Chapter 3

Application of the Eco-Epidemiological Method in the Study of Leishmaniasis Transmission Foci

Iván D. Vélez, Lina M. Carrillo, Horacio Cadena, Carlos Muskus and Sara M. Robledo

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66430

Abstract

The study of transmission foci involves the clinical characterization of disease within a focus using active surveillance of human cases, characterization of the population group that is most frequently infected or at highest risk of becoming infected, diagnosis and treatment of infected people and identification of the conceptions, attitudes, beliefs and practices related to the disease. An entomological survey is necessary to determine the geographical distribution of species and incrimination of vector species, the ecological characteristics of the zone (macro-focus), times of the year and day of highest biting activity and places in homes in which the most frequent contact between the people and the vectors occurs. A survey of peri-domestic and wild mammals in the area is advisable to try to identify potential reservoir hosts. Using this information, it is feasible to design specific and accurate prevention and effective, rational and economic control measures and define the times of the year and locations in which these measures must be applied. In this chapter, a description of the application of the eco-epidemiological method to the study of leishmaniasis transmission foci is provided. A special emphasis is placed on the methodology, multidisciplinary work and analysis of findings.

Keywords: Leishmaniasis, eco-epidemiology, reservoirs, *Leishmania*, *Lutzomyia*, factor risk

1. Introduction

Leishmaniases are a group of diseases caused by at least 20 species of parasites of the genus *Leishmania* that are transmitted to humans and other mammals by the bite of *Lutzomyia* species (in America) and *Phlebotomus* species (in the Old World). Reservoirs of these parasites include domestic and wild animals and, sometimes, humans; therefore, Leishmaniasis transmission

INTECH
open science | open minds

© 2017 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

may be zoonotic (from animals to humans) or anthroponotic (from one human to another). In humans, *Leishmania* infections cause a spectrum of illness that depends on the parasite species involved, inoculum size, and host immune response [1, 2], affecting skin, mucous tissues, or organs of the mononuclear phagocyte system and producing clinical symptoms of cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML), and visceral leishmaniasis (VL), respectively.

Leishmania spp. infections are acquired when an infected vector bites a mammal to consume its blood. In turn, the vector is infected when it feeds upon the blood of an infected reservoir species and ingests the transmitting parasites (as amastigotes). Transmission cycles of leishmaniasis have been found to have a focal distribution in specific geographic areas. These sites are called the natural foci of infection. The foci of infection are the places where the key elements necessary for transmission are present: vectors and reservoirs. The limits of infection foci are generally determined by the spatial distribution and relative density of the vector species. Hence, conducting entomological surveys and determining the behavior of vectors are important to clarify the epidemiological risk of infection. In turn, the presence of these elements, and especially of vectors, is conditioned by abiotic ecological factors such as climate, humidity, altitude, temperature, and vegetation [3, 4].

The study of the foci of Leishmaniasis transmission is complex due to the following factors: (1) the diversity of phlebotomine species; (2) the variety of *Leishmania* species; (3) the incrimination of a phlebotomine species requires that the vector meet certain criteria, including exhibiting anthropophilic behavior, being infected with the same species of *Leishmania* isolated from humans infected during the outbreak, and demonstrating geographical distribution consistent with the distribution of reported human cases; (4) the variety of methods required to incriminate potential mammalian reservoirs in an area, which involve capturing and analyzing many samples, isolating and identifying the species of *Leishmania* and determining the parasite prevalence and transmissibility; and (5) the challenge of diagnosing human cases with the clinical form of leishmaniasis, *Leishmania* species causing the disease, and locations in dwellings where transmission occurred [5, 6].

On the other hand, to facilitate the design and implementation of specific control measures, recognition of the transmission mode within a focus and determination of the eco-epidemiological risk of infection are required. Factors that must be determined include the following: (1) the geographical area associated with greatest risk of transmission, or "macrofocus"; (2) the population group at increased risk of becoming infected and developing the disease; (3) the time of year during which increased activity of phlebotomine species in the intradomicile environment occurs (nictemeral behavior); and (4) the place within the dwelling where contact between vectors and the population group at highest risk of infection, or "microfoci," are located [2]. Therefore, understanding the transmission of *Leishmania* infection, identifying the foci of transmission, and designing strategies and control measures requires a combination of different disciplines such as the health sciences, epidemiology, social sciences, entomology, cellular and molecular biology, and ecology, giving rise to eco-epidemiology. In addition to the inclusion of transmission (i.e., the vector), parasite, and reservoir dynamics as objects of eco-epidemiological study, this methodology also includes the study of associated ecological factors and human behaviors that affect the transmission of the disease.

http://dx.doi.org/10.5772/66430

As such, application of the eco-epidemiological methodology allows for determination of the following parameters: (1) identification of the species of *Leishmania* and phlebotomine in a given region, both overall and specifically those that serve as vectors, and determination of the role of domestic or wild reservoirs; (2) definition of the macrofocus (i.e., the geographical areas where the vector is present), its limits, and the environmental characteristics that allow the vector to develop, including ecological markers such as temperature, vegetation type, soil, altitude, and other aspects that characterize the macrofocus; (3) identification of both times during which there is a greater risk of infection (i.e., the times of night during which increased vector insect activity occurs) and locations in which vectors have a higher rate of natural infection with *Leishmania* and more contact with a population at risk using vector bionomics and behavior; (4) definition of the microfocus, which, as previously stated, is the location in a dwelling where contact between the human population and the infected vector occurs, that is, if this location is inside the home (intradomicile), around homes (peridomicile) or outside the home (extradomicile); (5) accurate determination of the population group most affected by the disease or at increased risk of infection, for example, if this population comprises men, children, or people who perform some activity (such as loggers, workers in mines, or some special type of farming or hunting) or if entire families are affected by the disease; and (6) identification of medical systems used by the community, i.e., how the population conceives the disease and its cause, from whom they seek treatment, and how the disease is treated. The identification of these parameters allows for the generation of elaborate risk maps and design of rational, economical, and effective prevention and control measures [7].

2. Activities to develop in a study of leishmaniasis transmission focus

When the study of a focus of transmission is initiated, it is necessary to carry out different activities involving all stakeholders involved in the transmission of infection, that is, the human population living in the area, possible vectors, and populations of mammals serving as potential reservoirs. Similarly, knowing the conceptions, attitudes, and practices of the people about the disease is critical to design appropriate control measures for each community. The following sections describe each of these activities.

2.1. Study of human population

Studies of resident populations are conducted by active case finding, conducting epidemiological surveys, and comparing the knowledge, conceptions, attitudes, and practices within a community against the prevalence of the disease and behaviors that facilitate being bitten by vector species.

Active surveillance for cases is performed to develop a detailed report describing the residents of a population, with a special emphasis on the identification of leishmaniasis lesions on both the skin and mucous membranes through clinical examination of the nasal-oropharyngeal region. Additionally, any signs or symptoms compatible with VL, such as fever and/or hepatosplenomegaly, should be identified, especially in children and adolescents. Examination of naso-oropharyngeal mucosa should also be performed on all people with a history of the disease or with compatible scars. During active case finding, it is possible to identify patients who have very advanced mucosal lesions causing destruction of the nose, mouth, pharynx, and lip. Because of their disfigurement, these individuals may not seek treatment at health centers or hospitals within the region, as they have lost all hope of being cured and have been isolated from the social environment due to their facial disfigurement. These people should be actively sought out using information provided by the community in order to obtain the necessary samples to make a diagnosis and initiate treatment. Samples may be then collected from the suspected cases of CL or ML, including scraping for smear and aspiration for culture in the NNN (Novy-MacNeal-Nicolle) medium. All samples should also be analyzed using PCR. In cases of ML, scraping for smear has been found to provide a useful sample [8]. In VL cases, a sample of spleen aspirate or bone marrow (for the smear, culture, and blood tests to detect specific antibodies against *Leishmania* spp.) may be collected; all these procedures have been established by the World Health Organization (WHO) [2].

It is very important to examine the largest possible number of residents in a study site to diagnose active cases and include patients presenting injuries in the healing process due to the use of empirical treatment (such as people with compatible scars and a clinical history that gives reason to suspect that the person had leishmaniasis) and record previous cases. In the analysis of this information, new and old cases may be compared by age, sex, profession, or occupation. Thus, population groups at increased risk of leishmaniasis may be individually identified.

It is also important to diagnose leishmaniasis early because it allows for the initiation of specific treatment as soon as possible. When treatment is initiated early, it is possible to control the progression of the disease, relieve the signs and symptoms of the disease and improve quality of life in patients who are exposed to great social stigma due to the physical "marks" (scars) left by leishmaniasis that make it easy to identify those who had or have the disease.

