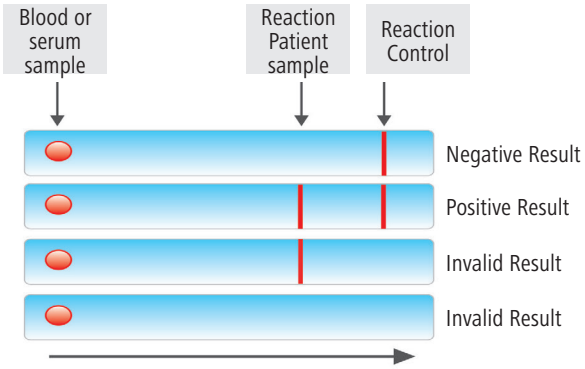


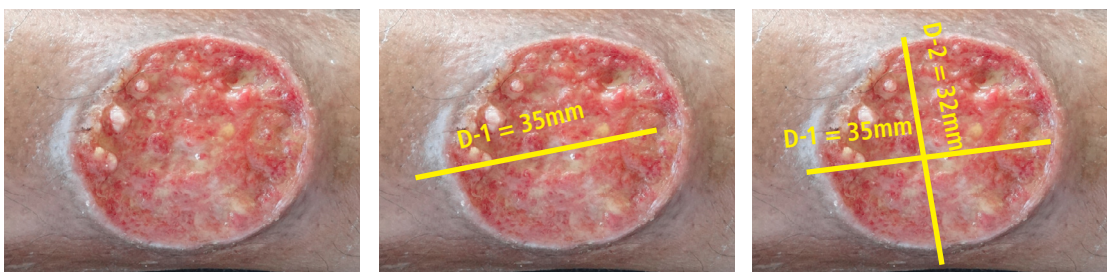
FIGURE 47 - Scheme for reading antibodies to the recombinant protein

ANNEX 8. SIZE OF THE LESION

Estimate of the size of the skin lesion for clinical assessment and follow-up to the therapeutic response

I. Estimate of the size of the skin lesion

Estimate of skin lesion size should be made while considering the scheme and figures below. It is important to mention that the proposed measurement does not imply specific mathematical formulas. Instead, its purpose is to estimate the size of the lesion to support clinical evaluation and decisions (intralesional injection of pentavalent antimony) and follow-up to treatment of lesions:



Typical ulcer | Diameter 1 = 35 mm | Diameter 2 = 32 mm

Area of the lesion: diameter 1 in mm x diameter 2 in mm
35 mm x 32 mm = 1,120 mm^2

FIGURE 48 - Estimate of the diameter of the lesion

Source: J. Soto: Funderma- Bolivia

Estimate of the amount of pentavalent antimonial for intralesional injection				
Area of the lesion in mm^2	x	0.008	=	Number of ml to inject
1120	x	0.008	=	8.96

Estimate of pentavalent antimonial for intralesional application

Source: J. Soto: Funderma- Bolivia

NOTE: The estimate will guide the amount of pentavalent antimonial to inject in the lesion. However, during the procedure, observe that the given injection quantity covers the entire lesion.

ANNEX 9. COSTS OF ANTILEISHMANIAL DRUGS

TABLE 19 - Prices of antileishmanial drugs in January 2019, Americas.

COMPOUND	BRAND NAME AND MANUFACTURER	PRICING INFORMATION [a, b]
Meglumine antimonate	Glucantime®, Sanofi Aventis Only source	Price negotiated by WHO: US$ 1.20 per ampoule in the box with 10 ampoules of 5 ml, 81 mg/ml Price negotiated by WHO: US$ 7.67 per ampoule in the box with 5 ampoules of 5 ml, 81 mg/ml
Liposomal amphotericin B	AmBisome®, Gilead (U.S.) Only source	Price negotiated by WHO: US$ 20.00 per vial of 50 mgb [b, c]
Sodium stibogluconate	Pentostam®, GSK	66.43 GBP per vial of 100 ml, 100 mg/ml [d]
Generic sodium stibogluconate	SSG, Albert David (India) Only source	5.65 EUR per vial of 30 ml, 100 mg/ml [d]
Pentamidine isethionate	Sanofi Aventis	Price reported by the PAHO/WHO Strategic Fund: US$ 38.11 per box with 5 vials, 300 mg.

Source: WHO TRS 949. Control of Leishmaniasis. Adapted and updated

[a] Prices and currency indicated by the manufacturer. Valid for governments, United Nations organizations, and nongovernmental organizations. Information on access to drugs at the prices negotiated by WHO is available on the WHO website: www.who.int

[b] The price depends on the volume of the order.

[c] Price established each year, with a ceiling of US$ 20 per vial.

[d] Price cited in the British National Formulary 59.

NOTE: the costs are those given by companies in the currencies indicated. They have been maintained as such to avoid potential variations.

