IMPROVED UNDERSTANDING, DIAGNOSIS, AND TREATMENT OF CUTANEOUS AND MUCOSAL LEISHMANIASIS IN RURAL ECUADOR

Jacob Machiel Bezemer

IMPROVED UNDERSTANDING, DIAGNOSIS, AND TREATMENT OF CUTANEOUS AND MUCOSAL LEISHMANIASIS IN RURAL ECUADOR

Jacob Machiel Bezemer

Colophon

IMPROVED UNDERSTANDING, DIAGNOSIS, AND TREATMENT OF CUTANEOUS AND MUCOSAL LEISHMANIASIS IN RURAL ECUADOR

This thesis was prepared at the Hospital del día Shell of the Fundación Misión Cristiana de Salud (Day care hospital of the Christian Health Mission Foundation).

Part of the research of this thesis was funded by the foundation Latin Link Nederland

Cover: figurine elaborated by Fanny Gualinga, photos from own collection, design by Mirjam Zwemer-Bezemer

Printed by ProefschriftMaken, Utrecht

Improved understanding, diagnosis, and treatment of cutaneous and mucosal leishmaniasis in rural Ecuador

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. ir. P.P.C.C. Verbeek
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op donderdag 7 september 2023, te 13.00 uur

door Jacob Machiel Bezemer
geboren te Reimerswaal

Promotiecommissie

Promotor:	prof. dr. H.J.C. de Vries	AMC-UvA
Copromotores:	dr. H.D.F.H. Schallig	AMC-UvA
	dr. M. Calvopiña Hinojosa	Universidad de Las Américas
Overige leden:	prof. dr. M. van Vugt	AMC-UvA
	prof. dr. C. Schultsz	AMC-UvA
	dr. R. Zonneveld	AMC-UvA
	dr. E.C. Hasker	ITG Antwerpen
	prof. dr. R. Reis	Universiteit Leiden
	prof. dr. M.W. Bekkenk	Vrije Universiteit Amsterdam

Faculteit der Geneeskunde

To Jesus my King

To my patients

To my marvelous children

Corné

Helen

Arianne

Jacoline

Richard

Erik

To my lovely wife Linda

Table of contents

Chapter 1

General introduction

Global leishmaniasis

Every year, up to one million new people are infected with the *Leishmania* parasite around the world [1]. Leishmaniasis is endemic in 99 tropical and subtropical countries [2]. This protozoan infects humans through the bite of female *Lutzomyia* or *Phlebotomus* sandflies (divided into more than 90 species). The sandfly species responsible for transmission differ depending on geographic region, reservoir host, and *Leishmania* species transmitted [3]. *Leishmania* infects the sandfly during blood meals on an infected host, multiplies in the midgut, and is transmitted to another host during a subsequent blood meal [4]. In most areas, *Leishmania* transmission is zoonotic (from animal to human) and involves domestic (e.g. dogs and cats), commensal (e.g. rats and foxes), and wild animals (e.g. rock hyraxes and forest rodents). Anthroponotic transmission (from human to human) occurs in Africa and Asia with the *L. tropica* and *L. donovani* species [5]. *Leishmania* causes three major diseases, depending on the interaction between the involved species and the host: visceral leishmaniasis (VL), which affects between 50.000 and 90.000 people annually, cutaneous leishmaniasis (CL) which affects between 600.000 and 1 million, and mucosal leishmaniasis (ML), whose global incidence is unknown [1, 6]. VL affects internal organs and is usually fatal if left untreated. More than 85% of reported cases originate in Asia (India), Africa (Eritrea, Ethiopia, Kenya, Somalia, South Sudan, and Sudan), and South America (Brazil) [2]. CL is endemic in 90 countries and primarily causes skin ulcers and nodules. More than 90% of the cases are reported from South and Central America, the Mediterranean, the Middle East, and Central Asia [2]. ML causes mucosal inflammation and ulceration of the nose, mouth, and throat [7]. The World Health Organization (WHO) estimates that more than 90% of ML cases are from South America (Bolivia, Brazil, and Peru) and Africa (Ethiopia) [2, 8]. ML, unlike CL, does not heal spontaneously, is difficult to diagnose due to parasite scarcity, and is more resistant to treatments [9, 10]. It can cause severe disease, including facial deformation, laryngeal obstruction, and death, if not treated promptly [11, 12]. In a CL patient infected with a high-risk species, the risk of developing ML is approximately 5% [13]. *L. braziliensis* is the main ML-causing species, followed by *L. guyanensis*, *L. panamensis*, *L. aethiopica,* and *L. amazonensis* [11, 14]. It is also associated with the male gender, older age, malnutrition, previous CL duration, and immunodeficiency [15-17]. The global burden of leishmaniasis is rising due to a number of factors, including climate change, socioeconomic vulnerability, and poverty [18]. The WHO has classified leishmaniasis as a Neglected Tropical Disease (NTD) [19]. In comparison to common diseases of the wealthy like diabetes, cancer, and cardiovascular diseases, NTDs are underrepresented on the global health agenda and receive almost no funding. This results from the fact that NTD-related scientific research is not profitable for businesses due to the poverty of the beneficiaries [20]. Lack of understanding of the epidemiology and disease characteristics, as well as a lack of adequate diagnostics, drugs, vaccines, and vector control strategies are the results [21].

Leishmania species distribution

The three human pathogenic subgenera that comprise the genus *Leishmania* are *Leishmania*, *Viannia*, and *Mundinia* [22]. *Viannia* is only found in South and Central America, but *Leishmania* and *Mundinia* are distributed all over the world [23]. The WHO recognizes 22 human pathogenic species within these three subgenera, 15 of which cause disease in the Americas [24]. Each *Leishmania* species is found in geographically defined areas and is associated with one or two of the three major diseases (VL, CL, and ML) [25]. Numerous animal reservoirs and sandfly vectors are involved in the transmission cycle, but many details remain unknown [26, 27].

Leishmania species determination

Leishmania species determination is important for the choice of treatment, the possible risk of mucosal leishmaniasis, and the comparison of research outcomes [9, 28]. It requires reference laboratories and cannot be accomplished with a microscope at the primary healthcare level [29]. For many years, culture followed by multilocus enzyme electrophoresis (MLEE) has been the gold standard but it is difficult, expensive, time-consuming, and unstandardized [8]. In 2015, van der Auwera and Dujardin reviewed the alternatives to MLEE: Monoclonal antibodies and mass spectrometry lack validation and, like MLEE, require culture to generate enough parasites. Polymerase chain reactions (PCR) constitute the foundation of current reliable methods. PCR detects a species or group of species directly in the sample or detects *Leishmania* in general and requires a second PCR to differentiate the species. After amplifying species DNA, it can be visualized with fluorescence detection or gel analysis. Gel-based analysis allows the comparison of the obtained amplicon size with reference samples. Additionally, gel can be used to visualize and compare fragment length polymorphisms (RFLP). RFLP applies enzymes to digest a PCR product at distinct (species-dependent) sites producing DNA fragments of varying lengths. RFLP is straightforward and may be done in any lab with PCR facilities. Sequencing the PCR product allows for polymorphism comparison at single nucleotide level, but requires additional equipment and training. Another technique is to analyze the melting curve (the temperature at which the strands separate) of DNA fragments, but this method lacks validation. Worldwide, hsp70 and cytochrome b gene sequencing may differentiate most species and are relatively well-validated [30].

Pathophysiology and clinical presentation of cutaneous and mucosal leishmaniasis

The *Leishmania* parasite has two natural forms: the flagellated promastigote stage within the sand fly vector gut and the amastigote mammalian host stage [31]. Human leishmaniasis commences with the inoculation of promastigotes into the dermis via a vector bite. Macrophages phagocytize but do not eliminate all promastigotes [32]. Intracellularly, promastigotes transform into amastigotes, replicate, and are released when the host cell dies [33]. Amastigotes remain at the inoculation site and can spread to nearby tissues or other body parts via hematogenous or lymphatic dislocation [34]. The incubation period is asymptomatic and lasts two to eight weeks after the bite, though the infection can remain subclinical for decades before developing into ML [35, 36]. The first clinical signs of the disease are nodules and pustules, which are occasionally accompanied by lymphadenopathy. These typically develop into ulcers or granulomas, which, except for ML, heal without therapy in months to years and leave visible scars [37]. The immune system generates specific T lymphocytes that produce Interferon-Gamma (IFNγ) and Tumor Necrosis Factor (TNF) in response to the amastigotes [38]. These stimulate macrophage intracellular parasite killing but may cause collateral tissue damage [39, 40]. The interaction between the parasite and the host immune system leads to the clinical spectrum of CL: Most cutaneous lesions are characterized by a moderately IFNγ-dominant response, an intermediate parasite burden, and limited localized tissue damage. Mucosal lesions are characterized by a strong IFNγ response, a low parasite burden, and extensive tissue damage [33].

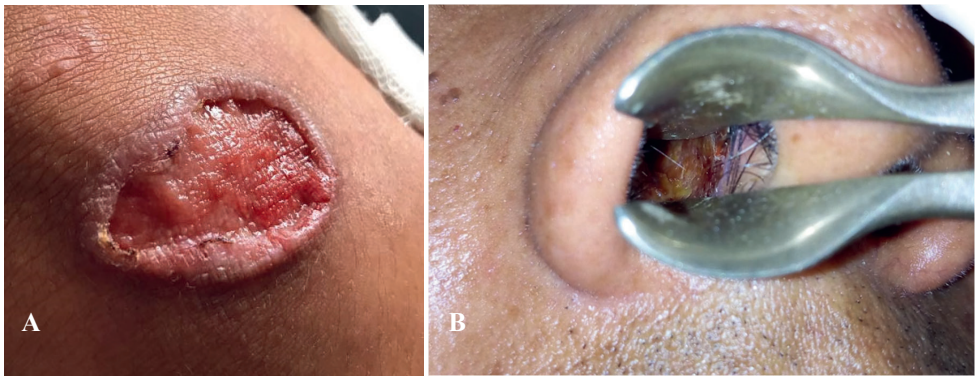


Figure 1. A) A typical ulcerative cutaneous leishmaniasis lesion on the right knee, with raised borders and a depressed center. B) Yellow crusts in the right nostril of a patient with nasal leishmaniasis. *Copyright © 2023 JM Bezemer, All rights reserved.*

Diagnosis of cutaneous and mucosal leishmaniasis

The diagnosis of CL and ML requires laboratory confirmation of the parasite [6, 10]. To obtain a sample from a suspected lesion, skin biopsies are frequently used, although they can cause pain, bleeding, infection, and scarring. Scrapings, fine needle aspirations, smears, and filter paper imprints cause less discomfort. The accuracy of tests using less invasive sampling methods is comparable, if not better in some cases. This is critical for patient well-being and adherence to research protocols [41, 42]. One of the non-invasive sampling methods, filter paper, absorbs liquid discharges when pressed against a lesion and, after drying at room temperature, reliably stores DNA for 12 months or longer [43]. A small piece of filter paper containing sample may yield material for PCR analysis after DNA extraction. This allows for the detection of *Leishmania* as well as species identification [44, 45]. The WHO generally recommends skin scrapings with smear slide microscopy: the border of a new lesion is cleaned with an antiseptic solution, scraped with a lancet, and the appearing lymphatic discharge is applied to a glass slide. A technician uses Giemsa to stain the slide and then examines it under a light microscope for the presence of *Leishmania* amastigotes [46]. The procedure is inexpensive and takes approximately an hour [47]. The WHO regularly trains public health technologists in the use of microscopy. This point-of-care testing in rural health clinics dictates treatment. The sensitivity of smear slide microscopy is affected by factors such as sample parasite load, lesion type, and technician experience [48, 49]. The overall detection rate of smear slide microscopy was reported to be 52% in a systematic review and meta-analysis of American CL and ML diagnostics that was published in 2020, leaving almost half of the patients undiagnosed. The aforementioned systematic review and meta-analysis reported on a couple of alternatives to smear slide microscopy, including culture, histopathology, immunohistochemistry, a rapid diagnostic test, Montenegro's intradermal reaction, Polymerase Chain Reaction (PCR), and indirect immunofluorescence reaction (IFR). The rapid diagnostic test detected the peroxidoxin antigen of *Leishmania* from a suspected ulcer with an immunochromatographic assay. Compared to microscopy, it showed very poor sensitivity (37%) [50]. From the remaining tests, only culture and PCR demonstrated sensitivity and specificity of >80% and >90%, respectively, making them suitable for clinical use [51]. *Leishmania* culture requires four weeks, a medium (Novy-MacNeal-Nicolle), and refrigeration. Apart from the lack of a regular cold chain for sample transportation in rural settings, the waiting time renders culture unsuitable for clinical practice [52]. PCR is limited to advanced laboratories due to the need for a consistent power supply, expensive equipment such as

a thermocycler, and advanced personnel training [8]. Nonetheless, methods that can be applied in remote settings have been developed: Loop-mediated amplification (LAMP) uses an intricate set of primers and a strand-displacing enzyme to form loops of DNA strands and continue amplifying target DNA at a constant temperature until the sample turns turbid and can be confirmed by the naked eye [53-55]. Recombinase Polymerase Amplification (RPA) applies three enzymes at a constant temperature to insert a forward and reverse primer followed by stabilization and displacement of strands, and amplification of target DNA or RNA. This process is repeated until exponential amounts of amplified product are generated which may be detected using gel electrophoresis and other techniques [56]. RPA for the detection of leishmaniasis is still in its early stages, and the number of studies is limited [57]. For LAMP, on the contrary, numerous studies have reported high sensitivity and specificity [50, 58]. Nonetheless, it has not been used in clinical practice in rural health clinics, possibly because it is too expensive; the cost of the kit, DNA extraction reagents, the incubator, and the labor required for one sample is much higher than the cost of microscopy. It could be useful for reference centers that process multiple samples at the same time [59].

Microscopy remains the gold standard for leishmaniasis diagnosis because of its high specificity, but its low sensitivity complicates the evaluation of alternative tests [60]. To resolve this, researchers use composite reference standards [61]. They assume that the combined tests have perfect specificity and that positive samples from any of the included tests can be added together. This may be appropriate for individual patients in clinical practice and is even recommended in guidelines, but it is insufficient for research [25]. Even if the specificity was previously reported to be 100%, false positives can occur with any diagnostic test. Adding the potential false positives of several tests increases the risk of errors in alternative tests' accuracy estimates [62]. Even worse is the assumption that combining several tests with imperfect sensitivities will result in perfect sensitivity. Low lesion parasite density, for example, increases the risk of false negative microscopy, culture, PCR, and histopathology results, and combining these tests is unlikely to result in 100% parasite detection. PCR could serve as a reference standard, but it has yet to be standardized [63]. The Latent Class Analysis (LCA) statistical method provides a solution for the lack of a gold standard. To calculate accuracy, the analysis takes into account the imperfect nature of each test. LCA is an important tool for improving the quality of CL and ML diagnostic test evaluations if applied with the correct assumptions [64, 65].

Obtaining parasite confirmation in CL and ML is so difficult that we may reconsider its utility. Syndromic diagnostic algorithms (SDA) are being used to make treatment decisions for infectious diseases such as tuberculosis and sexually transmitted infections [66, 67]. SDAs rely on clinical parameters that are readily available rather than pathogen confirmation in a laboratory. SDAs have been evaluated for CL in Colombia, yielding sensitivities above 90% but low specificities [68, 69]. No studies have been published on SDA for ML. The differential diagnosis of CL includes bacterial (e.g., furuncle, ecthyma, lepra, and tuberculosis), fungal (e.g., sporotrichosis and paracoccidioidomycosis), algal (e.g., protothecosis), viral (e.g., condyloma acuminata), and ectoparasitic (e.g., myiasis and tungiasis) infectious diseases. Some neoplastic diseases (e.g., basal cell carcinoma, squamous cell carcinoma, and lymphoma) as well as some autoinflammatory diseases (e.g., sarcoidosis and pyoderma gangrenosum) may also resemble CL [70]. The differential diagnosis of ML is similar but includes additional diseases such as bacterial (e.g., syphilis and actinomycosis), fungal (e.g., histoplasmosis, rhinosporidiosis, and aspergillosis), and viral (e.g., influenza) infections, autoinflammatory diseases (e.g., Wegener's granulomatosis), allergic (e.g., chronic rhinitis), trauma (e.g., self-induced, medical, or chemical), and neoplastic diseases (e.g., sarcoma) [12, 71]. The specificity of clinical parameters for CL and ML diagnosis may vary

depending on the regional prevalence of the aforementioned differential diagnoses. Low specificity is concerning because it may lead to unnecessary, costly, and toxic treatments [72]. Nevertheless, given the high percentage of patients missed by current first-line diagnostics and the potential of highly sensitive SDAs, adaptations per endemic regions should be considered, followed by implementation studies.

Cutaneous and Mucosal Leishmaniasis treatment

Health professionals in South America mostly treat CL and ML with intra-muscular injections of meglumine antimoniate (Glucantime) for 20-30 consecutive days, according to a 2019 WHO guideline [73]. The majority of patients experience generalized muscle pains, headaches, and gastrointestinal symptoms, among other side effects. Furthermore, it is hepatotoxic, nephrotoxic, cardiotoxic, and pancreas toxic in 7-39% of patients, and may cause sudden death [74, 75]. To avoid complications such as therapy failure and ML development, leishmaniasis therapy should ideally be started early in the course of the disease [17, 76]. Meglumine antimoniate resistance affects approximately 25% of CL and ML patients [77]. Alternatives include oral miltefosine and amphotericin B, which are either expensive or unavailable at the primary healthcare level [78]. The Pan American Health Organization´s 2022 guideline recommends intralesional injections for CL lesions less than 3 cm in diameter if the total number is less than four [9]. Intralesional therapy requires smaller total volumes of meglumine antimoniate, but it can have similar side effects as systemic therapy [79].

Health-related quality of life

Healthcare professionals, including researchers, promote health, which the WHO defined in 1946 as *"a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity"* [80, 81]. More than 100 traditional healers from southern Ecuador added the value of "*mental, emotional, physical, and spiritual balance"* as well as *"peacefulness and harmony with the community, nature, and oneself"* to the WHO definition [82]. Both definitions advocate for non-biomedical outcomes to be included in research. In response to that, researchers developed the concept of Quality of Life (QoL), defined by Theofilou in 2013 as "*how an individual measures the goodness of multiple aspects of their life"*[83]. This well-being is measured subjectively by oneself [84]. QoL is comprised of pleasant and unpleasant affects (e.g., joy, pride, sadness, and anger), life satisfaction (e.g., satisfaction with current life), and domain satisfaction (e.g., work, family, and health). Researchers have developed more than a thousand instruments to quantify QoL for research or care purposes [83]. The Health-Related Quality of Life (HRQL) instruments assess the specific impact of illness on QoL, whether generic (for general use) or disease-specific (for example, prostate cancer-specific). The majority of QoL tools allow for the numerical quantification of results and subsequent statistical analysis [85]. QoL varies depending on one's language, culture, and social background, and this should be considered when interpreting. Furthermore, measurement instruments should be studied for validity and reliability before being used in clinical practice or research [86]. The lack of explanatory information on outcomes is a limitation of HRQL quantification, and interpretation is dependent on qualitative information (e.g., open questions or observations) to clarify causes [87]. There are no leishmaniasis-specific QoL questionnaires. Researchers often use dermatology-specific instruments (such as the Dermatology Life Quality Index or Skindex-29) or generic tools [88, 89]. QoL studies are mostly limited to CL, and they show poor HRQL in Asian and African patients [90]. Hu reported significant HRQL impairment in patients with lower limb lesions in one of the few South American studies [91].

Leishmaniasis related stigma

Depending on the social and cultural context, leishmaniasis may result in stigma, social marginalization, and a subsequent decrease in quality of life [90]. The term "stigma" is derived from Greek and means "mark" [92]. Stigma occurs within a society when a sub-group is viewed negatively based on shared characteristics (stigmata or marks) [93]. Because of their visibility, skin diseases can easily provoke stigma [94]. Leprosy is a classic stigma-provoking skin disease that was recognized in the biblical law of Moses more than three thousand years ago [95]. When a person experiences social isolation, name-calling, or other forms of discrimination as a result of his condition, this is referred to as external or enacted stigma. Self-stigma or internal stigma occurs when a person anticipates external stigma or is ashamed of a particular characteristic [96, 97]. The latter, while hidden, may be the worst and is difficult to determine [98]. Oval infiltrative CL lesions and destructive ML lesions may resemble leprosy, and CL and ML are even referred to as "*mountain leprosy*" in some countries [99, 100]. As a result, we can anticipate the existence of leishmaniasis-related stigma. Several studies have found that this occurs in Asian and African countries, resulting in severely impaired HRQL [101, 102]. Surprisingly, during qualitative interviews with 205 CL patients in Surinam, anthropologist Ramdas found no stigma. Instead, the HRQL impairment was linked to physical discomfort caused by lesions [91, 103]. In a Colombian study, only 26% of CL patients reported stigma using the Explanatory Model Interview Catalogue, which was significantly lower than the 68% reported by leprosy patients [104]. After the peace agreement between the Colombian government and the Revolutionary Armed Forces of Colombia, Pinto Garcia interviewed soldiers, officers, guerrilla members, civilians, and healthcare professionals, among others. He describes the government's active stigmatization of leishmaniasis patients as "*guerrilla members*" emphasizing the importance of ongoing and region-specific stigma assessment [105]. This assessment is consistent with the WHO's emphasis on integrated approaches to reducing stigma associated with Neglected Tropical Skin Diseases (NTDs) [106].

Leishmaniasis in Ecuador

Ecuador is a small South American country with a population of 18 million people [107]. The Andes, an uninterrupted highland range, divides the Ecuadorian mainland from north to south in the Pacific (coastal), Highland, and Amazon regions (Figure 1) [99].

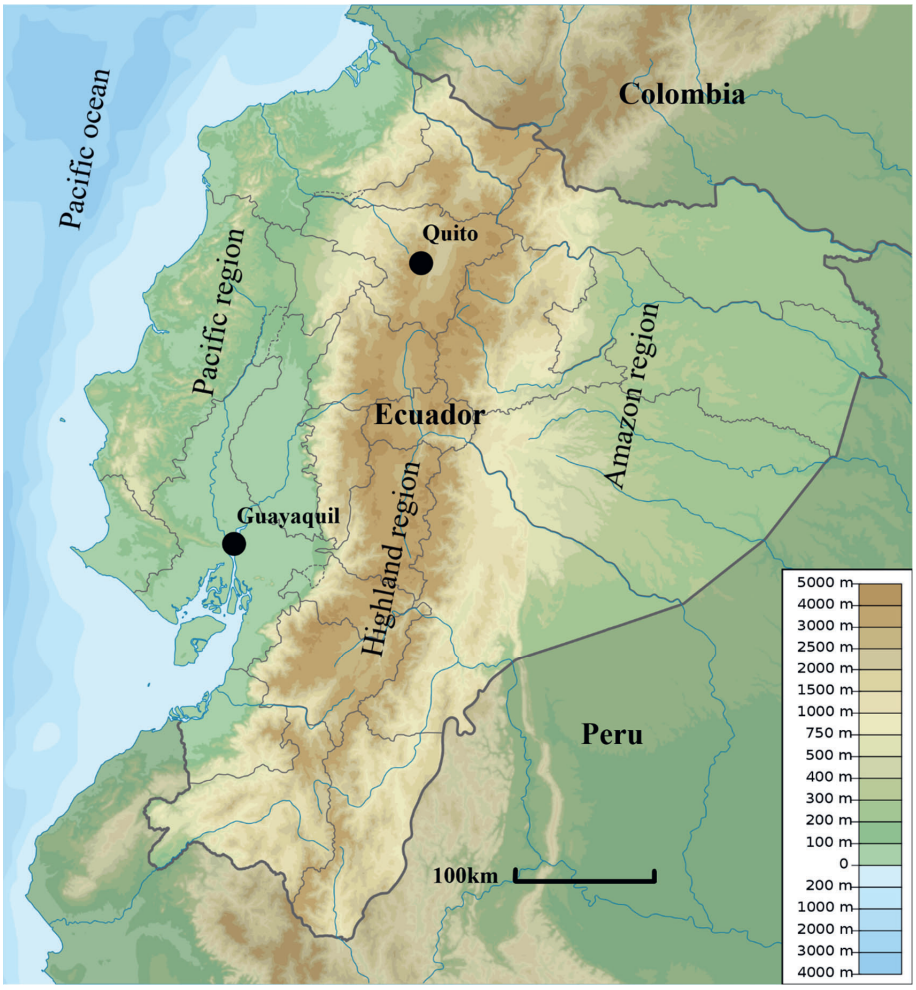


Figure 2. Altitude map of Ecuador with the coastal Pacific, Highland, and Amazon regions.
Copyright: The image is adapted from Wikipedia by the author and is available under the Creative Commons CC0 1.0 Universal Public Domain Dedication [108]

Leishmaniasis is uncommon above 1500 meters in the cold and dry climate of the highlands. CL is endemic in both the Pacific and Amazon regions, but ML is exclusively endemic in the Amazon [109, 110]. Spatial CL clusters occur in the subtropical northern Pacific region and throughout the tropical Amazon [111]. Even though leishmaniasis is a compulsorily notifiable disease, there is a three- to fivefold underreporting [1]. The estimated yearly incidence is between 30 and 49 per 100,000 people [78, 111]. CL is caused by eight species, principally *L. guyanensis* and *L. braziliensis* [99, 112]. *L. braziliensis* is the only species found in ML patients [7, 78]. Kato *et al.* applied the sequencing of a cytochrome B gene fragment for species differentiation. This method successfully distinguished all Ecuadorian species [112, 113]. Only a few Amazon patients have had the causative species identified, and it is not done routinely, even in the Pacific region [114]. In a 2016 study, *L. braziliensis* was found in 12% of 101 cases from the Pacific region; this finding suggests that this species is becoming more common, increasing the risk of ML [112]. Nevertheless, no follow-up was done on this study. The lack of current knowledge of the distribution of *Leishmania* species

complicates the surveillance of ML cases [115]. This is of special importance as the new guideline recommends local CL treatments that might increase the risk of ML [9, 116]. The Pacific region has better accessibility via roads and public transportation compared to the Amazon region [117]. Both the poverty level and analphabetism rate are relatively low [118]. More than 90% of its population is of Mestizo ethnic origin, who descend from European, Amerindian, and African ancestors [119]. Kichwa, Shuar, Achuar, Waorani, Sapara, Shiwiar, Andwa, Quijos, Siekopai, Siona, and Ai'Cofan are the 11 Amerindian ethnic groups that claim territory in the Amazon [120]. They have their languages and speak Spanish as a second or third tongue. Mestizos, who make up more than half of the Amazon population, discriminate against them [121]. Aside from overt discrimination, language barriers, cultural differences, and poverty, all impede access to health care [122, 123]. On top of that, public healthcare services in the majority of the Amazon are only accessible through expensive and time-consuming travel by plane, canoe, or foot [117]. Leishmaniasis research has been focused on the better-accessible Pacific region leaving a knowledge gap regarding the vulnerable Amazon population [78]. The diagnosis of leishmaniasis is based on smear slide microscopy, which has a sensitivity of 85-90% in patients who present within four months, according to the current guideline of the Ecuadorian Ministry of Health [46]. Diagnostic accuracy studies on smear slide microscopy reported lower sensitivities but had a high risk of bias [51]. Thus, high-quality methodological studies are urgently needed to assess the accuracy of current methods for CL confirmation [63]. Medical professionals in Ecuador treat leishmaniasis almost exclusively with meglumine antimoniate which has numerous and severe side effects. Moreover, the market price of meglumine antimoniate is 5-15 US dollars per day, there are frequent countrywide shortages, and the injections, containing 5-15 milliliters of the drug, are painful [78, 124]. As a result, if treatment is initiated, most patients do not complete it [125, 126]. Therefore, CL and ML patients urgently require effective, safe, oral, and readily available treatments. Weigel studied the HRQL of CL patients in Ecuador's Pacific region using a non-standardized questionnaire and found a negative effect [125]. This study has not been followed up and no studies were conducted in the Ecuadorian Amazon. The existence of a possible leishmaniasis-related stigma has never been investigated in Ecuador.

Aim of the thesis

In this thesis, I describe studies done in Ecuador with the following aims: To update and clarify knowledge on the distribution of *Leishmania* species in the Pacific and Amazon regions. To analyze regional differences in CL patient presentation and to identify determinants of health-seeking delay. Implement qPCR on DNA extracted from filter paper and estimate the diagnostic accuracy of both qPCR and smear slide microscopy for CL diagnosis. Assess the predictive values of demographic and clinical criteria for CL diagnosis. Explore the impact of CL-suspected lesions on the quality of life of patients in the subtropical Pacific and Amazon regions and the determinants of health-related quality of life. And understand the perceptions leading to CL and ML-related stigma expressions. In the broader South American context I describe a study that aims to explore the ML detection rates of clinical criteria, and in the worldwide context, a study that aims to assess the efficacy and safety of allylamines in CL and ML treatment and to define priorities for future research.

Outline of the thesis

The knowledge of the *Leishmania* species distribution and clinical presentation of CL patients in the Ecuadorian Pacific and Amazon regions is outdated and incomplete. Therefore, we describe the

identification of *Leishmania* species across the Pacific and Amazon regions, the clinical presentation of CL patients, and determinants of health-seeking delay in **Chapter 2**. Each year, the microscopy-based test and treat management of CL-suspected patients potentially leaves thousands without treatment; thus, in **Chapter 3**, we analyze the accuracy of smear slide microscopy and a qPCR on DNA extracted from filter paper. Because parasitological confirmation of *Leishmania* parasites is so complicated, in **Chapter 3** we assess the predictive values of demographic and clinical variables for CL diagnosis in suspected CL patients from Ecuador, and in **Chapter 4** we assess the detection rates of clinical criteria for ML diagnosis in South American patients reported in the literature. In **Chapter 5**, we conduct a systematic review of the worldwide literature to establish the safety and efficacy of allylamines as an oral or topical treatment for CL and ML, in response to the urgent need for therapeutic alternatives. In **Chapter 6**, we present the findings of a study on the Health-Related Quality of Life (HRQL) of suspected CL patients, which had not before been studied systematically in Ecuador. The stigma associated with leishmaniasis has never been studied in Ecuador. Therefore, we conduct a qualitative study to examine the perceptions that lead to leishmaniasis stigma and describe the findings in **Chapter 7**.

Study locations

The Shell Hospital of the Ecuadorian charity Fundación Misión Cristiana de Salud and 15 health centers from the Ministerio de Salud Pública (Ecuadorian Ministry of Health) recruited the patients for the studies described in **Chapter 2, 3, 6, and 7.** The research laboratory of the Universidad de las Americas in Quito Ecuador did all the molecular analyses (Figures 3, and 4). The Infectious Diseases Program of the Amsterdam Institute for infection and Immunity, and the Department of Epidemiology and Data Science of the Amsterdam UMC location University of Amsterdam the Netherlands, the OneHealth Research Group from the school of medicine at the Universidad de las Americas in Quito Ecuador, the Hospital Shell of the Fundación Misión Cristiana de Salud in Shell Ecuador, the School of Public Health of the College of Health Sciences of the Universidad de San Francisco de Quito Ecuador, the Department of Epidemiology, Biostatistics and Occupational Health of the McGill University in Montreal Canada, and the Department of Social and Cultural Anthropology of the Katholieke Universiteit Leuven Belgium collaborated with the planning, execution, and/or analysis of these studies.

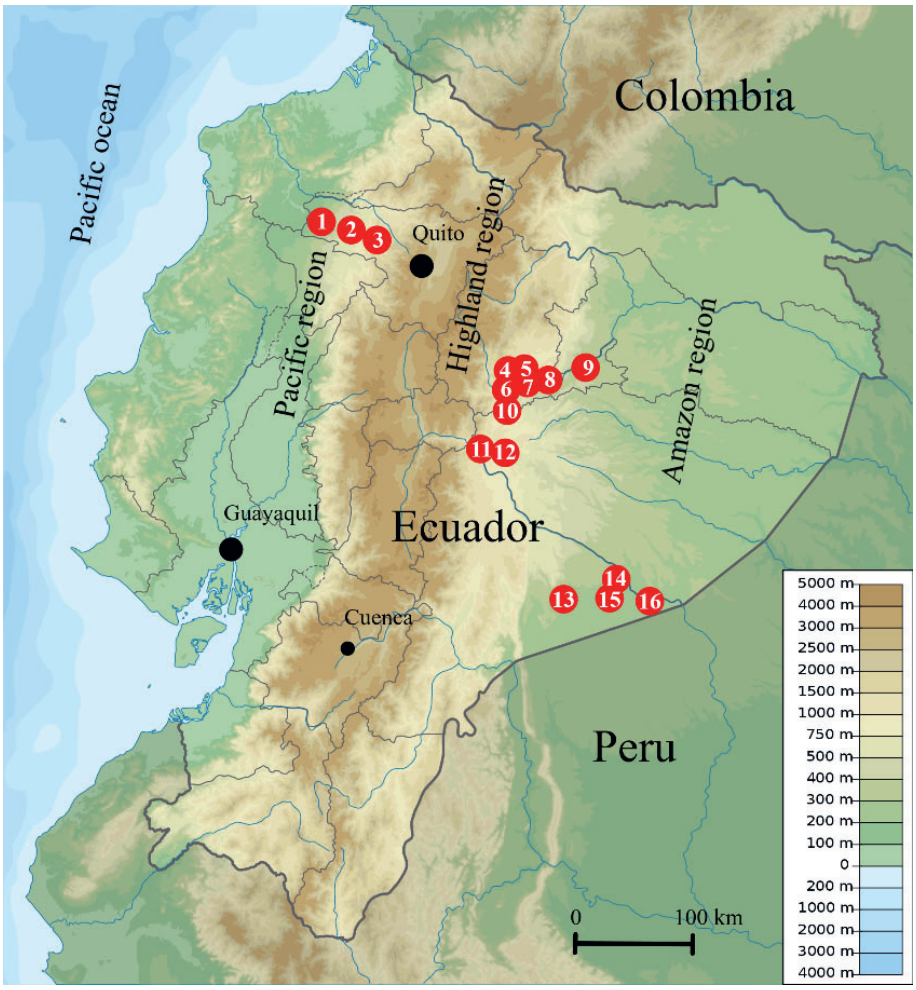


Figure 3. ***Ecuador altitude map with geographic regions and participating centers. Major cities are represented by black dots. Locations of participating health centers are indicated by red dots: In the Pacific region: 1: Puerto Quito, 2: Pedro Vicente Maldonado, 3: San Miguel de Los Bancos, and in the Amazon: 4: Tena Hospital, 5: Paushiyaku, 6: Satelital Tena, 7: Puerto Napo, 8: Misahualli, 9: Chontapunta, 10: Arosemena Tola, 11: Shell hospital, 12: Puyo hospital, 13: Tuutinentza, 14: Ipiak, 15: Wasakentsa, 16: Wachirpas.*** *Copyright: The image is adapted from Wikipedia by the author and is available under the Creative Commons CC0 1.0 Universal Public Domain Dedication [108].*

Figure 4. A) Shell hospital included the majority of the participants from the Amazon. B) The sequencer used to differentiate Leishmania species at the research laboratory of the Universidad de Las Americas in Quito. C) The thermocyclers used for qPCR to detect Leishmania DNA from samples dried on filter paper at the research laboratory of the Universidad de las Americas in Quito. *Image A: Copyright © 2022 Hospital Shell, All rights reserved., Image B and C: Copyright © 2023 Manuel Calvopiña, All rights reserved. All images were used with the permission of the copyright holders.*

Collaborations between the Infectious Diseases Program of the Amsterdam Institute for infection and Immunity, the Medical Library, and the Department of Epidemiology and Data Science of the Amsterdam UMC location University of Amsterdam the Netherlands, the OneHealth Research Group from the school of medicine at the Universidad de las Americas in Quito Ecuador, the Hospital Shell of the Fundación Misión Cristiana de Salud in Shell Ecuador, the Hospital San Miguel of the Fundación Quina Care in Puerto el Carmen Ecuador, the Serviço the Imunologia of the Hospital Universitário Prof. Edgar Santos of the Universidade Federal da Bahia Brazil, and the Department of Pediatrics of the Division of Infectious Diseases of the University of British Columbia in Vancouver Canada resulted in the literature reviews described in **Chapter 4 and 5**.