Since clinical and epidemiological findings are not pathognomonic of the disease, and it is necessary to obtain a laboratory diagnosis to verify clinical suspicion of leishmaniasis. This diagnosis is based on visualization of the parasite in spread (smear) or cultures obtained from lesion material (in cases with CL and ML) or material obtained from aspirate, biopsy of bone marrow, or the spleen (in cases with VL).

However, in some VL or ML cases, it is not always possible to visualize or isolate the parasite, and thus clinical diagnosis may be aided by the presence of specific antibodies against *Leishmania* spp. or molecular tests.

In the case of VL, the use of a clinical and epidemiological history with a positive rK-39 has been accepted as the criteria for diagnosis of the disease. For diagnosis of leishmaniasis, the following clinical laboratory tests may be performed:

1. Smear (or spread) is an easy, economical, and rapid procedure; with appropriate sampling and interpretation techniques, the sensitivity of this procedure can reach 90%. The test involves the collection of tissue from the active edge of a lesion and center of an ulcer for CL and ML. If a case has VL, the sample is taken from bone marrow, liver, or the spleen by aspiration, as described later. For cases with skin lesions, it is important to select lesions that are younger and do not display superimposed infection [9].

2. Cultivation technique consists of aspirating material from a lesion or the mucosa and cultivating the sample in the center of a biphasic NNN culture to encourage growth of the parasite to the promastigote stage. Cultures have been found to provide good diagnostic results, with a sensitivity of approximately 70% [9].

3. Polymerase chain reaction, or PCR, enables detection of the genetic material (DNA or RNA) of parasites in sampled lesions in patients, potential mammalian reservoirs, and phlebotomine vectors [10, 11]. The material analyzed using PCR can be scraped off or aspirated from the injury site; additionally, a blood sample or a small piece of biopsied tissue preserved in absolute alcohol or other media, such as NTE (NaCl, Tris-HCl and EDTA). For detection of parasites in phlebotomines, samples should include a pool of at least five insects or the anterior portion of an insect, including the first abdominal tergite area that includes parasites attached to the stomodeal valve [12, 13]. PCR can also distinguish between parasite species using a technique known as PCR-RFLP, which consists of determining the length of the restriction fragments of the amplified product when it is subjected to digestion by different restriction enzymes [14, 15].

There are variations of PCR, such as real-time PCR, that employ labeled probes to visualize the amplification reaction. This characteristic makes PCRs more sensitive and enables quantitative analysis of the parasites in a sample [16].

2.2. Epidemiological survey using the Montenegro skin test

This activity includes the application (to the general community) of the Montenegro skin test or "leishmanin" antigen test. This test causes an intradermal reaction that measures delayed cellular immunity response, and it has been commonly used in epidemiological studies for the identification of *Leishmania* exposure [17]. This delayed hypersensitivity test should be read 48 hours after being applied, and the pen method should be used to define the area of induration [18]. Although the Montenegro skin test is very sensitive and specific, it does not differentiate between current and past infections. The test is considered positive, or reactive, when the induration is more than 5 mm [2]. It is important to apply the test always on the same forearm (right or left) on each member of the population to facilitate reading, especially when people have negative reactions.

To interpret the results of an epidemiological survey, the common characteristics of people who have a positive test result (which can be a particular age group, profession, or occupation, without discrimination among household members) may be analyzed. For the analysis of these data, it is convenient to divide the population into four age groups: 0 to 4 years (children who usually stay inside the house), 5–14 years (children and adolescents who may have school activities and, in some rural regions, help their parents in agricultural work); 15–60 years (the economically active population); and 61 or more years (the population that most frequently stays at home). Thus, the population group that is at higher risk of infection (the population to which prevention and control measures should be prioritized) may be determined. For analysis purposes, it is helpful to determine the positivity rate, which is the number of positive tests out of the total tests performed in each subpopulation.

2.3. Conceptions, popular attitudes and practices (CAP)

Both rural and sylvan domestic endemic foci of leishmaniasis are characterized by poor and remote communities in urban centers with serious social conflict and little governmental health institution presence. In these areas, the conceptions of the cause of disease vary among population groups and are not necessarily associated with a parasite or the bite of sandflies. Communities have their own medical systems that include their conceptions and practices about the origin of the disease, facilities where they receive care when they are sick and procedures that are available for the diagnosis, treatment, cure, and prevention of the disease [19].

Identifying the conceptions, attitudes, and practices relating to leishmaniasis and medical systems operating in communities is an important component of the study of foci. This information is critical to the design and implementation of primary health education, social assistance programs, and control measures. It is therefore important to determine the locations where residents seek care when they have leishmaniasis, including healers, herbalists, pharmacists, physicians, or other healthcare providers. It is also necessary to determine the variety of treatments used, what residents believe causes disease, how residents refer to the vector and leishmaniasis, and what having leishmaniasis or skin ulcers means within their magical-religious conceptions through a respectful dialogue that enables the sharing of knowledge.

This type of research (the social type) is conducted mainly using qualitative methods but can be supplemented with quantitative methods. Within qualitative studies, techniques such as participant observations may be used. These studies require the constant presence of the researcher in the community, enabling a better approach to obtain this knowledge and facilitating the creation of bonds of trust that allow the collection of more credible information. It also allows researchers to construct an overview of the community, thereby knowing, for example, how people interrelate within the community itself and how these relationships (in one way or another) influence the CAP that have developed related to the disease. Other techniques include interviews with key stakeholders in the community, including herbalists and community leaders. Within the quantitative method exists the application of surveys with closed answers, which are most ideally conducted with all of the community members or with a statistically representative sample.

The use of social science methods allows researchers to characterize the material conditions that, in short, form a simple or complex network of social relations. These conditions influence a large number of people who are consolidated as a community with similar livelihoods and conceptions about disease. These methods also allow for the observance of human behaviors that may predispose individuals towards contact with insect vectors. Hence, there is a need for constant communication throughout the multidisciplinary group to share findings and refocus targeted observations; all together, these processes may help to determine the epidemiological risk of infection, which is the first step to design prevention and control measures that are economical and effective.

2.4. Study of the phlebotomine population

Leishmania vectors belong to the family *Psychodidae* and subfamily *Phlebotominae*. There has been no general agreement established as to the classification of phlebotomines into genus and more general taxonomic categories, but according to the most widely accepted categorization scheme, there are six genera: *Lutzomyia, Brumptomyia,* and *Warileya* in the New World and *Phlebotomus, Sergentomyia, and Chinius* in the Old World. The vector species of *Leishmania* belong to the genera *Phlebotomus* and *Lutzomyia* [2].

Although the biology of each species of phlebotomine is unique and complex, generally they are small nocturnal insects that range from 2 to 5 mm in length. By day, they remain at rest in burrows, caves, and hollow trees. The life cycle and behavior of phlebotomines are conditioned by abiotic factors (temperature, humidity, photoperiod) and biotic factors. Only the female is hematophagous, needing blood to develop eggs and maintain the gonotrophic cycle [20]. Phlebotomines are very fragile insects, and they are considered sedentary species with a short range of flight. Some studies using the techniques of capture, marking, and recapture with fluorescent powders have determined that the majority of recaptures occur within a range of less than 200 meters from the site of release [21].

The identification of phlebotomines is based on morphological characteristics in both male and female insects, especially the genitalia, alar indices, pharynx, and cibarium. Examination of morphological characteristics may help to solve problems associated with identification (usually cases where males can be distinguished but not females, which have the greatest epidemiological importance). Currently, taxonomic identification based on morphological characters may be corroborated by mitochondrial cytochrome c oxidase (COI) DNA gene-based molecular techniques, which can be provided as a barcode-specific marker for each phlebotomine species [22].

There are more than 800 currently described phlebotomine species. Approximately 465 of these species have been identified in the New World, and 375 of these species have been identified in the Old World; however, not all phlebotomine species are considered vectors [23]. A phlebotomine naturally infected with the promastigote forms of *Leishmania* is not necessarily capable of transmitting the parasite. For a species to be incriminated as a vector, certain criteria are required: an anthropophilic nature; contact with both humans and the disease reservoir; and infection by the same Leishmania strain identified in human cases. Additionally, the transmitted parasite must be able to develop in the vector, the vector must be able to be transmitted through the bite of the parasite, and the geographical distribution of the parasite must be compatible with that of the vector [2]. In America, approximately 400 species of *Lutzomyia* have been identified, but only 22 of these species have been implicated as vectors [24]. In Colombia, 153 species of *Lutzomyia* have been identified [25], but only the following species have been found to be naturally infected with *Leishmania* and incriminated as vectors: *Lu. trapidoi* and *Lu. gomezi* with *L. panamensis* [6, 21], *Lu. spinicrassa* and *Lu. gomezi* with *L. braziliensis* [26], *Lu. umbratilis* with *L. guyanensis* [27], *Lu. olmeca* with *L. mexicana* [28] and *Lu. longipalpis a*nd *Lu. evansi* with *L. chagasi* (=*L. infantum*) [29].