ANNEX 10. IDENTIFICATION OF *LEISHMANIA SP*. SPECIES

I. Introduction

The "gold standard" for the typification of *Leishmania* is the isoenzyme test. However, there are alternative methods such as reactivity to monoclonal antibody panels and PCR-based methods that still require standardization and validation at the regional level. This is a proposal that PAHO/WHO currently leads.

II. Indications for species identification

1. In outbreak situations.
2. In new transmission foci.
3. In endemic foci without previous knowledge about the circulating species.
4. In endemic foci with presence of an atypical epidemiological situation.
5. In special clinical situations in which species identification guides clinical management (e.g. immunosuppressed patient).

In situations in which species identification is required, the reference laboratory for the Region of the Americas (Fiocruz) should be contacted to evaluate the logistical feasibility of sample collection, conservation, and shipment following the recommendations already established in the official document sent to all countries.

TABLE 20 - Sample collection, conservation, and shipment for identification of *Leishmania* species in the regional reference laboratory.

Type of sample	Material	Temperature for immediate conservation and maximum time between sample collection and reception in the reference center	Long-term conservation	Typification method	Observation
Culture	Culture medium with promastigotes	24 °C to 26 °C 7 to 15 days (liquid or biphasic medium, respectively)	Does not apply	Isoenzymes, antibodies monoclonal, PCR, PCR-RFLP, sequencing	Both for typification with isoenzymes and with monoclonal antibodies, requires a large quantity of promastigotes (>1 x 108)
Aspirate	Syringe with aspirate	Refrigeration 4 °C to 8 °C 48 hours	In lysis buffer -20 °C to -80 °C	PCR, PCR-RFLP, sequencing	Avoid freezing or thawing due to degradation of nucleic acids.
Scraping	Sterile Eppendorf tube with scraping	Refrigeration 4 °C to 8 °C 48 hours	In lysis buffer -20 °C to -80 °C	PCR, PCR-RFLP, sequencing	Avoid freezing or thawing due to degradation of nucleic acids.
Swab	Swab with sample	Refrigeration 4 °C to 8 °C 72 hours	-20 °C to -80 °C	PCR, PCR-RFLP, sequencing	Avoid freezing or thawing due to degradation of nucleic acids.
Biopsy	Container with biopsy in conservation solution	OCT: -20 °C to -80 °C, until processing	< -80°C	PCR, PCR-RFLP, sequencing	Avoid freezing or thawing due to degradation of nucleic acids.
		70% alcohol, until processing	< -80°C		
		Paraffin at room temperature, until processing	Room temperature		

Source: PAHO/WHO, 2018, IOC/Fiocruz

ANNEX 11. VECTOR CONTROL. PHLEBOTOMINES

I. Introduction

The approach to vector control based on insecticides, which was successful and had a positive public health impact when it began, meant that environmental management and other alternative methods were not used or were even forgotten.

At the start of the new millennium, the Integrated Vector Management (IVM) strategy was proposed. IVM is based on a more flexible, rational and comprehensive approximation that considers the possibility of the simultaneous control of various transmitting insects of the principal vector-borne infections present in endemic places, the integration of different control methodologies and strategies, and intersectoral action.

Potential control methods can be environmental, mechanical, biological, or chemical. To ensure the appropriate selection of control measures, the following should be considered: the biology of the vector and its behavior; the advantages and disadvantages of the methods in local contexts; and community acceptance.

IVM is conceived as a flexible management system that can adapt to local changing conditions, following cyclical processes with multiple rounds of situation analysis, planning, design, implementation, monitoring, and evaluation, among other elements.

The foundational elements of IVM vector control methods presented below can be adopted for the control of phlebotomines.

TABLE 21 - Vector control methods.

ENVIRONMENTAL CONTROL	
Reorganization of the environment	- Housing improvement - Collection of waste and other materials - Urban planning
MECHANICAL /PHYSICAL CONTROL	
Focused on reducing human/vector contact	- Mosquito nets - Mesh netting for doors and windows - Adequate clothing
CHEMICAL CONTROL	
Focused on reducing vector density and increasing vector mortality	- Household residual spraying

Source: PAHO/WHO, VT/PREnt.

Historically, chemical control is one of the measures that has been shown to reduce cases of vector-borne diseases, especially in epidemic situations, when implemented in an adequate manner and with adequate intensity. However, today, diverse socio-environmental determinants should be considered for intervention models included in the IVM framework both in terms of effectiveness and sustainability. It should be highlighted that, regardless of the strategy, the use of insecticides should be rational and include systematic planning and supervision. Furthermore, control of vector-borne disease transmission in urban areas or endemic transmission situations is complex and laborious and results are not always satisfactory. Therefore, it is essential that alternative methods such as

environmental management and health education be strengthened and integrated, for routine use in health programs.

Community participation in vector control is a process that seeks to integrate popular knowledge, perceptions, and practices into IVM strategies. Health education permits the formation and internalization of accurate knowledge about disease transmission, primarily understanding of vector biology. It is expected that through participatory, communication and education activities, the population can act to prevent and control vectors in a more consistent, effective and sustainable way over time.