References

1. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PloS one. 2012;7(5):e35671.
2. World Health Organization. Leishmaniasis: WHO; 2023 https://www.who.int/data/gho/data/themes/topics/gho-ntd-leishmaniasis#:~:text=Out%20of%20200%20countries%20and,are%20endemic%20for%20CL%20only. Accessed: 26 January 2023.
3. Maroli M, Feliciangeli M, Bichaud L, Charrel R, Gradoni L. Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. Medical and veterinary entomology. 2013;27(2):123-47.
4. Serafim TD, Coutinho-Abreu IV, Dey R, Kissinger R, Valenzuela JG, Oliveira F, et al. Leishmaniasis: the act of transmission. Trends in parasitology. 2021;37(11):976-87.
5. Alemayehu B, Alemayehu M. Leishmaniasis: a review on parasite, vector and reservoir host. Health Science Journal. 2017;11(4):1.
6. Saeedi M. Leishmaniasis Geneva: WHO; 2022. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis. Accessed: 25 January 2022.
7. Calvopiña M, Angel G, Armijos R, Gomez E, Mimori T, Cooper P, et al. Clinical features of mucocutaneous leishmaniasis in the Amazonian region of Ecuador. Res Rep Ser. 2005;6:82-9.
8. de Vries HJ, Schallig HD. Cutaneous Leishmaniasis: A 2022 Updated Narrative Review into Diagnosis and Management Developments. American Journal of Clinical Dermatology. 2022:1-18.
9. Pan American Health Organization. Guideline for the treatment of leishmaniasis in the Americas. Second ed. Washington, DC: PAHO; 2022.
10. Cantanhêde LM, Mattos CB, Cruz AK, Ikenohuchi YJ, Fernandes FG, Medeiros E, et al. Overcoming the Negligence in Laboratory Diagnosis of Mucosal Leishmaniasis. Pathogens. 2021;10(9).
11. Cincura C, De Lima CMF, Machado PRL, Oliveira-Filho J, Glesby MJ, Lessa MM, et al. Mucosal leishmaniasis: A retrospective study of 327 cases from an endemic area of Leishmania (Viannia) braziliensis. American Journal of Tropical Medicine and Hygiene. 2017;97(3):761-6.
12. Rodríguez G, Sarmiento L, Hernández CA. Leishmaniasis mucosa y otras lesiones destructivas centrofaciales. Biomedica. 1994;14(4):215-29.
13. Van der Auwera G, Davidsson L, Buffet P, Ruf M-T, Gramiccia M, Varani S, et al. Surveillance of leishmaniasis cases from 15 European centres, 2014 to 2019: a retrospective analysis. Eurosurveillance. 2022;27(4):2002028.
14. Strazzulla A, Cocuzza S, Pinzone MR, Postorino MC, Cosentino S, Serra A, et al. Mucosal leishmaniasis: an underestimated presentation of a neglected disease. Biomed Res Int. 2013;2013:805108.
15. Alcais A, Abel L, David C, Torrez ME, Flandre P, Dedet JP. Risk factors for onset of cutaneous and mucocutaneous leishmaniasis in Bolivia. The American journal of tropical medicine and hygiene. 1997;57(1):79-84.
16. Llanos-Cuentas EA, Marsden PD, Cuba CC, Barreto AC, Campos M. Possible risk factors in development of mucosal lesions in leishmaniasis. Lancet (London, England). 1984;2(8397):295.
17. Machado-Coelho GL, Caiaffa WT, Genaro O, Magalhaes PA, Mayrink W. Risk factors for mucosal manifestation of American cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2005;99(1):55-61.
18. James SL, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2018;392(10159):1789-858.
19. Hotez PJ, Aksoy S, Brindley PJ, Kamhawi S. What constitutes a neglected tropical disease? : Public Library of Science San Francisco, CA USA; 2020. p. e0008001.
20. Dhar R, Kumar A, Karmakar S. Neglected tropical disease: Why we need to prioritize them more. Asian Journal of Medical Sciences. 2022;13(10):1-2.

21. Hotez PJ, Pecoul B, Rijal S, Boehme C, Aksoy S, Malecela M, et al. Eliminating the neglected tropical diseases: translational science and new technologies. PLoS neglected tropical diseases. 2016;10(3):e0003895.
22. Espinosa O, Serrano MG, Camargo E, Teixeira MMG, Shaw JJ. An appraisal of the taxonomy and nomenclature of trypanosomatids presently classified as Leishmania and Endotrypanum. Parasitology. 2018;145(4):430-42.
23. Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet (London, England). 2018;392(10151):951-70.
24. Organización Panamericana de la Salud. Leishmaniasis 2022 https://www.paho.org/es/temas/leishmaniasis. Accessed: 22 December 2022.
25. Aronson NE, Joya CA. Cutaneous Leishmaniasis: Updates in Diagnosis and Management. Infectious disease clinics of North America. 2019;33(1):101-17.
26. Hashiguchi Y, Gomez LE, Cáceres AG, Velez LN, Villegas NV, Hashiguchi K, et al. Andean cutaneous leishmaniasis (Andean-CL, uta) in Peru and Ecuador: the vector Lutzomyia sand flies and reservoir mammals. Acta tropica. 2018;178:264-75.
27. González U, Pinart M, Sinclair D, Firooz A, Enk C, Vélez ID, et al. Vector and reservoir control for preventing leishmaniasis. The Cochrane database of systematic reviews. 2015;2015(8):Cd008736.
28. Hodiamont CJ, Kager PA, Bart A, de Vries HJ, van Thiel PP, Leenstra T, et al. Species-directed therapy for leishmaniasis in returning travellers: a comprehensive guide. PLoS neglected tropical diseases. 2014;8(5):e2832.
29. Akhoundi M, Downing T, Votýpka J, Kuhls K, Lukeš J, Cannet A, et al. Leishmania infections: Molecular targets and diagnosis. Molecular aspects of medicine. 2017;57:1-29.
30. Van der Auwera G, Dujardin J-C. Species typing in dermal leishmaniasis. Clinical microbiology reviews. 2015;28(2):265-94.
31. das Chagas BD, Pereira TM, Cantanhêde LM, da Silva GP, Boité MC, Pereira LOR, et al. Interspecies and Intrastrain Interplay among Leishmania spp. Parasites. Microorganisms. 2022;10(10).
32. Gurjar D, Kumar Patra S, Bodhale N, Lenka N, Saha B. Leishmania intercepts IFN-γR signaling at multiple levels in macrophages. Cytokine. 2022;157:155956.
33. Scorza BM, Carvalho EM, Wilson ME. Cutaneous Manifestations of Human and Murine Leishmaniasis. International journal of molecular sciences. 2017;18(6).
34. Venazzi EA, Roberto AC, Barbosa-Tessmann IP, Zanzarini PD, Lonardoni MV, Silveira TG. Detection of Leishmania (Viannia) DNA in blood from patients with American cutaneous leishmaniasis. Experimental parasitology. 2007;115(4):399-402.
35. Amato VS, Tuon FF, Imamura R, de Camargo RA, Duarte MI, Neto VA. Mucosal leishmaniasis: description of case management approaches and analysis of risk factors for treatment failure in a cohort of 140 patients in Brazil. Journal of the European Academy of Dermatology and Venereology. 2009;23(9):1026-34.
36. Berberian D. Cutaneous leishmaniasis (oriental sore): II. incubation period. Archives of dermatology and syphilology. 1944;50(4):231-2.
37. Pearson RD, de Queiroz Sousa A. Clinical spectrum of leishmaniasis. Clinical infectious diseases. 1996:1-11.
38. Aguayo-Carreras P, Cuenca-Barrales C, Perez-López I, Ruiz-Villaverde R, Tercedor-Sanchez J. Successful response to liposomal amphotericin B in mucosal leishmaniasis in glans resistant to intralesional pentavalent. International journal of dermatology. 2019;58(7):e137-e8.
39. Nylén S, Eidsmo L. Tissue damage and immunity in cutaneous leishmaniasis. Parasite immunology. 2012;34(12):551-61.
40. Falcoff E, Giménez L, Bernabó J, Bottasso O. Interferon gamma as an immunological strategy for the treatment of human leishmaniasis. Medicina. 1994;54(3):265-71.
41. Boggild AK, Valencia BM, Espinosa D, Veland N, Ramos AP, Arevalo J, et al. Detection and species identification of Leishmania DNA from filter paper lesion impressions for patients with American cutaneous leishmaniasis. Clinical Infectious Diseases. 2010;50(1):e1-e6.

42. Boggild AK, Valencia BM, Veland N, Ramos AP, Calderon F, Arevalo J, et al. Non-invasive cytology brush pcr diagnostic testing in mucosal leishmaniasis: Superior performance to conventional biopsy with histopathology. PloS one. 2011;6 (10) (no pagination)(e26395).
43. Smit PW, Elliott I, Peeling RW, Mabey D, Newton PN. An overview of the clinical use of filter paper in the diagnosis of tropical diseases. The American journal of tropical medicine and hygiene. 2014;90(2):195.
44. Gomes CM, de Paula NA, Cesetti MV, Roselino AM, Sampaio RN. Mucocutaneous leishmaniasis: accuracy and molecular validation of noninvasive procedures in a L. (V.) braziliensis-endemic area. Diagnostic microbiology and infectious disease. 2014;79(4):413-8.
45. Miranda A, Saldaña A, González K, Paz H, Santamaría G, Samudio F, et al. Evaluation of PCR for cutaneous leishmaniasis diagnosis and species identification using filter paper samples in Panama, Central America. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2012;106(9):544-8.
46. Ministerio De Salud Pública. Manual de procedimientos del Subsistema alerta acción SIVE – ALERTA. Second ed. Quito: MSP; 2013.
47. Conteron E. Prevalencia de leishmaniasis en el area II de Pastaza, asociados a factores de riesgo que influyen en el desarrollo de la enfermedad. Ambato: Universidad Tecnica de Ambato; 2015.
48. Vega-López F. Diagnosis of cutaneous leishmaniasis. Current opinion in infectious diseases. 2003;16(2):97-101.
49. Chico ME, Guderian RH, Cooper PJ, Armijos R, Grogl M. Evaluation of a direct immunofluorescent antibody (DIFMA) test using Leishmania genus-specific monoclonal antibody in the routine diagnosis of cutaneous leishmaniasis. Revista da Sociedade Brasileira de Medicina Tropical. 1995;28:99-103.
50. Schallig HD, Hu RV, Kent AD, van Loenen M, Menting S, Picado A, et al. Evaluation of point of care tests for the diagnosis of cutaneous leishmaniasis in Suriname. BMC infectious diseases. 2019;19(1):1-6.
51. Pena HP, Belo VS, Xavier-Junior JCC, Teixeira-Neto RG, Melo SN, Pereira DA, et al. Accuracy of diagnostic tests for American tegumentary leishmaniasis: a systematic literature review with meta-analyses. Tropical medicine & international health : TM & IH. 2020;25(10):1168-81.
52. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clinical infectious diseases. 2016;63(12):e202-e64.
53. Celeste JLL, Caldeira RL, Pires SDF, Silveira KD, Soares RP, de Andrade HM. Development and evaluation of a loop-mediated isothermal amplification assay for rapid detection of Leishmania amazonensis in skin samples. Experimental parasitology. 2019;203:23-9.
54. Adams ER, Schoone GJ, El Safi S, Schallig HD. Development of a reverse transcriptase loop-mediated isothermal amplification (LAMP) assay for the sensitive detection of Leishmania parasites in clinical samples. The American journal of tropical medicine and hygiene. 2010;82(4):591.
55. Adams ER, Schoone G, Versteeg I, Gomez MA, Diro E, Mori Y, et al. Development and evaluation of a novel loop-mediated isothermal amplification assay for diagnosis of cutaneous and visceral leishmaniasis. Journal of Clinical Microbiology. 2018;56(7):e00386-18.
56. Lobato IM, O'Sullivan CK. Recombinase polymerase amplification: Basics, applications and recent advances. Trac Trends in analytical chemistry. 2018;98:19-35.
57. Bengtson M, Bharadwaj M, Franch O, van der Torre J, Meerdink V, Schallig H, et al. CRISPR-dCas9 based DNA detection scheme for diagnostics in resource-limited settings. Nanoscale. 2022;14(5):1885-95.
58. Vink MM, Nahzat SM, Rahimi H, Buhler C, Ahmadi BA, Nader M, et al. Evaluation of point-of-care tests for cutaneous leishmaniasis diagnosis in Kabul, Afghanistan. EBioMedicine. 2018;37:453-60.
59. Aerts C, Vink M, Pashtoon SJ, Nahzat S, Picado A, Cruz I, et al. Cost effectiveness of new diagnostic tools for cutaneous leishmaniasis in Afghanistan. Applied health economics and health policy. 2019;17(2):213-30.
60. de Vries HJ, Reedijk SH, Schallig HD. Cutaneous leishmaniasis: recent developments in diagnosis and management. American journal of clinical dermatology. 2015;16(2):99-109.

61. Valencia BM, Veland N, Alba M, Adaui V, Arevalo J, Low DE, et al. Non-invasive cytology brush PCR for the diagnosis and causative species identification of American cutaneous leishmaniasis in Peru. PloS one. 2012;7(11):e49738.
62. Schiller I, van Smeden M, Hadgu A, Libman M, Reitsma JB, Dendukuri N. Bias due to composite reference standards in diagnostic accuracy studies. Statistics in Medicine. 2016;35(9):1454-70.
63. Gomes CM, Mazin SC, Santos ER, Cesetti MV, Bächtold GA, Cordeiro JH, et al. Accuracy of mucocutaneous leishmaniasis diagnosis using polymerase chain reaction: systematic literature review and meta-analysis. Memorias do Instituto Oswaldo Cruz. 2015;110(2):157-65.
64. Dendukuri N, Schiller I, De Groot J, Libman M, Moons K, Reitsma J, et al. Concerns about composite reference standards in diagnostic research. bmj. 2018;360.
65. Cheung A, Dufour S, Jones G, Kostoulas P, Stevenson M, Singanallur N, et al. Bayesian latent class analysis when the reference test is imperfect. Revue Scientifique et Technique (International Office of Epizootics). 2021;40(1):271-86.
66. Banda H, Robinson R, Thomson R, Squire SB, Mortimer K. The 'Practical Approach to Lung Health' in sub-Saharan Africa: a systematic review. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2016;20(4):552-9.
67. World Health Organization. Sexually transmitted and other reproductive tract infections: a guide to essential practice: World Health Organization; 2005.
68. Weigle KA, Escobar M, Arias AL, Martinez F, Rojas C. A clinical prediction rule for American cutaneous leishmaniasis in Colombia. International Journal of Epidemiology. 1993;22(3):548-58.
69. Rubiano L, Alexander ND, Castillo RM, Martínez ÁJ, García Luna JA, Arango JD, et al. Adaptation and performance of a mobile application for early detection of cutaneous leishmaniasis. PLoS neglected tropical diseases. 2021;15(2):e0008989.
70. Handler MZ, Patel PA, Kapila R, Al-Qubati Y, Schwartz RA. Cutaneous and mucocutaneous leishmaniasis: Differential diagnosis, diagnosis, histopathology, and management. Journal of the American Academy of Dermatology. 2015;73(6):911-26; 27-8.
71. Agnihotri NT, McGrath KG. Allergic and nonallergic rhinitis. Allergy and asthma proceedings. 2019;40(6):376-9.
72. Brito NC, Machado de Assis TS, Rabello A, Cota G. Intralesional infiltration versus parenteral use of meglumine antimoniate for treatment of cutaneous leishmaniasis: A cost-effectiveness analysis. PLoS neglected tropical diseases. 2019;13(12):e0007856.
73. Ministerio de Salud Pública. Estrategia Nacional de Metaxénicas y Zoonóticas: MSP; 2019 https://www.salud.gob.ec/estrategia-nacional-de-metaxenicas-y-zoonoticas. Accessed: 23 December 2022.
74. Oliveira LF, Schubach AO, Martins MM, Passos SL, Oliveira RV, Marzochi MC, et al. Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. Acta tropica. 2011;118(2):87-96.
75. Chulay JD, Spencer HC, Mugambi M. Electrocardiographic changes during treatment of leishmaniasis with pentavalent antimony (sodium stibogluconate). The American journal of tropical medicine and hygiene. 1985;34(4):702-9.
76. Llanos-Cuentas A, Tulliano G, Araujo-Castillo R, Miranda-Verastegui C, Santamaria-Castrellon G, Ramirez L, et al. Clinical and parasite species risk factors for pentavalent antimonial treatment failure in cutaneous leishmaniasis in Peru. Clinical infectious diseases. 2008;46(2):223-31.
77. Pinart M, Rueda JR, Romero GA, Pinzón-Flórez CE, Osorio-Arango K, Silveira Maia-Elkhoury AN, et al. Interventions for American cutaneous and mucocutaneous leishmaniasis. The Cochrane database of systematic reviews. 2020;8:Cd004834.
78. Hashiguchi Y, Velez LN, Villegas NV, Mimori T, Gomez EAL, Kato H. Leishmaniases in Ecuador: Comprehensive review and current status. Acta tropica. 2017;166:299-315.
79. Fernandes HJ, da Silva RE, Ramalho DB, Aguiar MG, Silveira JN, Cota G. Safety profile of meglumine antimoniate intralesional infiltration for cutaneous leishmaniasis. Expert review of anti-infective therapy. 2020;18(4):381-7.

80. Walsh K, Grech C, Hill K. Health advice and education given to overweight patients by primary care doctors and nurses: A scoping literature review. Preventive Medicine Reports. 2019;14:100812.
81. World Health Organization. Constitution of the World Health Organization. New York: WHO; 1946.
82. Bautista-Valarezo E, Duque V, Verdugo Sánchez AE, Dávalos-Batallas V, Michels NR, Hendrickx K, et al. Towards an indigenous definition of health: an explorative study to understand the indigenous Ecuadorian people's health and illness concepts. International Journal for Equity in Health. 2020;19(1):1-8.
83. Theofilou P. Quality of Life: Definition and Measurement. Europe's Journal of Psychology. 2013;9:150-62.
84. Haraldstad K, Wahl A, Andenæs R, Andersen JR, Andersen MH, Beisland E, et al. A systematic review of quality of life research in medicine and health sciences. Quality of life Research. 2019;28:2641-50.
85. Solans M, Pane S, Estrada MD, Serra-Sutton V, Berra S, Herdman M, et al. Health-related quality of life measurement in children and adolescents: a systematic review of generic and disease-specific instruments. Value in health. 2008;11(4):742-64.
86. Jones-Caballero M, Peñas PF, García-Díez A, Badía X, Chren MM. The Spanish version of Skindex-29. International journal of dermatology. 2000;39(12):907-12.
87. Bryman A. Integrating quantitative and qualitative research: how is it done? Qualitative research. 2006;6(1):97-113.
88. Toledo Jr ACdC, da Silva RE, Carmo RF, Amaral TA, Luz ZMP, Rabello A. Assessment of the quality of life of patients with cutaneous leishmaniasis in Belo Horizonte, Brazil, 2009–2010. A pilot study. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2013;107(5):335-6.
89. Vares B, Mohseni M, Heshmatkhah A, Farjzadeh S, Safizadeh H, Shamsi-Meymandi S, et al. Quality of life in patients with cutaneous leishmaniasis. Archives of Iranian medicine. 2013;16(8):0-.
90. Bennis I, De Brouwere V, Belrhiti Z, Sahibi H, Boelaert M. Psychosocial burden of localised cutaneous Leishmaniasis: a scoping review. BMC Public Health. 2018;18(1):358.
91. Hu R, Ramdas S, Nieuwkerk P, Reis R, Lai AFRFM, de Vries HJC, et al. Body location of "New World" cutaneous leishmaniasis lesions and its impact on the quality of life of patients in Suriname. PLoS neglected tropical diseases. 2020;14(10):e0008759.
92. Simon B. Shame, Stigma, and Mental Illness in Ancient Greece. Stigma and mental illness. 1992:29.
93. World Health Organization. A guide to preventing and addressing social stigma associated with COVID-19: WHO; 2020 https://www.who.int/publications/m/item/a-guide-to-preventing-and-addressing-social-stigma-associated-with-covid-19. Accessed: 23 December 2022.
94. Wu JH, Cohen BA. The stigma of skin disease. Current opinion in pediatrics. 2019;31(4):509-14.
95. King James Bible. King James Bible Online 2020 https://www.kingjamesbibleonline.org. Accessed: 23 December 2022.
96. Scambler G. Stigma and disease: changing paradigms. The Lancet. 1998;352(9133):1054-5.
97. Weiss MG. Stigma and the social burden of neglected tropical diseases. PLoS neglected tropical diseases. 2008;2(5):e237.
98. Ritsher JB, Otilingam PG, Grajales M. Internalized stigma of mental illness: psychometric properties of a new measure. Psychiatry research. 2003;121(1):31-49.
99. Calvopina M, Armijos RX, Hashiguchi Y. Epidemiology of leishmaniasis in Ecuador: current status of knowledge -- a review. Memorias do Instituto Oswaldo Cruz. 2004;99(7):663-72.
100. Munguia Argenal I. Ministerio de Salud reporta un brote de lepra en las montanas de Nicaragua: La Prensa Nicaragua; 2018 https://www.laprensani.com/2018/01/25/nacionales/2366315-lepra-montanas-de-nicaragua#:~:text=Seg%C3%BAn%20la%20Organizaci%C3%B3n%20Mundial%20de,Leishmania%20acaban%20padeciendo%20la%20enfermedad. Accessed: 23 December 2022.
101. Kassi M, Kassi M, Afghan AK, Rehman R, Kasi PM. Marring leishmaniasis: the stigmatization and the impact of cutaneous leishmaniasis in Pakistan and Afghanistan. PLoS neglected tropical diseases. 2008;2(10):e259.
102. Bennis I, Thys S, Filali H, De Brouwere V, Sahibi H, Boelaert M. Psychosocial impact of scars due to cutaneous leishmaniasis on high school students in Errachidia province, Morocco. Infectious diseases of poverty. 2017;6(1):1-8.

103. Ramdas S, van der Geest S, Schallig HD. Nuancing stigma through ethnography: the case of cutaneous leishmaniasis in Suriname. Soc Sci Med. 2016;151:139-46.
104. Gómez LJ, van Wijk R, van Selm L, Rivera A, Barbosa MC, Parisi S, et al. Stigma, participation restriction and mental distress in patients affected by leprosy, cutaneous leishmaniasis and Chagas disease: a pilot study in two co-endemic regions of eastern Colombia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2020;114(7):476-82.
105. Pinto Garcia LB. Maraña: Leishmaniasis and the pharmaceuticalization of War in Colombia. 2020.
106. World Health Organization. Skin NTDs: prioritizing integrated approaches to reduce suffering, psychosocial impact and stigmatization Geneva: WHO; 2020 https://www.who.int/news/item/29-10-2020-skin-ntds-prioritizing-integrated-approaches-to-reduce-suffering-psychosocial-impact-and-stigmatization. Accessed: 23 December 2022.
107. Rodriguez A, Romero-Sandoval A, Sandoval BA, Romero N. Medical specialist distributions in Ecuador: a geographical and temporal analysis of data from 2000 to 2017. BMC Health Services Research. 2022;22(1):671.
108. Urutseg. Ecuador physical map 2014 https://en.m.wikipedia.org/wiki/File:Ecuador_relief_location_map.svg. Accessed: 20 January 2023.
109. Ronquillo TEF, Almeida F RT. Reporte de lesiones mucosas en leishmaniosis tegumentaria americana, en el litoral (Costa) Ecuatoriano. Rev patol trop. 2012;41(3):356-66.
110. Calvopina M, Guevara AG, Armijos RX, Hashiguchi Y, Davidson RN, Cooper PJ. Itraconazole in the treatment of New World mucocutaneous leishmaniasis. International journal of dermatology. 2004;43(9):659-63.
111. Henríquez-Trujillo AR, Coral-Almeida M, Hinojosa MC. The burden of cutaneous and mucocutaneous leishmaniasis in Ecuador (2014-2018), a national registry-based study. bioRxiv. 2019:751446.
112. Kato H, Gomez EA, Martini-Robles L, Muzzio J, Velez L, Calvopiña M, et al. Geographic Distribution of Leishmania Species in Ecuador Based on the Cytochrome B Gene Sequence Analysis. PLoS neglected tropical diseases. 2016;10(7):e0004844.
113. Kato H, Gomez EA, Seki C, Furumoto H, Martini-Robles L, Muzzio J, et al. PCR-RFLP analyses of Leishmania species causing cutaneous and mucocutaneous leishmaniasis revealed distribution of genetically complex strains with hybrid and mito-nuclear discordance in Ecuador. PLoS neglected tropical diseases. 2019;13(5):e0007403.
114. Kato H, Cáceres AG, Gomez EA, Tabbabi A, Mizushima D, Yamamoto DS, et al. Prevalence of Genetically Complex Leishmania Strains With Hybrid and Mito-Nuclear Discordance. Frontiers in cellular and infection microbiology. 2021;11:625001.
115. Crovetto-Martinez R, Aguirre-Urizar JM, Orte-Aldea C, Araluce-Iturbe I, Whyte-Orozco J, Crovetto-De la Torre MA. Mucocutaneous leishmaniasis must be included in the differential diagnosis of midline destructive disease: two case reports. Oral surgery, oral medicine, oral pathology and oral radiology. 2015;119(1):e20-6.
116. Solomon M, Sahar N, Pavlotzky F, Barzilai A, Jaffe CL, Nasereddin A, et al. Mucosal Leishmaniasis in Travelers with Leishmania braziliensis Complex Returning to Israel. Emerging infectious diseases. 2019;25(4):642-8.
117. Benabent Fernández de Córdoba M. El transporte público terrestre y la accesibilidad, instrumentos para el análisis funcional del sistema de asentamientos: el caso de Ecuador. Estoa Revista de la Facultad de Arquitectura y Urbanismo de la Universidad de Cuenca. 2017;6(11):99-122.
118. Villacis B, Carrillo D. País atrevido: la nueva cara sociodemográfica del Ecuador. Quito: Instituto Nacional de Estadística y Censos (INEC); 2012. Available from: https://www.ecuadorencifras.gob.ec/wp-content/descargas/Libros/Economia/Nuevacarademograficadeecuador.pdf.
119. Lopez G. Población que se Autoidentificó Indigena, Segú Provincia de Empadronamiento, Nacionalidad o Pueblo Indigena al que Pertenece y Sexo Quito: INEC; 2010 https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwi6per4kJv2AhVCNewKHUBlBewQFnoECBMQAQ&url=https%3A%2F%2Fwww.ecuadorencifras.gob.ec%2Fwp-content%2Fplugins%2Fdownload-

monitor%2Fdownload.php%3Fid%3D339%26force%3D0&usg=AOvVaw1sZMlqB_kh_8O316Yy4T8I. Accessed: 25 February 2022.
120. CONFENIAE. Nacionalidades Puyo: CONFENIAE; 2022 https://confeniae.net/nacionalidades. Accessed: 24 February 2022.
121. Rueda Miranda TM. Etnia y discriminación laboral; caso indígena en el Ecuador período 2010-2018: Riobamba, Universidad Nacional de Chimborazo; 2021.
122. Bautista-Valarezo E, Duque V, Verhoeven V, Mejia Chicaiza J, Hendrickx K, Maldonado-Rengel R, et al. Perceptions of Ecuadorian indigenous healers on their relationship with the formal health care system: barriers and opportunities. BMC Complementary Medicine and Therapies. 2021;21(1):1-10.
123. Carpio-Arias TV, Verdezoto N, Guijarro-Garvi M, Abril-Ulloa V, Mackintosh N, Eslambolchilar P, et al. Healthcare professionals' experiences and perceptions regarding health care of indigenous pregnant women in Ecuador. BMC Pregnancy and Childbirth. 2022;22(1):1-11.
124. Organización Panamericana de la Salud. Manual de procedimientos para vigilancia y control de las leishmaniasis en las Américas. Washington, DC: OPS; 2019.
125. Weigel MM, Armijos RX, Racines R, Zurita C, Izurieta R, Herrera E, et al. Cutaneous leishmaniasis in subtropical Ecuador: popular perceptions, knowledge, and treatment. Bulletin of the Pan American Health Organization (PAHO); 28 (2), jun 1994. 1994.
126. Weigel MM, Armijos RX. The traditional and conventional medical treatment of cutaneous leishmaniasis in rural Ecuador. Revista Panamericana de Salud Pública. 2001;10(6):395-404.

Chapter 2

Leishmania species and clinical characteristics of Pacific and Amazon cutaneous leishmaniasis in Ecuador and determinants of health-seeking delay: a cross-sectional study

Jacob M Bezemer, Byron P Freire-Paspuel, Henk D F H Schallig, Henry J C de Vries, Manuel Calvopiña

A revised version of this chapter has been published in: **BMC Infectious Diseases. 2023; 23(1):395**

Abstract

Background

Cutaneous Leishmaniasis (CL) affects up to 5.000 people in Ecuador each year. *L. guyanensis* and *L. braziliensis* are the most common of the eight CL-causing *Leishmania* species. Earlier CL research concentrated on the easily accessible Pacific region. This study aims to determine the distribution of *Leishmania* species in Pacific (Pichincha province) and Amazon (Napo, Pastaza, and Morona Santiago provinces) spatial CL clusters, compare CL patient presentation, and identify determinants of health-seeking delay.

Methods

All cases in this prospective cross-sectional study were diagnosed using smear slide microscopy, PCR, or both. Cytochrome B gene sequencing was used to identify the causative *Leishmania* species in qPCR-positive samples.

Results

This study included 245 patients, with 154 (63%) infected in the Pacific region and 91 (37%) infected in the Amazon. Causative *Leishmania* species were identified in 135 patients (73% of qPCR positives). The most common species was *L. guyanensis* (102/135, 76%), followed by *L. braziliensis* (26/135, 19%). The Pacific region had a low prevalence of *L. braziliensis* (5/89, 6%). For the first time, we report *L. guyanensis* from the provinces of Napo, Pastaza, and Morona Santiago, *L. braziliensis* from the province of Imbabura, and *L. lainsoni* from the provinces of Pichincha, Napo, Pastaza, and Morona Santiago. Amazon cases had a longer median health-seeking delay in months (2.0, IQR 3.0) than Pacific cases (1.0, IQR 1.5). Prolonged health-seeking delay was associated with older age, Amerindian ethnicity, infection at lower altitudes, non-ulcerative lesions, and lesions on the lower limbs.

Conclusions

In the Pacific region, a sustained low prevalence of *L. braziliensis* and relatively short health-seeking delay might explain why mucosal leishmaniasis remains uncommon. Limited access to health care and stigma might explain the prolonged health-seeking delay in the Amazon. We recommend routine species determination in Amazon CL cases, and additional regional research into diagnostic test accuracy. Furthermore, the determinants of health-seeking delay in Ecuador, as well as regional patient characteristics in neighboring countries Peru and Colombia should be investigated further.

Keywords

Leishmaniasis, cutaneous; Leishmaniasis, epidemiology; Phylogeny; Time-to-treatment; Ecuador

Introduction

Background

Leishmaniasis is a vector-transmitted parasitic disease that leads to either cutaneous (CL), visceral (VL), or mucosal (ML) lesions [1]. CL causes ulcers and nodular lesions of the skin that heal spontaneously with scarring over months to years. VL affects internal organs and is lethal if left untreated. ML ulcerates and deforms mucous membranes, does not heal spontaneously, and may be lethal [2]. The World Health Organization (WHO) classifies Leishmaniasis as a Neglected Tropical Disease (NTD) because it disproportionally affects poor and vulnerable populations, lacks funding, and requires research to improve understanding, diagnosis, treatment, and prevention (e.g. vaccines and vector control) of the disease [3, 4]. Twenty-two *Leishmania* species are pathogenic to humans with each primarily causing one or two of the disease manifestations CL, VL, or ML [5]. Their distribution is limited geographically depending on the interaction between the parasite, animal reservoir, and sand fly vector (*Phlebotomus* and *Lutzomyia*) [2, 6]. Every year, leishmaniasis affects 700.000 to 1 million people worldwide including an estimated 58.000 in South America of which 5.000 in Ecuador [7, 8]. The Andean Mountain range divides the Ecuadorian mainland into three regions: The Pacific on the west, the Highlands in the middle, and the Amazon on the east (Figure 1).