Although the incrimination of a species as a vector is a specialized job that requires the support of entomological research laboratories, a list of species incriminated as vectors in different countries has already been developed. Furthermore, by analyzing collected data, it is possible to identify species that potentially transmit the disease, thereby linking different elements of the study of transmission foci.

In the study of foci, one of the objectives is to determine the limits of the macrofocus, and this determination requires a combination of techniques, such as entomological transects, which involve the simultaneous capture of phlebotomines in different geographical locations, for example, along paths and roads and in houses and the extradomicile using sticky traps (which are traps with castor oil-impregnated paper). Five to 10 days later, the traps may be removed and sand flies be recovered from each sticky trap. This information can be used to determine the relative density per square meter of a particular vector species in a given geographical area. It is also possible to establish the area where the phlebotomines are present, thus specifying the limits of the focus.

To determine the risks within a microfocus, simultaneous captures of phlebotomines may be performed inside, around, and outside the home. Presence and relative density of the vector species may be then determined and correlated with other study data, such as Montenegro test positivity and the population group with the highest number of new and old cases of the disease.

To determine high activity hours during which the vector species is most likely to bite, catches may be made overnight from 18:00 in the evening to 6:00 the next day inside the dwelling (nictemeral monitoring). The specimens caught may then be stored for periods of 2 hours using a vial for each capture period (each properly labeled, indicating the hour of the capture and the place and date to be stored until identification). Additionally, captures should be performed during different seasons (rainy–summer) because the species composition and density may vary during different seasons.

To capture phlebotomines, a series of traps have been designed, among which we will mention the most frequently recommended for the study of foci.

- CDC traps with white light: These traps are installed between 6:00 pm and 6:00 am inside houses and in the peri- and extradomicile. These traps enable the capture of live specimens, which is useful for the isolation of parasites.

- Shannon trap: A Shannon trap is usually installed in the peri- or extradomicile and combines attraction to light with CO_2 emitted by people to capture insects that land on the fabric. Captures are performed using a "mouth aspirator," which consists of a glass or transparent acrylic tube connected to a rubber hose to which mouth suction is applied to suck the insect into the tube. This manual type of capture may be also used to collect insects that are dormant in housing walls, animal pens, rocks, caves, burrows, tree trunk, the buttresses of large trees, etc.

- Capture on protected "human bait": During capture, people are dressed properly and leave exposed only a portion of the lower extremities (legs); in their hands they charge a grabber and a flashlight and stay attentive to the sandflies landing. When a sand fly lands, it is captured as soon as possible thus avoid being bitten by the sand fly. This procedure has been approved by the Ethics Committee after reviewing the protocols. This technique allows for the identification of anthropophilic behavior in phlebotomine species that attempt to bite humans. During these captures, information regarding landing rates (# of sandflies/hour/man) can be obtained. Capture on protected human bait can be performed in the intra-, peri-, and extradomicile. When performed on animals, it can be used to infer the zoophilic behavior of a phlebotomine.

– Sticky traps: These traps are considered complementary to captures at rest and require less effort. Sticky traps consist of sheets of 20 by 25 cm bond paper that are fixed on a bamboo stick with a side that is castor oil-impregnated [29, 30]. Phlebotomines that settle on these traps can be used determine the density of each species per square meter trap, considering that a trap has an area of 0.01 square meter for capture. These traps are very useful for transects and to determine the spatial distribution of species and limits of foci of transmission. The traps are placed in the intra-, peri-, or extra-domicile and the number of phlebotomines captured at each point or "station" can vary, as can density because it is determined per square meter trap. When placing these traps, the geographical coordinates of the location and relationship to the domicile should be taken into consideration [6, 29–31].

The community can and should actively participate in entomological studies. The community may recognize *Lutzomyia* species using local popular names, which may vary in different regions of the country, and the community can help guide practitioners regarding the times when they see a greater abundance of phlebotomines. In Colombia, the highest phlebotomine density is frequently recorded after the onset of the rainy season. Thus, the community may inform researchers that they are being bitten more frequently inside or outside the house or at certain peak hours; however, this information must be corroborated by capture of specimens.

Entomological specimens captured using light, bait animal, resting, and human traps should be killed by introducing cotton soaked with ethyl acetate or other reagents in capture containers. Ethyl acetate must be handled carefully because of its toxicity. Subsequently, specimens should be deposited on a plate with a white background, and entomological tweezers should be used to separate the phlebotomines from bycatch species (other insects). Finally, phlebotomines should be deposited in vials, plastic bottles, or glasses with screw caps that are dry or contain 70% alcohol, according to the study objectives. The vials containing the phlebotomines should be marked with the capture source, date, method of collection, and person responsible, among other data.

2.5. Study of reservoirs

The ecology of *Leishmania* spp. is associated with their hosts; therefore, all factors affecting the survival and behavior of a host may affect the transmission cycle of a parasite. Most leishmaniases are zoonoses for which different species of animals may act as reservoirs of the human parasite. The transmission of the disease to humans requires interactions between a human being and the ecological niche of a vector and wild or domestic reservoirs [32].

It is understood that reservoirs provide an ecological system in which the infectious agent survives indefinitely. This system includes hosts, any intermediate host, or vector and any environmental component that is necessary to maintain the agent indefinitely [33]. Depending on the duration that hosts are able to maintain the parasite, they may be classified into primary (maintaining the infection for a long time), secondary, or accidental.

Reservoirs may be domestic, wild, or synanthropic, and for some species of the parasite, humans are the main reservoir. That is, the case of VL caused by *L. donovani donovani* and CL caused by *L. tropica tropica*. In the New World, leishmaniases are zoonoses; however, there is evidence of anthroponotic transmission during outbreaks, previously identified in the Andean region of the country [6, 34].

Generally, within a focus, there is a primary reservoir for each species of *Leishmani*a; however, other mammals in the same area can become infected, thereby becoming secondary or accidental hosts. Domestic, synanthropic, or wild (marsupial carnivores, rodents, Meshed, insectivores, and primates) reservoir species infected with *Leishmania* may or may not show obvious signs of infection. In general, reservoirs do not exhibit symptoms of the disease [2]. To be a source of transmission is not necessary to be a primary reservoir, and it is possible that different populations of mammals can maintain a continuous cycle of infection and become the source of infection or "primary reservoir" [35].

In America, approximately 310 species of mammals have been incriminated as possible reservoirs. More than half of these species have been incriminated just for having been detected as infected, but the majority of species have been studied and considered as wild reservoirs, such as species in the order Didelphimorphia, *Didelphis marsupialis* and *Didelphis albiventris*, which are important hosts of all Leishmania species, especially *L. amazonensis amazonensis* and *L. braziliensis*. Meanwhile the most important hosts of *L. mexicana* are *Peromyscus yucatanicus* and *Ototylomys phyllotis* rodents. For *L. panamensis* and *L. guyanensis*, an important reservoir has been identified to be *Choloepus hoffmanni* sloths [36].

The most important domestic reservoir is the dog; canine species are mainly a reservoir of *L. infantum*, but cases of infection by *L. braziliensis* and *L. panamensis* have also been observed [37, 38]. Donkeys and horses have also been incriminated as secondary reservoirs [2].

The study of reservoirs has been associated with decreased interest because of the difficulty in establishing incrimination. Briefly, to incriminate a host reservoir, the following criteria must be met: chronic maintenance of populations of parasites in each ecosystem, presentation of a parasitic load sufficient to ensure transmissibility, and identification of an appropriate population density (20% or more of studied wildlife) to provide opportunities for host-vector, host-environment, or host-host interactions depending on the type of transmission [32].

The activities used for identifying reservoir hosts include clinical, serological, and parasitological evaluations of domestic and wild mammals within a given locality. Performing these activities requires the participation of professionals specialized in the capture, identification, and sampling of domestic and wild mammals. For CL, it is very important to examine the snout, ears, genitals, appendages, and areas with less body hair (as *Lutzomyia* bites may occur on exposed areas of the skin) of a potential reservoir species and identify early CL lesions, or nodules, from which samples may be taken for direct examination, culture and/or PCR.