Environmental control is a tool designed to reduce human-vector contact and, as a result, new cases of the specific disease. Environmental changes such as cleaning, elimination of organic waste, pruning of trees, and reduction of sources of humidity are measures that hinder the development of immature forms of sand flies, which require organic matter, temperature, and moisture for their development. In this context, it is suggested that there is a direct impact on the vector population curve in the area where these activities are applied, demonstrating that this can be an effective tool for reducing vector populations.

II. Chemical control

Chemical control is one of the vector control measures recommended in the context of short-term collective mitigation. This measure is targeted only at adult insects and its purpose is to prevent or reduce contact between the transmitting insect and the human or animal population, consequently reducing the risk of disease transmission.

The success of chemical control will depend on, among other factors: technical aspects of the application, including how and with what (as described in the following sections); environmental aspects such as type of surface and exposure to light and rain; and biological and behavioral aspects of the vectors that determine where, when, how often, and for how long. In this regard, the residual insecticide application can be effective when the adults of the vector species eat and rest in closed household spaces or animal shelters (endophagic and endophilic); when females' reproductive potential is highest (given the disease incubation cycle during an outbreak, the peak of human cases can occur when vectors are no longer present or when the time for effective application has lapsed); and periodicity of the application will be defined by the species' biological cycle and extradomiciliary breeding sites. The most commonly used methodology is standardized indoor residual application (IRS) on the internal walls of the house, animal shelters and peridomestic constructions. In the case of the residence, insecticide is also applied on the external walls, although in this case, its residuality is much lower.

The use of insecticides for vector control should follow the recommendations of the WHO expert group (WHOPES or WHO Pesticide Evaluation Scheme). After exhaustively analyzing pesticides, WHOPES/WHO has facilitated countries with a list of products that can be used in public health (Table 22).

Insecticides used for the control of sand flies are from the pyrethroid (PY) class (Table 22). Alpha-cypermethrin SC 20% and deltamethrin are the most commonly used formulations in several of the Region's countries. Those insecticides, applied according to recommendations, can have a residual effect over an average period of 3 months. Hence, their application is indicated in intervals of 3 to 4 months. However, studies indicate that there is a relationship between residuality and the wall type sprayed, which means that residuality can decline. Furthermore, it is known that the external walls of the house are more greatly affected by environmental factors such as rain and sun and therefore have lower residuality than the internal walls.

TABLE 22 - Formulations of residual pesticides approved by WHOPES for household spraying.

MOLECULE AND FORMULATION	CHEMICAL GROUP	DOSE (g/m²)	MODE OF ACTION	RESIDUALITY (MONTHS)
DDT, WP	OC	1-2	Contact	>6
Malatión, WP	OF	2	Contact	2-3
Fenitrotión, WP	OF	2	Contact and aerial	3-6
Pirimifós-metilo, WP, EC	OF	2	Contact and aerial	2-3
Pirimifós-metilo, CS	OF	1-2	Contact and aerial	4-6
Bendiocarb WP, WP-SB	C	0.1-0.4	Contact and aerial	2-6
Propoxur WP	C	1-2	Contact and aerial	3-6
Alfa-cipermetrina, WP, SC	PY	0.02-0.03	Contact	4-6
Alfa-cipermetrina, WG-SB	PY	0.02-0.03	Contact	>4
Bifentrina, WP	PY	0.02-0.03	Contact	3-6
Ciflutrina, WP	PY	0.025-0.05	Contact	3-6
Deltametrina, SC-PE	PY	0.025-0.05	Contact	6
Deltametrina, WP, WG, WG-SB	PY	0.02-0.025	Contact	3-6
Etofenprox, WP	PY	0.01-0.3	Contact	3-6
Lambda-cihalotrina, WP, CS	PY	0.02-0.03	Contact	3-6

Source: WHOPES

The frequency and number of annual application cycles depend on the species, its seasonal population dynamic, and the climatic and environmental variables already described. Regardless, it is important to not carry out "empirical" chemical interventions, as determined by the routine operations of the leishmaniasis program or their effectiveness in other vector programs. Given the variability of the effectiveness of insecticide by species, practices associated with human exposure (site and timing), landscape, and climate, validation of effectiveness through controlled experiments and both entomological and epidemiological indicators is recommended. On the other hand, during an outbreak, a focus study should be implemented to confirm that the place and time in which the intervention is carried out are those with the greatest transmission risk and those in which it is feasible to impact the vector species population. This is since there are no recommendations for peridomiciliary or extradomiciliary spraying.

III. Equipment

EQUIPMENT

The selection of appropriate equipment for each situation is an important part of a vector control program. Materials should be in good condition, which requires periodic maintenance and personnel trained in the management of insecticides, equipment and biosafety. Among the equipment used, correct choice of nozzle and type of spraying equipment (knapsack or manual sprayer) are essential for this activity.