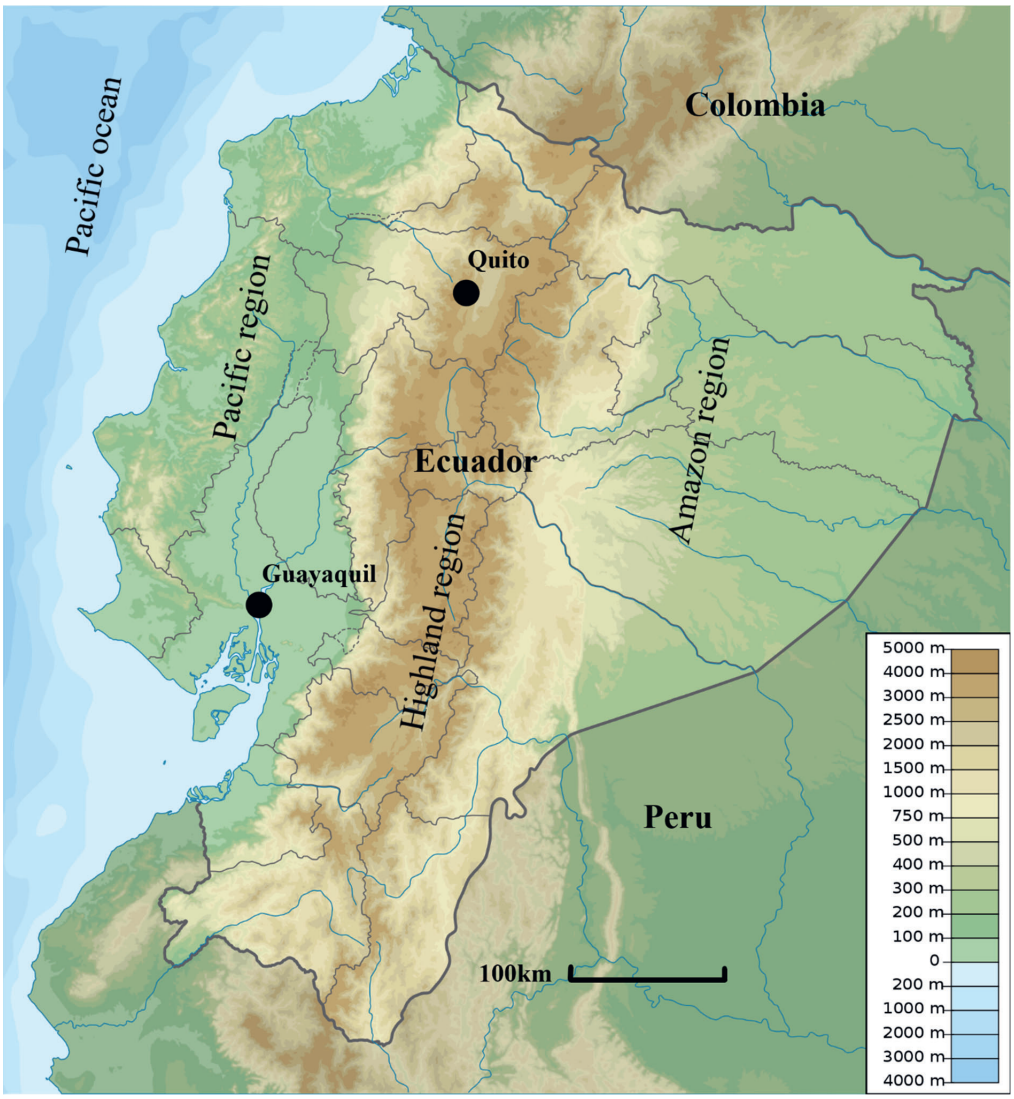


Fig 1. Altitude map of Ecuador with the coastal Pacific, Highland, and Amazon regions. *The image is adapted from Wikipedia by the authors and is available under the Creative Commons CC0 1.0 Universal Public Domain Dedication [9].*

No VL cases have been reported in Ecuador [10]. CL is endemic (1.7-46.4/1.000 inhabitants yearly) in 12 cantons in the Pacific region with a total surface of 11.000 Km^2 and an estimated 749.000 inhabitants but ML is uncommon. Leishmaniasis is not endemic in the Highland region. In the Amazon, CL, and ML are endemic (1.7-21.2/1.000 inhabitants yearly) in all the cantons with a total surface of 120.000 Km^2 and an estimated 957.000 inhabitants [8, 11-13]. *Leishmania guyanensis* and *L. braziliensis* are dominant among eight recorded species. *L. guyanensis* is only found in CL patients but *L. braziliensis* in both CL and ML patients. Studies on *Leishmania* species in Ecuador have primarily focused on the accessible Pacific region and included only a few patients from the remote Amazon [14, 15]. They reported a predominance of *L. guyanensis* in the Pacific and *L. braziliensis* in the Amazon. Kato *et al.* reported an increase in *L. braziliensis* cases in the Pacific in

2016, possibly increasing the risk of ML [14]. Because this study was not followed up on, the need for active surveillance of ML cases remains unclear. In Ecuador, the reservoirs and vectors of *L. guyanensis* and *L. braziliensis* are unknown.

Most of the Pacific region's population belongs to the Mestizo group (descendants from Europeans mixed with Amerindians), with intermediate levels of poverty, relatively low rates of analphabetism, and relatively high accessibility via public transportation. The Amazon population auto-identifies for almost 50% as Amerindian, has high levels of poverty, high rates of analphabetism, and low accessibility via public transportation [16-18]. The Amerindian population is marginalized and lives partially in the lowland rainforest with only air travel access [19-21]. Regional differences in *Leishmania* species and human population characteristics might influence clinical characteristics (e.g., age, gender, lesion location, lesion types, health-seeking delay), and response to treatment [22, 23]. Nevertheless, no studies have compared the presentation of CL cases in the Pacific and Amazon regions hampering surveillance, prevention, timely diagnosis, and subsequent treatment.

Objectives

The objectives of this paper are to elucidate the distribution of *Leishmania* species in Pacific (Pichincha province) and Amazon (Napo, Pastaza, and Morona Santiago provinces) spatial CL clusters. To analyze regional differences in CL patient presentation and to identify determinants of health-seeking delay. The first objective was specified in the research protocol. The emergence of regional differences in the clinical and laboratory characteristics during data analysis led to the conception of the second and third objectives.

Methods

Participants

This study was part of a prospective cross-sectional project in CL patients that included diagnostic test evaluations, a quantitative health-related quality of life questionnaire, qualitative interviews, and species determination. Patients were included in private and public primary health care centers and hospitals from the subtropical Pacific region of the Pichincha province and the Amazon Napo, Pastaza, and Morona Santiago provinces. Patients were included from January 2019 through June 2021. All participants or their legal representatives provided written informed consent. The research protocol was approved by the ethical committee of the `Universidad Internacional del Ecuador' registration number: UIDE-FCM-EDM-COM-18-0069, and the Ecuadorian Ministry of Health, registration number: MSPCURI000284-3, prior to its initiation. All the methods were carried out in agreement with the guidelines of the Ministry of Health of Ecuador and in accordance with the declaration of Helsinki. The participating centers provided free leishmaniasis treatment (intramuscular meglumine antimoniate) in accordance with the national protocol of the Ecuadorian Ministry of Health [24].

All patients sent for a microscopic smear slide examination of a skin lesion suspected of CL in the participating centers were eligible. Patients were included by the doctor, nurse, or laboratory technician during normal workflow before lesion sampling. Patients were excluded from the study if the *Leishmania* infection could not be confirmed either by Polymerase Chain Reaction (PCR) or microscopy.

Laboratory tests

An experienced laboratory technician took a skin scraping from the inner border of the lesions to perform a smear slide. In the participating health centers, smear slides were Giemsa stained according to the protocol of the Ecuadorian Ministry of Health and read with light microscopy [25, 26]. The result of microscopy was reported as "positive" when *Leishmania* amastigotes were identified. A quality check was performed on all positive samples. Immediately after the skin scraping for smear slide, a filter paper, 903 Protein Saver Card (Whatman, Newton Center, MA), was pressed three times on the scraping site and dried at room temperature. Filter papers were processed at the `Universidad de las Americas' research laboratory in Quito, Ecuador. DNA was extracted from the filter paper using Chelex 100 (Sigma-Aldrich, USA) [27]. A 2x2mm piece was cut from each paper and transferred to a 1.5mL tube containing 200µL of 10% (wt/vol) Chelex and 10µL of Proteinase K (Invitrogen, USA). Samples were strongly vortexed for 5 min and subsequently incubated at 56°C for 60 min and 96°C for 20 min in an Eppendorf ThermoMixer C. The supernatant containing the DNA was separated from the Chelex resin and transferred to a new 1.5 mL tube. The presence of *Leishmania* DNA in the extracted samples was evaluated by probe-based real-time PCR (qPCR) following the protocol described by Bezemer *et al.* [28]. Identification of samples that showed amplification of *Leishmania* 18S rDNA by qPCR was subsequently performed by nested PCR and sequencing of the cytochrome B (*Cyt* B) The first PCR reaction, using outer primers L.cyt-AS and L.cyt-AR (Table 1), was prepared in a total volume of 15µL containing: 0.06U/µL of Platinum Taq DNA Polymerase (Invitrogen, USA), 0.5µM of each primer, 2.5mM of $MgCl_2$, 0.3mM of dNTP mix, and 2µL of DNA sample. The following thermal cycler protocol was used: 1 cycle at 94°C for 2 min; 35 cycles at 95°C for 30 sec, 55°C for 30 sec, 72°C for 1 min; 1 cycle at 72°C for 5 min. Then, 1µL of the product of the first PCR was reamplified in the second PCR using primers L.cyt-S and L.cyt-R following the same protocol [14]. Differentiation between *L. braziliensis* and *L. peruviana* was performed by sequencing the Mannose Phosphate Isomerase (MPI) gene using MPI-S and MPI-R primers [29, 30]. The sequencing reaction was prepared using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and capillary electrophoresis were run in a 3500 Genetic Analyzer (Applied Biosystems, USA). The resulting sequences were compared with the NCBI database by applying the Basic Local Alignment for species determination [31]. Additionally, a phylogenetic tree was obtained using the Geneious R11 software and the Tamura-Nei model. The following species' reference sequences described by Kato *et al.* [29] were included in the tree: *L. braziliensis* (GenBank accession number AB095966), *L. guyanensis* (AB095969), *L. lainsoni* (AB433280*), L. naiffi* (AB433279), *L. panamensis* (AB095968), *L. shawi* (AB433281) and *L. peruviana* (AB433282) [32]. In addition to the qPCR, an endpoint PCR was performed that differentiated between the *Viannia* and *Leishmania* subgenus, having a sensitivity of only 50% as published elsewhere [33]. The endpoint PCR was discontinued after the first 100 samples due to human resources problems as a result of the COVID-19 pandemic.

Table 1. Primers used for *Leishmania* identification.

Target	Primer	Sequence
Cyt b	L.cyt-AS	5'-GCG GAG AGR ARG AAA AGG C-3'
	L.cyt-AR	5'-CCA CTC ATA AAT ATA CTA TA-3'
	L.cyt-S	5'-GGT GTA GGT TTT AGT YTA GG-3'
	L.cyt-R	5'-CTA CAA TAA ACA AAT CAT AAT ATR CAA TT-3'
MPI	MPI-S	5'-GCT CTT CCT GTC GGA CAG CGA GC-3'
	MPI-R	5'-TCA CTC TCG AAG GGA GTT CG-3'

Study variables

The following demographic data of study participants were recorded: Age in years, gender (male or female), ethnicity as recognized by the Ecuadorian government [34], presumed place of infection, health-seeking delay in months (defined as the time since onset of symptoms to inclusion in the study and based on recall), lesion type (ulcer, nodular, or other), number of skin lesions, and the location of lesion(s) (indicated with pencil on a person image by the health professional). GPS coordinates of the presumed place of infection were estimated with Google Maps [35]. Altitude in meters was defined as the altitude of the airstrip as reported by the general directorate of civil aviation for hinterland villages [36]. Altitudes of other places were estimated with topographic-map.com [37]. We compared the clinical characteristics of CL patients and *Leishmania* species prevalence between the Pacific and Amazon regions as these regions are geographically separated. Patient ethnicity was grouped into Mestizo and Amazon Amerindian to allow analysis. Lesion location was categorized into four major groups for analysis: Head, Trunk, Upper limbs, and Lower limbs. The sample size was calculated using the average annual number of CL tests performed by the participating centers in the five years preceding the start of the study. With a 24-months inclusion period, the study was expected to include 600 (50%) cases to achieve the maximum number of *Leishmania* species determinations. When the first nationwide COVID-19 quarantine started, the number of inclusions fell dramatically as patients feared to visit healthcare centers, leaving lesions unattended that may have been cured spontaneously, or to give informed consent. The research team had funding and personnel to prolong the inclusion period from 24 months to 29 months until obtaining more than half of the expected number of inclusions and had to stop then.

Analysis

Two independent investigators entered collected data into an electronic data capture system and the data were computer validated [38]. Allocation to the Pacific or Amazon group was based on the infection region. All calculations were done in SPSS Statistics version 28, considering $P < 0.05$ as statistically significant [39]. Associations of CL patient clinical characteristics with the region of infection were tested with the independent samples T, proportions (Wald), Fishers exact (when proportions <1), or Mann-Whitney U test. The patient was excluded from a particular comparison if data were missing for that comparison. The continuous variable `Health-seeking delay' was transformed into a binary variable `Health-seeking delay ≥ one month' for analysis of its determinants. The one-month cut was chosen because bacterial lesions, which are the most common differential diagnosis, mostly heal in two weeks, leaving 14 days for early health-seeking. The following determinants of health-seeking delay were deemed independent and included in Block 1 of a multivariate binary logistic regression model: Age (years), Male gender, Amerindian

ethnicity, Altitude of the place of infection (hectometers), Number of lesions, and Lesion location head and neck. Age was included because it influences illness knowledge and attitudes [40, 41]. Gender because it may influence the moment of health-seeking as well as the decision-making process [42]. Amerindian ethnicity because cultural values and practices as well as language barriers may influence the moment of health-seeking [43]. Altitude because it determines the distance to referral centers [17]. Number of lesions because they may increase the perceived severity. Lesion location head and neck because there it's difficult to hide lesions and differences might indicate stigma [44]. The variable 'Infected in the Amazon' was added to Block 2. The Omnibus Tests of Model Coefficients was applied to assess if 'infected in the Amazon' improved this model indicating that non-included confounders in that region influence health-seeking delay. Lesion types (ulcer, nodular, or other) were not included in this model because of sparse results in at least one cell in the cross-tabulations. This article was written by the STROBE checklist for cross-sectional studies [45].

Results

Leishmania species distribution

Informed consent was signed by 324 cases but four had no cutaneous lesions and were therefore excluded from further examination in the context of the present study whilst other adequate medical care was provided. The presence of *Leishmania* parasites was confirmed with PCR and/or microscopy in 245 patients who were included in this study. Infecting *Leishmania* species could be determined in 135 (73%) of the qPCR-positive samples. The mean age of patients with species determination was 23.2 years and 84 (62%) were male. 102 (76%) patients were from the Mestizo ethnic group and 33 (24%) Amerindian. 20 (77%) of the *L. braziliensis* infected patients were Amazon Amerindian compared to 9 (9%) of the *L. guyanensis* infected patients. More than three-quarters of the *L. braziliensis* infected lesions (24/28, 86%) were on the upper or lower limbs compared to two-thirds (67/115, 58%) of the *L. guyanensis* infected lesions. In the Pacific region, *L. guyanensis*, *L. braziliensis*, and *L. lainsoni* were determined in 83 (93%), five (6%), and one (1%) patient respectively. In the Amazon, *L. braziliensis, L. guyanensis,* and *L. lainsoni* were determined in 21 (46%), 19 (41%), and six (13%) patients respectively. All *L. braziliensis* samples belonged to a single clade in the phylogenetic tree of the Cyt B sequences that included *L. peruviana*. The *L. guyanensis* samples were divided into a subclade with 76 (99%) Pacific samples and a subclade with 18 (72%) Amazon samples, see Tables 2 and 3, Additional file 1, and Figure 2. The MPI gene could be sequenced in 18/26 (69%) *L. braziliensis* samples. The presence of *L. peruviana* in the group of samples analyzed was ruled out since the "G" allele, characteristic of *L. peruviana*, was not found at location 1082 in the MPI gene. However, in addition to the "C" allele of *L. braziliensis*, the novel C1082A polymorphism was identified in several samples. The frequencies of the genotypes identified at position 1082 in the MPI gene were: "CC" N=13 (72%), "CA" N=4 (22%), and "AA" N=1 (6%).

Table 2. Baseline characteristics of 135 CL patients with confirmed *Leishmania* species.

Patient characteristic	*L. guyanensis*	*L. braziliensis*	*L. lainsoni*	Total
Number of Patients (%)	102 (76)	26 (19)	7 (5)	135 (100)
General characteristics				
Mean age in years (SD)	21.5 (15.8)	25.6 (16.4)	38.1 (10.2)	23.2 (16.0)
Males (%)	63 (62)	18 (69)	3 (43)	84 (62)
Mestizo (%)	93 (91)	6 (23)	3 (43)	102 (76)
Amazon Amerindian (%)	9 (9)	20 (77)	4 (57)	33 (24)
Median altitude of place of infection in hectometers (IQR)	5.5 (3.9)	3.2 (1.3)	5.2 (1.5)	5.1 (3.7)
Clinical presentation				
Median health-seeking delay in months (IQR)	1 (2)	2 (4)	1 (2)	1 (1)
Lesion type: ulcer (%)	99 (97)	26 (100)	7 (100)	132 (98)
Median number of lesions (IQR)	1 (1)	1 (1)	1 (0)	1 (1)
Body location of the lesion[a]				
Total number of body locations with lesions	115 (100)	28 (100)	8 (100)	151 (100)
Head and neck (%)	35 (31)	2 (7)	0 (0)	37 (25)
Trunk (%)	13 (11)	2 (7)	2 (25)	17 (11)
Upper limbs (%)	44 (38)	15 (54)	4 (50)	63 (42)
Lower limbs (%)	23 (20)	9 (32)	2 (25)	34 (22)

SD = Standard Deviation, IQR = Interquartile Range

[a]Patients with lesions on different body regions were counted more than once.

Table 3. Provincial distribution of infecting *Leishmania* species in 135 patients with cutaneous lesions from the Ecuadorian subtropical Pacific and Amazon regions. Total (%)

	L. guyanensis	*L. braziliensis*	*L. lainsoni*	Total
Pacific provinces	**83 (93)**	**5 (6)**	**1 (1)**	**89 (100)**
Pichincha	78 (94)	4 (5)	1 (1)	83 (100)
Imbabura	1 (50)	1 (50)	0 (0)	2 (100)
Santo Domingo	2 (100)	0 (0)	0 (0)	2 (100)
Manabi	1 (100)	0 (0)	0 (0)	1 (100)
Guayas	1 (100)	0 (0)	0 (0)	1 (100)
Amazon Provinces	**19 (41)**	**21 (46)**	**6 (13)**	**46 (100)**
Sucumbios	2 (100)	0 (0)	0 (0)	2 (100)
Napo	1 (25)	0 (0)	3 (75)	4 (100)
Orellana	1 (50)	1 (50)	0 (0)	2 (100)
Pastaza	13 (39)	18 (55)	2 (6)	33 (100)
Morona Santiago	2 (40)	2 (40)	1 (20)	5 (100)
Total	**102 (76)**	**26 (19)**	**7 (5)**	**135 (100)**

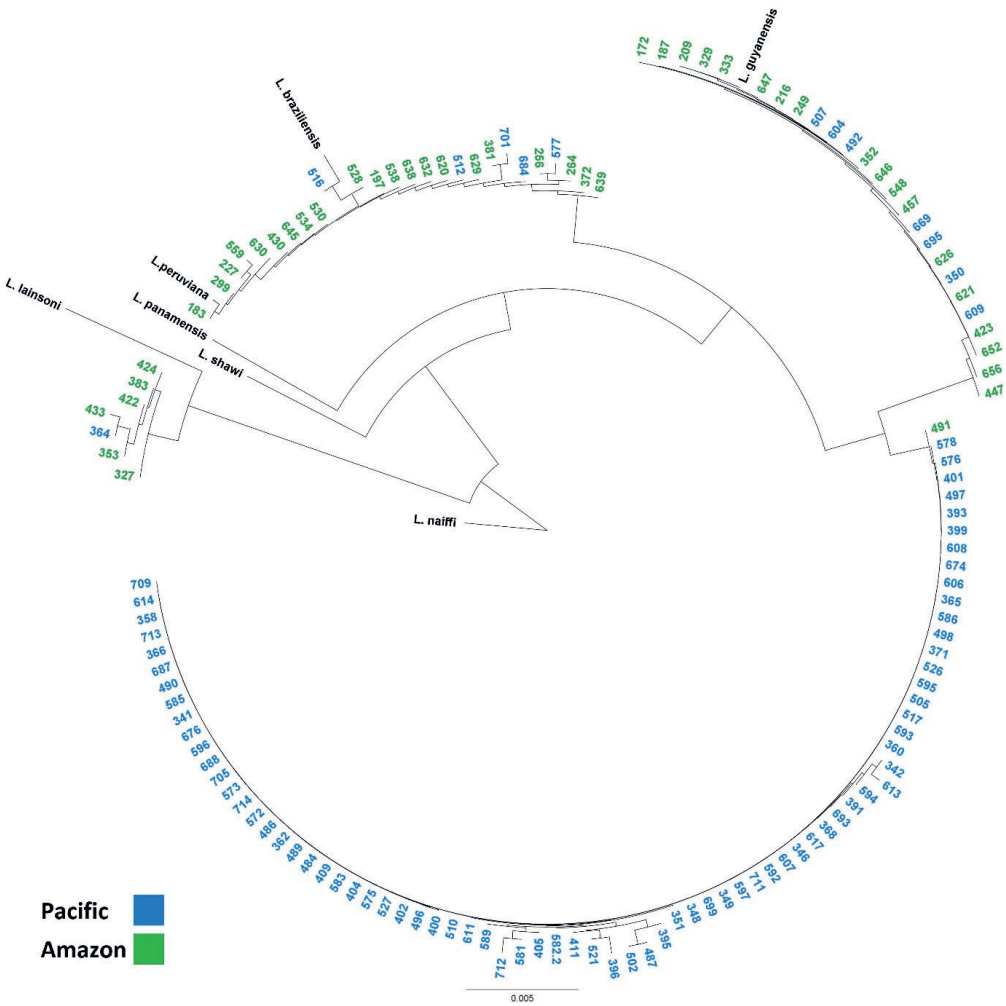


Fig 2. Phylogenetic tree of a cytochrome B gene fragment from 135 Ecuadorian CL patient samples.

Regional comparison of clinical characteristics

Of the 245 confirmed cases, 154 (63%) were infected in the subtropical Pacific region and 91 (37%) in the Amazon. The mean age was 25,5 years and patients infected in the Pacific were an average of 8.4 years younger (P<0.01) than in the Amazon. Most of the patients were male (57%). Overall, most included patients were mestizos (73%) but in the Amazon, the majority were Amazon Amerindian (73%). The median altitude of the place of infection was significantly higher in the Pacific region (5.5 hectometers) than in the Amazon (3.8 hectometers). The median health-seeking delay was significantly lower in the Pacific region (1 month) compared to the Amazon (2 months). Patients from the Pacific had significantly more often lesions on the head and neck (29%) compared to the Amazon (11%), see Table 4 and Additional file 1.

Table 4. Clinical characteristics of 245 cutaneous leishmaniasis patients from the Pacific and Amazon regions.

Patient characteristic (N missing for variable)	Pacific	Amazon	Two-sided P[a]	Total
Number of Patients (%)	154 (63)	91 (37)		245 (100)
General characteristics				
Mean age in years (SD)	22.4 (17.4)	30.8 (20.4)	<0.01[b]	25.5 (19.0)
Males (%)	83 (54)	57 (63)	0.18	140 (57)
Mestizo (%)	154 (100)	25 (27)		179 (73)
Amazon Amerindian (%)	0 (0)	66 (73)	<0.01[b]	66 (27)
Median altitude of place of infection in hectometers (IQR)	5.5 (5.0)	3.8 (3.2)	0.03[b]	4.6 (4.6)
Clinical presentation (1)				
Median health-seeking delay in months (IQR)	1.0 (1.5)	2.0 (3.0)	<0.01[b]	1.0 (1.5)
Lesion type: ulcer (%)	143 (93)	87 (96)	0.39	230 (94)
Median number of lesions (IQR)	1 (1)	1 (0)	0.48	1 (1)
Body location of the lesion[c]				
Total number of body locations with lesions	169 (100)	99 (100)		268 (100)
Head and neck (%)	45 (27)	10 (10)	<0.01[b]	55 (21)
Trunk (%)	18 (11)	15 (15)	0.31	33 (12)
Upper limbs (%)	67 (39)	43 (43)	0.57	110 (41)
Lower limbs (%)	39 (23)	31 (32)	0.15	70 (26)

N = Number, P = Probability, SD = Standard Deviation, IQR = Interquartile Range

[a] Comparing Pacific to Amazon with the Independent samples T, Wald, Fishers exact, or Mann-Whitney U test.

[b] Statistically significant.

[c] Patients with lesions on different body regions were counted more than once.

Determinants of health-seeking delay

The duration of health-seeking delay was known for 244 of 245 confirmed patients. Patients with health-seeking delay ≥ one month were significantly older (27.7 compared to 21.9), more often had Amerindian ethnicity (34% compared to 14%), were infected in the Amazon (45% compared to 14%), presented with no ulcer (92% compared to 98%), were less often infected on the head or neck (15% compared to 32%) and more often on the lower limbs (32% compared to 15%) (Table 5). The multivariate binary logistic model of health-seeking delay ≥ one month did not improve significantly after the addition of the determinant 'Infected in the Amazon' to 'age', 'gender', 'Amerindian ethnicity', 'altitude of place of infection', and 'lesion on the head or neck' (Table 6).

Table 5. Patient characteristics and prolonged health-seeking delay (≥ one month) in 244 confirmed cutaneous leishmaniasis patients in Ecuador.

Patient characteristic (N missing for variable)	Health-seeking delay <1 month	Health-seeking delay ≥ 1 month	Two-sided P[a]	All patients
Number of Patients (%)	88 (36)	156 (64)		244 (100)
General characteristics				
Mean age in years (SD)	21.9 (17.1)	27.7 (19.7)	0.02[b]	25.6 (18.9)
Males (%)	52 (59)	87 (56)	0.62	139 (57)
Mestizo (%)	76 (86)	103 (66)		179 (73)
Amazon Amerindian (%)	12 (14)	53 (34)	<0.01[b]	65 (27)
Characteristics of the area of infection				
Pacific region (%)	69 (78)	85 (55)		154 (63)
Amazon region (%)	19 (22)	71 (45)	<0.01[b]	90 (37)
Median altitude of place of infection in hectometers (IQR)	6.3 (6.0)	3.7 (3.5)	<0.01[b]	4.7 (4.6)
Clinical presentation				
Total number of lesion types presented (%)	89 (100)	156 (100)		245 (100)
Lesion type: ulcer (%)	87 (98)	142 (92)	0.01[b]	229 (93)
Lesion type: nodule (%)	2 (2)	7 (4)	0.50	9 (4)
Lesion type: other (%)	0 (0)	7 (4)	0.05	7 (3)
Median number of lesions (IQR)	1 (0)	1 (1)	0.11	1 (1)
Body location of the lesion[c]				
Total number of body locations with lesions	94 (100)	173 (100)		267 (100)
Head and neck (%)	30 (32)	25 (15)	<0.01[b]	55 (21)
Trunk (%)	14 (15)	18 (10)	0.33	32 (12)
Upper limbs (%)	36 (38)	74 (43)	0.33	110 (41)
Lower limbs (%)	14 (15)	56 (32)	<0.01[b]	70 (26)

N = Number, P = Probability, SD = Standard Deviation, IQR = Interquartile Range

[a] Comparing Health-seeking delay <1 month and ≥ 1 month with the independent samples T, Wald, Fishers exact, or Mann-Whitney U test.

[b] Statistically significant.

[c] Patients with lesions on different body regions were counted more than once.

Table 6. Blockwise addition of the variable 'infected in the Amazon´ to a multivariate model of health-seeking delay ≥ one month in 244 confirmed cutaneous leishmaniasis patients in Ecuador.

	Block 1	Block 2	P[a]
Overall significance	<0.01	<0.01	
Nagelkerke R^2	27.3%	28.1%	
Percentage correctly classified	63.9%	74.6%	
Variable	**Odds ratios (95% CI)**	**Odds ratios (95% CI)**	
Age in years	1.02 (1.00-1.03)	1.01 (1.00-1.03)	
Male gender	0.83 (0.46-1.51)	0.80 (0.44-1.47)	
Amerindian ethnicity	1.85 (0.87-3.92)	1.00 (0.30-3.32)	
Altitude of place of infection in hectometers	0.77 (0.69-0.85)	0.76 (0.69-0.85)	
Number of lesions	1.26 (0.94-1.68)	1.26 (0.93-1.70)	
Lesion location: Head and neck	0.44 (0.22-0.90)	0.47 (0.23-0.95)	
Infected in the Amazon		2.04 (0.70-5.92)	0.18

CI = Confidence Interval, NA = Not Applicable

[a]Omnibus Tests of Model Coefficients comparing Block 1 to Block 2

Discussion

This study reports the infecting *Leishmania* species in 135 Ecuadorian CL cases. Additionally, the clinical presentation and determinants of health-seeking delay of over 240 confirmed CL cases were compared between the Pacific and Amazon regions. For the first time, we report *L. guyanensis* in the provinces of Napo, Pastaza, and Morona Santiago, *L. braziliensis* in the province of Imbabura, and *L. lainsoni* in the provinces of Pichincha, Napo, Pastaza, and Morona Santiago. Amazon cases had a twice as long median health-seeking delay as Pacific cases. Prolonged health-seeking delay (≥ one month) was associated with older age, Amerindian ethnicity, infection at lower altitudes, non-ulcerative lesions, and lesions on the lower limbs.

This is the first study to report the causative *Leishmania* species in over 130 Ecuadorian CL patients, including a representative group from the Amazon region [10]. Former studies on a limited number of CL samples from the northern Ecuadorian Amazon provinces Sucumbíos and Orellana reported the mixed presence of *L. guyanensis*, *L. braziliensis*, and *L. lainsoni* whilst suggesting that *L. braziliensis* was the only causing species in the south [14, 15]. Our study, however, discovered that non-*L. braziliensis* species cause lesions in approximately half of the CL patients infected in the southern Amazon provinces. Therefore, we recommend *Leishmania* species determination for all Amazon-infected CL patients to allow long-term follow-up of the *L. braziliensis* cases for the development of mucosal lesions. Early detection of ML would allow timely treatment preventing destructive malformations and resistance to treatment [46, 47]. This is of special importance as the new guideline of the Pan American Health Organization recommends non-systemic treatments for most American CL lesions which might increase the risk of ML [48, 49].

We report only a couple of *L. braziliensis*-caused CL cases from the Pacific region and do not confirm an increase as suggested by Kato *et al.* In that region, the health-seeking delay was

relatively short, allowing rather prompt initiation of treatment and a subsequent decrease in the risk of ML [47]. Thus, a sustained low *L. braziliensis* prevalence combined with prompt initiation of CL treatment possibly explains why mucosal leishmaniasis is rare in the Pacific region and we do not recommend routine follow-up of CL cases for ML [50, 51].

L. braziliensis was indistinguishable from *L. peruviana* during the comparison of a Cyt B gene fragment with the NCBI database and in the obtained phylogenetic tree. This has been reported before in Peruvian samples, but not in Ecuador [29]. Therefore, we recommend that the Cyt B sequencing for species determination in Ecuador, as proposed by Kato *et al.,* should be complemented with the sequencing of an MPI gene fragment [14]. The phylogenetic tree divided *L. guyanensis* samples into a Pacific and Amazon predominant subclade. This suggests that a region-specific mutational development has taken place and is in agreement with Calvopiña *et al.* who reported region-specific zymodeme variations in Ecuador that seemed to be associated with the clinical presentation of patients [52]. Such variations underscore the importance of a regional analysis of CL case presentation, as done in this paper, as well as diagnostic test accuracy, resistance to treatment, and the risk of ML.

Five *L. braziliensis* samples showed at least one "A" allele at location 1082 of the MPI gene [30]. This mutation has not been described before and seems to be Ecuadorian. Genetically complex *Leishmania* strains have been described in Ecuador before and show the importance of continuously validating and updating species determination methods [53].

This study compares a low (Pacific) with a high (Amazon) *L. braziliensis* endemic area. Younger age and a higher percentage of lesions on the head of patients infected in the Pacific are in agreement with former studies [54, 55]. This might be explained by different reservoirs and transmitting vectors in the Pacific region compared to the Amazon, but evidence on both is absent. The current hypothesis is that *Leishmania* transmission is peri-domestic in the Pacific region and occupational (agriculture, military, and hunting amongst others) in the Amazon [10, 15, 25]. The vectors, who tend to fly low to the ground, might bite children at younger ages during peri-domestic transmission in the Pacific region with a higher risk of bites on the head [56, 57].