Some larger lesions, such as ulcerated or plate-type lesions, may also be identified [39]. In the case of VL, it is important to examine dogs for the previously defined clinical signs of VL such as peri-orbital waxing, increase in popliteal lymph node size, growing nails (onychogryphosis), emaciation (cachexia), peeling, and, sometimes, keratitis. For serology, dog blood should be drawn from the cephalic vein to facilitate collection; however, other blood vessels, including the jugular and saphenous veins, may also be used. It is important to take samples for culture, which can also be obtained by puncturing the popliteal lymph node; this sampling requires, removal of hair from the sampling area and good skin scraping [40].

Skin biopsies can be used for both pathological study and culture and PCR. It is inadvisable to use aspirate samples because pollution levels are usually very high; however, a high percentage of antibiotic and antimycotic can be used on this media [41].

2.6. Determination of the epidemiological risk of infection

Studying the eco-epidemiology of infection risk involves the identification of different segments of the population who are at risk of becoming infected with *Leishmania*. Through this methodology, the spatial, temporal, and population risk may be evaluated. Spatial risk corresponds to the macrofocus, or geographically and ecologically defined area in which the transmission process occurs, as defined by Pavlovsky [42] and developed for use in the study leishmaniasis by Rioux et al. [43]. A macrofocus can be recognized through the identification of ecological indicators such as the presence of certain plants, altitude, or average rainfall [3, 4, 7, 30].

Geographic information systems and spatial analysis of vector-borne diseases are currently very useful tools for the depiction of regions with a high prevalence of vector-borne diseases, allowing for the identification of risk factors for infection in a given area. From the human occupation perspective, establishment of specific schematic (noso-ecological) maps is the last phase in the determination of risk areas; in these maps, research groups may depict the boundaries and internal organization of the foci. These maps are a tool for the study of human ecology but may also provide a practical guide to the health authorities responsible for the implementation and maintenance of programs for the prevention and control of disease.

Maps should have a small scale of one-billionth or 1 in 500,000, and in these maps, large zones of risk may be identified, that is, the macrofoci. The maps can be used to set parameters for vector density, that is, the spatial or geographical distribution of a vector at an appropriate density, or determine the correlation between the bioclimatic or phytoclimatic zones, and living areas or vegetation types in the areas where the vector species inhabits. Within these small-scale maps, the transmission areas for each *Leishmania* species and areas in which each vector inhabits can be determined [44].

Temporal risk corresponds to the period of greatest risk and can be defined in different time scales: daily, seasonal, or one or more years. Generally, it corresponds to the periods during which there is a higher density of vectors and greater risk of parasitic infection.

3. Findings of the application of eco-epidemiological method in some focus studies on Colombia

In Colombia, leishmaniasis has been identified in all its clinical forms. It may occur in the endemic form, causing natural outbreaks throughout almost all the national territory areas with altitudes lower 2000 meters above sea level, including wilderness areas, tropical dry forests, the Andean region where coffee is grown and plains and deserts areas located in the inter-Andean region, eastern region, and Guajira Peninsula.

Our group has studied the natural infection patterns of leishmaniasis in different foci of transmission, and these studies have demonstrated that women and children are affected by leishmaniasis with equal or greater frequency than men. This finding is in opposition with the belief that leishmaniasis is more common in men (previously considered as an occupational disease that affected men during the performance of rural labor activities in forested areas inhabited by the insect vector). However, studies have shown that women (adults and children) have less access to proper diagnosis and treatment, leading to infrequent case reporting. These studies have also shown that due the domestic adaptation of vector species, the occurrence of epidemic peaks of CL in different regions may affect entire families. It was observed that the presence of gender differences depended on the locations in which the consultations took place; however, no changes in the activities performed by women in the field were identified that could explain the increased frequency of contact with the vector observed [45].

In the different studied foci, three major leishmaniasis transmission cycles have been identified: sylvan, domestic/rural, and urban.

- Sylvatic cycle: Humans are infected when they enter into the proximity of forests or jungles and are bitten by the insect vectors; in this case, humans are an accidental host who are not involved in the transmission cycle. In Colombia, military members, miners, loggers, and indigenous communities are the most affected by this transmission cycle.

- Rural/domestic cycle: This cycle occurs mainly when intra-domiciliary transmission of the disease occurs (in which vectors are inside homes and affect the entire family without discrimination by age or sex). In foci, such as Montebello and San Roque (Antioquia) [6, 28, 33] and areas with traditional coffee cultivation [46], children under the age of 5 years have been found to have the highest incidence of leishmaniasis; in these areas, the most frequently incriminated vector has been identified as *Lu. gomezi*, which has increased biting activity during the early hours of the night. When an outbreak occurs in the epidemic form, the data have shown that humans are part of the transmission cycle and act as a reservoir [29, 34].

- Urban cycle: Similar to the rural/domestic cycle, in this cycle, vectors enter the peridomicile or home and may transmit the infection to the entire family; in this cycle, a higher rate of infection in children has been observed. Additionally, in this transmission cycle, humans can be accidental hosts not involved in the transmission cycle or act as reservoirs.

In Colombia, sylvatic and domestic-rural endemic foci are characterized by locations that are generally in remote and impoverished areas of cities with great social inequities and little state health institution presence. Because the lesions are visible (usually located on the exposed areas of the skin), these lesions may become chronic and disfigure the skin or mucous membranes, and indigenous peoples have developed their own medical systems for the disease.

For the evaluation of medical systems in foci of transmission, qualitative ethnographic methods have been used in which participant observation techniques (requiring the presence of researchers within the community) are applied. Interviews also allow for the establishment of a personal dialogue with the community and key stakeholders such as healers and community leaders. Quantitative methods have also been applied through the use of surveys conducted by the investigator with questions designed in consultation with community members. Comparisons of CL cases seeking care at urban centers with cases identified through active surveillance have also shown that inhabitants in each region have developed their own conceptions about the origin and management of disease. These medical systems were found to vary between indigenous and peasant communities, such as those described below.

3.1. Medical systems in indigenous communities: Zenú, Emberá and Tikuna

The indigenous community Zenú, an endemic focus of VL in Colombia, is located in a tropical dry forest region on the Caribbean coast. The Emberá community, an endemic focus of CL, is located in a tropical wet forest region of the Colombian Pacific coast, while the Tikuna community lies within the Amazon rainforest. Despite differences in their culture and the ecological characteristics of the places they inhabit, the medical systems in these communities are similar. However, two types of disease that differ in these communities have been identified:

The first type of disease is called "bush illnesses," "Indian illnesses" or "*Esperajai*" in Emberá. The second type of disease includes Western diseases or "White diseases" that are treated by Western doctors and a type of facultative medicine called "Kapuniajai" in Emberá.

The "bush illnesses" or "Indian illnesses" are distinguished according to their etiology and include three types of diseases: (i) diseases caused by supernatural beings such as "Jais," which populate the jungle, "charms," "chimpine" or the spirits of ancestors or other evil spirits; (ii) diseases caused by jinxes resulting from an enemy administering a potion, usually in food, obtained from a sorcerer, healer or herbalist; and (iii) diseases caused by natural causes and due to a sudden contact between hot and cold matter.

"Bush illnesses" or "Indian illnesses" that relate to VL and are observed in the Zenú community include the following:

1. Diseases caused by supernatural beings: In this type of disease, it is believed that a "mushroom" or "wind mushroom" is produced by evil winds from the world of the dead. The symptoms of this disease include fever, body aches, headache, and possible unconsciousness or coma. This disease is also known as "wind sickness" or "mountain view" and is thought to be the separation of the "soul" from the body. When afflicted by this disease,

the spirit is believed to wander aimlessly, sometimes resulting in death in sick children. The "soul" is thought to leave the body when a mother leaves the house with a child in her arms without providing any protection to prevent the evil wind that roams the mountains from taking over the child's soul while walking. The hours most conducive to this phenomenon are believed to occur at sunrise and sunset. When the mother takes the sleeping child from home to home, it is thought that the soul stays in the first location. This spirit loss may result in crying, loss of appetite, fever, hair loss, diarrhea, edema, and appearance of a "bun" or ball on the left side of the stomach.

2. This feature is important because 100% of interviewed mothers who had children with VL consulted a healer as their first medical resource, with all cases of diseases diagnosed as "wind illness" or "milkbun illness" [19, 47].

3. Diseases caused by natural causes: These diseases are believed to be the result of sudden contact between hot and cold matter. For example, indigestion is thought to be caused by walking barefoot or bathing in hot weather. These diseases include the "milkbun illness" or "child in *Chime*," which is believed to occur when a pregnant mother breastfeeds another child. Through this process, breast milk is damaged, and the child that drinks the milk suffers from constant fever, loss of appetite, and stomach swelling (liver, spleen). A "bun" or ball is then believed to grow in the navel, and then the child dies. Elderly people say that this disease is very old, and many children have died from fever because they would not "refresh" and had a swollen abdomen.