NOZZLES

Nozzles are classified by the type of energy used to fragment and propel insecticide particles. Types of energy include gaseous, centrifugal, and hydraulic, with the latter representing the most commonly used energy type for residual spraying in public health.

The specific recommended type of hydraulic energy nozzle is the "HSS TeeJet 8002E," which means: "HSS" refers to the type of manufacture material; "TeeJet" refers to the discharge of particles in a "jet" of fan-shaped thin covering; "80" refers to the angle of the fan's opening; "02" refers to the nozzle flow (0.2 U.S. gallons/minute = 757 ml/min); and "E" refers to the uniform deposit of material on the sides of the jet.

Nozzles that present a flow higher than 900 ml/minute due to erosion should be discarded.

SPRAYER - PUMP EQUIPMENT

For equipment, 10-liter, variable-pressure sprayers or constant, manual pumps are used. Constant pressure pumps have a manometer coupled with reading at a mean pressure of 40 lb/in^2 (82 kg/cm^2, 2.72 atmospheres) and a range from 25 to 55 lb/in^2. It should be pointed out that for the use of variable pressure pumps, it is necessary to observe the change in the fan formed on the surface since it shows the reduction in the pump's pressure, in turn indicating the need to "pump" the pump's lateral lever.

To date, there are no studies that prove the effectiveness of insecticide applied using ultra-low volume (ULV) in leishmaniasis vector control. This means that the impact of the insecticide needs to occur with the vectors in flight and as a result, the use of ULV is not recommended for leishmaniasis control programs.

APPLICATION TECHNIQUE

To obtain the expected results, it is indispensable to consider the factors that can directly influence the application and deposit of the insecticide on surfaces. Key factors include: the injector consumption; the operating pressure that affects insecticide flow; insecticide concentration and speed of application, which influence the quantity of insecticide deposited on the surface; and the distance from the end of the nozzle to the wall, which influences the size of the fan generated by the nozzle, with a closer distance resulting in a smaller fan.

Given the aforementioned parameters, a pump flow of 757 ml, ranging from 700 to 850 ml, is recommended. The distance from the nozzle to the surface should be 45 cm, which guarantees the angle of 80^o. The application speed in a 19m^2 area should be one minute to ensure the deposit of the correct quantity of insecticide on the surface.

INDICATIONS FOR CHEMICAL CONTROL

As previously described in the vector surveillance and control section, chemical control can only be indicated in specific situations. However, the information below presents a brief summary of the indications for VL and CL.

A. Visceral leishmaniasis (VL)

Rural environment:

a) Human or canine cases of VL in areas with no history of transmission: If the focus investigation confirms that the first cases are autochthonous, a chemical blockade should take place in the focus investigation area or locality, as justified by the distribution and density of residences.

b) Outbreaks of human VL in areas with known transmission: If there is a concentration of cases and presence of vectors (according to the entomological survey), chemical blockade should take place in the locality, as justified by the distribution of cases and the density of residences. It is necessary to evaluate the impact of chemical control through periodic surveys and domestic reservoir incidence studies.

Peri-urban/urban environment:

a) Human or canine cases of VL in areas with no history of transmission: If the focus investigation confirms that the first cases are autochthonous, chemical blockade should take place in the focus investigation area.

b) Outbreaks of human VL in areas with known transmission: The relevance of carrying out a chemical intervention will be evaluated when there is a high concentration of incident human cases and an increase in vector abundance. The intervention should be implemented in limited areas of a certain size to be able to guarantee the operational quality and feasibility of the intervention in a short time.

c) Areas with moderate, high, intense, or very intense transmission: When chemical control is implemented, it is necessary to carry out pre- and post-control entomological surveys using an impact study design and canine serological surveys at least once a year.

B. Cutaneous leishmaniasis (CL)

Intervened sylvatic environment or rural or peri-urban environment:

a) Cases or outbreaks of CL in areas with no history of transmission: If the focus investigation confirms autochthony, carry out chemical blockade of the focus investigation area and provide recommendations on prevention and environmental management.

b) Outbreaks of CL in areas with low transmission: When the focus investigation demonstrates that there is domiciliary/peridomiciliary transmission and that it is still occurring, carry out chemical blockade of the area where the CL cases are concentrated and provide recommendations on prevention and environmental management.

c) Moderate, high, intense, or very intense endemic transmission:

1. When the indices of transmission cases in children and/or women are higher than the country average and the entomological survey indicates that there is high vector abundance in domiciliary/peridomiciliary areas that are far from primary/ secondary vegetation. In the presence of atypical CL, differentiate between the groups under analysis when calculating the transmission indices.
2. When the periodic entomological survey indicates an increase in vector abundance in domiciliary/peridomiciliary areas that are far from primary/secondary vegetation.

When chemical control takes place, the guidance is to carry out pre- and post-control surveys using an impact study design and to consider epidemiological indicators.