A longer health-seeking delay for Amazon CL patients is a new finding though not unexpected as the geographical distances and physical barriers to travel to health centers in the Amazon are higher compared to the Pacific region. In the Amazon, the road network starts in the highlands and descends into the rainforest lowlands until 700 to 250m above sea level in the provinces that included patients for this study. This results in limited access to health care in the lowlands and might be an explanation for prolonged health-seeking delay [17]. Older age, Amerindian ethnicity, infection at lower altitudes, and lesions more often on the lower limbs (that are easier to cover than lesions on the head) are significantly associated with the Amazon region and may all contribute to the prolonged health-seeking delay in the Amazon. These findings might be triggered by the stigma expressions towards Amazon CL patients that were found by our team during qualitative interviews and observations that are being published separately [58, 59] as well as determinants not included in this study such as occupation, educational level, income, marital status, time to the nearest health center, visit to a traditional healer, and possible doctors delay [60]. We recommend a future study on CL health-seeking delay in the Amazon that includes those additional factors. Non-ulcerative lesions are not associated with the Amazon region and the association with prolonged health-seeking delay might be explained by a decreased recognition by the patient population as well as by health professionals which should be clarified in a future study.

Former studies have shown that parasite load is inversely related to the duration of the lesion [61-63]. Lesion parasite paucity lowers the possibility of finding amastigotes in smear slides (currently the gold standard in Ecuador). Therefore, the longer health-seeking delay in the Amazon might influence CL test accuracy and we recommend the evaluation of smear slide microscopy in the Amazon. In addition to the lesion type, number, and body location of skin lesions, this study might have included the diameter, the aspect (wet or dry), and smell which could have differed per region and or impacted health-seeking delay.

As this study included patients without restrictions from both private and public health centers from main Ecuadorian CL clusters providing free CL treatment, we consider that the results may be generalizable for the CL patient population of included areas and in a lower degree for the entire country.

Conclusion

Our study on 245 confirmed Ecuadorian CL patients, including the causative species determination in 135 samples, shows a sustained low prevalence of *L. braziliensis* in the Pacific region. Additionally, it discovers the presence of *L. guyanensis* in the Napo, Pastaza, and Morona Santiago provinces, *L. braziliensis* in the Imbabura province, and *L. lainsoni* in the Pichincha, Napo, Pastaza, and Morona Santiago provinces. The longer health-seeking delay and a genetically different *L. guyanensis* subclade in the Amazon compared to the Pacific region reveal the need for region-specific analysis of CL test accuracy. Prolonged health-seeking delay was associated with older age, Amerindian ethnicity, infection at lower altitudes, non-ulcerative lesions, and lesions on the lower limbs which possibly result from limited access to health care and stigma. We recommend routine species determination in Amazon CL cases, regional studies of determinants of health-seeking delay in Ecuador, and regional studies of patient characteristics in neighboring countries Peru and Colombia.

List of abbreviations

CL: Cutaneous Leishmaniasis

Cyt B: Cytochrome B

hTNF: human Tumor Necrosis Factor

ML: Mucosal lesions

MPI: Mannose Phosphate Isomerase

qPCR: real-time PCR

rDNA: ribosomal DNA

Declarations

Ethics approval and consent to participate

Before the initiation and patient recruitment, the project was approved by the ethical committee of the `Universidad Internacional del Ecuador', registration number: UIDE-FCM-EDM-COM-18-0069, and the Ecuadorian Ministry of Health, registration number: MSPCURI000284-3. All patients or their legal representatives gave written informed consent in Spanish before inclusion. All the methods were carried out in agreement with the guidelines of the Ministry of Health of Ecuador and in accordance with the declaration of Helsinki.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The cytochrome B sequences were submitted to GenBank with accession numbers: OQ608467-OQ608601. The MPI sequences were submitted to GenBank with accession numbers: OQ608603-OQ608620 [32].

Competing interest

The authors declare that they have no competing interests.

Funding

Foundation Latin Link Nederland provided funding for the current study. The funding body had no role in the study design, collection, analysis, and interpretation of data, nor in writing the manuscript.

Authors contributions

JB contributed to the conception and design of the work, data acquisition, analysis, and interpretation, and drafted the work.

BF contributed to data acquisition, analysis, interpretation, and substantial revision.

HS contributed to the conception and design of the work, data analysis, interpretation, and substantial revision.

HdV contributed to the conception and design of the work, data analysis, interpretation, and substantial revision.

MC contributed to the conception and design of the work, data acquisition, data analysis, interpretation, and substantial revision.

All authors read and approved the submitted version. All authors agreed both to be personally accountable for their contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved and the resolution documented.

Acknowledgments

We thank all the study staff and patients for their contributions and participation.

References

1. de Vries HJ, Schallig HD. Cutaneous Leishmaniasis: A 2022 Updated Narrative Review into Diagnosis and Management Developments. American Journal of Clinical Dermatology. 2022:1-18.

2. Saeedi M. Leishmaniasis Geneva: WHO; 2022. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis. Accessed: 25 January 2022.
3. Hotez PJ, Pecoul B, Rijal S, Boehme C, Aksoy S, Malecela M, et al. Eliminating the neglected tropical diseases: translational science and new technologies. PLoS neglected tropical diseases. 2016;10(3):e0003895.
4. Hotez PJ, Aksoy S, Brindley PJ, Kamhawi S. What constitutes a neglected tropical disease? : Public Library of Science San Francisco, CA USA; 2020. p. e0008001.
5. Organización Panamericana de la Salud. Leishmaniasis 2022 https://www.paho.org/es/temas/leishmaniasis. Accessed: 22 December 2022.
6. Alemayehu B, Alemayehu M. Leishmaniasis: a review on parasite, vector and reservoir host. Health Science Journal. 2017;11(4):1.
7. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PloS one. 2012;7(5):e35671.
8. Henríquez-Trujillo AR, Coral-Almeida M, Hinojosa MC. The burden of cutaneous and mucocutaneous leishmaniasis in Ecuador (2014-2018), a national registry-based study. bioRxiv. 2019:751446.
9. Urutseg. Ecuador physical map 2014 https://en.m.wikipedia.org/wiki/File:Ecuador_relief_location_map.svg. Accessed: 20 January 2023.
10. Hashiguchi Y, Velez LN, Villegas NV, Mimori T, Gomez EAL, Kato H. Leishmaniases in Ecuador: Comprehensive review and current status. Acta tropica. 2017;166:299-315.
11. Encalada E. Población, Superficie (KM2), Densidad Poblacional a nivel Parroquial Quito: INEC; 2010 https://www.ecuadorencifras.gob.ec/wp-content/plugins/download-monitor/download.php?id=311&force=1. Accessed: 08 June 2022
12. INEC. Proyección de la Poblacion Ecuatoriana, por Años Calendario, Según Regiones, Provincias y Sexo Quito: INEC; 2010 https://www.ecuadorencifras.gob.ec//wp-content/descargas/Boletines/Proyecciones_poblacionales_cantonales/PROYECCION+PROVINCIAS,+SEXOS+Y+AREAS+2010+-+2020.xlsx. Accessed: 08 June 2022
13. INEC. Proyección de la Poblacion Ecuatoriana, por Años Calendario, Según Cantones Quito: INEC; 2010 https://www.ecuadorencifras.gob.ec/documentos/web-inec/Poblacion_y_Demografia/Proyecciones_Poblacionales/proyeccion_cantonal_total_2010-2020.xlsx. Accessed: 08 June 2022
14. Kato H, Gomez EA, Martini-Robles L, Muzzio J, Velez L, Calvopiña M, et al. Geographic Distribution of Leishmania Species in Ecuador Based on the Cytochrome B Gene Sequence Analysis. PLoS neglected tropical diseases. 2016;10(7):e0004844.
15. Calvopina M, Armijos RX, Hashiguchi Y. Epidemiology of leishmaniasis in Ecuador: current status of knowledge -- a review. Memorias do Instituto Oswaldo Cruz. 2004;99(7):663-72.
16. Lopez G. Población que se Autoidentificó Indigena, Segú Provincia de Empadronamiento, Nacionalidad o Pueblo Indigena al que Pertenece y Sexo Quito: INEC; 2010 https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwi6per4kJv2AhVCNewKHUBlBewQFnoECBMQAQ&url=https%3A%2F%2Fwww.ecuadorencifras.gob.ec%2Fwp-content%2Fplugins%2Fdownload-monitor%2Fdownload.php%3Fid%3D339%26force%3D0&usg=AOvVaw1sZMlqB_kh_8O316Yy4T8I. Accessed: 25 February 2022.
17. Benabent Fernández de Córdoba M. El transporte público terrestre y la accesibilidad, instrumentos para el análisis funcional del sistema de asentamientos: el caso de Ecuador. Estoa Revista de la Facultad de Arquitectura y Urbanismo de la Universidad de Cuenca. 2017;6(11):99-122.
18. Villacis B, Carrillo D. País atrevido: la nueva cara sociodemográfica del Ecuador. Quito: Instituto Nacional de Estadística y Censos (INEC); 2012. Available from: https://www.ecuadorencifras.gob.ec/wp-content/descargas/Libros/Economia/Nuevacarademograficadeecuador.pdf.
19. Rueda Miranda TM. Etnia y discriminación laboral; caso indígena en el Ecuador período 2010-2018: Riobamba, Universidad Nacional de Chimborazo; 2021.

20. Siguencia Ibadango SK. Discriminación salarial por etnia en el período 2010-2017 en el Ecuador: PUCE-Quito; 2018.
21. CONFENIAE. Nacionalidades Puyo: CONFENIAE; 2022 https://confeniae.net/nacionalidades. Accessed: 24 February 2022.
22. Romero GA, de Farias Guerra MV, Paes MG, Macêdo VdO. Comparison of cutaneous leishmaniasis due to Leishmania (Viannia) braziliensis and L.(V.) guyanensis in Brazil: clinical findings and diagnostic approach. Clinical Infectious Diseases. 2001;32(9):1304-12.
23. Aronson NE, Joya CA. Cutaneous Leishmaniasis: Updates in Diagnosis and Management. Infectious disease clinics of North America. 2019;33(1):101-17.
24. Espin CJT, Procel MC. Leishmaniasis en el Ecuador: revisión bibliográfica. Mediciencias UTA. 2021;5(3):2-11.
25. Conteron E. Prevalencia de leishmaniasis en el area II de Pastaza, asociados a factores de riesgo que influyen en el desarrollo de la enfermedad. Ambato: Universidad Tecnica de Ambato; 2015.
26. Ministerio De Salud Pública. Manual de procedimientos del Subsistema alerta acción SIVE – ALERTA. Second ed. Quito: MSP; 2013.
27. Bastidas CA, Villacrés-Granda I, Navarrete D, Monsalve M, Coral-Almeida M, Cifuentes SG. Antibiotic susceptibility profile and prevalence of mecA and lukS-PV/lukF-PV genes in Staphylococcus aureus isolated from nasal and pharyngeal sources of medical students in Ecuador. Infection and Drug Resistance. 2019;12:2553.
28. Bezemer JM, Merckx J, Freire Paspuel BP, Calvopiña M, de Vries HJC, Schallig HDFH, et al. Diagnostic accuracy of qPCR and microscopy for Cutaneous Leishmaniasis in rural Ecuador: A Bayesian latent class analysis. 2023.
29. Kato H, Cáceres AG, Hashiguchi Y. First evidence of a hybrid of Leishmania (Viannia) braziliensis/L.(V.) peruviana DNA detected from the phlebotomine sand fly Lutzomyia tejadai in Peru. PLoS neglected tropical diseases. 2016;10(1):e0004336.
30. Tsukayama P, Lucas C, Bacon DJ. Typing of four genetic loci discriminates among closely related species of New World Leishmania. International journal for parasitology. 2009;39(3):355-62.
31. National Library of Medicine. Basic Local Alignment Search Tool. 2022 https://blast.ncbi.nlm.nih.gov/Blast.cgi. Accessed: 02 December 2022.
32. National Library of Medicine. GenBank. 2023 https://www.ncbi.nlm.nih.gov/genbank/. Accessed: 10 March 2023.
33. Carrera F, Alejandro D. Identificación y diferenciación molecular por la técnica de la Reacción de la Cadena Polimerasa (PCR) múltiple de subgéneros de Leishmania (Viannia y Leishmania) en el Ecuador. Quito: Universidad de las Américas; 2019.
34. CODENPE. Modulo 3: Interculturalidad. Quito: CODENPE; 2011.
35. GOOGLE. Google Maps. 2022 https://www.google.com/maps. Accessed: 02 December 2022.
36. DGAC. Internet Flight Information Service. 2022 http://www.ais.aviacioncivil.gob.ec/. Accessed: 02 December 2022.
37. topographic-map.com. Mapa topográfico Ecuador. 2022 https://es-ec.topographic-map.com/. Accessed: 02 December 2022.
38. CASTOR. CASTOREDC. 2022 https://www.castoredc.com/. Accessed: 02 December 2022.
39. IBM. SPSS Statistics for Windows. Version 28.0. Armonk. New York: IBM; 2021.
40. Mackenzie CS, Heath PJ, Vogel DL, Chekay R. Age differences in public stigma, self-stigma, and attitudes toward seeking help: A moderated mediation model. Journal of Clinical Psychology. 2019;75(12):2259-72.
41. Paz-Soldan VA, Alban RE, Dimos Jones C, Powell AR, Oberhelman RA. Patient reported delays in seeking treatment for tuberculosis among adult and pediatric TB patients and TB patients co-infected with HIV in Lima, Peru: a qualitative study. Front Public Health. 2014;2:281.
42. Alcalde-Rubio L, Hernández-Aguado I, Parker LA, Bueno-Vergara E, Chilet-Rosell E. Gender disparities in clinical practice: are there any solutions? Scoping review of interventions to overcome or reduce gender bias in clinical practice. International journal for equity in health. 2020;19(1):1-8.

43. Bautista-Valarezo E, Duque V, Verhoeven V, Mejia Chicaiza J, Hendrickx K, Maldonado-Rengel R, et al. Perceptions of Ecuadorian indigenous healers on their relationship with the formal health care system: barriers and opportunities. BMC Complementary Medicine and Therapies. 2021;21(1):1-10.
44. Hu R, Ramdas S, Nieuwkerk P, Reis R, Lai AFRFM, de Vries HJC, et al. Body location of "New World" cutaneous leishmaniasis lesions and its impact on the quality of life of patients in Suriname. PLoS neglected tropical diseases. 2020;14(10):e0008759.
45. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Annals of internal medicine. 2007;147(8):573-7.
46. Llanos-Cuentas A, Tulliano G, Araujo-Castillo R, Miranda-Verastegui C, Santamaria-Castrellon G, Ramirez L, et al. Clinical and parasite species risk factors for pentavalent antimonial treatment failure in cutaneous leishmaniasis in Peru. Clinical infectious diseases. 2008;46(2):223-31.
47. Machado-Coelho GL, Caiaffa WT, Genaro O, Magalhaes PA, Mayrink W. Risk factors for mucosal manifestation of American cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2005;99(1):55-61.
48. Solomon M, Sahar N, Pavlotzky F, Barzilai A, Jaffe CL, Nasereddin A, et al. Mucosal Leishmaniasis in Travelers with Leishmania braziliensis Complex Returning to Israel. Emerging infectious diseases. 2019;25(4):642-8.
49. Pan American Health Organization. Guideline for the treatment of leishmaniasis in the Americas. Second ed. Washington, DC: PAHO; 2022.
50. Ministerio de Salud Publica. Subsistema de Vigilancia SIVE-ALERTA Enfermedades Transmitidas por Vectores Ecuador 2021. Quito: Ministerio de Salud Publica; 2022.
51. Ronquillo TEF, Almeida F RT. Reporte de lesiones mucosas en leishmaniosis tegumentaria americana, en el litoral (Costa) Ecuatoriano. Rev patol trop. 2012;41(3):356-66.
52. Calvopina M, Armijos RX, Marco JD, Uezato H, Kato H, Gomez EA, et al. Leishmania isoenzyme polymorphisms in Ecuador: relationships with geographic distribution and clinical presentation. BMC infectious diseases. 2006;6:139.
53. Kato H, Cáceres AG, Gomez EA, Tabbabi A, Mizushima D, Yamamoto DS, et al. Prevalence of Genetically Complex Leishmania Strains With Hybrid and Mito-Nuclear Discordance. Frontiers in cellular and infection microbiology. 2021;11:625001.
54. Armijos R, Weigel M, Izurieta R, Racines J, Zurita C, Herrera W, et al. The epidemiology of cutaneous leishmaniasis in subtropical Ecuador. Tropical Medicine & International Health. 1997;2(2):140-52.
55. Amunárriz M. Estudios sobre patologías tropicales en la Amazonia ecuatoriana: Cicame; 1991.
56. Faiman R, Kirstein O, Moncaz A, Guetta H, Warburg A. Studies on the flight patterns of foraging sand flies. Acta tropica. 2011;120(1-2):110-4.
57. Gaglio G, Brianti E, Napoli E, Falsone L, Dantas-Torres F, Tarallo VD, et al. Effect of night time-intervals, height of traps and lunar phases on sand fly collection in a highly endemic area for canine leishmaniasis. Acta tropica. 2014;133:73-7.
58. Vargas V, Bezemer J, Calvopina M, Schallig H, Ortega F, Salazar N, et al. Perceptions of American tegumentary leishmaniasis in Ecuador: questioning the visuality of aesthetic stigma. 2022.
59. Bezemer JM, Calvopiña Hinojosa M, Corral Zabala AE, Ortega Pérez F, Vargas Román VC, Schallig HDFH, et al. Quality of life of cutaneous leishmaniasis suspected patients in the Ecuadorian Pacific and Amazon regions: a cross sectional study. BMC infectious diseases. 2022;22(1):1-9.
60. Henry M, GalAn N, Teasdale K, Prado R, Amar H, Rays MS, et al. Factors contributing to the delay in diagnosis and continued transmission of leprosy in Brazil–an explorative, quantitative, questionnaire based study. PLoS neglected tropical diseases. 2016;10(3):e0004542.
61. Jara M, Adaui V, Valencia BM, Martinez D, Alba M, Castrillon C, et al. Real-time PCR assay for detection and quantification of Leishmania (Viannia) organisms in skin and mucosal lesions: exploratory study of parasite load and clinical parameters. Journal of clinical microbiology. 2013;51(6):1826-33.

62. Pereira LOR, Moreira RB, de Oliveira MP, Reis SO, de Oliveira Neto MP, Pirmez C. Is Leishmania (Viannia) braziliensis parasite load associated with disease pathogenesis? International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2017;57:132-7.
63. Chico ME, Guderian RH, Cooper PJ, Armijos R, Grogl M. Evaluation of a direct immunofluorescent antibody (DIFMA) test using Leishmania genus-specific monoclonal antibody in the routine diagnosis of cutaneous leishmaniasis. Revista da Sociedade Brasileira de Medicina Tropical. 1995;28:99-103.

Additional files

Additional file 1. Excel spreadsheet.xlsx. Patient data. Individual patient variables and laboratory results.

This file can be accessed with the following link:
https://osf.io/5c6qs/?view_only=83a155666fa2407f88cc00a0253356eb

Chapter 3

Diagnostic accuracy of qPCR and microscopy for cutaneous leishmaniasis in rural Ecuador: A Bayesian latent class analysis

Jacob M Bezemer[¶], Joanna Merckx[¶], Byron P Freire Paspuel, Manuel Calvopiña, Henry J C de Vries, Henk D F H Schallig, Mariska M G Leeflang, Nandini Dendukuri

[¶]These authors contributed equally to this work.

Submitted to: **PLoS Neglected Tropical Diseases**

Abstract

Background

Clinical and laboratory diagnosis of cutaneous leishmaniasis (CL) is hampered by under-ascertainment of direct microscopy.

Methods

This study compared the diagnostic accuracy of qPCR on DNA extracted from filter paper to the accuracy of direct smear slide microscopy in patients presenting with a cutaneous lesion suspected of leishmaniasis to 16 rural healthcare centers in the Ecuadorian Amazon and Pacific regions, from January 2019 to June 2021. We used Bayesian latent class analysis to estimate test sensitivity, specificity, likelihood ratios (LR), and predictive values (PV) with their 95% credible intervals (95%CrI) and assessed the diagnostic yield of using either or both tests. The impact of sociodemographic and clinical characteristics on predictive value was assessed as a secondary objective.

Results

Of 320 participants, paired test results were available for 129 from the Amazon and 185 from the Pacific region. We estimated sensitivity of 68% (95%CrI 49% to 82%) and 73% (95%CrI 73% to 83%) for qPCR, and 51% (95%CrI 36% to 66%) and 76% (95%CrI 65% to 86%) for microscopy in the Amazon and Pacific region, respectively. Health-seeking delay did not explain the regional difference in microscopy sensitivity. In the Amazon, with an estimated disease prevalence among participants of 73%, negative PV for qPCR was 54% (95%CrI 5% to 77%) and 44% (95%CrI 4% to 65%) for microscopy. In the Pacific, (prevalence 88%) the negative PV was 34% (95%CrI 3% to 58%) and 37% (95%CrI 3% to 63%). The addition of qPCR parallel to microscopy in the Amazon increases the observed prevalence from 38% to 64% (+26 (95%CrI 19 to 34) percentage points).

Conclusion

The accuracy of either qPCR on DNA extracted from filter paper or microscopy for CL diagnosis as a stand-alone test is unsatisfactory and region-dependent. The incremental diagnostic yield due to qPCR is clinically relevant.

Author Summary

Cutaneous leishmaniasis is caused by the parasite *Leishmania* and is treated when a microscopy test confirms the presence of the parasite in a sample of the lesion. This test, however, is known to miss patients with cutaneous leishmaniasis. DNA diagnostic tests (like PCR) that detect the parasite's genetic material in the lesion, have been proposed to improve diagnosis. Filter paper preserves DNA at room temperature and allows samples to be transported from remote health centers to the PCR laboratory. The ability of microscopic and DNA testing to recognize leishmaniasis patients in real-life, rural situations in two Ecuadorian regions – the Amazon and the Pacific – is complex to evaluate. We compared the performance of both tests using a statistical method that can evaluate both tests simultaneously without assuming that either test works perfectly. We found that PCR will be positive around 70% of the time in a patient with leishmaniasis in both Ecuadorian regions. In the Amazon, microscopy detects one out of every two cases, while it does in three out of every four cases in the Pacific. The addition of the PCR test can improve the number of patients with a diagnosis of leishmaniasis, mostly in the Amazon region.

Keywords

Cutaneous leishmaniasis, Latent class analysis, Diagnostic accuracy, Polymerase chain reaction, Microscopy, Ecuador

Introduction

Background

Protozoan parasites of the genus *Leishmania* are the causative agent of cutaneous, mucosal, and visceral human leishmaniasis. The mainstay method for leishmaniasis confirmation is the combination of clinical characteristics and microscopy [1, 2]. Worldwide, cutaneous leishmaniasis (CL) is the most common clinical manifestation of leishmaniasis affecting 600.000 to 1 million patients annually [3]. Ecuadorian CL manifests mainly as localized skin ulcers and nodules and has an estimated prevalence of 3905-6415 cases or 30-49 per 100.000 inhabitants per year. It leads to an estimated health loss of 0.32 Disability Adjusted Live Years (DALY) per 100.000 people per year in the country and affects poor and indigenous populations disproportionally, probably as a result, among others, of CL-related stigmatization [4, 5].

According to the Ecuadorian Ministry of Health (MoH), direct microscopy observation of the parasite (smear or biopsy) is the "gold standard" to diagnose CL in Ecuador. Culture, immunological and molecular tests are not available at public health centres as in most endemic regions [6]. The diagnostic accuracy of smear slide microscopy provided in Ecuador has however not been systematically evaluated. The existing small, limited number of peer-reviewed studies (of 14 to 90 patients) show low sensitivities ranging from 14% to 51% and specificities of near 100%, however based on a comparison to composite reference standards [7-11] which might give a biased result. Patients are nevertheless provided anti-leishmanial treatment for free by the MoH, conditional on having received a positive microscopy diagnosis [12]. Given the aforementioned estimated low sensitivity of the diagnostic test, this might leave several thousands of patients without treatment every year.

Molecular methods are promising for CL diagnosis because of their reported high diagnostic accuracy compared to microscopy [13]. Centralization of molecular tests would save costs, but transportation generally requires a cold chain that is not available. Hashiguchi *et al*. proposed filter paper imprints of CL lesions as a solution for the transport challenges [12]. Filter paper allows prolonged sample DNA conservation without the need for a cold chain [14]. An alternative approach to improve the diagnostic yield is the use of epidemiological, demographic, and clinical characteristics in the diagnostic process [6, 15]. Weigle *et al*. reported high sensitivity of the use of a clinical diagnostic algorithm for Colombian CL, but the estimated specificity was low. A corresponding algorithm, however, has to be adapted and validated regionally before its implementation [16]. Of special clinical interest is the predictive value of the tests in use to evaluate the likelihood the patient has or does not have the disease after including the test result [17].

The lack of a reliable reference standard test or thus the absence of a true "gold standard" for CL diagnosis makes it challenging to assess the accuracy of any new diagnostic method and to investigate the true accuracy of the tests in use [18]. Two commonly proposed solutions to this problem are the use of 1) a composite reference standard, and 2) Bayesian Latent Class Analysis (LCA). Diagnostic test accuracy estimates based on a composite reference standard may be biased because combining multiple imperfect tests does not make one perfect reference standard [19], nor can the accuracy of the tests in the composite be evaluated. LCA, in comparison, applies a statistical model that simultaneously analyzes the results of the different tests observed while taking the imperfect nature of each test into account. Provided the assumptions of the model are correct, LCA allows unbiased estimation of the test accuracies, without depending on a perfect reference test [20, 21].

In this prospective cross-sectional study, we used Bayesian LCA to estimate the diagnostic accuracy

(sensitivity and specificity) of qPCR on DNA extracted from filter paper and microscopy for the diagnosis of CL in Ecuador. As a secondary objective, we assessed the predictive values of a specific set of demographic and clinical criteria.

Methods

Ethical considerations

This prospective study was approved by the ethical committee of the `Universidad Internacional del Ecuador' (registration number: UIDE-FCM-EDM-COM-18-0069) and by the Ecuadorian MoH (registration number: MSPCURI000284-3). All participants signed a written consent and received free treatment for leishmaniasis according to the Ecuadorian MoH guidelines. The full study protocol can be accessed upon reasonable request to the corresponding author.

Participants, data source, and data collection

This is a cross-sectional study of patients suspected of CL. Any case with a suspected cutaneous lesion for whom a physician practicing at a participating health center ordered CL testing was eligible for inclusion. Participants were included at three public primary health care centers in the Pacific subtropical region of the Pichincha province and from public and private primary health care centers and hospitals in the Napo, Pastaza, and Morona Santiago provinces in the Amazon (Total N=16, See S1 Fig). Patients were identified and enrolled consecutively between the 1st of January 2019 and the 30th of June 2021 at the centers, and during community visits, by the physician, nurse, or laboratory technician, just before routine sampling for CL. The sample size was determined by convenience. All centers had the laboratory capacity to perform smear slide microscopy and offered free treatment for CL-confirmed patients (intramuscular meglumine antimoniate for 20 consecutive days) or treatment for alternative diagnoses. The demographics of participants and clinical characteristics of cutaneous lesions were recorded before sampling. Age was recorded in years, ethnicity according to the classifications described in the Ecuadorian law [22], gender as binary (male/female), health-seeking delay was defined as the time since lesion onset as mentioned by the patient, number of lesions was counted by the professional as the number of lesions separated by healthy skin, and body location was drawn on a person figure by the health professional. The geographical location of the patient was recorded to estimate the altitude of the place of infection in meters from the altitude of the airstrip of the nearest village (http://www.ais.aviacioncivil.gob.ec/) or with topographic-map.com (https://es-ec.topographic-map.com/maps/6ogw/Ecuador/) and was dichotomized as well in the Amazon and the Pacific region. All the study data were collected on paper forms and entered into an electronic data capture system (https://www.castoredc.com/). Data entry was done in duplicate by two independent investigators and computer validated. We used the STARD-BLCM guidelines for the reporting of the study [23].

Sample collection and diagnostic tests

The sample for microscopy was collected by scraping the inner border of the cutaneous lesion. The resulting material was collocated on a glass slide and Giemsa stained following the Ecuadorian MoH guidelines [24]. Smear slide microscopy was performed by WHO-trained and experienced microscopists at the local center and sequentially at the central facility for all primary positive smears for diagnosis confirmation. The final results are reported as positive or negative (binary) because Ecuadorian laboratory technicians are not trained in grading parasite density [24]. A

positive microscopy test meant it had been read positive on two occasions (on-site and at the central laboratory). Technicians were aware of the clinical characteristics of the patients but unaware of the results of qPCR testing. To collect the sample for qPCR, the local laboratory technician pressed a filter paper (903 Protein Saver Card (Whatman, Newton Center, MA)) three times, for at least one second each, on the inner border of the suspected lesion, immediately after scraping for the smear slide. Filter papers were dried and sent under uncontrolled conditions to the research laboratory of the `Universidad de las Americas' in Quito by canoe, plane, bus, private car, and/or postal service. They were stored at room temperature in the including centers, during transport, and in the research laboratory in Quito. DNA was extracted from the filter paper according to a Chelex and Proteinase K based protocol [25] that is non-time consuming and cheap and therefore preferable for resource-restricted settings [26, 27]. A piece of 2*2mm with visible material was separated from the filter paper and placed in a sterile 1.5mL tube containing 200µL of 10% (wt/vol) Chelex 100 (Sigma-Aldrich, USA) and 10µL (≥4 units) of Proteinase K (Invitrogen, USA). Samples were vortexed for 5 min, incubated at 56°C for 60 min, and then at 96°C for 20 min. Finally, samples were centrifuged at 10,000 g (earth's gravitational force) for 5 min, and the supernatant containing extracted DNA (approximately 150µL*)* was removed to another sterile tube. Extracted DNA was quantified with the NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA). *Leishmania* DNA was detected by duplex real-time PCR (qPCR) of the *Leishmania* 18S ribosomal (rDNA) gene, which was validated for South American *Leishmania* species [28] and the human Tumor Necrosis Factor (hTNF) gene as internal control [29]. *Leishmania* rDNA and hTNF probes were labeled with FAM and HEX as reporters, respectively. The qPCR reaction was prepared with 2µL of extracted DNA and 13µL of master mix containing: 1X TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 500nM of each primer, 250nM of each probe, and nuclease-free water to complete a final volume of 15µL. The reaction was run in a CFX96 Dx Thermal Cycler (Bio-Rad, USA) following this protocol: 95°C for 10 min; 40 cycles of 95°C for 15 sec and 58°C for 60 sec (detection). The last step was optimized with an alignment/extension temperature gradient maintaining the temperature with the highest fluorescence and lowest Ct values. Other conditions were according to the manufacturer's recommendations. The detection limit of the qPCR reaction was determined directly on a 10-fold dilution series of a 441bp synthetic DNA fragment (IDT, USA) containing the *Leishmania* rDNA and hTNF target sequences and did not include DNA extraction. The DNA extraction and qPCR were repeated once when the hTNF Ct value was >32 or negative. Results were considered invalid if the hTNF probe did not amplify. Detection of amplified *Leishmania* rDNA resulted in defining the sample positive, except for Ct values >40, which were classified as negative. The technicians that performed the qPCR in Quito were unaware of the clinical characteristics of patients and the microscopy results. Species determination was done by sequencing cytochrome B and MPI (to differentiate *L. braziliensis* from *L. peruviana* gene fragment) as described elsewhere [30, 31].

Data analysis

Descriptive statistics (proportions, means, and medians, as appropriate) were used to describe the study population. We drew a directed acyclic graph (DAG) to illustrate the relation between the diagnostic tests that were used (i.e., smear slide microscopy and qPCR), the quantity of the pathogen itself, i.e. the amastigotes, or their genetic material (DNA) in the samples, and the target condition, being CL (S2 Fig). Based on this, we reasoned that the available data could be used to construct a two-class latent model with the two classes being "presence of CL" and "absence of CL". We used Bayesian latent class models, adjusted for conditional dependence, to estimate the sensitivity and specificity of qPCR and microscopy for the diagnosis of CL. We also estimated the

likelihood ratios, the prevalence of CL in the study population correcting for their diagnostic accuracy and comparing to the observed prevalence, and the predictive values (negative and positive) with their 95% credible intervals (95%CrI). We used an informative prior for the specificity of microscopy in (Beta (99,1) distribution with median of 99% (95%CrI 96%, 100%)) based on the known high specificity of the microscopy results. We also used an informative prior for qPCR specificity (Beta (97,3) with median of 97% (95%CrI 93%, 99%)) based on available evidence [13]. We present the sensitivity and specificity for both tests separately by region, the Amazon, and the Pacific (Model 1), after noticing the significant difference in sensitivity values between the two regions, not reflected in the pooled estimates (S1 Table). Sequentially, and as part of our secondary aim, we assessed the impact on diagnostic accuracy of pre-defined covariates or thus within prespecified subgroups, i.e., defined by the socio-demographic and clinical variables. More specifically we investigated if the covariate health-seeking delay (cut-off 4 weeks) was an effect measure modifier versus confounder for the accuracy between the regions. We, therefore, allowed the model to estimate the sensitivity by covariate level (Model 2), while allowing for conditional dependence among CL positive subjects, in the subpopulations and the pooled sample test specificities. We further assessed the impact on diagnostic performance within prespecified subgroups, i.e., defined by age (cut-off 20 years of age), gender, altitude of infection (cut-off 500m), body location of lesion (head and neck versus other location), assessing the predictive values in the subgroups. It was hypothesized that sensitivity for a single test would be clinically insufficient (threshold set at 80%) and that the NPV, which is dependent on the prevalence, would differ by 20 percentage points between the two regions. For all statistics, medians and 95% CrIs were reported. Statistical significance was determined by a CrI of differences not including 0. To conclude and as a comparison, the accuracy of qPCR (and its 95% confidence interval (CI)) was estimated using microscopy as the reference standard from a two-by-two contingency table by region. We used R version 4.0 (R Foundation for Statistical Computing, Vienna, Austria, 2020), more specifically for carrying out Bayesian inference on the latent class model we used the rjags package.