Given these elements, it can be concluded that the VL is recognized in the Zenú medical system within the context of "bush illnesses" but is explained as originating from two different causes: the first being of supernatural origin and the second being due to natural reasons. For diagnosis, the patient's family may seek care from a "healer" or traditional doctor and provide a urine sample from the patient. Following observation of the urine, the healer diagnoses the patient with a disease and establishes a plant-based treatment that is administered and accompanied by a special diet for the child that includes chicken broth.

Medicinal plants used by a "healer" may be classified as "hot" or "cool." Hot plants include cinnamon (*Cinamomum zeilanicum*), pennyroyal (*Bistropogon mollis*), coriander (*Coriandrum sativum*), and "the happy" (*Lantana* spp.). "Cool" plants include basil (*Ocimum basilicum*), flaxseed (*Linum usitatis simum*), corn (*Zea mays*) and soursop leaves (*Annona muricata*). Some of these plants grow in the reserve, and others may be purchased at market places in nearby cities. The leaves are prepared in an infusion to drink or a concoction of leaves, stems and roots for baths, compresses, and poultices.

The Emberá Indians do not recognize CL as an individual clinical entity but consider it to be part of the skin conditions called "Aidá" in their language [47]. This disease is believed to occur in people who violate the social norms associated with menarche or widowhood. During menarche or widowhood, a person acquires a temporary taboo status that, if violated, is believed to then cause the disease. This disease is also thought to occur due to chance encounters with the "Jais" of the jungle, which are the forces that act on and control human well-being and comprise the spirits of ancestors (tutelary, protective and/or aggressive "Jais")

http://dx.doi.org/10.5772/66430

and of prey animals, which are almost always aggressive “Jais” and agents of diseases who want to exact revenge on hunters [48].

In the Emberá community, a patient may be taken to the Jaibaná (shaman) who is in charge of controlling both evil and beneficial spiritual forces. Through ritual singing to the “Jai,” the Jaibaná is thought to “see” the agent that has possessed the body of the victim and look for a way to exorcise it and heal the person. The Jaibaná also provides answers and defines the disease. According to this diagnosis, the Jaibaná can cure the patient or sends him/her to the herbalist or hospital to receive treatment. The agents causing “Aidá,” represented as the “Jais,” are thought to be small worms found in the forest. When treatment is provided by an herbalist, he or she may diagnose the disease by examining the urine of the patient. The herbalist may then add a plant to the urine, which may produce the typical signs that result when someone has made a curse on a person. When treatment is provided by a Jaibaná, he or she uses the songs of the “Jai” and plants, purification rituals or daily allowances, depending on what the “Jais” advises. Meanwhile, herbalists also prescribe a special diet without sweet or salty foods.

For Tikunas Indians, CL is thought to be produced by an encounter inside the forest with the sloths (*Choloepus hoffmanni*) that transmit the disease by staring at the person, and this belief is consistent with the epidemiological findings of higher disease prevalence in hunters and the presence of vector insects that live in the forest. For treatment, the doctor uses indigenous macerated bark of a tree, which is highly caustic and helps to heal the lesions.

3.2. Medical systems in rural communities

The first descriptions of CL in Colombia were made during the last century under the name “sows” and attributed to poor hygiene. This view persists in rural eastern Colombia, where the cause of the disease is attributed to the bite of flies from pig pens known as “pigflies.” Meanwhile, the peasants of northwestern Colombia believe that the disease is caused by the bite of the “pito,” which is the designated name for both CL and for some herbivorous and hematophagous hemipteran, and specifically reduviid, insects that live in logs and decaying timbers found near houses. These insects cause skin lesions by biting and defecating on people. Additionally, this disease is recognized by the name “vine” in these communities due to the bite of small animals that have a thread-like appearance and are called “vines” or “I saw you”; these animals have been found to often bite people while in the forests and in the branches of trees. Peasants in northwestern Colombia say that if a person sees this animal, he or she can shout, “Saw you,” and the animal is paralyzed and does not cause itching or disease [46]. In these communities, the diagnosis of this condition is made by examining the lesion or urine.

Other communities believe that the etiology of the disease is due to the action of the “warty louse,” a small parasite of *Lachesis muta* snakes, known as rattlesnakes or “warty” snakes. People believe that when they kill these snakes, they are exposed to and bitten by these “lice” thus causing the disease.

Treatment of CL in rural communities varies according to the community's conception of the origin of the disease and the degree of antiquity of the human settlement. Despite sharing the same geographic area, the CAP of rural populations may be very different. For example, populations that have been settled in this area for a longer period are of African descent. These populations fear the disease and strongly prefer to avoid jobs that involve entering the jungle to prevent infection. People in these communities also believe that leishmaniasis is a "hallmark of the jungle" and that having the disease means that the jungle is welcoming your arrival; therefore, they have no preventive measures against it.

Another population, known as "paisas," is a recent settlement of people from coffee cultivating areas who came to this region over the last 30 years; this region has an endemic focus of CL produced by *L. panamensis*, and people in this population use caustic agents and plants to treat the disease. Treatment with caustic agents consists of the local application of various substances such as silver nitrate, sulfuric acid, hot water, and hot brown sugar and, frequently, cauterization of the lesion with a spoon or the tip of a machete that has been placed in a fire and is applied without anesthesia to the ulcer, leaving a smooth scar [49]. Treatment with plants consists of the local application of macerated leaves or bark of various trees and shrubs and some latex from vines, many with leishmanicidal action previously demonstrated in the laboratory [50]. In recent settlement communities, no preference for a plant or group of plants exists, and a wide range of plants is used; however, moxa is one of the most popular.

Conceptions of the disease are closely associated with not only culture but also social relations. For example, gender relations are represented by the provision of feminine or masculine attributes to ulcers, the papular, silent type of ulcers that are not easily observed and generally isolated and difficult to treat are called "male pito" and the ulcerous lesions that are often numerous, "weep" (produce fluid), and more easily observed and treated are called "female pito" [49].

Nonspecific medications are often used, including antifungal drugs, ointments, salves, local application antibiotics, and veterinary medicines; these medications are generally self-made or prescribed by doctors who have had no possibility to confirm the diagnosis with laboratory tests. Among the most commonly used veterinary drugs is ivermectin, which has been recently approved for human use and has been found to be effective in some studies [51].

In our studies conducted in indigenous communities, it was not possible to verify the use of urine as a healing element, which has been described in black communities in central San Juan [52]; in these communities, in addition to using urine for diagnosis, it has been used for treatment of various skin diseases, rheumatism, and snakebites and smeared on the skin or drank.

It is interesting to compare the observations described here with the Igun model [53], which showed that the selection of a given treatment or system of health care is determined by personal, family, and sociocultural conceptualizations related to the cause, severity, and potential consequences of the diseases as well as the effectiveness and cost and difficulty in obtaining different types of health care (traditional or modern).

3.3. Characterization of the macro-, meso- and microfoci

Although the first record of leishmaniasis in Colombia was reported in 1872 by Indalecio Camacho, it was not until the early 1980s that the study of the macrofoci of leishmaniasis began. During this decade, leishmaniasis was defined as a sylvan disease affecting men working in affected areas as loggers, soldiers, and hunters. One of the first eco-epidemiologic studies was conducted in Montebello in 1986; in this mesofocus, the risk of infection in homes (microfocus) as opposed to in the extradomiciliary environment was characterized for the first time because the vectors were identified in the intradomicile and much of the affected and at risk populations was identified to be women and children under the age of 5 [6, 54].

In the indigenous community of San Andres de Sotavento-Cordoba on Colombia's Caribbean coast, a microfocus for VL has been identified in homes that were located near the gallery forest. In these cases, *Lu. evansi* species were found to be able to easily access homes and bite entire families, including the population group that is most at risk of developing VL, children under the age of 5 years. In the same region, a high rate of infected dogs with *Leishmania* has been identified, as have vectors, which have even been found to be present in areas with a low incidence of VL; these infected vectors and reservoirs have been found in the extradomicile, but *Lu. evansi* has not been found to enter homes, and therefore VL cases have not been identified. In the evening hours when increased biting activity of *Lu. evansi* has been identified, children are often inside homes and within the reach of infected vectors. However, adults that have been bitten in the extradomicile have not been found to develop the disease [7].