NOTE: In all epidemiological situations, recommendations on prevention and environmental management will be made with the goal of reducing vectors' reproductive success and minimizing vectors' contact with humans or reservoirs. Furthermore, in the case of VL, it is necessary to intensify guidance on responsible pet ownership and reproduction. Chemical control should always be part of IVM, as applicable in the situations specified in each scenario and according to procedures that are already validated or will be validated through impact action-research with both entomological indicators for the species involved at the site and epidemiological indicators.

ANNEX 12. PHLEBOTOMINE COLLECTION FORM

Model

Pan American Health Organization — World Health Organization, Regional Office for the Americas

FORM FOR COLLECTION OF PHLEBOTOMINES

***Complete 1 form for each vector collection point**

Form code: _____

Country: _____ District: _____

Department: _____ Province: _____

Geographical coordinates: _____ Latitude _____ Longitude

Name and surname: _____

Address: _____

A) Reason for the work:

		CL	VL
1 - Vulnerable area			
2 - Receptive area			
3 - Human cases CL/VL			
4 - Canine cases VL			

B) Methodology

	Entomological survey
	Focus investigation
	Monitoring

C) Information about data collection:

INTRADOMICILIARY AREA — **Identification of CDC trap:** _____

Date	Schedule	Temp.	Humidity	Rain	Wind		Phases of the moon	
	Start:				1 - Still		1 - Waxing	
	End:				2 - Light		2 - Full	
	Start:				3 - Moderate		3 - New	
	End:				4 - Strong		4 - Waning	
	Start:				5 - Gale			
	End:							

PERIDOMICILIARY AREA — **Identification of CDC trap:** _____

Date	Schedule	Temp.	Humidity	Rain	Wind		Phases of the moon	
	Start:				1 - Still		1 - Waxing	
	End:				2 - Light		2 - Full	
	Start:				3 - Moderate		3 - New	
	End:				4 - Strong		4 - Waning	
	Start:				5 - Gale			
	End:							

EXTRADOMICILIARY — **Identification of CDC trap:** _____

Date	Schedule	Temp.	Humidity	Rain	Wind		Phases of the moon	
	Start:				1 - Still		1 - Waxing	
	End:				2 - Light		2 - Full	
	Start:				3 - Moderate		3 - New	
	End:				4 - Strong		4 - Waning	
	Start:				5 - Gale			
	End:							

Collectors: _____

_____

Site _____ **Date** _____

ANNEX 13. DEMOGRAPHIC AND VETERINARY FORM

Model Part 1

Pan American Health Organization — World Health Organization, Regional Office for the Americas

DEMOGRAPHIC FORM

Date: _____ Form code: _____ Municipality: _____

Department: _____ Locality: _____

Data collection: [ ] Census [ ] Sample [ ] Individual

IDENTIFICATION OF OWNER:

Name and surname: _____

Address: _____

How long has the owner's family lived in this house? years _____ months _____

How many people from the owner's family live in this house? Sex: ( ) Male ( ) Female

Have you heard of human visceral leishmaniasis? ( ) Yes ( ) No

Have you heard of canine visceral leishmaniasis? ( ) Yes ( ) No

Do you know how this disease is transmitted? ( ) Yes ( ) No

Have you heard of the vector (bites at night, is smaller, has extended wings, moves in leaps and short flights)? ( ) Yes ( ) No

Where are there more insects or where do they bite more? ( ) Inside the house ( ) Outside of the house ( ) In the forest

What type of pets do you have? How many of each type?

[ ] Dogs
[ ] Hens
[ ] Cats
[ ] Pigs
[ ] Cows
[ ] Horses
[ ] Others. Which? _____

Does anyone have these symptoms ?

[ ] No
[ ] Fever
[ ] Swollen belly
[ ] Weight loss
[ ] Defined, non-ulcerated, chronic lesion

Has anyone had the disease? ( ) Yes ( ) No

Did the person receive treatment? ( ) Yes ( ) No

Description of the environment surrounding the house:

[ ] Pasture
[ ] Stubble
[ ] Forest
[ ] Other. Which? _____

Comments: _____

Name of person responsible: _____

Signature of person responsible: _____

Model Part 2

Pan American Health Organization — World Health Organization, Regional Office for the Americas

VETERINARY FORM

Inclusion criteria - rapid test	Exclusion criteria - rapid test
1. Dogs that have lived in the work area for more than 6 months;	1. Aggressive dogs;
2. Dogs that are equal to or older than 8 months of age;	2. Stray dogs;
	3. Dogs that have received any type of anti-*Leishmania* treatment;

Date: _____ Form code: _____

Data collection: ☐ Census ☐ Sample ☐ Individual

IDENTIFICATION OF ANIMAL:

Name of animal: _____ Sex: ( ) Male ( ) Female

Breed: ( ) Undefined ( ) Other. Which? _____ Age: _____

Coat color: Coat length: ( ) Short ( ) Long

Residence: ( ) Domiciled ( ) Semi-domiciled ( ) Street

Time in house: ( ) Less than 1 year ( ) 1 to 2 years ( ) Longer than 2 years

Was it born in this neighborhood? ( ) Yes ( ) No

CLINICAL EXAMINATION

General State: ( ) Good ( ) Average ( ) Poor	**Gestation:** ( ) Yes ( ) No
Condition of Body: ( ) Very thin ( ) Thin ( ) Ideal ( ) Obese ( ) Overweight	**Ectoparasites:** ( ) None ( ) Fleas ( ) Ticks ( ) Others. Which? _____
Mucous membranes: ( ) Hypocolored ( ) Normal ( ) Hyperemic ( ) Jaundiced	**Apathy:** ( ) Yes ( ) No
Skin lesion: ( ) Yes ( ) No	**Num. of lesions:** _____
Site of lesions: ( ) Ear ( ) Muzzle ( ) Scrotum ( ) Others. Which? _____	**Onset of lesions**: _____
Loss of appetite: ( ) Yes ( ) No	**Weight loss:** ( ) Yes ( ) No
Local alopecia: ( ) Yes ( ) No	**General alopecia**: ( ) Yes ( ) No
Alteration of eyes: ( ) Yes ( ) No	**Onychogryphosis (long nails):** ( ) Yes ( ) No
Dull coat: ( ) Yes ( ) No	**Epistaxis:** ( ) Yes ( ) No
Increased general nodes: ( ) Yes ( ) No	**Increased local nodes:** ( ) Yes ( ) No
Material collected: ( ) Blood ( ) Skin ( ) Bone marrow ( ) Lymph node	**Cachexia:** ( ) Yes ( ) No

Comments. _____

Name of person responsible: _____

Signature of person responsible: _____

ANNEX 14. SAMPLE COLLECTION FOR THE PARASITOLOGICAL EXAMINATION OF CANINE VISCERAL LEISHMANIASIS

Parasitological diagnosis is a method based on the analysis of the parasite obtained from biological material in: liver, lymph node, spleen, and bone marrow punctures; and skin biopsies. The sensitivity varies greatly (30-80%) depending on the sample collection conditions, parasitemia, the animal's clinical symptoms, the type of material, and technician experience. The tissue samples that have higher sensitivity and lower risk for the animal are bone marrow, skin, and lymph node punctures (Almeida et al., 2013). These procedures should be carried out by a trained veterinarian given that they are invasive methods that require total or local sedation of the animal. Before carrying out total sedation of the animal, the following should be evaluated: risks such as the animal's age, general condition and gestation; available inputs; and a favorable environment for providing any additional needed support.

Utilization of these methods by public health services is indicated only for confirmation of the case and identification of the species of *Leishmania*. This should occur with the emergence of the first autochthonous case of canine VL in an area previously considered to have no history of VL transmission.

The steps for sample collection of biological material for the parasitological diagnosis of canine VL are as followed:

1. Carry out a clinical evaluation with the dog duly muzzled and physically contained and complete the veterinary form with information about the animal and its owner.
2. Obtain the owner's authorization to sedate the animal. Use standard sedation protocols. Example: intramuscular ketamine chloral hydrate (10mg/kg) associated with acepromazine (0.2 mg/kg).
3. Collect fragments of whole skin through biopsy:
    a. In the scapular region, carry out the trichotomy with a disposable stainless-steel blade, using local sedation with 2% lidocaine chloral hydrate, without vasoconstriction, making an anesthetic button in the center of the site where the biopsies will be carried out.
    b. Carry out antisepsis with sterile gauze with 2% chlorhexidine, iodized alcohol, and 70% alcohol – in this order – at least 3 times for every reagent.
    c. Take four whole skin fragments using the 4 mm "dermatological biopsy punch" for parasitological, histopathological and molecular examinations.
    d. For the four fragments:
        i. Two are stored in 0.2 ml plastic tubes with screw tops that contain sterile saline solution with an antibiotic (penicillin and streptomycin) and antimycotic (fluorocytosine) for the cultures.
        ii. One is stored in a 0.2 ml plastic tube with screw top that contains 10% buffered formalin for histological examinations.

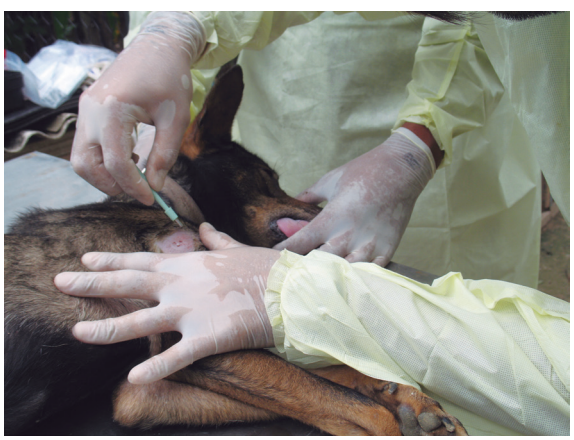


FIGURE 49 - Collection of whole skin fragments using biopsy.
Source: Laboratory of Clinical Research on Dermatozoonosis in Domestic Animals, Evandro Chagas National Institute of Infectious Diseases (Lapclin Dermozoo/INI), FIOCRUZ.

iii. One is stored in a 0.2 ml plastic tube with screw top without reagents for molecular examinations. Samples can be kept refrigerated until reaching the laboratory, where they should be stored at -20 ºC until carrying out molecular tests.

e. When there are skin lesions, carry out the same procedure used for whole skin to collect samples from the lesion.

f. Confirm that there is no bleeding. If there is, carry out procedures to control and/or stop the bleeding and apply a scarring cream, while maintaining the animal with repellent.