Results

Participants

Participating centers enrolled 324 (50%) out of 646 eligible patients in the study (Fig 1). Four initially included patients were excluded because they had no cutaneous lesions. A total of 188 (59%) patients from the Pacific and 132 (41%) patients from the Amazon region were analyzed (Table 1). The mean age of the included patients was 26.8 years (range 0.1-88 years) and was higher in the Amazon (31.2 years) compared to the Pacific (23.7 years) region. A total of 100/188 (53%) patients included from the Pacific region were male compared to 85/132 (64%) from the Amazon. No Amerindian patients were included from the Pacific region compared to 87 (66%) patients from the Amazon. Median health-seeking delay in the Amazon was one month longer than in the Pacific (median 1 month). Two hundred ninety-three (91%) lesions were ulcerated. The median altitude of the presumed place of infection was 455m, which was similar in the Pacific and Amazon regions. CL suspected lesions were most frequently on the upper limbs (36%) and lower limbs (30%). Amazon patients had fewer lesions on the head and neck (13%) compared to Pacific patients (25%). None of the participants had initiated anti-leishmanial treatment prior to the sampling.

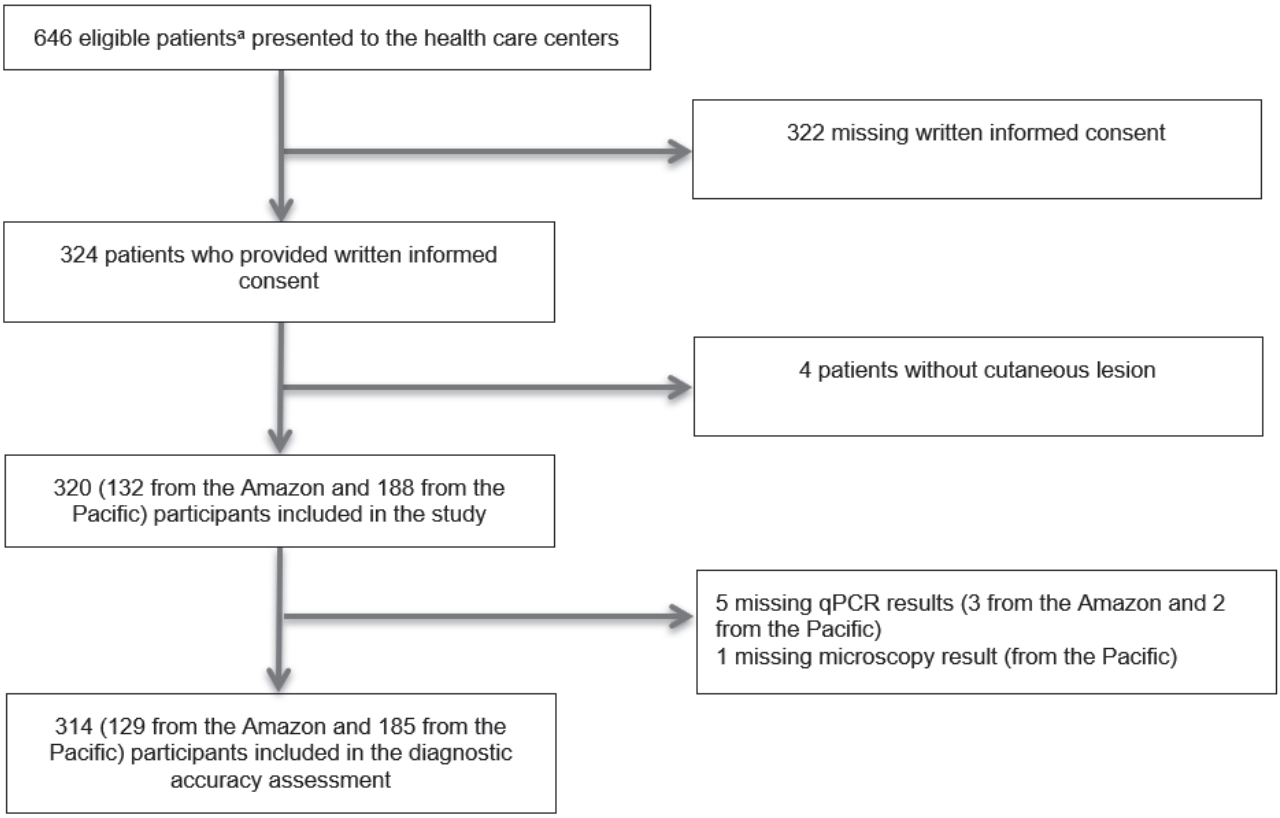


Fig 1. Flow chart of the study population

[a]An eligible participant was defined as an individual presenting to one of the 16 participating healthcare posts with a cutaneous lesion instructed to sample for the diagnosis of cutaneous leishmaniasis.

Table 1. Baseline characteristics of 320 suspected cutaneous leishmaniasis patients from the Ecuadorian Pacific and Amazon regions. Total number (%).

Patient characteristic (N missing for variables)	Pacific	Amazon	Total
Number of Cases (%)	188 (59)	132 (41)	320 (100)
General characteristics (1)			
Mean age in years (SD)	23.7 (18.8)	31.2 (20.1)	26.8 (19.7)
Male (%)	100 (53)	85 (64)	185 (58)
Mestizo (%)	188 (100)	45 (34)	233 (73)
Amerindian (%)	0 (0)	87 (66)	87 (27)
Clinical presentation (3)			
Median health-seeking delay in months (IQR)	1.0 (0.5-2.0)	2.0 (0.9-4.0)	1.0 (0.5-2.0)
Total number of lesion types presented (%)[a]	189 (59)	133 (41)	322 (100)
Ulcer (%)	170 (90)	123 (92)	293 (91)
Nodule (%)	9 (5)	10 (8)	19 (6)
Other (%)	10 (5)	0 (0)	10 (3)
Median number of lesions (IQR)	1 (1-2)	1 (1-1)	1 (1-2)
Median altitude of suspected place of infection in meters above sea level (IQR)	532 (219-779)	396 (278-647)	455 (274-739)
Body location of the lesion[b]			
Total number of body locations with lesions (%)	204 (59)	144 (41)	348 (100)
Head and neck (%)	51 (25)	18 (13)	69 (20)
Trunk (%)	22 (11)	25 (17)	47 (14)
Upper limbs (%)	77 (38)	49 (34)	126 (36)
Lower limbs (%)	54 (26)	52 (36)	106 (30)

N = Number, SD = Standard Deviation, IQR = Inter Quartile Range

[a]The denominator is lesion types, patients with several lesion types were counted more than once

[b]The denominator is body locations, patients with lesions on different body regions were counted more than once

Laboratory results and species identification

A total of 186/320 (58%) qPCR samples tested positive (49% in the Amazon and 64% in the Pacific), and 5 samples had an invalid hTNF internal test control, compared to 176/320 (55%) microscopy positive samples, with one invalid microscopy test. No adverse events took place during or after the sampling of the specimens. Ct values by region and microscopy result are presented in S2 Table. The paired test results of 314 patients are included in the further description and following diagnostic accuracy analysis. Discordant results, microscopy positive while qPCR negative, were present in 14% and 17% of the cases in the Amazon and the Pacific; microscopy negative while qPCR positive results were present in 26% and 15% in the same regions. The qPCR detected $1x10^{-9}$ ng/μL of synthetic DNA, which is equivalent to 4 copies of DNA in each 15uL reaction (S1 File). In 135/185 (73%) of the qPCR positive samples (71% Amazon, 74% Pacific) the causative *Leishmania* species could be determined, or in 46 (36%) and 89 (48%) of the total number of paired samples investigated. Table 2 provides information on the species distribution by region, the sample internal control Ct values, and Ct values by species. Microscopy positivity varied by species, with 84% of *L. guyanensis* positive and only 42% of *L. braziliensis* positive.

Table 2. Laboratory results in 135 cases with *Leishmania* species determination

	L. guyanensis (N=102)[a]	*L. braziliensis* (N=26)[a]	*L. lainsoni* (N=7)[a]	All samples (N=135)
Median hTNF Ct (IQR)[b]	29,4 (27,3-31,0)	27,1 (25,7-28,9)	30,6 (29,9)	29,0 (26,8-30,8)
Median *Leishmania* rDNA Ct (IQR)[b]	29,7 (27,7-32,5)	31,7 (29,8-35,2)	34,4 (27,7-35,3)	30,3 (27,8-33,3)
Pacific (%)	83 (81)	5 (19)	1 (14)	89 (66)
Microscopy positive (%)				
Total	86 (84)	11 (42)	6 (86)	103 (76)
Amazon	13 (68)	7 (33)	5 (83)	25 (54)
Pacific	73 (88)	4 (80)	1 (100)	78 (88)

hTNF: human Tumor Necrosis Factor, Ct: Cycle Threshold, IQR: Interquartile Range

[a] *Leishmania* species was determined in *Leishmania* 18SrDNA positive samples by sequencing a gene fragment that codes for the *Leishmania* Cytochrome B enzyme.

[b] hTNF was applied as internal control for sample taking and DNA extraction in a duplex qPCR together with *Leishmania* rDNA. Ct values have a logarithmic relationship with DNA concentrations and lower Cts indicate more sample DNA.

Diagnostic accuracy estimates of qPCR on DNA extracted from filter paper and microscopy

Applying our model (Model 1) to the population of the Amazon region, we found a sensitivity of qPCR of 68% (95%CrI 49;82) with a specificity of 97% (95%CrI 93;99), and a sensitivity of 51% (95%CrI 36;66) with a specificity of 99% (95%CrI 96;100) for microscopy. In the Pacific region, qPCR sensitivity and specificity were 73% (95%CrI 63;83) and 97% (95%CrI 93;99), respectively. Microscopy sensitivity was 76% (95%CrI 65;86) with a specificity of 99% (95%CrI 96;100) (Table 3). Microscopy sensitivity was statistically significantly lower in the Amazon compared to the Pacific (-24.9 percentage points (95%CrI -43.5; -6.7). Differences between qPCR and microscopy sensitivity were also largest in the Amazon (-16 percentage points (95%CrI -31;-2) and statistically significant. Estimating the diagnostic accuracy of qPCR and microscopy by health-seeking delay (Model 2) separately for the two regions, there is no evidence of an effect in the Amazon on qPCR or microscopy and a non-statistically significant effect on the microscopy in the Pacific region, with 8.1 percentage points (95%Cr -26.9;10.2) lower sensitivity in cases presenting more than 4 weeks after reported lesion appearance (Table 4). Using microscopy as the perfect reference standard, we estimated a sensitivity of 48% (95% confidence Interval (CI) 35;61) and specificity of 72% (95% CI 59;82) for qPCR in the Amazon and a sensitivity of 78% (95%CI 69;85) and specificity of 52% (95% CI 39;64) in the Pacific (Table 3).

Table 3: Model 1: Diagnostic accuracy estimates with their 95% credible interval for qPCR and microscopy using latent class analysis and two-by-two table calculation (95% confidence interval)

	Model 1[a] in subpopulation of the Amazon	Model 1[a] in subpopulation of the Pacific	two-by-two-table in subpopulation of the Amazon[b]	two-by-two-table in subpopulation of the Pacific[c]
Sensitivity qPCR	68.0% (49.1;82.4)	73.4% (62.7;82.7)	63.3% (48.3;76.6)	75.2% (66.7;82.5)
Sensitivity Microscopy	51.2% (35.9;65.5)	76.4% (65.0;85.6)		
Difference sensitivity[d]	*-16.0 (-31.4;-1.7)*	*2.9 (-6.5;12.2)*		
Specificity qPCR	97.2% (92.6;99.4)	97.2% (92.8;99.4)	57.5% (45.9;68.5)	55.0% (41.6;67.9)
Specificity Microscopy	99.3% (96.1;100)	99.3% (96.1;100)		
Difference specificity[d]	*1.8 (-1.7;6.6)*	*1.8 (-1.7;6.4)*		
LR+ qPCR	24 (9;107)	27 (10;113)	1.5 (1.1;2.2)	1.7 (1.2;2.3)
LR+ Microscopy	71 (13;1852)	105 (19;2400)		
LR- qPCR	0.3 (0.2;0.5)	0.3 (0.2;0.4)	0.6 (0.4;1)	0.5 (0.3;0.7)
LR- Microscopy	0.5 (0.3;0.6)	0.2 (0.1;0.4)		

LR+: Positive Likelihood Ratio: true-positive proportion/false-positive proportion; LR-: false-negative proportion/true-negative proportion. A test with a LR+ of >10 is considered useful to rule in a diagnosis when a test is positive, while a test with a LR- <0.1 is considered useful to exclude a diagnosis when a test is negative.

[a]Latent class analysis with two latent classes, using the data of one joint population and informative prior for specificity microscopy with a beta distribution (99,1) and informative prior for specificity qPCR with a beta distribution (97,3). The model allows for conditional dependency between the two tests' sensitivities and specificities.

[b]Two-by-two contingency table of samples from the Amazon region, using microscopy as the reference standard: true positives N=31; false positives N=34, false negatives N=18, and true negatives N=46.

[c]Two-by-two contingency table of samples from the Pacific region, using microscopy as the reference standard: true positives N=94; false positives N=27, false negatives N=31 and true negatives N=33.

[d]Difference between the estimate for qPCR and microscopy in percentage points

Table 4: Model 2: Diagnostic accuracy estimates by delay in presentation for qPCR and microscopy using latent class analysis and two-by-two table calculation

LR+: Positive Likelihood Ratio: true-positive proportion/false-positive proportion; LR-: false-negative proportion/true-negative proportion. A test with a LR+ of >10 is considered useful to rule in a diagnosis when a test is positive, while a test with a LR- <0.1 is considered useful to exclude a diagnosis when a test is negative.

[a]Latent class analysis with two latent classes, using the data of two subpopulations, i.e., the binary variable delay in presentation, allowing for differing sensitivities by stratum, and informative prior for specificity microscopy with a beta distribution (99,1) and informative prior for specificity qPCR with a beta distribution (97,3). The model allows for conditional dependency between the two tests sensitivities and between the subpopulation sensitivities, and two tests specificities.

	Model 2[a] in subpopulation of the Amazon, by delay in presentation		**Model 2[a] in subpopulation of the Pacific, by delay in presentation**	
	Delay (lesion present >4 weeks)	*No delay* (lesion present up to 4 weeks)	*Delay* (lesion present >4 weeks)	*No delay* (lesion present up to 4 weeks)
Sensitivity qPCR	66.7% (47.8;82.4)	69.5% (45.6;87.8)	74.2% (59;86.7)	72.7% (61.0;82.8)
Difference between delay and no delay	*-3.0 (-30.0; 26.7)*		*1.5 (-17.2; 19.0)*	
Sensitivity Microscopy	54.7% (38.2;71.2)	47.3% (29.1;66.9)	70.7% (55.5;84.2)	79.0% (66.8; 88.9)
Difference between delay and no delay	*7.2 (-18.5;32.0)*		*-8.1 (-26.9;10.2)*	
Difference sensitivity[b]	*-11.6 (-30.1; 7.5)*	*-21.2 (-45; 2.5)*	*-3.4 (-19.6;13.2)*	*6.2 (-5.2;17.6)*
Specificity qPCR	97.2% (92.7; 99.4)		97.2% (92.5;99.4)	
Specificity Microscopy	99.3% (96.1; 100)		99.3% (96.4;100)	
Difference specificity[b]	*1.9 (-1.9; 6.5)*		*1.9 (-1.6;6.7)*	
LR+ qPCR	24 (8;106)	24 (8;110)	26 (10;113)	26 (10;112)
LR+ Microscopy	73 (14; 2309)	63 (11; 1971)	97 (18; 2724)	110 (21; 3057)
LR- qPCR	0.3 (0.2; 0.5)	0.3 (0.1; 0.6)	0.3 (0.1; 0.4)	0.3 (0.2; 0.4)
LR- Microscopy	0.5 (0.3; 0.6)	0.5 (0.3; 0.7)	0.3 (0.2; 0.4)	0.2 (0.1; 0.3)

[b]Difference between the estimate for qPCR and microscopy in percentage points

Prevalence estimation, PPV, NPV, and effect of demographic and clinical characteristics on diagnostic performance

The prevalence of CL in the sampled cases was 73% (95%CrI 58;97) in the Amazon region and 88% (95%CrI 78;99) in the Pacific region using Model 1, correcting for test accuracy. In the Amazon, the addition of qPCR test positive cases to those identified by microscopy (observed prevalence microscopy positives: 38.0%) can increase the observed prevalence with 26.4 (95%CrI 19.3;34.4) percentage points. In the Pacific, the prevalence increases 14.6 (95%CrI 10.1;20.2) percentage points, also improving the diagnostic yield (Table 5). PPV and NPV are presented by region and by the different covariates age, altitude of infection, and lesion body location in with their estimates in the S3 Table, together with the estimates of covariate stratified sensitivities and specificities by region. NPV in the Amazon region was overall 54% (95%CrI 5;77) and 44% (95%CrI 4;65) for qPCR and microscopy respectively while being 34% (95%CrI 3;58) and 37 (95%CrI 3;63) in the Pacific region.

Table 5: Prevalence estimates in the sampled population correcting for the diagnostic accuracy of qPCR and microscopy (true prevalence); prevalence observed using qPCR and microscopy separately and the potential additional prevalence detected by adding qPCR positive cases to microscopy negative cases, by region.

	Amazon	Pacific
Estimated true prevalence (Model 1)[a]	72.6% (57.9; 97.3)	87.7% (78.1;99.1)
Observed prevalence of microscopy positive	38.0% (30.0;46.6)	67.6% (60.6;74.0)
Observed prevalence of qPCR positive	50.4% (41.8;58.9)	65.4% (58.4;72.0)
Observed prevalence of either microscopy or qPCR positive[b]	64.3% (55.8;72.2)	82.3% (76.2;87.2)
Additional prevalence (in percentage points) diagnosed by adding qPCR to microscopy prevalence (microscopy negatives, qPCR positives)	26.4 (19.3;34.4)	14.6 (10.1;20.2)

[a]The prevalence is estimated taking both test results and their imperfect accuracy into account and provides the estimated true prevalence in the study sample.
[b]The sum is lower than the true prevalence estimates, given this is a prevalence based on observed positives and not corrected for imperfect accuracy. In this scenario, both false positive microscopy cases and false positive qPCR cases contribute to the prevalence and cases with positive qPCR and microscopy agreement are not double counted.

Discussion

In this diagnostic accuracy study, using latent class analysis in patients presenting with suspected CL diagnosis in two regions in Ecuador, we found that the sensitivity of qPCR on DNA extracted from filter paper and more profoundly, of smear slide microscopy is considerably lower in the Amazon region than in the Pacific region. This difference could not be explained by differences in health-seeking delay. In addition, different patients were diagnosed with either test. The specificity point estimates were 97% for qPCR and 99% for microscopy, using informative priors. The NPV reached its highest value in the lower prevalence region of the Amazon (prevalence 73%) both for qPCR and microscopy with a point estimate of 54% and 44% respectively, still lower than needed to confidentially consider the negative test as the proof of absence of CL in the patient. Adding the qPCR test and including qPCR positive cases as confirmed cases of CL would however increase the detection of cases with 15 to 26 percentage points, depending on the region. Other socio-demographic or clinical characteristics did not provide evidence to change the posterior probability of disease, more particularly, disease absence.
This study has several limitations. First, recruitment and thus the sample size suffered from the study being performed during the COVID-19 pandemic. During the COVID-19 pandemic, treatment supply at national level was interrupted temporarily and therefore the care for suspected CL patients was postponed in an unknown percentage of the patients. Additionally, individuals might have been afraid or had competing interests leading to lower participation rates than anticipated. We do not assume that participation was differential to qPCR or microscopy results, however. Because of the heterogeneous effect by region, the stratified sample sizes are relatively small, resulting in wider CrI. Secondly, with only two tests performed, we cannot further refine our model or assess the underlying mechanisms of the disagreement between the microscopy tests and qPCR. Third, the study population assessed might not be transportable to other environments, most

specifically concerning their distribution of covariates. Additionally, the distribution of the covariates in the study population is conditional on the testing strategies that are in use in the different zones in Ecuador, which can lead to selection bias. Prevalence distributions can shift when the pre-test probabilities change over time or by center, alike the probability that an individual presenting with a lesion has an alternative diagnosis compared to CL. Fourth, the microscopic observations were made by different lab technicians leading possibly to a bias in the results, with most likely introduction of more false negatives, thereby decreasing the sensitivity of microscopy.
We found heterogeneity in the sensitivity of microscopy by region. Ecuador's mainland is divided from north to south by the Andean highlands where, at its heights, CL is rare. CL clusters occur in the northwestern (Pacific) and the entire eastern (Amazon) region [4]. The probable reservoir, vector, and infecting *Leishmania* species differ by these regions. *L. guyanensis* is the prevalent species in patients from the Pacific region and a mix of *L. guyanensis*, *L. braziliensis, L. lainsoni*, and *L. naiffi* species is prevalent in the Amazon [4, 12, 31]. Detailed surveillance data by region are however not available. Additionally, patient presentation (age, health-seeking delay, and body location of lesions) and quality of life of CL-suspected patients are region dependent [5, 12]. Patients from the Pacific region were included in the three cantons with Ecuador's highest burden of CL (212-464 cases per 10.000 inhabitants). The Amazon patients were included from cantons with lower CL burden (17-212 cases per 10.000 inhabitants) [4]. The differences in patient presentation (lower age, shorter health-seeking delay, and more lesions on the head and neck in the Pacific region) and estimated CL prevalence as found in this study are in agreement with former publications [32, 33]. However, this is the first publication to reflect on the diagnostic accuracy of both tests by region. The diagnostic program for leishmaniasis is Ecuador wide with uniform training procedures for microscopy technicians, as was the training of technicians involved in the filter paper sampling for this study [6]. Nevertheless, the lower prevalence of leishmaniasis in the Amazon may have resulted in fewer samples per technician, less experience, and, as a result, a lower sensitivity of smear slide microscopy [4]. Additional information about the lesions (diameter, wetness, infected or not) was not collected in this study, which could have helped to explain differences [34]. The causative *Leishmania* species, a prolonged period of health-seeking delay, and/or patient variables such as lesion location and age could all have influenced test performance [35]. This study's strength is that it relied on existing diagnostic structures and thus reflects actual clinical practice. This has the limitation of not allowing us to draw conclusions about the determinants of test accuracies which should be addressed in future research. Because CL is more prevalent in the Pacific than in the Amazon, professionals in the Pacific (more experienced) may have sent fewer non-CL patients for *Leishmania* testing, resulting in a higher true prevalence. The differences in NPV depend on both test accuracy and estimated true prevalence and they remained below 80% in both the Amazon and Pacific, making it impossible to exclude CL after receiving a negative test from a suspected case.
In this study, we aimed at answering a diagnostic question relevant to the diagnosis of CL where no good reference standard is available and where there remains a vacuum of functional diagnostic tools for its diagnosis. The use of the LCA allowed the estimation of the sensitivity and specificity for qPCR and microscopy in the same data set, therefore estimating the accuracy of the test in use and the potential alternative or add-on test. By using an LCA we did not assume that microscopy has perfect sensitivity or specificity. We used priors for the specificity of both tests. While single microscopy tests can suffer from artifacts being recognized as amastigotes, the results, as in the current practice, are only positive when the presence of amastigotes is confirmed by the central laboratory, through second reading.
When compared to several other studies assessing the accuracy of PCR on DNA extracted from

filter paper, our study found a lower sensitivity and lower positive agreement with microscopy [27, 36]. Our findings, on the other hand, are consistent with a study of >700 Palestinian CL suspects that found a limited sensitivity of PCR on DNA extracted from filter paper and disagreement with smear slide microscopy [37]. Such a disagreement may be caused by a number of issues: First, because amastigotes are distributed unevenly across skin layers, the sample *Leishmania* DNA concentration may be affected by the sampling technique [38]. In our study, the sample for microscopy was obtained by scraping and the sample for qPCR by imprinting the lesion on filter paper. In other studies, lesion imprints on filter paper followed by PCR have shown better accuracy in comparison to scrapings followed by microscopy, however, also failed to detect *Leishmania* DNA in 8-17% of the proven microscopy positive samples [39-41]. A direct comparison of lesion imprints and scrapings on filter paper could reveal whether the sampling technique resulted in false negatives. Second, the sampling site may cause heterogeneity, but in this study, both the filter paper imprints and scrapings were taken from the lesion's inner border [42]. Third, the handling and transport of the filter papers in remote tropical forest areas under uncontrolled conditions may still have affected the DNA quality. Fourth, when compared to other methods, the in our study used chelex-based DNA extraction can result in more DNA extracted [43]. This may help detect *Leishmania* DNA, but abundant non-*Leishmania* DNA and contamination may also interfere with amplification [36, 43]. Chelex has been applied in a limited number of studies to extract DNA from filter paper for the detection of CL, so an optimization study is recommended. Fifth, the *Leishmania* 18S qPCR used in this study has been validated in South American CL samples and is expected to detect the Ecuadorian endemic species [44]. Nonetheless, because the qPCR lacks a reverse transcriptase step, it does not amplify RNA, which may be more abundant in the samples than DNA. We recommend that in the future, the qPCR includes a reverse transcriptase step, as described by van der Meide *et al.* [28]. Finally, grading of the parasite density of *Leishmania* positive microscopy slides might have clarified an association between parasitemia and qPCR false negativity in our study and is recommended for future studies.

The use of socio-demographic and clinical characteristics and their integration in the diagnostic pathway has been suggested, given this information is easily available also in very remote regions, is known on presentation, and does not require costly funding. Assessing the sensitivity and specificity of these characteristics, however, does not inform us how to interpret a positive or negative result of a qPCR or microscopy test and make a more informed treatment decision. Estimating the predictive values and likelihood ratios does allow their use in clinical decision-making. We showed that the sociodemographic and clinical characteristics as assessed do not improve the posterior probability to exclude the diagnosis of CL. While, for example, exposure to the vector, i.e., in Ecuador the sandfly of the subfamily Phlebotominae genus *Lutzomyia,* has historically been defined by sex and/or gender, we did not see a difference in the prevalence of CL cases in males versus females who were tested for CL. As an additional remark, the covariates were dichotomized, which will result in information loss and might obscure real differences if we had chosen different cut-offs or had used the continuous variable.

Lessons learned from the results of this study for clinical practice and use of the diagnostic tests qPCR and microscopy are the following: First, a larger study is necessary to be able to decrease the uncertainty around the estimates. Second, data on the first microscopy and agreement with the confirmative reading will provide additional information. Our analysis can only be part of the general approaches to assess the true accuracy of tests used in the clinical diagnosis of CL. The addition and inclusion of direct PCR sampling and different sampling procedures can however optimize the specimen for PCR and its diagnostic yield, as is also the recommended diagnostic strategy as published in the IDSA guidelines [45]. Using microscopy solely for diagnosis maintains

the status quo of under ascertainment and thus under treatment of patients with CL.

Conclusions

The accuracy of the diagnostic tests evaluated for the diagnosis of CL is unsatisfactory. Smear slide microscopy suffers from being insufficiently sensitive and being region dependent while having good specificity. None of the investigated tests, qPCR nor microscopy alone, has a sufficient performance to confidently rule out CL in the presenting patients. This leads inevitably to increased morbidity and a sustained burden of DALY's related to CL, due to patients who remained without a diagnosis and therefore untreated. An additional diagnostic test, either microscopy or qPCR, seems necessary to improve the overall sensitivity of the diagnostic strategy.

Declarations

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

None declared

Financial Disclosure

Foundation Latin Link Nederland http://www.latinlink-nederland.nl/ provided funding for the current study.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding was received for this study.

Author Contributions

Jacob Bezemer: Conceptualization, Data Curation, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Visualization, Writing-Original Draft Preparation
Joanna Merckx: Conceptualization, Formal analysis, Methodology, Project Administration, Resources, Visualization, Writing-Original Draft Preparation
Byron Freire: Investigation, Resources, Validation, Writing-Review and Editing
Manuel Calvopina: Conceptualization, Investigation, Resources, Supervision, Writing-Review and Editing
Henry de Vries: Conceptualization, Resources, Supervision, Writing-Review and Editing
Henk Schallig: Conceptualization, Resources, Supervision, Writing-Review and Editing
Mariska Leeflang: Conceptualization, Resources, Supervision, Writing-Review and Editing
Nandini Dendukuri: Conceptualization, Resources, Supervision data analysis, Validation, Writing-Review and Editing

References

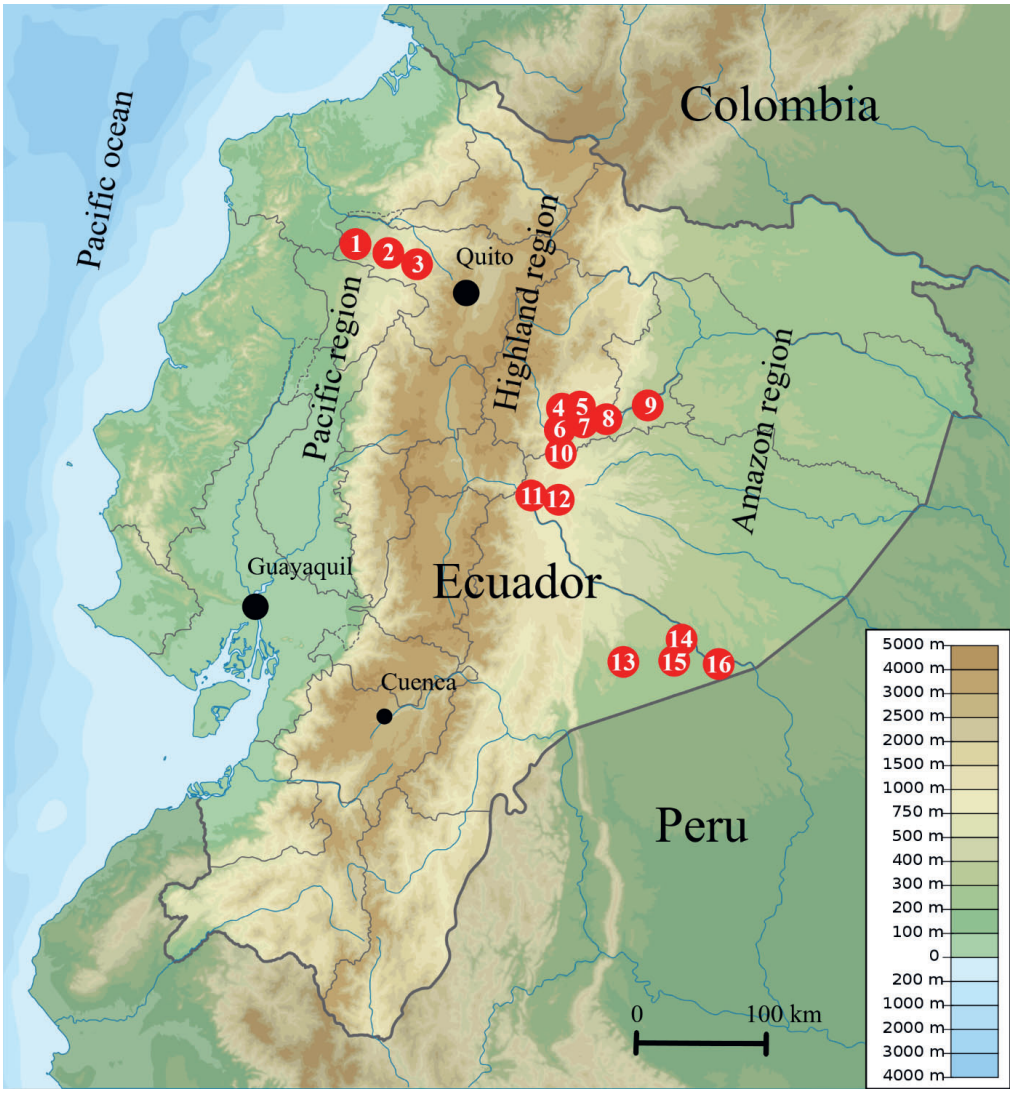
1. Kumari D, Perveen S, Sharma R, Singh K. Advancement in leishmaniasis diagnosis and therapeutics: An update. European Journal of Pharmacology. 2021;910:174436.
2. de Vries HJ, Schallig HD. Cutaneous Leishmaniasis: A 2022 Updated Narrative Review into Diagnosis and Management Developments. American Journal of Clinical Dermatology. 2022:1-18.