Humans play a modulatory role in facilitating or hindering the risk of transmission. For example, in the Magdalena River valley region, deforestation and rangeland establishment have confined the CL insect vectors to the forest. These species have been found to not reach the home when a paddock belt is more than 100 meters away. However, deforestation, while increasing the distance from some vector species, also creates favorable environments for the establishment of others. Such is the case of *Lu. longipalpis*, a VL vector in the Magdalena River valley region, which has been found to invade new areas and create new risks for populations living in these regions.

Characterization of microfoci has helped the creation of proposals for prevention and control.

3.4. Contributions to the knowledge of phlebotomines

As a result of the study of foci, valuable information about the presence and distribution of *Lutzomyia* species in Colombia has been obtained. Some species have been reported as novel to Colombia, such as *Lu. suapiensis* [55] and *Lu.a Franca* in the Amazon region [56], or described as novel species in general, such as *Lu. velezi* [57]. Others have been newly reported in certain areas, such as *Lu. reburra* in Antioquia [58] and *Lu. ovallesi* in Amazonas (unpublished data).

However, knowledge has not only been generated about the presence of a species in a focus but also on biodistribution, behavior, and location relative to home of these species. Thus, changes in the geographical distribution of some vector species over time have been detected that require adaptability to both urban environment and novel areas. Such is the case of *Lu. longipalpis*, which had been reported at a maximum altitude of 1100 meters above sea level until 2006, when we identified this species in the Andean region of the country (Caldas) at 1387 meters above sea level; this was the second global report of the prevalence of this species at a higher altitude [59]. *Lu. panamensis*, one of the main vector species of CL in Colombia, has become the predominant species in the most urbanized areas, showing its high capacity for adaptation, unlike other species present in the jungle areas of Darien Colombia (Department Choco) (Carillo LM, unpublished data).

While the biting activity of insect vectors has been found to be crepuscular and nocturnal, these species have variable hours of activity. For example, in Montebello and Antioquia, *Lu. gomezi* has been found to have greater biting activity from 6 to 8 pm. In the municipality of La Guaira, Valle del Cauca, *Lu. youngi* has generally been identified as having increased biting activity between 7:00and 9:00 pm [60]. *Lu. evansi* in San Andres de Sotavento has been found to have increased biting activity from 11:00 pm to 1 am [7]. Specifying the peak hours of biting is important for the selection of the control measure to be used.

Seasonal variations in the density of vectors have been identified. For example, in sub-tropical countries, the period of greatest activity of a vector has been found to correspond to the warmer months of the year. In France, *Ph. ariasi* has been found to be active from June to September, with its highest density identified at the end of July [5, 60, 61]; however, after examining the physiological age of the insects, it was found that the highest proportion of calved females and natural infections with *Leishmania* occurred in August and early September. In tropical regions and notably in Colombia, we have found that the period of greatest risk corresponds to the rainy season. In San Andres de Sotavento (*Lu. evansi*) [27], Montebello (*Lu. gomezi*) [6] and Choco (*Lu. panamensis* and *Lu. trapidoi*) (Carrillo LM, unpublished data) a significant increase in vector density during the rainy season has been identified. At the end of this period, the rate of calved females and natural *Leishmania* infections has also been determined to be significantly higher. In the area of Urabá during the phenomenon "*El Niño*" a tendency towards decreased vectors has been identified, contrary to what was reported by Cardenas et al. [62].

Over longer periods, disease outbreaks (e.g., Montebello, San Roque, and Saiza) have been observed that correspond to the confluence of multiple factors such as the presence of infected reservoirs and vector abundance; in all of these outbreaks, it was found that transmission occurred in the intra- and peridomicile, with children highly affected and no gender difference identified. Additionally, humans were identified as a possible reservoir due to the large number of active lesions present at any given time and high number of bites (see the photo of the girl with bites and photos of extensive lesions). Climatic factors, such as a phenomenon of "*La Niña*," characterized in Colombia by the rainy season, could favor higher vector densities and outbreaks; however, studies confirming this hypothesis are needed.

4. Conclusions and recommendations

Leishmaniasis is a complex disease of multifactorial origin in which different species of parasites, reservoirs, vectors, and ecological and social factors interact. The focal nature of the transmission of leishmaniasis in each endemic area has been found to be associated with the particular environmental conditions in a given area, such as climate, humidity, altitude, temperature, and vegetation, that favor the vector's presence. Like other vector-borne diseases, leishmaniasis has been found to be strongly influenced by global warming and climate variability, which affect the dynamics of the vectors, reservoirs and human populations. The use of eco-epidemiological assessments provides a multidisciplinary approach to understanding heterogeneities and dynamics that occur in the foci of leishmaniasis transmission.

The application of the eco-epidemiological method then enables the following processes to occur in the natural foci of transmission: (a) identification of the species of *Leishmania* and role of phlebotomines and wild and domestic reservoirs; (b) definition of the geographical areas or macrofoci and description of the environmental characteristics that may become ecological markers of the presence of a vector, such as temperature, vegetation, soil type, and altitude; (c) establishment of the time of the year (spatio-temporal) associated with increased risk of transmission based on the rates of natural infection and biting activity; (d) establishment, with respect to housing (microfocus), of the risk of infection for inhabitants in the intra-, peri- and extradomicile; (e) determination of the different age groups in a community that are most affected or at risk of becoming ill; and (f) identification of the conceptions, attitudes, and practices in communities related to the disease. The improvement of eco-epidemiological surveillance for leishmaniasis should be based on the identification of these parameters to facilitate the design and implementation of cost-effective prevention and control measures. In addition, the sustainability of these measures is dependent on intersector collaboration and support (municipal/Department of Health) and active participation of the community.

The results of different studies have shown that in a focus, transmission may be intra-, peri- and extradomiciliary, and while women and children may be as substantially affected by leishmaniasis as men, women have less access to healthcare. These data have also shown that official statistics are not a good reflection of the true epidemiology of leishmaniasis in the rural areas where a greater number of cases are recorded.

The fact that women less frequently seek care causes the prevalence in this population to be underestimated, and therefore there are inaccurate estimates of the gender-specific rate of infection. Implementation of better governmental strategies will be required to correct the inequality in access to the treatment for women, and strategies for controlling the disease that are oriented towards preventing intra- and peridomiciliary transmission are also required. There is also a need to increase the focus within health departments towards correcting the unequal access to health services in women (regarding leishmaniasis), as this disparity can reflect other problematic situations in the rural health setting.

Based on these different findings, it is suggested that the design of control strategies take into account the following considerations:

When it is determined (through the study of a focus) that transmission is occurring in the interior of a domicile, then measures for vector control and the prevention of leishmaniasis can be used to prevent people from becoming infected with the parasite by preventing bites from the infected vectors.

Control measure should be applied before the period of greatest risk and aimed at the protecting people at higher risk in the geographical area in which there has been transmission, whether that area is intradomiciliary or extradomiciliary. Additionally, the hours of greatest risk should be considered. The rainy season has been identified as the time when there is a greater risk transmission in foci and a higher density of vectors; therefore, control measures should be implemented before the rainy season begins. However, these findings must be validated in each foci.

Various vector control measures are available, including the use of bed nets or curtains impregnated with insecticides (even hammocks) and spraying of insecticides inside houses (in those foci where transmission is intra- and peridomiciliary).

In the case of bed nets, it is necessary to take into account the type of material and size of the holes and educate the community so they will not wash the mosquito net and damage the residual effect and nature of the material (regardless of whether it is impregnated). For example, nylon tulle or polyester bed nets are most effective because the residual effects of the insecticide are much higher; additionally, in these bed nets, the holes are very small and prevent *Lutzomyia* passing through the mosquito net. While bed nets are generally well accepted by most communities, acceptance must be considered individually in each community because it can vary from one community to another. For example, color has been identified as one factor that may affect the degree of acceptance of bed nets. If a mosquito net is not impregnated with insecticide, *Lutzomyia* species may get through even very small holes. However, when it is impregnated, vectors will not go through the bed net.

In foci where transmission occurs in an intra- and/or peridomicile, the infection of pets (and people with lesions) has been hypothesized to demonstrate that in these foci, the disease affects both women and men. Proper treatment of people may serve as a control measure of transmission in foci where humans are reservoirs and provide support for the implementation of better methods of control.

Finally, implementing primary health education to inform the community about issues related to the disease will increase knowledge about why the disease occurs, what causes it, what transmits it and how it heals. Recognizing the parasite that causes the disease (causative agent), transmitting insect, its behavioral habits, clinical manifestations of the disease, and mucosal compromise will help to promote disease control.

Individuals in these communities need to understand that it is necessary to perform some diagnostic procedures before initiating treatment and that there are options for treating the disease. These treatments are provided free of charge by the Ministry of Health through the sectional health services for all people with a diagnosis of leishmaniasis with any of its clinical presentations. Additionally, explaining the need to complete treatment to prevent the emergence of drug resistance, relapse, and complications in the mucosae and educating community members about some important measures that can contribute to avoiding contact with insect vectors are crucial.