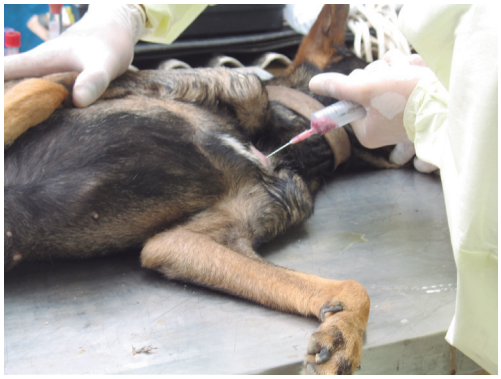


FIGURA 50 - Collection of bone marrow aspirate through puncture of the sternal manubrium.
Source: Lapclin Dermozoo/INI – FIOCRUZ.

4. Collection of bone marrow aspirate:

   a. Puncture the sternal manubrium with a 20 ml syringe and a 40x12 mm (18 gauge) needle, after carrying out the trichotomy, anesthesia, and antisepsis described in the previous paragraph.

   b. Place the material obtained for the isolation of *Leishmania* in a tube for extraction of blood using ethylenediaminetetraacetic acid (EDTA). The next step is a laboratory analysis under a hood or using a Bunsen burner to create sterile growing conditions directly in Novy-MacNeal-Nicolle (NNN) and Schneider culture media with 10% fetal bovine serum.

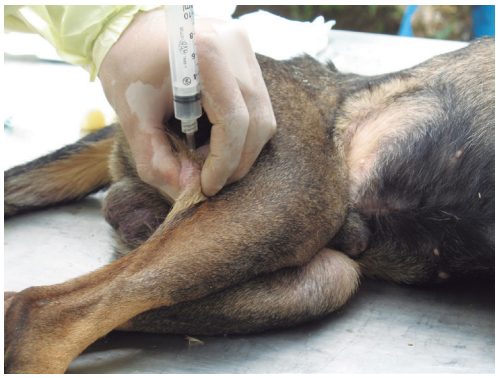


FIGURA 51 - Collection of popliteal lymph node aspirate.
Source: Lapclin Dermozoo/INI – FIOCRUZ.

5. Collection of lymph node aspirate:

   a. Puncture the popliteal lymph node (easy execution and less pain), although this procedure can also be carried out in other nodes based on the professional's evaluation (select enlarged nodes). Use a 5 ml syringe with a 0.7x25 mm (22 gauge) needle, after carrying out the trichotomy and antisepsis described above.

   b. Store the material obtained in 0.2 ml plastic tubes with screw tops that contain sterile saline solution with an antibiotic (penicillin and streptomycin) and antimycotic (fluorocytosine) for the cultures, as described in the previous paragraph.

The collected tissues will be cultivated in a biphasic medium at a temperature of 22 ºC to 28 ºC for isolation of promastigote forms of the parasite (Evans, 1989). The combination of in vitro culture of *Leishmania sp*. with isoenzymatic characterization using electrophoresis is a method that has a specificity of 100%. It is considered to be the "gold standard" reference method for the identification of species of *Leishmania* (Almeida *et al*., 2011).

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ABBREVIATIONS AND ACRONYMS

ACL	Atypical cutaneous leishmaniasis
ADR	Adverse drug reaction
Ag	Antigen
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine transaminase
AME	Adverse medical events
Amp	Ampoules
ASAT	Aspartate aminotransferase
CL	Cutaneous leishmaniasis
DALY	Disability-adjusted life year
DCL	Diffuse cutaneous leishmaniasis
DIF	Direct immunofluorescence
DNA	Deoxyribonucleic acid
EKG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
GRADE	Grading of Recommendations Assessment, Development and Evaluation
h	Hours
HIV	Human immunodeficiency virus
ICTRP	International Clinical Trials Registry Platform
IDRM	Intradermal reaction of Montenegro
IFN- ɣ	Interferon gamma
IgG	Immunoglobulin G
IIF	Indirect immunofluorescence
IVM	Integrated vector management
kg	Kilogram
LILACS	Latin American and Caribbean Health Sciences Literature
LTh1	Type 1 T helper lymphocyte
Medline/PubMed	Medical Literature Analysis and Retrieval System Online
mg	Milligram
ml	Milliliters
mL	Mucosal leishmaniasis
mm	Millimeters
MRP	Medication-related problem
MRUP	Medication-related utilization problem
PAHO	Pan American Health Organization
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
qPCR	Real-time (quantitative) polymerase chain reaction
RFLP	Restriction fragment length polymorphism, which refers to specific nucleotide sequences in DNA
rK39 antigen	Antigen specific to recombinant protein k39
SOP	Standard operating procedure
RPL	Regional Program for Leishmaniasis
Sb5+	Pentavalent antimonial
SciELO	Scientific Electronic Library Online Latin America
SisLeish	PAHO/WHO Leishmaniasis Regional Information System
Tc	Cytotoxic T lymphocyte
TDM	Therapeutic drug monitoring
TNF	Tumor necrosis factor
TOR	Toxic oxygen radicals
UNICEF	United Nations Children's Fund
VL	Visceral leishmaniasis
WHO	World Health Organization