3. Saeedi M. Leishmaniasis Geneva: WHO; 2022. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis. Accessed: 25 January 2022.
4. Henríquez-Trujillo AR, Coral-Almeida M, Hinojosa MC. The burden of cutaneous and mucocutaneous leishmaniasis in Ecuador (2014-2018), a national registry-based study. bioRxiv. 2019:751446.
5. Bezemer JM, Calvopiña Hinojosa M, Corral Zabala AE, Ortega Pérez F, Vargas Román VC, Schallig HDFH, et al. Quality of life of cutaneous leishmaniasis suspected patients in the Ecuadorian Pacific and Amazon regions: a cross sectional study. BMC infectious diseases. 2022;22(1):1-9.
6. Ministerio De Salud Pública. Manual de procedimientos del Subsistema alerta acción SIVE – ALERTA. Second ed. Quito: MSP; 2013.
7. Aviles H, Belli A, Armijos R, Monroy FP, Harris E. PCR detection and identification of Leishmania parasites in clinical specimens in Ecuador: a comparison with classical diagnostic methods. The Journal of parasitology. 1999:181-7.
8. Calvopina M, Guevara A, Gomez E, Guderian R, Mimori T, Hashiguchi Y. A comparative study of conventional and PCR-based diagnosis of cutaneous leishmaniasis in Ecuador. Research Report Series; 1997.
9. Matsumoto T, Hashiguchi Y, Gomez E, Calvopina M, Nonaka S, Saya H, et al. Comparison of PCR results using scrape/exudate, syringe-sucked fluid and biopsy samples for diagnosis of cutaneous leishmaniasis in Ecuador. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1999;93(6):606-7.
10. Chico ME, Guderian RH, Cooper PJ, Armijos R, Grogl M. Evaluation of a direct immunofluorescent antibody (DIFMA) test using Leishmania genus-specific monoclonal antibody in the routine diagnosis of cutaneous leishmaniasis. Revista da Sociedade Brasileira de Medicina Tropical. 1995;28:99-103.
11. Mimori T, Matsumoto T, Calvopiña M, Gomez E, Saya H, Katakura K, et al. Usefulness of sampling with cotton swab for PCR-diagnosis of cutaneous leishmaniasis in the New World. Acta tropica. 2002;81(3):197-202.
12. Hashiguchi Y, Velez LN, Villegas NV, Mimori T, Gomez EAL, Kato H. Leishmaniases in Ecuador: Comprehensive review and current status. Acta tropica. 2017;166:299-315.
13. Pena HP, Belo VS, Xavier-Junior JCC, Teixeira-Neto RG, Melo SN, Pereira DA, et al. Accuracy of diagnostic tests for American tegumentary leishmaniasis: a systematic literature review with meta-analyses. Tropical medicine & international health : TM & IH. 2020;25(10):1168-81.
14. Smit PW, Elliott I, Peeling RW, Mabey D, Newton PN. An overview of the clinical use of filter paper in the diagnosis of tropical diseases. The American journal of tropical medicine and hygiene. 2014;90(2):195.
15. Bezemer JM, Meesters K, Naveda CL, Machado PR, Calvopiña M, Leeflang MM, et al. Clinical criteria for Mucosal Leishmaniasis diagnosis in rural South America: A systematic literature review. PLoS neglected tropical diseases. 2022;16(8):e0010621.
16. Weigle KA, Escobar M, Arias AL, Martinez F, Rojas C. A clinical prediction rule for American cutaneous leishmaniasis in Colombia. International Journal of Epidemiology. 1993;22(3):548-58.
17. Altman DG, Bland JM. Statistics Notes: Diagnostic tests 2: predictive values. Bmj. 1994;309(6947):102.
18. Cantanhêde LM, Mattos CB, Cruz AK, Ikenohuchi YJ, Fernandes FG, Medeiros E, et al. Overcoming the Negligence in Laboratory Diagnosis of Mucosal Leishmaniasis. Pathogens. 2021;10(9).
19. Dendukuri N, Schiller I, De Groot J, Libman M, Moons K, Reitsma J, et al. Concerns about composite reference standards in diagnostic research. bmj. 2018;360.
20. MacLean EL, Kohli M, Köppel L, Schiller I, Sharma SK, Pai M, et al. Bayesian latent class analysis produced diagnostic accuracy estimates that were more interpretable than composite reference standards for extrapulmonary tuberculosis tests. Diagnostic and prognostic research. 2022;6(1):1-10.
21. Boelaert M, Aoun K, Liinev J, Goetghebeur E, Van Der Stuyft P. The potential of latent class analysis in diagnostic test validation for canine Leishmania infantum infection. Epidemiology & Infection. 1999;123(3):499-506.
22. CODENPE. Modulo 3: Interculturalidad. Quito: CODENPE; 2011.

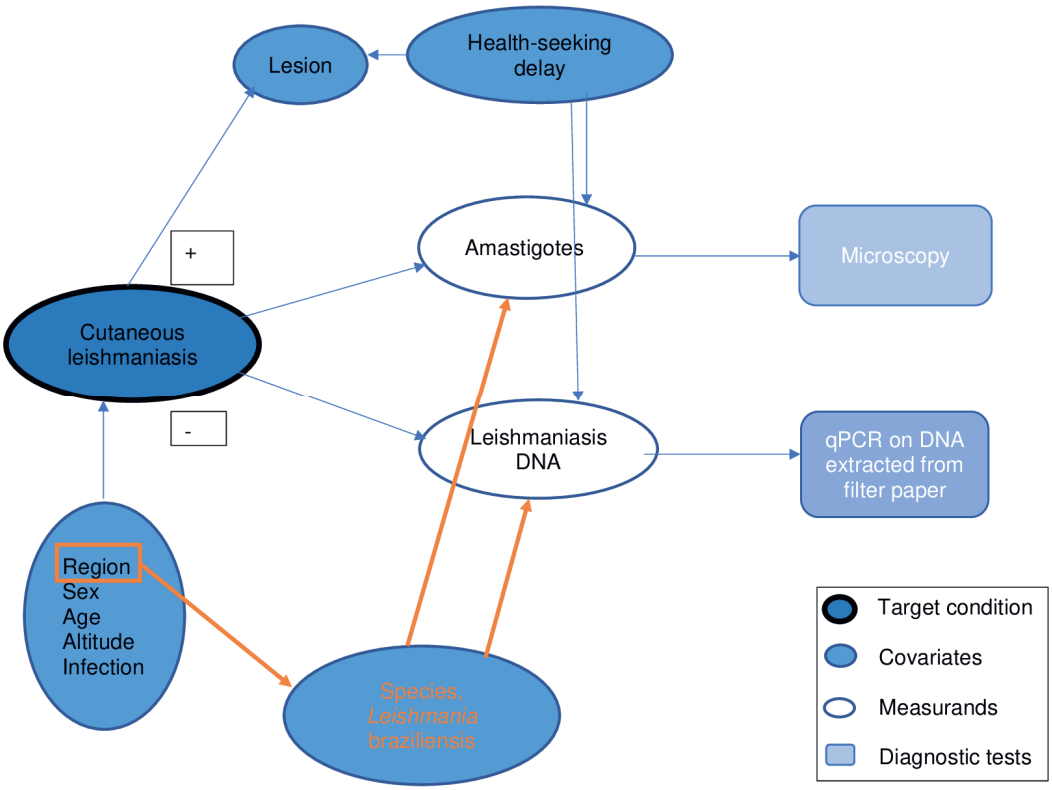
23. Kostoulas P, Nielsen SS, Branscum AJ, Johnson WO, Dendukuri N, Dhand NK, et al. STARD-BLCM: Standards for the Reporting of Diagnostic accuracy studies that use Bayesian Latent Class Models. Preventive veterinary medicine. 2017;138:37-47.
24. Conteron E. Prevalencia de leishmaniasis en el area II de Pastaza, asociados a factores de riesgo que influyen en el desarrollo de la enfermedad. Ambato: Universidad Tecnica de Ambato; 2015.
25. Bastidas CA, Villacrés-Granda I, Navarrete D, Monsalve M, Coral-Almeida M, Cifuentes SG. Antibiotic susceptibility profile and prevalence of mecA and lukS-PV/lukF-PV genes in Staphylococcus aureus isolated from nasal and pharyngeal sources of medical students in Ecuador. Infection and Drug Resistance. 2019;12:2553.
26. Panda BB, Meher AS, Hazra RK. Comparison between different methods of DNA isolation from dried blood spots for determination of malaria to determine specificity and cost effectiveness. Journal of Parasitic Diseases. 2019;43(3):337-42.
27. Miranda A, Saldaña A, González K, Paz H, Santamaría G, Samudio F, et al. Evaluation of PCR for cutaneous leishmaniasis diagnosis and species identification using filter paper samples in Panama, Central America. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2012;106(9):544-8.
28. van der Meide W, Guerra J, Schoone G, Farenhorst M, Coelho L, Faber W, et al. Comparison between quantitative nucleic acid sequence-based amplification, real-time reverse transcriptase PCR, and real-time PCR for quantification of Leishmania parasites. Journal of Clinical Microbiology. 2008;46(1):73-8.
29. Weirather JL, Jeronimo SM, Gautam S, Sundar S, Kang M, Kurtz MA, et al. Serial quantitative PCR assay for detection, species discrimination, and quantification of Leishmania spp. in human samples. Journal of clinical microbiology. 2011;49(11):3892-904.
30. Kato H, Cáceres AG, Hashiguchi Y. First evidence of a hybrid of Leishmania (Viannia) braziliensis/L.(V.) peruviana DNA detected from the phlebotomine sand fly Lutzomyia tejadai in Peru. PLoS neglected tropical diseases. 2016;10(1):e0004336.
31. Kato H, Gomez EA, Martini-Robles L, Muzzio J, Velez L, Calvopiña M, et al. Geographic Distribution of Leishmania Species in Ecuador Based on the Cytochrome B Gene Sequence Analysis. PLoS neglected tropical diseases. 2016;10(7):e0004844.
32. Calvopina M, Armijos RX, Hashiguchi Y. Epidemiology of leishmaniasis in Ecuador: current status of knowledge -- a review. Memorias do Instituto Oswaldo Cruz. 2004;99(7):663-72.
33. Amunárriz M. Estudios sobre patologías tropicales en la Amazonia ecuatoriana: Cicame; 1991.
34. Boggild AK, Ramos AP, Valencia BM, Veland N, Calderon F, Arevalo J, et al. Diagnostic performance of filter paper lesion impression PCR for secondarily infected ulcers and nonulcerative lesions caused by cutaneous leishmaniasis. Journal of clinical microbiology. 2011;49(3):1097-100.
35. Romero GA, de Farias Guerra MV, Paes MG, Macêdo VdO. Comparison of cutaneous leishmaniasis due to Leishmania (Viannia) braziliensis and L.(V.) guyanensis in Brazil: clinical findings and diagnostic approach. Clinical Infectious Diseases. 2001;32(9):1304-12.
36. Mota CA, Venazzi EAS, Zanzarini PD, Aristides SMA, Lonardoni MVC, Silveira TGV. Filter paper performance in PCR for cutaneous leishmaniasis diagnosis. Revista da Sociedade Brasileira de Medicina Tropical. 2020;54.
37. Al-Jawabreh A, Dumaidi K, Ereqat S, Nasereddin A, Azmi K, Al-Jawabreh H, et al. A comparison of the efficiency of three sampling methods for use in the molecular and conventional diagnosis of cutaneous leishmaniasis. Acta tropica. 2018;182:173-7.
38. Sevilha-Santos L, Dos Santos Júnior ACM, Medeiros-Silva V, Bergmann JO, da Silva EF, Segato LF, et al. Accuracy of qPCR for quantifying Leishmania kDNA in different skin layers of patients with American tegumentary leishmaniasis. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2019;25(2):242-7.
39. Boggild AK, Valencia BM, Espinosa D, Veland N, Ramos AP, Arevalo J, et al. Detection and species identification of Leishmania DNA from filter paper lesion impressions for patients with American cutaneous leishmaniasis. Clinical Infectious Diseases. 2010;50(1):e1-e6.

40. de Morais RCS, de Melo MGN, de Goes TC, e Silva RP, de Morais RF, de Oliveira Guerra JA, et al. Duplex qPCR for Leishmania species identification using lesion imprint on filter paper. Experimental parasitology. 2020;219:108019.
41. Mendez-Bejarano CP, Perez-Rico JJ, Rodriguez-Angarita O, Velandia-Rodriguez DA, Romero-Barbosa YA, Alvarado-Flechas MT, et al. the imprint as a non-invasive method of sampling for the molecular diagnosis of cutaneous leishmaniasis in Colombia's Military population. Revista Med. 2018;26(2):15-21.
42. Thomaz C, de Mello CX, Espindola OdM, Shubach AdO, Quintella LP, de Oliveira RVC, et al. Comparison of parasite load by qPCR and histopathological changes of inner and outer edge of ulcerated cutaneous lesions of cutaneous leishmaniasis. PloS one. 2021;16(1):e0243978.
43. Simon N, Shallat J, Williams Wietzikoski C, Harrington WE. Optimization of Chelex 100 resin-based extraction of genomic DNA from dried blood spots. Biology Methods and Protocols. 2020;5(1):bpaa009.
44. Adams ER, Gomez MA, Scheske L, Rios R, Marquez R, Cossio A, et al. Sensitive diagnosis of cutaneous leishmaniasis by lesion swab sampling coupled to qPCR. Parasitology. 2014;141(14):1891-7.
45. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clinical infectious diseases. 2016;63(12):e202-e64.
46. Urutseg. Ecuador physical map 2014 https://en.m.wikipedia.org/wiki/File:Ecuador_relief_location_map.svg. Accessed: 20 January 2023.

Supporting Information



S1 Fig. Altitude map of Ecuador with geographic regions and participating health centers (N=16). *Black dots indicate major cities. Red dots indicate participating health center locations in the Pacific region: 1: Puerto Quito, 2: Pedro Vicente Maldonado, 3: San Miguel de Los Bancos, and in the Amazon region: 4: Tena Hospital, 5: Paushiyaku, 6: Satelital Tena, 7: Puerto Napo, 8: Misahualli, 9: Chontapunta, 10: Arosemena Tola, 11: Shell hospital, 12: Puyo hospital, 13: Tuutinentza, 14: Ipiak, 15: Wasakentsa, 16: Wachirpas. Copyright: The image is adapted from Wikipedia by the authors and is available under the Creative Commons CC0 1.0 Universal Public Domain Dedication [46].*



S2 Fig. Directed acyclic graph (DAG) - Graphical representation of the study question.
The target condition under study is cutaneous leishmaniasis. Amastigotes or their genetic material (DNA) in the wound under suspicion are the measurands. The tests under evaluation are qPCR on DNA extracted from filter paper and direct microscopy after staining of a sample taken by wound scraping. The figure represents a DAG of the study question. We define two latent classes in this accuracy question, being (i) cutaneous leishmaniasis disease positive and (ii) disease status negative. Covariates potentially associated with a difference in prevalence of disease were assessed. Geographical region (Amazon versus Pacific region) was found an important factor. Other covariates were investigated separately by geographical region. Altitude of infection (500 m cut-off), body location of the lesion (head or neck versus elsewhere), health-seeking delay (cut-off 4 weeks) and age (younger or older than 20 years old) were only minimally different. Disease was equally distributed among both sexes.

S1 Table. Diagnostic accuracy estimates for qPCR and microscopy using latent class analysis using region pooled data.

	Model 3[a]
Sensitivity qPCR	71.6% (58.5;81.3)
Sensitivity Microscopy	67.2% (54.6;77.0)
Difference sensitivity[b]	*-4.3 (-12.4;3.8)*
Specificity qPCR	97.3% (92.7;99.4)
Specificity Microscopy	99.3 (96.4;100)
Difference specificity[b]	*1.9 (-1.4;6.5)*
LR+ qPCR	26 (9.5;112)
LR+ Microscopy	98 (19;2624)
LR- qPCR	0.3 (0.2;0.5)
LR- Microscopy	0.3 (0.2;0.4)

LR: likelihood ratio; LR+: positive likelihood ratio; LR-: negative likelihood ratio
[a]Latent class analysis with two latent classes, using the data of one joint population and informative prior for specificity microscopy with a beta distribution (99,1) and for specificity qPCR with a beta distribution (97,3).
[b]Difference between estimate for qPCR and for microscopy in percentage points

S2 Table. Characteristics of filter paper samples of 314 further analyzed cases.

	Pacific (N=185)	Amazon (N=129)	Microscopy positive AND *Leishmania* qPCR positive (N=125)	Microscopy negative AND *Leishmania* qPCR positive (N=61)	Microscopy positive AND *Leishmania* qPCR negative (N=49)	Time between sampling and DNA extraction >1 year (N=93)	All samples (N=314)
Median hTNF CT (IQR)[a]	30,0 (27,7-31,6)	29,4 (28,0-31,6)	29,5 (27,8-31,3)	30,0 (28,0-32,2)	30,7 (29,0-32,1)	30,6 (28,7-32,4)	29,8 (27,8-31,6)
Median time in months between sampling and DNA extraction (IQR)	9,2 (7,7-11,8)	6,2 (3,4-22,5)	9,1 (5,0-11,8)	8,7 (4,4-22,9)	9,1 (7,7-19,7)	23,3 (21,3-24,5)	8,9 (4,7-20,5)
***Total Leishmania* species determinations (%)[b]**	89 (48)	46 (36)	103 (82)	32 (52)			135 (43)
***L. guyanensis* (%)**	83 (93)	19 (41)	86 (83)	16 (50)	0 (0)	29 (31)	102 (76)
***L. braziliensis* (%)**	5 (6)	21 (46)	11 (11)	15 (47)			26 (19)
***L. lainsoni* (%)**	1 (1)	6 (13)	6 (6)	1 (3)			7 (5)
Microscopy positive AND *Leishmania* qPCR negative (%)	31 (17)	18 (14)	0 (0)	0 (0)	49 (100)	14 (15)	49 (16)
Microscopy negative AND *Leishmania* qPCR positive (%)	27 (15)	34 (26)	0 (0)	61 (100)	0(0)	24 (26)	61 (19)

qPCR: quantitative Polymerase Chain Reaction, hTNF: human Tumor Necrosis Factor, CT: Cycle Threshold, IQR: Interquartile Range

[a] hTNF was applied as internal control for sample taking and DNA extraction in a duplex qPCR together with *Leishmania* rDNA. CT values have a logarithmic relationship with DNA concentrations and lower CTs indicate more sample DNA.

[b] *Leishmania* species was determined in *Leishmania* 18SrDNA qPCR positive samples by sequencing a gen fragment that codes for the *Leishmania* cytochrome B enzyme.

S3 Table. Prevalence, PPV and NPV with 95% credible Interval, by covariate using LCA[a].

	Sensitivity qPCR/microscopy	Specificity qPCR/microscopy	Prevalence CL	PPV qPCR	PPV microscopy	NPV qPCR	NPV microscopy
Amazon region	68.0 (49.1;82.4)/ 51.2 (35.9;65.5)	97.2 (92.6;99.4) / 99.3 (96.1;100)	72.6 (57.9; 97.3)	98.7 (95.1; 99.9)	99.6 (96.9; 100)	54.0 (5.2; 77.4)	43.9 (4.3; 64.5)
Pacific region	73.4 (62.7;82.7) / 76.4 (65.0;85.6)	97.2 (92.8;99.4) / 99.3 (96.1;100)	87.7 (78.1; 99.1)	99.6 (98.2; 100)	99.9 (99.2; 100)	34.1 (2.5; 57.5)	37.3 (2.7; 62.6)
Females							
Amazon	73.9 (51.9; 90.5) / 45.5 (28.7; 64.1)	97.2 (92.7; 99.3) / 99.3 (96.0; 100)	74.5 (55.1; 97.6)	98.9 (95.2; 99.9)	99.5 (96.3; 100)	57.1 (5.5; 85.7)	38.9 (3.8; 64.2)
Pacific	68.7 (55.2; 80.9) / 73.4 (59.4; 85.4)	97.2 (92.6; 99.3) / 99.3 (96.2; 100)	87.0 (74.3; 99.1)	99.5 (97.7; 100)	99.9 (99.0; 100)	32.1 (2.3; 58.5)	36.2 (2.6; 65.3)
Males							
Amazon	64.4 (44.6; 81.1) / 54.6 (36.9; 71.8)	97.2 (92.7; 99.3) / 99.3 (96.0; 100)	71.1 (54.3; 97.2)	98.5 (94.2; 99.9)	99.6 (96.6; 100)	53.4 (5.1; 77.7)	47.7 (4.6; 71.2)
Pacific	76.7 (64.1; 87.1) / 78.3; 65.6; 88.7)	97.2 (92.6; 99.3) / 99.3 (96.2; 100)	88.1 (76.5; 99.1)	99.6 (98.2; 100)	99.9 (99.2; 100)	36.6 (2.8; 64.4)	38.7 (3.0; 68.3)
<=20 years							
Amazon	75.4 (55.8; 90.2) / 44.9 (29.7; 61.4)	97.2 (92.7; 99.4) / 99.3 (96.1; 100)	79.8 (62.1; 98.3)	99.2 (96.5; 100)	99.7 (97.2; 100)	51.0 (4.3; 81.4)	31.7 (2.7; 55.8)
Pacific	76.2 (63.6; 86.5) / 78.8 (66.1; 89.1)	97.2 (92.6; 99.4) / 99.3 (96.1; 100)	88.3 (77.1; 99.3)	99.6 (98.2; 100)	99.9 (99.2; 100)	35.6 (2.3; 62.9)	38.9 (2.5; 68.3)
>20 years							
Amazon	62.2 (40.8; 80.0) / 57.1 (36.9; 75.6)	97.2 (92.7; 99.4) / 99.3 (96.1; 100)	66.8 (49.2; 96.3)	98.1 (92.6; 99.8)	99.5 (96.1; 100)	56.9 (6.5; 80.1)	54.3 (6.2; 77.6)
Pacific	69.3 (55.6; 81.6) / 72.8 (58.5; 84.9)	97.2 (92.6; 99.4) / 99.3 (96.1; 100)	86.6 (73.2; 99.0)	99.5 (97.6; 100)	99.9 (98.9; 100)	35.6 (2.3; 62.9)	38.9 (2.5; 68.3)
Infection altitude up to 500m							
Amazon	72.6 (54.5; 86.9) / 45.2 (31.3; 59.8)	97.1 (92.4; 99.3)/ 99.3 (96.3; 100)	77.9 (62.2; 98.0)	99.0 (96.1; 99.9)	99.6 (97.1; 100)	50.9 (47.6;78.1)	34.2 (3.2; 55.9)
Pacific	72.7 (56.9; 85.7) / 68.9 (53.4; 82.4)	97.2 (92.7; 99.3) / 99.3 (96.3; 100)	82.3 (68.5; 98.6)	99.3 (97.1; 100)	99.8 (98.6; 100)	43.9 (3.5; 71.4)	41.2 (3.2; 68.2)
Infection altitude >500m							
Amazon	59.2 (36.0; 80.2) / 64.1 (38.9; 86.8)	97.1 (92.4; 99.3)/ 99.3 (96.3; 100)	62.8 (42.7; 95.7)	97.6 (89.7; 99.8)	99.4 (95.9; 100)	59.5 (69.3;83.0)	63.1 (73.7; 88.5)
Pacific	73.8 (63.0; 83.4) / 82.6 (71.5; 91.1)	97.2 (92.7; 99.3) / 99.3 (96.3; 100)	92.1 (82.4; 99.5)	99.7 (98.7; 100)	99.9 (99.5; 100)	24.3 (1.6; 49.3)	33.1 (2.1; 64.8)
Head or neck lesion							
Amazon	42.4 (18.7; 71.2) / 70.6 (37.5; 95.5)	97.1 (92.4; 99.3) / 99.3 (96.2; 100)	61.7 (33.8; 95.3)	96.3 (79.5; 99.7)	99.4 (95.0; 100)	52.0 (67.6;80.7)	68.9 (8.9; 96.3)
Pacific	81.4 (67.3; 92.0) / 79.3 (65.1; 90.5)	97.2 (92.5; 99.3) / 99.3 (96.3; 100)	91.4 (78.7; 99.5)	99.7 (98.6; 100)	99.9 (99.4; 100)	33.3 (2.2; 69.2)	31.5 (2.1; 66.3)
Lesion other location than head or neck							
Amazon	72.3 (52.4; 86.5) / 49.2 (34.6; 63.5)	97.1 (92.4; 99.3) / 99.3 (96.2; 100)	73.3 (58.8; 97.4)	98.8 (95.4; 99.9)	99.6 (96.7; 100)	56.9 (55.6;80.6)	42.1 (41.4; 62.2)
Pacific	69.8 (57.6; 80.5) / 74.8 (61.9; 85.6)	97.2 (92.5; 99.3) / 99.3 (96.3; 100)	86.0 (74.6; 98.9)	99.4 (97.7; 100)	99.9 (99.0; 100)	34.8 (2.9; 58.8)	39.6 (3.2; 66.4)
Health-seeking delay							
Amazon	69.6 (45.6;87.8)/ 47.3 (29.1; 66.9)	97.2 (92.7;99.4) / 99.3 (96.1;99.4)	68.5 (49.1;96.5)	98.4 (93.5; 99.9)	99.4 (95.5; 100)	60.1 (6.7; 86.1)	46.9 (5.3;71.0)
Pacific	72.7 (61.0;82.8) / 79.0 (66.8; 88.9)	97.2 (92.5;99.4) / 99.3 (96.4;100)	88.4 (77.7; 99.2)	99.6 (98.1;100)	99.9 (99.2; 100)	32.7 (2.4; 57.5)	39.5 (2.9; 68.0)
No delay							
Amazon	66.7 (47.8;82.4)/ 54.7 (38.2;71.2)	97.2 (92.7;99.4) / 99.3 (96.1;99.4)	75.3 (58.5;97.7)	98.8 (95.2; 99.9)	99.6 (97.3; 100)	50.3 (5.0; 76.1)	42.8 (4.2; 66.9)
Pacific	74.2 (59;86.7) / 70.7 (55.5;84.2)	97.2 (92.5;99.4) / 99.3 (96.4;100)	85.9 (71.8; 98.9)	99.5 (97.6; 100)	99.9 (98.9; 100)	38.5 (2.7; 68.8)	36.0 (2.6; 65.7)

[a]Models use a beta distribution for the priors for sensitivity and specificity, including the informative prior for microscopy specificity of 99% and qPCR specificity of 97%.

S1 File. Limit of detection and standard curve of the qPCR reaction.

This file can be accessed with the following link:
https://osf.io/5c6qs/?view_only=83a155666fa2407f88cc00a0253356eb

S2 File. Study data of 320 participants.

This file can be accessed with the following link:
https://osf.io/5c6qs/?view_only=83a155666fa2407f88cc00a0253356eb

S3 File. STARD BLCM checklist.

This file can be accessed with the following link:
https://osf.io/5c6qs/?view_only=83a155666fa2407f88cc00a0253356eb

Chapter 4

Clinical criteria for Mucosal Leishmaniasis diagnosis in rural South America: a systematic literature review

Jacob M Bezemer, Kevin Meesters, Cristhian L Naveda, Paulo R L Machado, Manuel Calvopiña, Mariska M G Leeflang, Henk D F H Schallig, Henry J C de Vries

PLoS Neglected Tropical Diseases. 2022;16(8):e0010621

Abstract

Background

Mucosal Leishmaniasis (ML), a neglected tropical disease caused by *Leishmania* parasites, impairs the quality of life of under-resourced populations in South America. If not treated promptly, this disease progresses to facial deformities and death. The low sensitivity of microscopy results and the unavailability of other accurate tests hamper the diagnosis. As clinical criteria are readily available in any setting, these may be combined in a syndromic algorithm, which in turn can be used as a diagnostic tool. We explore potential clinical criteria for a syndromic diagnostic algorithm for ML in rural healthcare settings in South America.

Methodology / Principal findings

The protocol for this systematic review was pre-registered in PROSPERO with the number: CRD42017074148. In patients with ML, described in case series identified through a systematic retrieval process, we explored the cumulative ML detection rates of clinical criteria. Participants: all patients with active mucosal disease from an endemic area in South America. Any original, non-treatment study was eligible, and case reports were excluded. PUBMED, EMBASE, Web of Science, SCIELO, and LILACS databases were searched without restrictions. The risk of bias was assessed with the JBI checklist for case series. We included 10 full texts describing 192 ML patients. Male gender had the highest detection rate (88%), followed by ulcer of the nasal mucosa (77%), age >15 (69%), and symptom duration >4 months (63%).

Significance

Within this selection of patients, we found that the male gender, ulcer of the nasal mucosa, age >15, and symptom duration >4 months lead to the highest detection rates. However, higher detection comes -naturally- with a higher rate of false positives as well. As we only included ML patients, this could not be verified. Therefore, the criteria that we found to be most promising should be validated in a well-designed prospective study.

Author Summary

Mucosal leishmaniasis, a disease caused by Leishmania parasites, is transmitted from animals to humans by sandflies. It is a forgotten disease that affects under-resourced populations in South America. Without treatment, this disease mutilates the face and can even be fatal. Diagnosing mucosal leishmaniasis is challenging. The only available testing in rural areas is the use of either a lesion smear or biopsy for light microscopy, however, this is unreliable. Many patients suffer for years before receiving treatment. Syndromic algorithms use patient characteristics, such as age, gender, and symptoms to identify patients for treatment. This method has been promoted to manage infectious diseases, such as tuberculosis and sexually transmitted diseases, in low resource settings. We explore clinical criteria for a new algorithm to diagnose mucosal leishmaniasis in patients described in the medical literature. We searched the literature for reports written in any language and identified 10 studies describing 192 patients with mucosal leishmaniasis. We found that male gender, ulcer of the nasal mucosa, age >15, and symptom duration >4 months lead to acceptable detection rates. Therefore, diagnostic algorithms might improve the detection of patients with mucosal leishmaniasis but need prospective studies in clinical practice to prove their true potential.

Keywords

Systematic review, Mucosal Leishmaniasis, Diagnosis, Diagnostic algorithm, Histopathology, Smear slide, sensitivity, Resource limited settings, Clinical characteristics

Introduction

Background

Mucosal leishmaniasis (ML), a disease caused by the *Leishmania* parasites, is a Neglected Tropical Disease (NTD) that affects under-resourced populations mainly in remote, rural areas of the South American continent [1, 2]. In most cases, ML is caused by *Leishmania braziliensis* and, less commonly by *L. guyanensis, L. panamensis,* or *L. amazonensis*. The parasites are transmitted through the bite of an infected female sandfly (genus *Lutzomyia*). Following a bite, most patients develop cutaneous ulcers or nodules, referred to as cutaneous leishmaniasis (CL). ML can develop simultaneously with CL or start months to decades after a healed skin lesion. ML is caused by the dissemination of parasites to the oral, nasal, pharyngeal, and laryngeal mucosa. However, not all new ML patients report a history of CL [3, 4]. Unfortunately, the spontaneous cure of ML is rare. Furthermore, if untreated, ML may progress to nasal septum perforation and destruction, severe facial deformities, airway obstruction, and ultimately, death [5-7]. Antimonials and amphotericin-B are the recommended treatments for ML but both are associated with severe side effects and have to be injected [8-10]. Miltefosine is an expensive systemic agent for oral administration, but has limited efficacy, potentially severe side effects, and is not universally available [9, 11]. Therefore, accurate diagnosis is essential to justify ML treatment. However, diagnosing ML is challenging on clinical grounds alone, as there is a significant number of differential diagnoses such as common rhinitis, chronic sinusitis, banal nasal septum perforation, midline lymphoma, paracoccidioidomycosis, tuberculosis, rhinosporidiosis, nasal scleroma, Wegener´s granulomatosis, histoplasmosis, sporotrichosis, Hansen´s disease, squamous cell carcinoma, and chronic nasal cocaine use, among others [12, 13]. Given the significant harms of not treating ML, a high index of suspicion is warranted in all patients from endemic areas with chronic nasal, oropharyngeal, or laryngeal symptoms. Additionally, serology and the Montenegro skin test can indirectly support the diagnosis of ML, but these are neither sensitive nor specific and often unavailable in rural settings [2, 6, 14, 15]. Therefore, mucosal tissue smear slide or histopathology is recommended for a more precise diagnosis. However, the diagnostic accuracy of both these testing methods is extremely variable but usually low, even when performed in specialized centers, due to the paucity of amastigotes in the mucosal tissue [7, 16, 17]. In addition, even molecular diagnostics, such as polymerase chain reaction techniques, fail to confirm the diagnosis in more than a quarter of patients and are usually unavailable in resource-limited endemic areas [18, 19].

Syndromic algorithms for the diagnosis of infectious diseases have shown effectivity, and are often the only option in resource-limited settings [20-22]. To the best of our knowledge, little evidence exists on the application of syndromic algorithms for ML diagnosis. However, it could increase access to therapy in resource-limited populations. This study explores clinical criteria for syndromic ML diagnosis in low-resource settings in South America. Before designing a prospective diagnostic accuracy study to evaluate a syndromic algorithm, we planned to assess the accuracy of a syndromic algorithm in the existing literature. However, this requires meticulous reporting of patient characteristics and test results, as done in case series and diagnostic accuracy studies (containing patients with and without ML). As no diagnostic accuracy studies of any syndromic algorithm for ML diagnosis were available, we aimed to investigate the ML detection rates of predefined clinical characteristics and test results. Hereto, case series of ML patients were retrieved through a systematic literature review and the presence of predefined characteristics and test results in these case series were recorded.

Objective

Objective: To explore the ML detection rates of clinical criteria in participants from endemic areas in South America.

Methods

Protocol

The protocol for this systematic review was pre-registered on August 10, 2017, in the PROSPERO International prospective register of systematic reviews with registration number: CRD42017074148, 2017 [23] and is available from; https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42017074148. This systematic review followed the Preferred Reporting Items for a Systematic Review and Meta-analysis (PRISMA) guideline [24].

General study eligibility criteria

Study eligibility criteria for inclusion in this systematic review are described in Table 1. Case series were included without date or language limitations. Treatment studies and studies reporting on less than five ML patients were excluded to avoid selection bias.

Table 1. General study eligibility criteria for inclusion in this systematic review.

Inclusion criteria
1. Case series with any publication date or language
2. Report on patients with a history of a stay in an ML epidemic area of South America
3. Report on patients with active nose, throat, or oral disease (e.g. obstruction, hyperemia, erosion, ulceration, or granulomatous lesions)
4. Present clinical information at individual patient level.
Exclusion criteria:
1. Narrative reviews
2. Studies reporting on <5 ML patients
3. Treatment studies
4. Duplicate publication on the same individual patient or patient group
5. Patients without a history of a visit to South America

ML: Mucosal Leishmaniasis

General study identification

PUBMED, EMBASE, Web of Science, SCIELO, and LILACS databases were searched without restrictions, with the last search on the 14th of April 2022. The following search string was applied In PUBMED: ¨human AND (mucocutan* OR mucos* OR mucous OR tegument* OR nasal) AND (leishmanias* OR leishmanios*)¨. The annotation of the search string was adjusted for each literature database. In addition, reference lists of studies included for full-text analysis were searched for additional papers.

Study selection

Title and abstract screening were performed using the Rayyan QCR software [25]. Full texts of included studies were either retrieved electronically or requested manually by the medical library of the University of Amsterdam. Full texts were assessed using a predefined checklist (S1 Table) and included if they matched the eligibility criteria (Table 1). Each step of the study selection was done independently by JB and either KM or CN. Differences were resolved through consensus, or with help of HdV or HS.