It is important to emphasize during primary education that control measures (mosquito nets) should be provided to the populations at greatest risk. Specifically, children should be protected, as they are the population at highest risk of visceral leishmaniasis. It is also necessary to protect the family unit in general in the intra-domicile through the use of mosquito nets every night.

Through the use of primary health education, the community itself may help to guide control efforts by reporting increases in the intradomiciliary density of *Lutzomyia* species. Informing health providers to recognize early disease (leishmaniasis) is also important. A provider may then diagnose the patient or refer the patient for diagnosis (in early cases). Through community participation, it is possible for this disease become a priority issue for community activities.

Acknowledgements

The authors are grateful for the strong support of the TDR program at World Health Organization, the University of Antioquia and Colciencias (Grant CT-695-2014).

Author details

Iván D. Vélez[1,*], Lina M. Carrillo[1, 2], Horacio Cadena[1], Carlos Muskus[1] and Sara M. Robledo[1]

*Address all correspondence to: ivan.velez@udea.edu.co

1 PECET, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia

2 Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellín, Colombia

References

[1] Liew FY, O'Donnell CA. Immunology of leishmaniasis. Adv Parasitol. 1993;32:161–259. Review.

[2] World Health Organization. Control of the leishmaniases. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22–26 March 2010. Technical report series 949. World Health Organization, Geneva, Switzerland; 2010. 201 p.

[3] Rioux JA, Lanotte G, Petter F et al. Cutaneous leishmaniasis of the western Mediterranean basin. From enzymatic identification to eco-epidemiological analysis. The example of three foci, Tunisian, Moroccan and French. In: Leishmania. Taxonomy and phylogeny. Eco-epidemiological Applications (ed JA Rioux) IMEEE, Montpellier, France; 1986a. pp. 365–395. French.

[4] Rioux JA, Lanotte G & Pratlong F. Leishmania killicki n.sp. (Kinetoplastida-Trypanosomatidae). In: Leishmania. Taxonomy and phylogeny. Eco-epidemiological applications. (ed JA Rioux) IMEEE, Montpellier, France; 1986b. pp. 139–142. French.

[5] Killick-Kendrick R, Rioux JA, Bailly M, Guy MW, Wilkes TJ, Guy FM, Davidson I, Knechtli R, Ward RD, Guilvard E, et al. Ecology of leishmaniasis in the south of France. 20. Dispersal of *Phlebotomus ariasi* Tonnoir, 1921 as a factor in the spread of visceral leishmaniasis in the Cévennes. Ann Parasitol Hum Comp. 1984;59(6):555–72.

[6] Velez ID, Wolff M, Valderrama R, Escobar JP, Osorio L. Community and environmental risk factors associated with cutaneous leishmaniasis in Montebello, Antioquia, Colombia. Leishmaniasis control strategies. Proceedings of a Workshop. International Development Research Center. Canada. 1992.

[7] Vélez ID, Gallego JI, Adarve JC, Llano R, Trujillo GA, Alzate AM, Montoya M, Travi BL. Ecoepidemiological delimitation of visceral leishmaniasis in the Caribbean Colombian Coast. Bol de Dir Malariol Saneam Amb. 1995;35(S1):359–370.

[8] Mimori T, Matsumoto T, Calvopiña MH, Gomez EA, Saya H, Katakura K, et al. Usefulness of sampling with cotton swab for PCR-diagnosis of cutaneous leishmaniasis in the New World. Acta Trop. 2002; 81(3):197–202.

[9] Velez ID, Agudelo SP. Manual of procedure for diagnosis of American cutaneous leishmaniasis. Editorial University of Antioquia. 1996.

[10] Vivero RJ, Torres-Gutierrez C, Bejarano EE, Peña HC, Estrada LG, Florez F, Ortega E, Aparicio Y, Muskus CE. Study on natural breeding sites of sand flies (Diptera: Phlebotominae) in areas of Leishmania transmission in Colombia. Parasit Vectors. 2015;8:116. doi: 10.1186/s13071-015-0711-y.

[11] Montalvo AM, Fraga J, Rodríguez O, Blanco O, Llanos-Cuentas A, García AL, Valencia BM, Muskus C, Van der Auwera G, Requena JM. Detection of Leishmania spp. based on the gene encoding HSP20. Rev Peru Med Exp Salud Publica. 2014;31(4):635–43. Spanish.

[12] de Paiva BR, Secundino NF, Pimenta PF, Galati EA, Andrade Junior HF, Malafronte Rdos S. Standardization of conditions for PCR detection of Leishmania spp. DNA in sand flies (Diptera, Psychodidae). Cad Saude Publica. 2007;23(1):87–94. Portuguese.

[13] Santamaría E, Ponce N, Puerta C, Ferro C. Validation of PCR as a tool for the detection of Leishmania (Vianna) spp. parasites in the Lutzomyia (Diptera:Psychodidae) vector. Biomedica. 2005;25(2):271–9. Spanish.

[14] Montalvo AM, Monzote L, Fraga J, Montano I, Muskus C, Marín M, de Doncker S, Vélez ID, Dujardin JC. PCR-RFLP and RAPD for typing neotropical *Leishmania*. Biomedica. 2008;28(4):597–606. Spanish.

[15] Montalvo Alvarez AM, Nodarse JF, Goodridge IM, Fidalgo LM, Marin M, Van Der Auwera G, Dujardin JC, Bernal ID, Muskus C. Differentiation of *Leishmania (Viannia) panamensis* and *Leishmania (V.) guyanensis* using BccI for hsp70 PCR-RFLP. Trans R Soc Trop Med Hyg. 2010;104(5):364–7. doi: 10.1016/j.trstmh.2009.12.002.

[16] Nath-Chowdhury M, Sangaralingam M, Bastien P, Ravel C, Pratlong F, Mendez J, Libman M, Ndao M. Real-time PCR using FRET technology for Old World cutaneous leishmaniasis species differentiation. Parasit Vectors. 2016;9(1):255. doi: 10.1186/s13071-016-1531-4

[17] Montenegro JA. Cutis reaction in leishmaniasis. Ann. Fac. Med. Sao Paulo. 1926; 1:323-330.

[18] Sokal JE. Measurement of delayed skin-test responses. N Engl J Med. 1975;29(10)3: 501–502.

[19] Turbay SM. Elements of religiosity in the indigenous shelter of San Andrés de Sotavento. Univ. Hum. 1987;27(27):35–44.

[20] Detinova TS. Physiological changes of ovaries in female Anophelesmaculipennis. Med Parazitol. 1949;18:410.

[21] Alexander JB, Young DG. Dispersal of phlebotomine sand flies (Diptera: Psychodidae) in a Colombian focus of *Leishmania (Viannia) braziliensis*. Mem Inst Oswaldo Cruz. 1992;87(3):397–403.

[22] Hebert PDN, Cywinska A, Ball SL, de Waard JR. Biological identifications through DNA barcodes. Proc R Soc Lond B. 2003; 270(1512):313–321.

[23] Galati EAB. Classificacao de Phlebotominae. In: Rangel ER, Lainson R, editors. Flebotomineos do Brasil. Editora Fiocruz, Rio de Janeiro, Brazil. 2003; pp. 23–52. 367 p.

[24] Young DG, Arias JR. Phlebotomine Sandflies in the Americas. Pan American Health Organization, 1991. Technical paper 33. World Health Organization, Geneva, Switzerland. 26 p.

[25] Contreras Gutiérrez MA, Vivero RJ, Vélez ID, Porter CH, Uribe S. DNA barcoding for the identification of sand fly species (Diptera, Psychodidae, Phlebotominae) in Colombia. PLoS One. 2014;9(1):e85496. doi:10.1371/journal.pone.0085496.

[26] Young DG, Morales A. New species and records of phlebotomine sand flies from Colombia (Diptera: Psychodidae). J Med Entomol. 1987 Nov;24(6):651–65.

[27] Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M; WHO Leishmaniasis Control Team.. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7(5):e35671. doi: 10.1371/journal.pone.0035671.

[28] Grimaldi G Jr, Tesh RB, McMahon-Pratt D. A review of the geographic distribution and epidemiology of leishmaniasis in the New World. Am J Trop Med Hyg. 1989; 41(6):687-725.

[29] Travi BL, Vélez ID, Brutus L, Segura I, Jaramillo C, Montoya J. Lutzomyia evansi, an alternate vector of Leishmania chagasi in a Colombian focus of visceral leishmaniasis. Trans R Soc Trop Med Hyg 1990; 84(5):676-677.