FIGURES

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GRAPHIC PROJECT
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Pan American
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Organization

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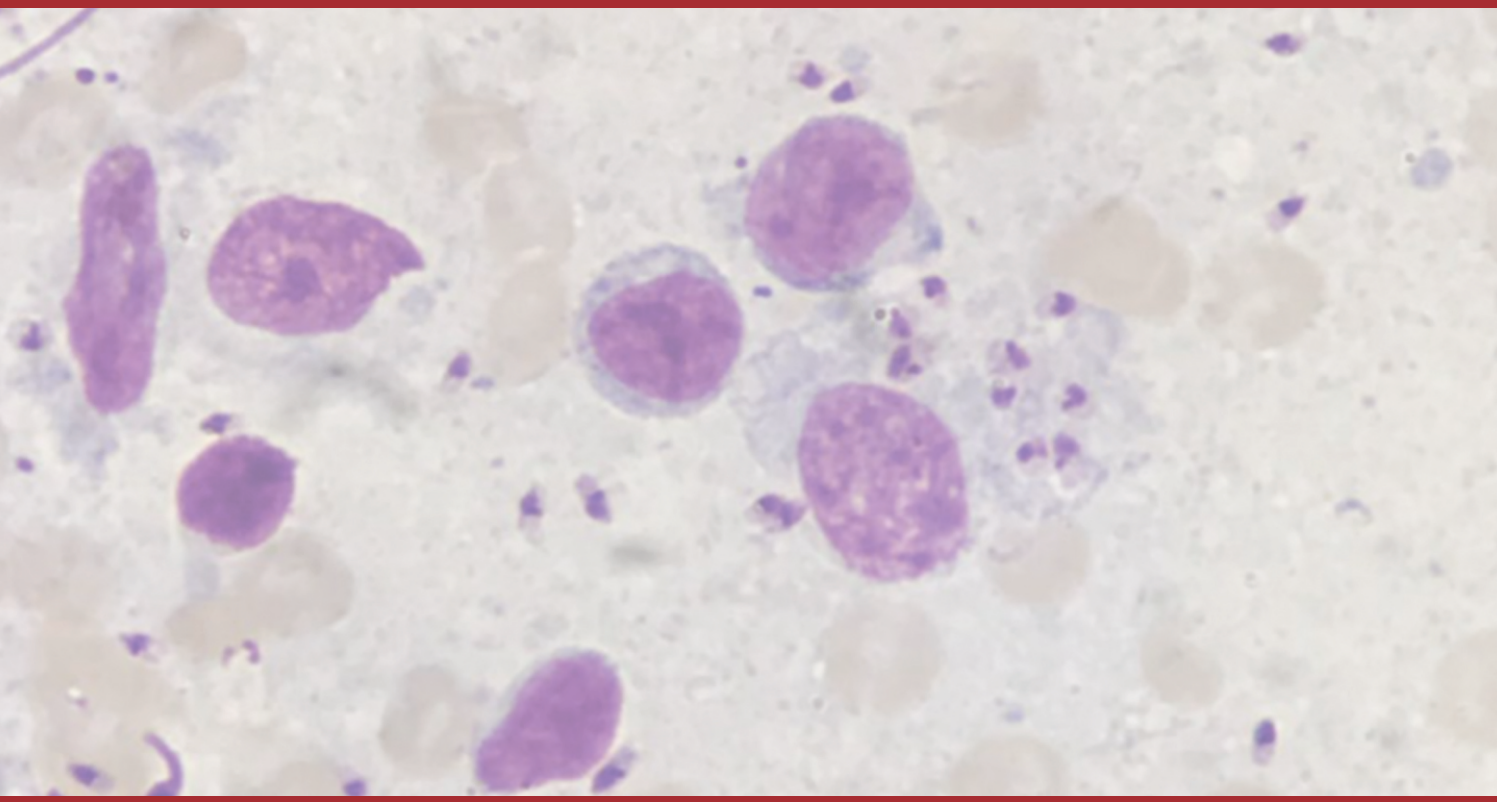
REGIONAL OFFICE FOR THE Americas



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