General data collection

All steps in data collection were individually performed by JB and either KM or CN. Disputes were resolved through consensus. Using a pre-defined form, the following information was extracted from individual patients in the included papers: identifiers of the patient, presence of concomitant CL, diagnostic method(s) used, HIV status, other concomitant illnesses, results of histopathology, and smear slide microscopy (both defined as positive exclusively in case of amastigote visualization), causing *Leishmania* species, medications used before diagnosis, stage of disease according to Lessa *et al*. [6], presenting symptoms; epistaxis, dysphagia or odynophagia, voice changes, CL-scar, ulceration of the nasal mucosa, nasal deformation, oropharyngeal lesions, and symptom duration.

Risk of bias assessment

Risk of bias assessment was done with the JBI checklist for case series [26]. Because this study retrieved data at the individual patient level, the question on the appropriateness of the statistical analysis was obviated from the JBI checklist. The following question was added to assess the possible risk of bias through the inclusion of a specific patient population: `Did the case series avoid exclusion based on clinical characteristics?'. Risk of bias assessment was individually performed by JB and KM. Disputes were resolved through discussion.

Data analysis

All included studies were investigated for 12 predefined binary clinical criteria that are frequently mentioned in the literature and are easily available in clinical practice in rural settings, as our ultimate goal is to develop an algorithm for syndromic management (See Table 2). Cumulative detection rates were calculated per patient with Microsoft Excel 2018 software [27]. For cumulative detection rate calculation, the criteria were arranged from the highest absolute number of patients positive to the lowest. Non-reported criteria were interpreted as negative. Cumulative detection rates were calculated separately for males and females to avoid gender-based selection of patients by a diagnostic algorithm. Because of the low quality of the included studies and the exploratory nature of this paper, a meta-analysis was not done.

Table 2. Twelve rurally available clinical criteria assessed for diagnostic accuracy in this study.

Criterion	Reason
Male	Risk factor for ML [28]
Age >15 years	Risk factor for ML [28, 29]
Symptom duration >4 months	Differentiation from acute viral syndromes [30]
Ulcer of the nasal mucosa	Present from stage 2 of the disease [6]
Epistaxis	Present from stage 2 of the disease [6]
Oropharyngeal lesion	Worse prognosis of the disease[31]
Dysphagia or odynophagia	Sign of severe disease [6]
Nasal deformation	Sign of severe disease [6]
CL scar	Risk factor for ML [4, 7]
Concomitant CL	Risk factor for ML [4, 32]
Histopathology	Current confirmative test [33]
Smear slide microscopy	Current confirmative test [14]

Results

Study selection and data obtained

After the removal of duplicates, 4377 reports were retrieved through the searches in different databases. Of these, 10 were included that reported on a total of 192 ML patients [5, 6, 16, 34-40]. 160 full texts were excluded because they reported on less than five ML patients and seven because they presented no data at the individual patient level. The reasons for full text exclusions are summarized in Fig 1.

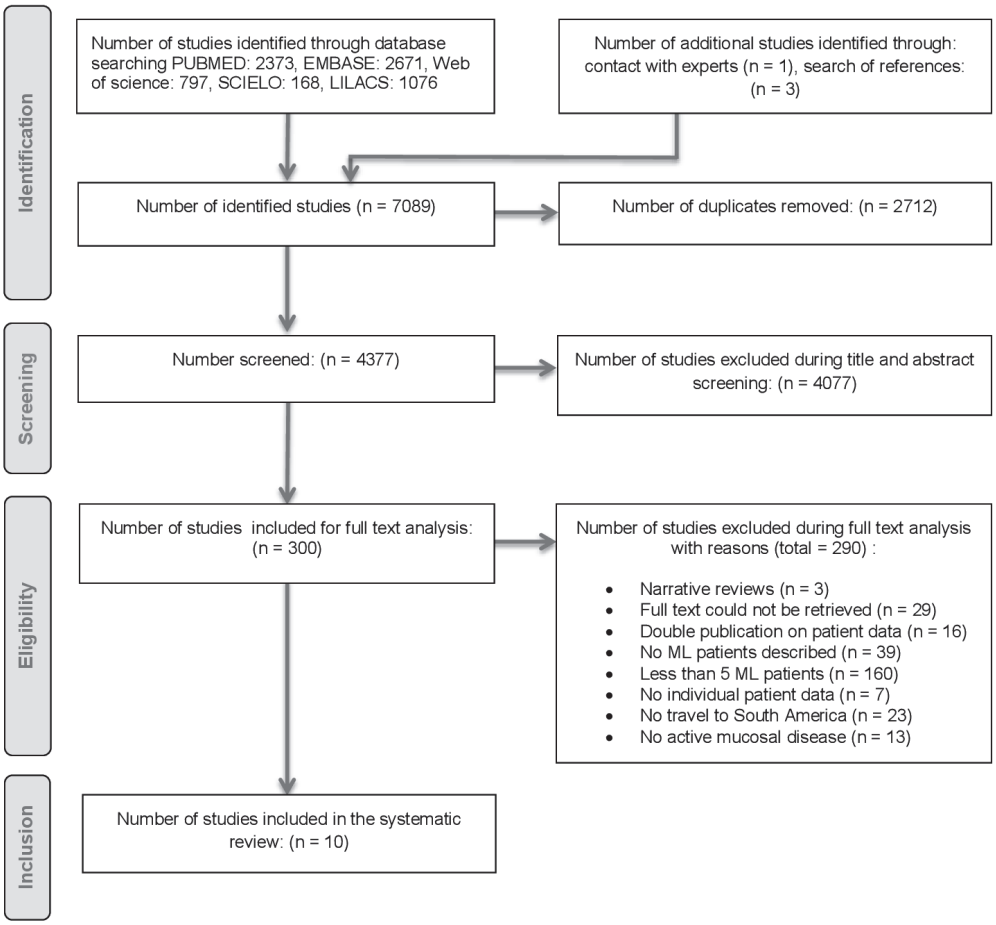


Fig 1. PRISMA literature assessment flow diagram.

General study characteristics

This review included 10 case series that were all published between 1968 and 2019. Most studies reported on patients diagnosed in Brazil (n = 6), followed by Peru (n = 2). The median number of reported ML cases per publication was 13, ranging from 5 to 50. Most patients were diagnosed with several methods, including PCR, Montenegro skin test, culture, and histopathology. *L. braziliensis* was reported as the most common species in ML lesions. However, the species was unknown in two (20%) of the included studies (Table 3 and S1 Table).

Table 3. Characteristics of the 10 case series reporting on 192 ML patients included in the systematic review.

Study characteristic		
Year published (median, range)	2007	1968-2019
Number of ML patients (median, range)	13	5-50
Country of diagnosis	**Number of studies**	**Number of patients (%)**
Brazil	6	101 (53)
Peru	2	73 (38)
Ecuador	1	13 (7)
United Kingdom	1	5 (3)
Diagnostic method for inclusion in study[a]		
PCR	7	64 (33)
Montenegro skin test	8	115 (60)
Culture	4	22 (11)
Histopathology	9	51 (27)
Serology	3	27 (14)
Smear slide microscopy	1	13 (7)
Cure with antimonial treatment	2	6 (3)
Reported causative *Leishmania* species[b]		
L. braziliensis	8	70 (36)
L. amazonensis	2	6 (3)
L. viannia complex	2	12 (6)
Unknown	2	104 (54)

[a]The majority of studies applied several diagnostic methods to every patient
[b]Several studies reported mixed causative species and the species was unknown in a part of the patients.

Risk of bias assessment

Assessment of the risk of bias in the 10 case series included in this systematic review, using the modified JBI checklist for case series, revealed a high risk of bias in all the included studies. Six studies lacked clear inclusion criteria and three studies included patients on the basis of clinical characteristics: Boaventura *et al*. included patients if they had concomitant CL and Falcao *et al*. and Motta *et al*. included patients if they had oral lesions. Four studies did not describe ML diagnostic methods clearly. Six studies applied indirect methods (skin test, serology, or cure on antimonial treatment) for ML diagnosis. Only two studies included patients consecutively and completely. Patient demographics (age, gender, and duration of symptoms) were unclearly described by one study. No study reported completely on the clinical criteria explored for ML detection rates. Follow-up was unclear in two studies and demographic data of the study site was unclear in three. Results of the risk of bias assessment are summarized in Fig 2.

Study	Barral	Boaventura	Boggild	Calvopiña	de Almeida	Falcao	Lawn	Lessa	Motta	Reyes Aragon
Were there clear criteria for inclusion in the case series?	☹	☺	☺	☹	☹	?	☺	☺	☹	☹
Did the case series avoid exclusion based on clinical characteristics?	☺	☹	☺	☺	☺	☹	☺	☺	☹	☺
Was the condition measured in a standard, reliable way for all participants included in the case series?	☺	☹	☺	☺	☺	☹	☺	☺	☹	?
Were valid methods used for identification of the condition for all participants included in the case series?	☺	☹	☺	☹	☺	☹	☺	☹	☹	☹
Did the case series have consecutive inclusion of participants?	?	☺	☺	?	☹	☹	☺	?	☹	☹
Did the case series have complete inclusion of participants?	?	☺	☺	?	☹	☹	?	☹	☹	☹
Was there clear reporting of the demographics of the participants in the study?	☺	☺	☺	☺	☺	☺	☺	☺	☺	☹
Was there clear reporting of clinical information of the participants?	☹	☹	☹	☹	☹	☹	☹	☹	☹	☹
Were the outcomes or follow up results of cases clearly reported?	NA	NA	NA	☺	NA	☺	☺	NA	☹	☹
Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	☹	☺	☺	☺	☹	☺	☺	☺	☺	☹

Fig 2. Risk of bias assessment of the included studies with the modified JBI checklist for case series.

Findings

All of the study participants` genders were known, with 88% of them being male. Information was incomplete for the other criteria. The male gender resulted in the highest number of patients positive, followed by ulcer of the nasal mucosa, age >15, and symptom duration >4 months. Results of histopathology and smear slide microscopy were unknown for the majority of patients and were positive in 55 and 41% of reported patients respectively (Table 4).

Table 4. Arrangement of the clinical criteria from the highest absolute number of patients positive to the lowest.

Nr.	Criterion	Reported in N patients (%)	N positive (% of reported)	Detection rate
1	Male	192 (100)	168 (88)	0,88
2	Ulcer of the nasal mucosa	159 (83)	147 (92)	0,77
3	Age>15	141 (73)	133 (94)	0,69
4	Symptom duration >4 months	148 (77)	121 (82)	0,63
5	Oropharyngeal lesion	155 (81)	88 (57)	0,46
6	Epistaxis	63 (33)	58 (92)	0,30
7	Histopathology	93 (48)	51 (55)	0,27
8	Dysphagia or odynophagia	42 (22)	40 (95)	0,21
9	CL scar	57 (30)	36 (63)	0,19
10	Concomitant CL	68 (35)	31 (46)	0,16
11	Nasal deformation	86 (45)	30 (34)	0,16
12	Smear slide microscopy	32 (17)	13 (41)	0,07

N = Number

The cumulative detection rates of clinical criteria for males and females are shown in Tables 5 and 6. Two or more positives out of the three criteria `ulcer of the nasal mucosa', `age >15', and `symptom duration >4 months' had a cumulative detection rate of 84% in males and 79% in females. Three or more positives out of the six criteria `ulcer of the nasal mucosa', `age >15', `symptom duration >4 months', `oropharyngeal lesion', `epistaxis', and `histopathology positive' had a cumulative detection rate of 75% in males and 54% in females.

Table 5. Cumulative detection rates of clinical criteria in 168 male Mucosal Leishmaniasis (ML) patients.

Nr.	Criterion	Cumulative detection rates at individual patient level			
	Cut-off score	≥1	≥2	≥3	≥4
2	Ulcer of the nasal mucosa (%)	139 (83)	0 (0)	0 (0)	0 (0)
3	Age>15 (%)	160 (95)	80 (48)	0 (0)	0 (0)
4	Symptom duration >4 months (%)	168 (100)	141 (84)	42 (25)	0 (0)
5	Oropharyngeal lesion (%)	168 (100)	162 (96)	88 (52)	14 (8)
6	Epistaxis (%)	168 (100)	163 (97)	104 (62)	40 (24)
7	Histopathology positive (%)	168 (100)	163 (97)	126 (75)	60 (36)
8	Dysphagia or odynophagia (%)	168 (100)	163 (97)	130 (77)	75 (45)
9	CL-scar (%)	168 (100)	164 (98)	144 (86)	82 (49)
10	Concomitant CL (%)	168 (100)	165 (98)	148 (88)	88 (52)
11	Nasal deformation (%)	168 (100)	165 (98)	153 (91)	99 (59)
12	Smear slide microscopy positive (%)	168 (100)	165 (98)	153 (91)	102 (61)

Table 6. Cumulative detection rates of clinical criteria in 24 female Mucosal Leishmaniasis (ML) patients.

Nr.	Criterion	Cumulative detection rates at individual patient level			
	Cut-off score	≥1	≥2	≥3	≥4
2	Ulcer of the nasal mucosa (%)	18 (75)	0 (0)	0 (0)	0 (0)
3	Age>15 (%)	24 (100)	16 (67)	0 (0)	0 (0)
4	Symptom duration >4 months (%)	24 (100)	19 (79)	7 (29)	0 (0)
5	Oropharyngeal lesion (%)	24 (100)	22 (92)	8 (33)	3 (13)
6	Epistaxis (%)	24 (100)	23 (96)	9 (38)	6 (25)
7	Histopathology positive (%)	24 (100)	23 (96)	13 (54)	6 (25)
8	Dysphagia or odynophagia (%)	24 (100)	23 (96)	14 (58)	7 (29)
9	CL-scar (%)	24 (100)	23 (96)	18 (75)	7 (29)
10	Concomitant CL (%)	24 (100)	23 (96)	20 (83)	11 (46)
11	Nasal deformation (%)	24 (100)	23 (96)	20 (83)	11 (46)
12	Smear slide microscopy positive (%)	24 (100)	23 (96)	20 (83)	11 (46)

Discussion

The objective of this study was to explore the ML detection rates of clinical criteria combinations. In our systematic review, we included 10 case series reporting on more than 190 ML patients in South America.

Our main finding is the acceptable ML detection rate of clinical criteria and promising combinations for ML diagnostic algorithms. As accurate reference tests are often unavailable in lower resource settings, such as many centers in South America [14, 15], algorithms for syndromic diagnosis for ML would be highly desirable to select patients for the, often toxic, treatment.

Adverse effects, such as musculoskeletal pain and gastrointestinal disturbances, are very common

after administration of antimonials and severe complications such as arrhythmias, leucopenia, hepatitis, and pancreatitis occur in up to 14% of treated patients. Occasionally, even deaths are reported from patients under antimonial treatment [8]. To avoid drug toxicities in patients who do not have ML, it is at least as important to know the proportion of false positive results as the proportion of true positives. However, the proportion of false positives (one minus the specificity) requires a study sample including participants without ML and these were not included in this study. Therefore, we have no estimates of the specificity of clinical criteria combinations and we cannot rule out that the proportion of false positive results of syndromic algorithms may be too high.

The absence of an established universal reference test for ML diagnosis limits the current study. We included ML patients diagnosed with any of the currently applied tests in South America, including the Montenegro skin test, serology, and cure with antimonial treatment. This results in the possible inclusion of non-ML patients in this study and the overestimation of detection rates.

Risk of bias evaluation revealed significant flaws in all the studies leading to a high risk of bias. The incomplete reporting of clinical criteria limits this study and leads to a possible underestimation of the detection rates as non-reported criteria were interpreted as negative. The selection of concomitant CL or oral ML patients by three studies possibly leads to an overestimation of the detection rates of concomitant CL and oropharyngeal lesions.

Former studies have reported that the male gender is a risk factor for leishmaniasis in its cutaneous (CL), mucosal (ML), and visceral expressions [28, 41]. The underlying processes could be sex-specific biological, differences in vector exposure, variances in health-seeking behavior, and marginalization of female patients in health care and publications [42-44]. Therefore, it is not unexpected that the majority of the patients in this study are men. As the methodological quality of the included studies is low, we cannot exclude that the gender difference was at least partially caused by biased patient inclusion or publication. That also applies to the criterion `age >15'. We emphasize that the clinical criteria combinations shown in this paper have to be adapted according to the results of a well-designed prospective study (including non-ML patients) that should avoid the exclusion of patients based on gender, age, ethnicity, and other personal identifiers and include an evaluation of health-seeking behavior in its protocol.

The ML detection rates of clinical criteria combinations explored in this review reached levels comparable to the performance of the Montenegro skin test [7, 45]. Their application would be rapid, cheap, and feasible in any rural clinical setting located in endemic regions and thus of potential clinical value.

Conclusion

We present an exploration of the detection rates of clinical criteria in 192 ML patients reported in case series. Within this selection of patients, we found that male gender, ulcer of the nasal mucosa, age >15, and symptom duration >4 months lead to the highest detection rates. They could improve diagnosis and hence prompt treatment of ML in vulnerable groups in resource-limited settings where diagnostic confirmation cannot be obtained. Therefore, the criteria that we found to be most promising, should be validated in a well-designed prospective study.

Declarations

Data Availability

All relevant data are within the manuscript and its Supporting Information files.

Competing interests

I have read the journal's policy and the authors of this manuscript have the following competing interests: JB is a volunteer for Latin Link Nederland and receives a monthly volunteer allowance from this organization. There are no patents, products in development or marketed products associated with this research to declare. This does not alter our adherence to PLOS NTD policies on sharing data

Financial Disclosure

Funding: JB received a monthly volunteer allowance from Latin Link Nederland http://www.latinlink-nederland.nl/, which helped fund the study. Latin Link had no role in study design, data collection and analysis, decision to publish, preparation of the manuscript. No additional external funding was received for this study.

Author Contributions

Conceptualization: Jacob M. Bezemer, Kevin Meesters, Paulo R. L. Machado, Manuel Calvopiña, Henk D. F. H. Schallig, Henry J. C. de Vries.
Data curation: Jacob M. Bezemer.
Formal analysis: Jacob M. Bezemer, Kevin Meesters, Cristhian L. Naveda.
Investigation: Jacob M. Bezemer, Kevin Meesters, Cristhian L. Naveda.
Methodology: Jacob M. Bezemer, Kevin Meesters, Mariska M. G. Leeflang.
Project administration: Jacob M. Bezemer.
Resources: Henry J. C. de Vries.
Software: Jacob M. Bezemer.
Supervision: Henk D. F. H. Schallig, Henry J. C. de Vries.
Validation: Cristhian L. Naveda, Henk D. F. H. Schallig, Henry J. C. de Vries.
Visualization: Jacob M. Bezemer.
Writing – original draft: Jacob M. Bezemer.
Writing – review & editing: Kevin Meesters, Paulo R. L. Machado, Manuel Calvopiña, Mariska M. G. Leeflang, Henk D. F. H. Schallig, Henry J. C. de Vries.

References

1. WHO. Investing to Overcome the Global Impact of Neglected Tropical Diseases: Third WHO Report on Neglected Tropical Diseases 2015. Geneva: World Health Organization; 2015.
2. WHO. Control of the leishmaniases: report of a meeting of the WHO Expert Commitee on the Control of Leishmaniases, Geneva, 22-26 March 2010. Geneva: World Health Organization; 2010. Contract No.: 949.
3. Bennett JE, Dolin R, Blaser MJ. Principles and Practice of Infectious Diseases. Philadelphia: Elsevier Health Sciences; 2019.
4. Cincura C, De Lima CMF, Machado PRL, Oliveira-Filho J, Glesby MJ, Lessa MM, et al. Mucosal leishmaniasis: A retrospective study of 327 cases from an endemic area of Leishmania (Viannia) braziliensis. American Journal of Tropical Medicine and Hygiene. 2017;97(3):761-6.
5. Calvopiña M, Angel G, Armijos R, Gomez E, Mimori T, Cooper P, et al. Clinical features of mucocutaneous leishmaniasis in the Amazonian region of Ecuador. Res Rep Ser. 2005;6:82-9.
6. Lessa HA, Lessa MM, Guimaraes LH, Lima CM, Arruda S, Machado PR, et al. A proposed new clinical staging system for patients with mucosal leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2012;106(6):376-81.
7. Amato VS, Tuon FF, Imamura R, de Camargo RA, Duarte MI, Neto VA. Mucosal leishmaniasis: description of case management approaches and analysis of risk factors for treatment failure in a cohort of 140 patients in Brazil. Journal of the European Academy of Dermatology and Venereology. 2009;23(9):1026-34.
8. Oliveira LF, Schubach AO, Martins MM, Passos SL, Oliveira RV, Marzochi MC, et al. Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. Acta tropica. 2011;118(2):87-96.
9. Pinart M, Rueda JR, Romero GA, Pinzón-Flórez CE, Osorio-Arango K, Silveira Maia-Elkhoury AN, et al. Interventions for American cutaneous and mucocutaneous leishmaniasis. The Cochrane database of systematic reviews. 2020;8:Cd004834.
10. Santos CR, Tuon FF, Cieslinski J, de Souza RM, Imamura R, Amato VS. Comparative study on liposomal amphotericin B and other therapies in the treatment of mucosal leishmaniasis: A 15-year retrospective cohort study. PloS one. 2019;14(6):e0218786.
11. Sunyoto T, Potet J, Boelaert M. Why miltefosine-a life-saving drug for leishmaniasis-is unavailable to people who need it the most. BMJ global health. 2018;3(3):e000709.
12. Rodríguez G, Sarmiento L, Hernández CA. Leishmaniasis mucosa y otras lesiones destructivas centrofaciales. Biomedica. 1994;14(4):215-29.
13. Agnihotri NT, McGrath KG. Allergic and nonallergic rhinitis. Allergy and asthma proceedings. 2019;40(6):376-9.
14. Hashiguchi Y, Velez LN, Villegas NV, Mimori T, Gomez EAL, Kato H. Leishmaniases in Ecuador: Comprehensive review and current status. Acta tropica. 2017;166:299-315.
15. Braz LMA. Tegumentary leishmaniasis diagnosis: what happened with MST (Montenegro Skin Test) in Brazil? Revista do Instituto de Medicina Tropical de Sao Paulo. 2019;61:e17.
16. Boggild AK, Valencia BM, Veland N, Ramos AP, Calderon F, Arevalo J, et al. Non-invasive cytology brush pcr diagnostic testing in mucosal leishmaniasis: Superior performance to conventional biopsy with histopathology. PloS one. 2011;6 (10) (no pagination)(e26395).
17. Zajtchuk JT, Casler JD, Netto EM, Grogl M, Neafie RC, Hessel CR, et al. Mucosal leishmaniasis in Brazil. The Laryngoscope. 1989;99(9):925-39.
18. Gomes CM, Mazin SC, Santos ER, Cesetti MV, Bächtold GA, Cordeiro JH, et al. Accuracy of mucocutaneous leishmaniasis diagnosis using polymerase chain reaction: systematic literature review and meta-analysis. Memorias do Instituto Oswaldo Cruz. 2015;110(2):157-65.
19. Calvopina M, Armijos RX, Hashiguchi Y. Epidemiology of leishmaniasis in Ecuador: current status of knowledge -- a review. Memorias do Instituto Oswaldo Cruz. 2004;99(7):663-72.

20. Banda H, Robinson R, Thomson R, Squire SB, Mortimer K. The 'Practical Approach to Lung Health' in sub-Saharan Africa: a systematic review. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2016;20(4):552-9.
21. Marais BJ, Gie RP, Hesseling AC, Schaaf HS, Lombard C, Enarson DA, et al. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. Pediatrics. 2006;118(5):e1350-9.
22. WHO. Sexually transmitted and other reproductive tract infections: a guide to essential practice: World Health Organization; 2005.
23. Bezemer J, Meesters K, Calvopiña M, Machado P, de Vries H, Schallig H. Syndromic management of mucosal leishmaniasis in south america: a systematic review and meta-analysis. PROSPERO. 2017:CRD42017074148.
24. Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. Bmj. 2021;372.
25. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. Systematic reviews. 2016;5(1):210.
26. Munn Z, Barker TH, Moola S, Tufanaru C, Stern C, McArthur A, et al. Methodological quality of case series studies: an introduction to the JBI critical appraisal tool. JBI evidence synthesis. 2020;18(10):2127-33.
27. Microsoft. Microsoft Excel. Internet: Microsoft Corporation; 2018.
28. Machado-Coelho GL, Caiaffa WT, Genaro O, Magalhães PA, Mayrink W. Risk factors for mucosal manifestation of American cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2005;99(1):55-61.
29. Ampuero J, Macedo V, Marsden P. Clinical findings of tegumentary leishmaniasis in children under five years of age in an endemic area of Leishmania (Viannia) braziliensis. [Portuguese]. Revista da Sociedade Brasileira de Medicina Tropical. 2006;39(1):22-6.
30. Jaume F, Valls-Mateus M, Mullol J. Common Cold and Acute Rhinosinusitis: Up-to-Date Management in 2020. Curr Allergy Asthma Rep. 2020;20(7):28.
31. Costa DCSD, Palmeiro MR, Moreira JS, Martins ACDC, Da Silva AF, De Fatima Madeira M, et al. Oral manifestations in the American tegumentary leishmaniasis. PloS one. 2014;9 (11) (no pagination)(e109790).
32. Canário A, Queiroz M, Cunha G, Cavalcante T, Riesz V, Sharma R, et al. Presence of parasite DNA in clinically unaffected nasal mucosa during cutaneous leishmaniasis caused by Leishmania (Viannia) braziliensis. Clinical Microbiology and Infection. 2019;25(4):515. e5-. e7.
33. Cantanhêde LM, Mattos CB, Cruz AK, Ikenohuchi YJ, Fernandes FG, Medeiros E, et al. Overcoming the Negligence in Laboratory Diagnosis of Mucosal Leishmaniasis. Pathogens. 2021;10(9).
34. Lawn SD, Whetham J, Chiodini PL, Kanagalingam J, Watson J, Behrens RH, et al. New world mucosal and cutaneous leishmaniasis: an emerging health problem among British travellers. QJM : monthly journal of the Association of Physicians. 2004;97(12):781-8.
35. Boaventura VS, Cafe V, Costa J, Oliveira F, Bafica A, Rosato A, et al. Short report: Concomitant early mucosal and cutaneous leishmaniasis in Brazil. American Journal of Tropical Medicine and Hygiene. 2006;75(2):267-9.
36. Falcão G, Lins-Kusterer L, Leite-Ribeiro PM, Sarmento VA. Orofacial manifestations of mucocutaneous leishmaniasis: a case series from Brazil. F1000Research. 2019;8:756.
37. Motta ACF, Lopes MA, Ito FA, Carlos-Bregni R, De Almeida OP, Roselino AM. Oral leishmaniasis: A clinicopathological study of 11 cases. Oral Diseases. 2007;13(3):335-40.
38. Barral A, Pedral-Sampaio D, Grimaldi Jr G, Momen H, McMahon-Pratt D, Ribeiro De Jesus A, et al. Leishmaniasis in Bahia, Brazi: Evidence that Leishmania amazonensis produces a wide spectrum of clinical disease. American Journal of Tropical Medicine and Hygiene. 1991;44(5):536-46.
39. de Almeida OP, Sanchez-Romero C, Junior HM, Matta VLRd, Soares CdM, Mariano F, et al. Immunohistochemical and Molecular Diagnosis of Mucocutaneous and Mucosal Leishmaniasis. International journal of surgical pathology. 2019.
40. Reyes Aragon J. [Skin leishmaniasis. II. Otorhinolaryngologic lesions]. Acta oto-rino-laringologica ibero-americana. 1968;19(2):97-121 contd.

41. Valero NNH, Uriarte M. Environmental and socioeconomic risk factors associated with visceral and cutaneous leishmaniasis: a systematic review. Parasitology research. 2020;119(2):365-84.
42. Cloots K, Burza S, Malaviya P, Hasker E, Kansal S, Mollett G, et al. Male predominance in reported Visceral Leishmaniasis cases: Nature or nurture? A comparison of population-based with health facility-reported data. PLoS neglected tropical diseases. 2020;14(1):e0007995.
43. Lockard RD, Wilson ME, Rodríguez NE. Sex-Related Differences in Immune Response and Symptomatic Manifestations to Infection with Leishmania Species. Journal of immunology research. 2019;2019:4103819.
44. Dahal P, Singh-Phulgenda S, Olliaro PL, Guerin PJ. Gender disparity in cases enrolled in clinical trials of visceral leishmaniasis: A systematic review and meta-analysis. PLoS neglected tropical diseases. 2021;15(3):e0009204.
45. Weigle KA, De Davalos M, Heredia P. Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: A comparison of seven methods. American Journal of Tropical Medicine and Hygiene. 1987;36(3):489-96.

Supporting information

S1 Appendix. PRISMA 2020 abstract checklist for systematic reviews

This file can be accessed with the following link:
https://doi.org/10.1371/journal.pntd.0010621.s001

S2 Appendix. PRISMA 2020 checklist for systematic reviews

This file can be accessed with the following link:
https://doi.org/10.1371/journal.pntd.0010621.s002

S1 Table. Characteristics of included studies

This file can be accessed with the following link:
https://doi.org/10.1371/journal.pntd.0010621.s003

S2 Table. Characteristics of 192 individually assessed patients

This file can be accessed with the following link:
https://doi.org/10.1371/journal.pntd.0010621.s004

Chapter 5

Safety and efficacy of allylamines in the treatment of cutaneous and mucocutaneous leishmaniasis: a systematic review

Jacob M Bezemer, Jacob van der Ende, Jacqueline Limpens, Henry J C de Vries, Henk D F H Schallig

PloS One. 2021; 16(4):e0249628.

Abstract

Cutaneous and mucocutaneous leishmaniasis affect a million people yearly, leading to skin lesions and potentially disfiguring mucosal disease. Current treatments can have severe side effects. Allylamine drugs, like terbinafine, are safe, including during pregnancy. This review assesses efficacy and safety of allylamines for the treatment of cutaneous and mucocutaneous leishmaniasis. It followed the PRISMA statement for reporting and was preregistered in PROSPERO(CRD4201809068). MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials, the Global Health Library, Web of Science, Google Scholar, and clinical trial registers were searched from their creation to May 24th, 2020. All original human, animal, and *in vitro* studies concerning allylamines and cutaneous or mucocutaneous leishmaniasis were eligible for inclusion. Comparators — if any — included both placebo or alternative cutaneous or mucocutaneous leishmaniasis treatments. Complete cure, growth inhibition, or adverse events served as outcomes. The search identified 312 publications, of which 22 were included in this systematic review. There were one uncontrolled and two randomised controlled trials. The only well-designed randomised controlled trial that compared the treatment efficacy of oral terbinafine versus intramuscular meglumine antimoniate in 80 Leismania tropica infected patients showed a non-significant lower cure rate for terbinafine vs meglumine antimoniate (38% vs 53%). A meta-analysis could not be performed due to the small number of studies, their heterogeneity, and low quality. This systematic review shows that there is no evidence of efficacy of allylamine monotherapy against cutaneous and mucocutaneous leishmaniasis. Further trials of allylamines should be carefully considered as the outcomes of an adequately designed trial were disappointing and *in vitro* studies indicate minimal effective concentrations that are not achieved in the skin during standard doses. However, the *in vitro* synergistic effects of allylamines combined with triazole drugs warrant further exploration.

Keywords

Systematic review, Tegumentary leishmaniasis, Cutaneous leishmaniasis, Mucocutaneous leishmaniasis, Human, Animal, *In vitro*, Allylamine, Terbinafine, Butenafine, Naftifine, Treatment, Oral, Topical

Introduction

Cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL), classified by the World Health Organization (WHO) as emerging neglected diseases, affect more than one million people yearly [1, 2]. CL manifests as skin lesions and MCL as potentially disfiguring mucosal disease of the nose, mouth, and larynx [3]. At least 20 different *Leishmania* parasite species can cause CL and MCL with differing clinical manifestations and responses to treatment [4]. Depending on the infecting *Leishmania* species, multiple treatment options are available but pentavalent antimonials (e.g., sodium stibogluconate and meglumine antimoniate) are still the most frequently used for American CL and MCL [5] and frequently used for old world leishmaniasis [6]. Yet, antimonial therapy is painful and requires multiple intralesional, intravenous, or intramuscular injections up to 30 days [5, 6]. Miltefosine, the oral alternative for systemic CL and MCL therapy, is not widely available and very expensive, limiting its use in clinical practice [7]. Moreover, pentavalent antimonials can result in hepatotoxicity, renal insufficiency, pancreatitis, cardiac arrest, and other serious side effects and there is no safe alternative systemic drug for use in pregnant women [8, 9]. Furthermore, depending on the region and species, poor treatment responses exist for pentavalent antimonials and miltefosine [10]. Consequently, there is a pressing need to identify alternative oral, safe, available, affordable, and efficacious treatment options for CL and MCL.

Thirty years ago, Goad *et al.*, reported an inhibitory effect of terbinafine on cultured promastigotes of the *Leishmania mexicana* complex species [11]. Terbinafine is a member of the allylamine drug group, together with butenafine and naftifine.

Allylamines inhibit squalene-2,3-epoxidase causing accumulation of squalene and depletion of sterols in *Leishmania* amastigotes, resulting in growth inhibition and parasite death [12].

Terbinafine is used as a first line oral treatment for fungal infections and is the preferred systemic treatment for toenail infections in elderly people for safety reasons. Because of its use as antifungal, terbinafine is widely available in pharmacies all over the world at reasonable prices in oral and topical formulations [13].Terbinafine might be a safe systemic option in pregnancy, as no teratogenic side effects have been described [14, 15].

Since allylamines might be an attractive alternative CL and MCL treatment option, a systematic literature review was performed to assess the efficacy and safety of allylamines in CL and MCL treatment and to define priorities for future investigations. All original human, animal, and *in vitro* studies concerning allylamines and CL or MCL were eligible for inclusion. Comparators — if any — included both placebo or alternative CL and ML treatments. Cure rate in humans, change in lesion diameter in animals, promastigote and amastigote viability and growth, and adverse events served as outcome.