[30] Rioux JA, Velez ID, Denial M, Dereure J, Perières J, Lanotte G, el Mellouki W. [Presence in Morocco of Phlebotomus (Paraphlebotomus) kazeruni Theodor and Mesghali, 1964]. Ann Parasitol Hum Comp. 1986c;61(4):473-81. French.

[31] Vélez ID, Travi BL, Gallego J, Palma GI, Agudelo SP, Montoya J, et al. Eco-epidemiological evaluation of visceral leishmaniasis in the Zenú indigenous community of San Andrés de Sotavento, Córdoba: First step for its control. Rev Col Entomol. 1995; 21(3):111–122.

[32] Ashford RW. Leishmaniasis reservoirs and their significance in control. Clin Dermatol. 1996;14(5):523–32.

[33] Ashford RW. The leishmaniases as model zoonoses. Ann Trop Med Parasitol. 1997 Oct;91(7):693–701. Review.

[34] Vélez ID, Ospina S, Henao G, LePape P, Correa M, Wolff M, Jaramillo L. Epidemiology of cutaneous leishmaniasis in San Roque, Antioquia 1987. Bol Epidemiol Antioquia. 1987; 12(4): 354–359.

[35] Haydon DT, Cleaveland S, Taylor LH, Laurenson MK. Identifying reservoirs of infection: a conceptual and practical challenge. Emerg Infect Dis. 2002;8(12):1468–73

[36] Roque AL, Jansen AM. Wild and synanthropic reservoirs of *Leishmania* species in the Americas. Int J Parasitol Parasites Wildl. 2014;3(3):251–62.

[37] Travi BL, Tabares CJ, Cadena H. *Leishmania (Viannia) braziliensis* infection in two Colombian dogs: a note on infectivity for sand flies and response to treatment. Biomedica. 2006;26 Suppl 1:249–53.

[38] Vélez ID, Carrillo LM, López L, Rodríguez E, Robledo SM. An epidemic outbreak of canine cutaneous leishmaniasis in Colombia caused by *Leishmania braziliensis* and *Leishmania panamensis*. Am J Trop Med Hyg. 2012;86(5):807–11.

[39] Sasani F, Javanbakht J, Samani R, Shirani D. Canine cutaneous leishmaniasis. J Parasit Dis. 2016;40(1):57–60.

[40] Gharbi M, Mhadhbi M, Rejeb A, Jaouadi K, Rouatbi M, Darghouth MA. Leishmaniosis (*Leishmania infantum* infection) in dogs. Rev Sci Tech. 2015;34(2):613–26.

[41] Mäser P, Grether-Bühler Y, Kaminsky R, Brun R. An anti-contamination cocktail for the *in vitro* isolation and cultivation of parasitic protozoa. Parasitol Res. 2002;88(2):172–4.

[42] Pavlovsky EN. Natural nidality of transmissible diseases. Peace Publishers, Moscow; 1964. 246 p.

[43] Rioux JA, Golvan YJ, Croset H, Houin R. Leishmanioses in the Mediterranean "Midi": results of an ecologic survey. Bull Soc Pathol Exot Filiales. 1969;62(2):332–3. French.

[44] Eisen L, Eisen RJ. Using geographic information systems and decision support systems for the prediction, prevention, and control of vector-borne diseases. Annu Rev Entomol. 2011;56:41–61. doi: 10.1146/annurev-ento-120709-144847. Review.

[45] Velez ID, Hendrickx E, Robledo SM, del Pilar Agudelo S. Gender and cutaneous leishmaniasis in Colombia. Cad Saude Publica. 2001;17(1):171–80.

[46] Alexander B, Agudelo LA, Navarro JF, Ruiz JF, Molina J, Aguilera G, Klein A, Quiñones ML. Relationship between coffee cultivation practices in Colombia and exposure to infection with Leishmania. Trans R Soc Trop Med Hyg. 2009;103(12):1263–8. doi: 10.1016/j.trstmh.2009.04.018

[47] Velez ID. Leishmaniasis in Colombia. Conceptions, Attitudes and practices in indigenous and peasant communities. In: Proceedings 3rd Chilean Congress of Anthropology. Tomo I. Lom editions. Santiago, Chile. 2000.

[48] Morales DM. Illness, Healing and Jaibanism. Embera conceptions on the most common diseases. The French Institute of Andean Studies. 1994.

[49] Carrillo-Bonilla LM, Trujillo JJ, Alvarez-Salas L, Vélez-Bernal ID. Study of knowledge, attitudes, and practices related to leishmaniasis: evidence of government neglect in the Colombian Darién. Cad Saude Publica. 2014;30(10):2134–44.

[50] Weniger B, Robledo S, Arango GJ, Deharo E, Aragón R, Muñoz V, Callapa J, Lobstein A, Anton R. Antiprotozoal activities of Colombian plants. J Ethnopharmacol. 2001;78(2-3):193–200.

[51] dos Santos AR, Falcão CA, Muzitano MF, Kaiser CR, Rossi-Bergmann B, Férézou JP. Ivermectin-derived leishmanicidal compounds. Bioorg Med Chem. 2009;17(2):496–502.

[52] Peñuela-Uricoechea M. Therapeutic uses of urine in the black communities of the middle San Juan, Departamento del Chocó. Javeriana University. 1989.

[53] Igun UA. Stages in health-seeking: a descriptive model. Soc Sci Med. 1979; 13A(4):445-456

[54] Reyes G. Some aspects of Leishmania in the Hospital of San Juan de Dios in Bogotá. Rev Fac Med Universidad Nacional. 1957;25(9-12):31–39.

[55] Contreras MA, Vivero RJ, Bejarano EE, Carrillo LM, Vélez ID. New records of phlebotomine sand flies (Diptera: Psychodidae) near the Amoya River in Chaparral, Tolima. Biomedica. 2012;32(2):263–8.

[56] Bejarano Eduar E, Castro M, Pérez-Doria A, Hernández-Oviedo E, Vélez A, Vélez ID. First report of *Lutzomyi*a França in the department of Guainía, Amazonian Colombia, and of *Brumptomyia mesai* Sherlock (Diptera: Psychodidae) in the Colombian Caribbean Coast. Neotrop Entomol. 2007;36(6):990–3.

[57] Bejarano EE, Vivero RJ, Uribe S. Description of *Lutzomyia velezi*, a new species of phlebotomine sand fly (Diptera: Psychodidae) from the Department of Antioquia, Colombia. Mem Inst Oswaldo Cruz. 2010;105(3):322–5.

[58] Vergara D, Carrillo LM, Bejarano EE, Vélez ID. First report of *Lutzomyia yuilli* Young & Porter, 1972 and *Lutzomyia triramula* (Fairchild & Hertig1952) (Diptera: Psychodidae) in the department of Caldas, Colombia. Biota Neotropica. 2008;8(3):251–253.

[59] Acosta LA, Mondragón-Shem K, Vergara D, Vélez-Mira A, Cadena H, Carrillo-Bonilla L. Expansion of the distribution of Lutzomyia longipalpis (Lutz & Neiva, 1912) (Diptera: Psychodidae) in the department of Caldas: increased risk of visceral leishmaniasis. Biomedica. 2013;33(2):319–25.

[60] Alexander B, Usma MC, Cadena H, Quesada BL, Solarte Y, Roa W, et al. Phlebotomine sandflies associated with a focus of cutaneous leishmaniasis in Valle del Cauca, Colombia. Med Vet Entomol. 1995;9(3):273–8.

[61] Alten B, Maia C, Afonso MO, Campino L, Jiménez M, González E, Molina R, Bañuls AL, Prudhomme J, Vergnes B, Toty C, Cassan C, Rahola N, Thierry M, Sereno D, Bongiorno G, Bianchi R, Khoury C, Tsirigotakis N, Dokianakis E, Antoniou M, Christodoulou V, Mazeris A, Karakus M, Ozbel Y, Arserim SK, Erisoz Kasap O, Gunay F, Oguz G, Kaynas S, Tsertsvadze N, Tskhvaradze L, Giorgobiani E, Gramiccia M, Volf P, Gradoni L. Seasonal dynamics of Phlebotomine sand fly species proven vectors of Mediterranean leishmaniasis caused by *Leishmania infantum*. PLoS Negl Trop Dis. 2016;10(2):e0004458. doi: 10.1371/journal.pntd.0004458.

[62] Cardenas R, Sandoval CM, Rodríguez-Morales AJ, Franco-Paredes C. Impact of climate variability in the occurrence of leishmaniasis in northeastern Colombia. Am J Trop Med Hyg. 2006;75(2):273–7.

View publication stats