Methods

Search strategy

This systematic review, registered in PROSPERO (registration number CRD42018090687, 2018) and available at: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=90687, followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [16]. A medical information specialist (JL) searched the following electronic databases for studies on leishmaniasis and allylamines, using controlled terms and text words, from their creation to May 24th, 2020: MEDLINE (OVID), EMBASE (OVID), the Cochrane Central Register of Controlled Trials (CENTRAL), The Global Health Library, Web of Science, Google Scholar (1st 150 hits) and the clinical

trial registers, ClinicalTrials.gov and WHO_ICTRP. No language, date or other restrictions were applied. The complete search strategies are presented in the S1 File. Reference lists and the citing articles of the identified relevant papers were cross-checked in Web of Science for additional relevant studies. The records retrieved were imported and de-duplicated in EndNote.

Study eligibility

All original human, animal, and *in vitro* studies were eligible if they examined the effects of systemic or topical allylamines with the following endpoints: cure rate in humans, skin lesion diameter in animals and promastigote or amastigote *in vitro* growth or viability in the laboratory. The presence of *Leishmania* parasites had to be confirmed in the study by either microscopy, culture, or molecular techniques. If one *Leishmania* species was known to cause >90% of the CL or ML cases in the study area in human studies, this species might be assumed as the causative species in all patients.

Study selection

JB and JvdE independently screened the identified studies using EndNote and resolved differences through discussion or consultation with a third reviewer (HS). Studies included during title and abstract screening were subsequently assessed as full text. Authors of conference abstracts were contacted to request unpublished data. If the full report was written in another language than English, Spanish, German, Dutch, French, or Portuguese authors were requested to provide a translation.

Data extraction

The following data from all included studies were entered in Excel: study setting, study population, probable *Leishmania* species, allylamine studied and treatment combinations. From human studies the following information was recorded: age, gender, lesion type and duration, drug presentation, treatment scheme, cure rates, adverse events, and information for assessment of risk of bias. Cure rates were calculated according to intention to treat analysis and cure was defined as complete epithelialization of ulcers or decrease in induration size > 75% of nodules at last available follow up. From animal studies the following information was recorded: age, gender, lesion type and duration, drug presentation, treatment scheme, effect on lesion diameter, adverse events, and information for assessment of risk of bias. From *in vitro* studies the following information was recorded: drug concentrations, promastigote or amastigote growth or viability, and culture cytotoxicity. JB and JvdE extracted data in duplicate and resolved differences through discussion.

Risk of bias assessment

JB and JvdE independently assessed the quality of the clinical trials and resolved differences through discussion. Randomised controlled trials were assessed using the revised Cochrane collaborations tool (RoB2) and non-randomised controlled trials with the Cochrane tool for non-randomised controlled trials (ROBINS-1) [17, 18]. Animal studies were assessed with the SYRCLE´s risk of bias assessment tool [19]. *In vitro* studies were assessed with the tool developed by the United States national toxicology program [20]. Results of risk of bias assessments were visualized using the Cochrane risk-of-bias visualization tool [17].

Results

Studies included

The literature search identified 312 manuscripts of which 75 were included for full text assessment after screening of titles and abstract. After full text examination, 22 studies were included. Major reasons for exclusion were: `different topic´ and `textbook or review´. The data of two conference abstracts, could not be retrieved by contacting the authors [21, 22], and were therefore excluded from the study. The authors of a study presented in Chinese and another study in Farsi could not provide the data or the English translation of the report and these studies were therefore excluded [23, 24] (Fig 1).

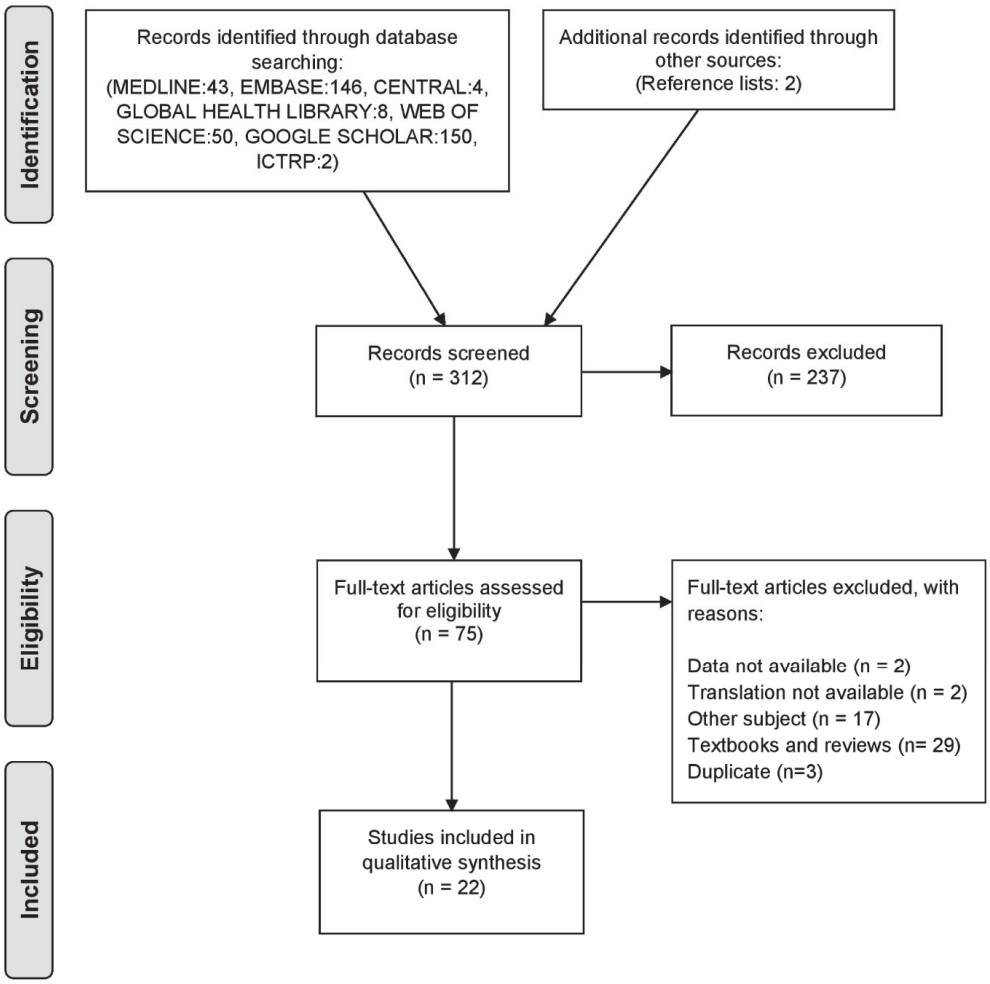


Fig 1. PRISMA literature assessment flow diagram.

Characteristics of the included human trials (n = 3) [25-27], mice studies (n = 2) [28, 29], and

amastigote and promastigote studies (n = 12) [11, 30-40] are presented in Tables 1 and 2. The case reports (n = 5) [41-45] are presented in S1 Table.

Table 1. Characteristics of human and mice trials reporting treatment of cutaneous leishmaniasis with terbinafine

First Author	Farajzadeh [25]	Farajzadeh [26]	Bahamdan [27]	Zakai [28]	Sampaio [29]
Year	2015	2016	1997	2000	2003
***Leishmania* species**	*tropica*	*tropica*	*tropica*	*major*	*amazonensis*
Number of participants	40	44	27	20	15
Number of controls	40	44	NA	40	29
Presentation	systemic	topical	systemic	systemic	systemic
Combination	cryotherapy	meglumine antimoniate	NA	NA	NA
Dose / day	125-500mg	32,25-75.5mg	500mg	0,2mg	100mg/kg
Days treated	28	20	28	28	20
Control 1 treatment	meglumine antimoniate + cryotherapy	meglumine antimoniate + placebo	NA	untreated	placebo
Control 2 treatment	NA	NA	NA	Itraconazole	sodium stibogluconate
Mean Lesion diameter (mm)	NA	NA	NA	5[c]	35[b]
Control 1 mean lesion diameter	NA	NA	NA	7	36
Control 2 mean lesion diameter	NA	NA	NA	1[c]	28[c]
Cure rate[a]	0,38	0,14	0,15	NA	NA
Control cure rate[a]	0,53	0,20	NA	NA	NA
Adverse event rate	none	none	none	none	none

NA: Not Applicable

[a] Defined as complete epithelialization of ulcers or decrease in induration size > 75% of nodules at last available follow up and calculated according to intention to treat analysis

[b] no significant difference with untreated controls

[c] significant difference with untreated controls

Table 2. Characteristics of *in vitro* studies reporting on effects of allylamines in cutaneous and mucocutaneous *Leishmania* species

First Author	Andrade Neto [30]	Andrade Neto [31]		Andrade Neto [32]	Beach [33]	Berman [34]		Bezerra Souza [35]		Chance [36]		Goad [11]	Rangel [37]	Tariq [38]	Vannier-Santos [39]		Zakai [40]	
Year *Leishmania* species	2013 *amazonensis*	2011 *amazonensis*		2009 *amazonensis*	1989 multiple	1987 *major*		2016 *amazonensis / braziliensis*		1999 *amazonensis*		1985 *mexicana*	1996 *mexicana / braziliensis*	1994 *tropica*	1995 *amazonensis*		2003 *major*	
Allylamine	terbinafine	terbinafine		terbinafine	terbinafine	terbinafine		butenafine	naftifine	terbinafine		terbinafine	terbinafine	terbinafine	terbinafine		terbinafine	
Combination	LBqT01 / imipramine	NA	imipramine	NA	NA	NA	NA	NA	NA	NA	NA	NA	ketoconazole / D0870	NA	ketoconazole	NA	NA	NA
***Leishmania* model**	amastigote	promastigote	promastigote	promastigote	promastigote	amastigote	promastigote	amastigote	amastigote	promastigote	promastigote	promastigote	promastigote	promastigote	amastigote	promastigote	promastigote	promastigote
Amastigote host cell type	mice peritoneal macrophages	NA	NA	NA	NA	human monocyte derived macrophages	human monocyte derived macrophages	mice peritoneal macrophages		NA	NA	NA	NA	NA	NA	NA	mice	NA
Host cell toxic concentration (µM)	80	NA	NA	NA	NA	>110	>110	CC50: 98		NA	NA	NA	NA	NA	NA	NA	NA	NA
Effective concentration (µM)	23	15	8	4-9	27	31	45	30 -38		34-81	34	34	5-15	1373	0,001 [a]	1	6	No effect
Parameter of effectivity	IC50	IC50	IC50	IC50	26-93% growth inhibition	ED50	ED50	ED50		ED50	>2-fold increase of squalene	MIC	MIC	MIC	MIC	MIC	IC50	NA

NA: Not Applicable, IC50: Half maximal inhibitory concentration, ED50: Median effective dose, MIC: Minimum inhibitory concentration

[a] combined with 0,001µM Ketoconazole

Risk of bias assessment

Two randomised controlled clinical trials [25, 26], a one arm non randomised trial [27], and two animal trials [28, 29] were assessed for risk of bias. Farajzadehs randomised controlled trial in 2015 had an acceptable risk of bias [25]. The study of Farajzadeh from 2016 lost 73% of patients to follow up [26] and Bahamdans study had severe deviations from intended interventions and 48% loss to follow up [27], leading to an overall judgement of high risk of bias for both. The two mice studies suffered from high risk of bias in various domains including allocation concealment and blinding of outcome assessment [28, 29]. The 12 *in vitro* studies presented minor methodological risks of bias (Figs 2-5).

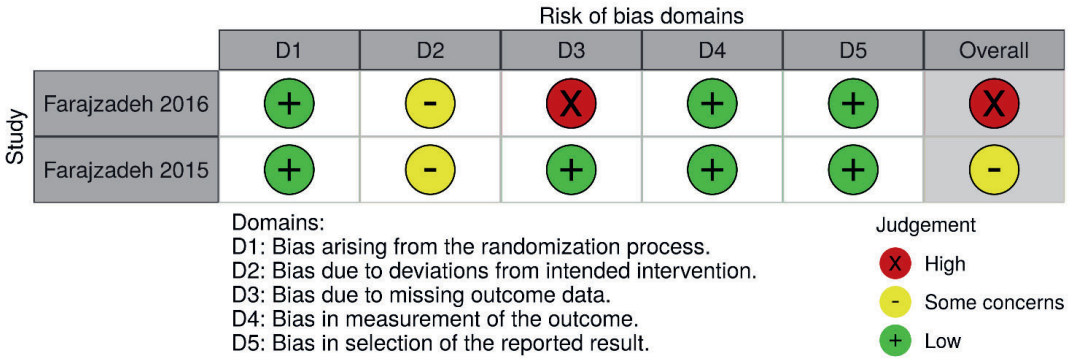


Fig 2. Risk of Bias assessment of randomised controlled trials. The Revised Cochrane risk-of-bias tool for randomised trials (RoB2) was applied for the evaluation.

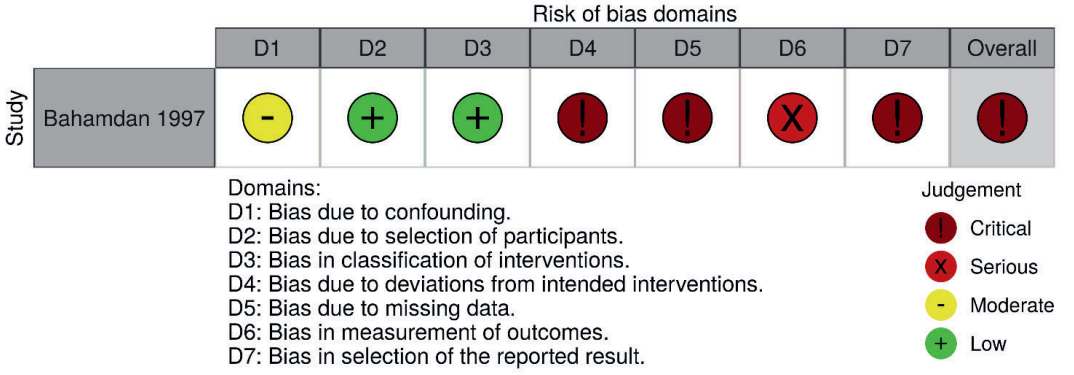


Fig 3. Risk of Bias assessment of a non-randomised study in humans. The Risk Of Bias In Non-randomised Studies – of Interventions (ROBINS-I) assessment tool was applied for the evaluation.

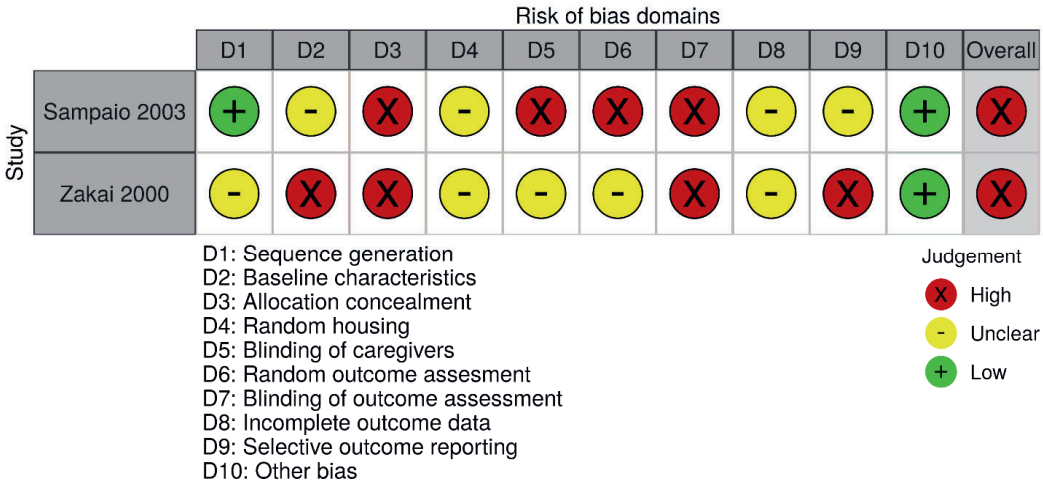


Fig 4. Risk of Bias assessment of animal trials. SYRCLE's risk-of-bias tool for animal studies was applied for the evaluation.

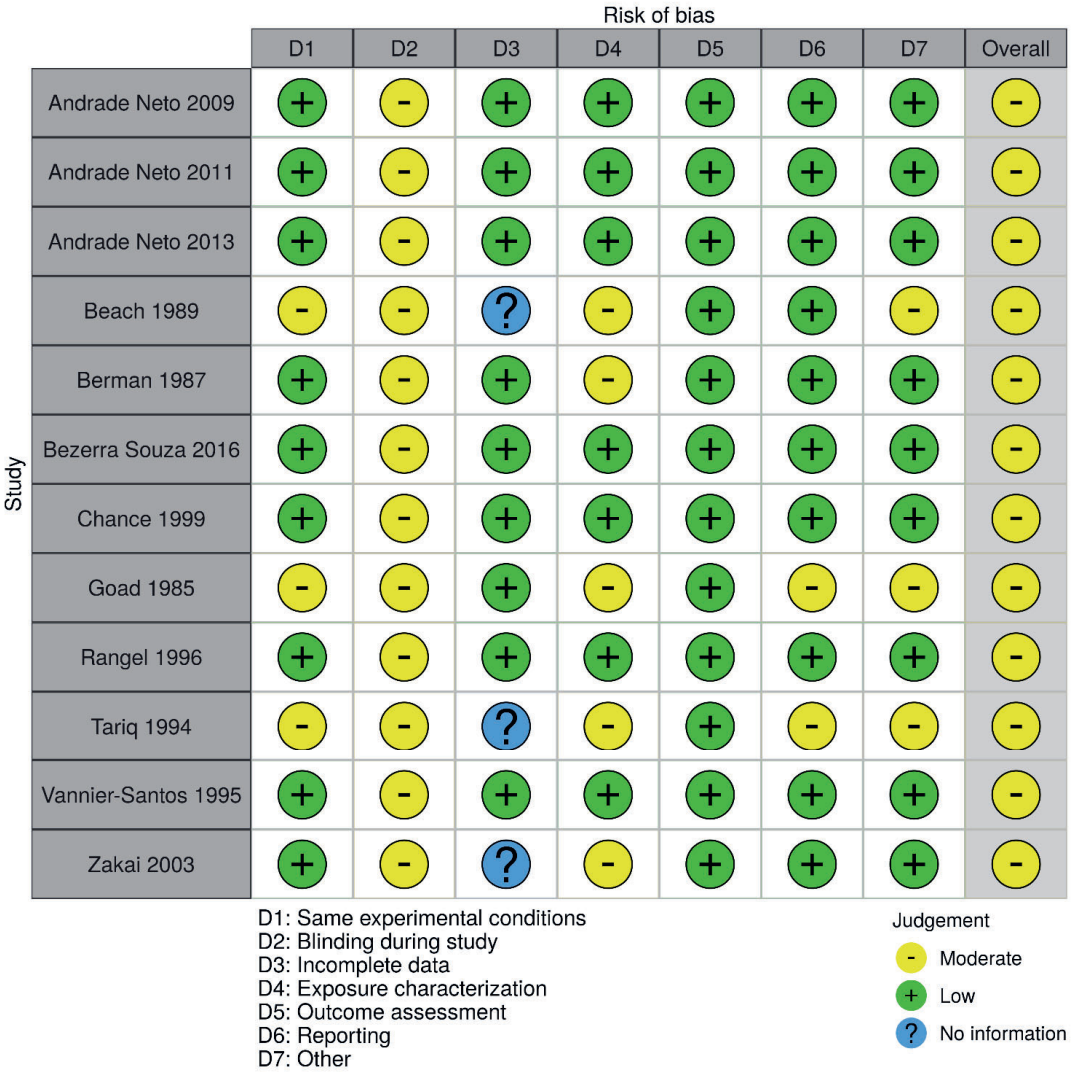


Fig 5. Risk of Bias assessment of in vitro studies. The risk-of-bias tool to address in vitro studies developed by the United States national toxicology program was applied for the evaluation.

It was not possible to perform a meta-analysis of the study outcomes, due to the small number of studies, their heterogeneity and low quality.

Efficacy of terbinafine on *L. tropica* human infections and adverse events

The clinical trial of Farajzadeh 2015 [25] included 80 *L. tropica* infected patients randomised between two different treatment groups: 1) oral terbinafine 125-500mg (weight dependent) daily during four weeks, combined with cryotherapy every two weeks (n = 40) and 2) meglumine antimoniate 15mg/kg/day for three weeks combined with cryotherapy every two weeks (n = 40). Complete follow up was achieved for all patients at three months. Contrary to the hazard ratios

presented by Farajzadeh, in this review the endpoint was the complete cure rate. In the terbinafine arm 15/40 (38%) patients were cured and in the meglumine antimoniate arm 21/40 (53%) cases were cured, a difference that was not statistically significant in Kaplan Meier analysis (p = 0,39). None of the in vivo studies reported adverse events [25-29].

Species specific effectivity of allylamine treatments

Growth inhibition of terbinafine in promastigote *in vitro* studies was reported for the *L. major*, *L. tropica*, *L. mexicana*, *L. braziliensis*, and *L. guyanensis* complexes. An interspecies comparison in promastigote cultures with terbinafine 27µM showed higher inhibition levels in old world (*L. major*, *L. tropica*, and *L. aethiopica*) species (Table 3).

Table 3. Overview of clinical and *in vitro Leishmania* species specific results of terbinafine in cutaneous leishmaniasis

Leishmania Complex	*Leishmania* Species	Growth inhibition in promastigotes at 27µM	Effective doses in promastigotes	Effective doses in amastigotes	Cure rate in clinical study [a]
major	*major*	52-90%	IC50: 6µM	ED50: 31µM	NA
tropica	*tropica*	92-93%	MIC: 1373µM	NA	38%
	aethiopica	90%	NA	NA	NA
mexicana	*mexicana*	26%	no inhibition / MIC: 15-34µM	NA	NA
	amazonensis	74%	IC50: 4 - 9µM / MIC: 1µM	IC50: 23µM / MIC: 0,001µM [b]	NA
braziliensis	*braziliensis*	72%	MIC: 1-5µM	NA	NA
guyanensis	*guyanensis*	49%	NA	NA	NA
	panamanensis	41%	NA	NA	NA

IC50: Half IC50: maximal inhibitory concentration, ED50: Median effective dose, MIC: Minimum inhibitory concentration, NA: Not Applicable

[a] Defined as decrease in induration size > 75% of lesions at last available at follow up and calculated according to intention to treat analysis

[b] combined with 0,001µM Ketoconazole

Treatment with butenafine killed *in vitro* cultured amastigotes of *L. amazonensis* and *L. braziliensis* at a mean effective dose of 30-38µM compared to the median cytotoxic concentration of 98µM [35]. Naftifine killed *L. major* amastigotes with mean effective dose of 45µM whilst cytotoxicity levels were more than 110µM [34].

Case reports reporting cure with terbinafine

There were five case reports showing a curative effect of terbinafine. In two case reports terbinafine cured a *L. tropica* infected patient although the reason to start terbinafine was unclear [41, 42]. An HIV positive patient infected in Colombia and initially diagnosed with a skin mycosis, was treated, and cured with terbinafine when CL was diagnosed eventually. The causative *Leishmania* species was unknown [43]. In another case, terbinafine 250mg daily combined with a Crotamiton 10% + Sulphur 2% cream in the absence of other CL treatments cured a Kenyan patient with CL; the causative *Leishmania* species was unknown [44]. Terbinafine 500mg combined with itraconazole 200mg daily for six months was started without evident reason in a patient suffering from MCL, visceral leishmaniasis, and liver cirrhosis caused by *L. infantum*. Terbinafine proved

surprisingly effective resulting in the cure of the nasal mucosal inflammation and improvement of the liver function [45].

Terbinafine drug combination treatment

Various *in vitro* studies evaluated the combination of terbinafine with drugs from the triazole group. Up to 300-fold improvement was demonstrated of the inhibition of *L. braziliensis* promastigotes when combining ketoconazole with terbinafine [37]. Another study reported that ketoconazole and terbinafine had a synergistic effect on the inhibition of *L. amazonensis* amastigotes resulting in a minimally inhibitory concentration of 0,001µM (Table 4) [39].

Table 4. Results of *in vitro* and clinical studies on the combination of terbinafine with other treatment in cutaneous and mucocutaneous leishmaniasis

Study	*Leishmania* species	Target	Combined therapy	Result
Andrade-Neto 2013[30]	*L. amazonensis*	promastigote	LBqT01	synergistic effect[a]
Andrade-Neto 2013	*L. amazonensis*	promastigote	imipramine	additive effect[b]
Andrade-Neto 2013	*L. amazonensis*	amastigote	LBqT01	no significant effect
Andrade-Neto 2013	*L. amazonensis*	amastigote	imipramine	no significant effect
Vannier Santos 1995 [39]	*L. amazonensis*	amastigote	ketoconazole	synergistic effect[c]
Rangel 1996 [37]	*L. braziliensis*	promastigote	ketoconazole	synergistic effect[d]
Rangel 1996	*L. braziliensis*	promastigote	D0870	synergistic effect[d]
Vellin 2005[45]	*L. infantum*	MCL	itraconazole	complete epithelialization
Mawenzi 2018[44]	unknown	CL	crotamiton + sulfur	complete epithelialization
Farajzadeh 2015[25]	*L. tropica*	CL	cryotherapy	no significant effect
Farajzadeh 2016[26]	*L. tropica*	CL	meglumine antimoniate	no significant effect

[a] Synergism defined as fractional inhibitory concentration index (FICI) ≤ 0,5
[b] Additive effect defined as: 0,5 < FICI < 4
[c] Synergism defined as total fractional inhibition higher than expected from adding up the fractional inhibition of each individual drug
[d] Synergism defined as 300-fold reduction of the Minimum Inhibitory Concentration of ketoconazole with 1µM terbinafine.

Discussion

This systematic review assesses efficacy and safety of allylamines for the treatment of CL and MCL. It comprises an exhaustive search of eight electronic databases and trial registers. It assesses the risk of bias of two randomised controlled trials, a non-controlled trial, two animal studies, and 12 *in vitro* studies and summarizes the available evidence including five case reports. Generally, the quality of evidence was low and human studies were done only in *L. tropica*.
The only well-designed randomised controlled trial of Farajzadeh *et al*. that compared the treatment efficacy of oral terbinafine versus intramuscular meglumine antimoniate showed a non-significant lower cure rate for terbinafine (38% vs 53% of treated patients) [25].
Farajzadeh [26] and Bahmdans [27] clinical trials with terbinafine reported cure rates of 14% and 15% respectively, but the findings of these studies should be interpreted with caution due to high rates of loss to follow up. Two animal studies lacked allocation concealment and did not blind

outcome assessment and therefore should be interpreted with caution [28, 29].

The *in vitro* studies showed that terbinafine, butenafine, and naftifine eliminated amastigotes at concentrations between 23 and 45µM, that is approximately five times higher than the terbinafine levels achieved in the skin during terbinafine treatment [46-48]. Therefore, we conclude that allylamines are not promising for CL and MCL treatment

Farajzadeh recommends terbinafine as an alternative to meglumine antimoniate in the case of allergy or resistance [25]. Although the work reports on hazard ratios and time to healing, it does not mention the complete cure rates in the abstract and conclusion sections. The cure rate of 38% was not significantly lower than the 53% cure rate with meglumine antimoniate, and we consider it too low to propose it as a new alternative treatment. The lack of significance of the lower cure rate of terbinafine compared to meglumine antimoniate could be explained by a low effectivity of the latter.

Whilst this review shows that there is no evidence for efficacy of terbinafine in the treatment of CL and MCL, it is highly effective in the treatment of mycotic skin disease. The difference may be due to the high sensitivity of skin fungus to terbinafine compared to *Leishmania* amastigotes. Terbinafine eliminates skin fungus *in vitro* at a mean concentration of 0,014µM [49], thus is approximately 2500 times more effective than the elimination of *Leishmania* amastigotes. Promastigote cultures are relatively easy and cheap to maintain but are not very reliable as predictors of in vivo effectivity as they represent the infective mosquito stage of the parasite whilst human infection is sustained by intracellular amastigotes [50, 51]. Therefore, the results of the *in vitro* study of Beach *et al*. that indicates effective concentrations of 1-34µM of terbinafine in *L. braziliensis* and *L. amazonensis* promastigotes should be interpreted with caution. Results of promastigote studies must be confirmed in amastigote studies.

Although triazole monotherapy does not seem effective as treatment of CL patients, results from *in vitro* studies indicate terbinafine combined with triazole drugs may be effective through a synergistic effect. Terbinafine combined with ketoconazole eliminated *L. amazonensis* amastigotes at levels of 0,001µM of both drugs. Terbinafine would reach those levels with an oral dose of 250mg but the best combination with a triazole drug still has to be defined [46]. Triazole drugs like ketoconazole and fluconazole are inhibitors of the enzymes CYP 2C9 and CYP 3A4, involved in terbinafine metabolism, and may cause significant rise in terbinafine plasma concentrations. Secondary effects of terbinafine combined with triazoles have not been studied extensively and would require large clinical studies before implementation [52, 53].

Conclusion

Based on a systematic review of available literature we conclude that there is no evidence for the efficacy of allylamine monotherapy against CL and MCL. Further trials of allylamines as a treatment for CL and MCL should be carefully considered as the outcomes of an adequately designed trial were disappointing and *in vitro* studies indicate minimal effective concentrations that are not achieved in the skin during standard doses of 250-1000mg oral terbinafine/day. However, the *in vitro* synergistic effects of allylamines combined with triazole drugs against amastigotes, warrant more investigation starting with high quality animal studies to define optimal doses and safety profiles and followed by well-designed trials in humans in case of positive findings.

Declarations

Availability of data and materials

All relevant data are within the paper and its Supporting Information files.

Competing interest

JB is a volunteer for Latin Link Nederland and receives a monthly volunteer allowance from this organization. JvdE is a volunteer for Fundación Quina Care Ecuador and receives a monthly volunteer allowance from this organization. There are no patents, products in development or marketed products associated with this research to declare. This does not alter our adherence to PLOS ONE policies on sharing data.

Funding

JB received a monthly volunteer allowance from Latin Link Nederland http://www.latinlink-nederland.nl/, which helped fund the study. JvdE is a volunteer for Fundación Quina Care Ecuador and receives a monthly volunteer allowance from this organization which also helped fund the study. Latin Link and Fundación Quina Care Ecuador had no role in study design, data collection and analysis, decision to publish, preparation of the manuscript. No additional external funding was received for this study.

Author contributions

Conceptualization: Jacob M. Bezemer, Jacob van der Ende, Jacqueline Limpens, Henry J. C. de Vries, Henk D. F. H. Schallig.
Formal analysis: Jacob M. Bezemer, Jacob van der Ende, Jacqueline Limpens.
Investigation: Jacob M. Bezemer, Jacob van der Ende, Jacqueline Limpens.
Methodology: Jacob M. Bezemer, Jacob van der Ende, Jacqueline Limpens.
Project administration: Jacob M. Bezemer.
Resources: Henry J. C. de Vries, Henk D. F. H. Schallig.
Software: Jacqueline Limpens.
Supervision: Henry J. C. de Vries, Henk D. F. H. Schallig.
Validation: Jacqueline Limpens, Henk D. F. H. Schallig.
Writing – original draft: Jacob M. Bezemer.
Writing – review & editing: Jacob van der Ende, Jacqueline Limpens, Henry J. C. de Vries, Henk D. F. H. Schallig.

References

1. Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS ONE. 2012;7(5):e35671.
2. Molyneux DH, Savioli L, Engels D. Neglected tropical diseases: progress towards addressing the chronic pandemic. Lancet (London, England). 2017;389(10066):312-25.
3. Cincura C, De Lima CMF, Machado PRL, Oliveira-Filho J, Glesby MJ, Lessa MM, et al. Mucosal leishmaniasis: A retrospective study of 327 cases from an endemic area of Leishmania (Viannia) braziliensis. American Journal of Tropical Medicine and Hygiene. 2017;97(3):761-6.
4. Hodiamont CJ, Kager PA, Bart A, de Vries HJ, van Thiel PP, Leenstra T, et al. Species-directed therapy for leishmaniasis in returning travellers: a comprehensive guide. PLoS neglected tropical diseases. 2014;8(5):e2832.
5. Pinart M, Rueda JR, Romero GA, Pinzón-Flórez CE, Osorio-Arango K, Silveira Maia-Elkhoury AN, et al. Interventions for American cutaneous and mucocutaneous leishmaniasis. The Cochrane database of systematic reviews. 2020;8:Cd004834.
6. Heras-Mosteiro J, Monge-Maillo B, Pinart M, Lopez Pereira P, Reveiz L, Garcia-Carrasco E, et al. Interventions for Old World cutaneous leishmaniasis. The Cochrane database of systematic reviews. 2017;12(12):Cd005067.
7. Sunyoto T, Potet J, Boelaert M. Why miltefosine-a life-saving drug for leishmaniasis-is unavailable to people who need it the most. BMJ global health. 2018;3(3):e000709.
8. Oliveira LF, Schubach AO, Martins MM, Passos SL, Oliveira RV, Marzochi MC, et al. Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. Acta tropica. 2011;118(2):87-96.
9. Coelho DR, De-Carvalho RR, Rocha RC, Saint'Pierre TD, Paumgartten FJ. Effects of in utero and lactational exposure to SbV on rat neurobehavioral development and fertility. Reproductive toxicology (Elmsford, NY). 2014;50:98-107.
10. de Vries HJ, Reedijk SH, Schallig HD. Cutaneous leishmaniasis: recent developments in diagnosis and management. American journal of clinical dermatology. 2015;16(2):99-109.
11. Goad LJ, Holz GG, Jr., Beach DH. Effect of the allylamine antifungal drug SF 86-327 on the growth and sterol synthesis of Leishmania mexicana mexicana promastigotes. Biochem Pharmacol. 1985;34(20):3785-8.
12. Petranyi G, Ryder NS, Stutz A. Allylamine derivatives: new class of synthetic antifungal agents inhibiting fungal squalene epoxidase. Science. 1984;224(4654):1239-41.
13. Kreijkamp-Kaspers S, Hawke K, Guo L, Kerin G, Bell-Syer SE, Magin P, et al. Oral antifungal medication for toenail onychomycosis. Cochrane Database Syst Rev. 2017;7:Cd010031.
14. Kaul S, Yadav S, Dogra S. Treatment of Dermatophytosis in Elderly, Children, and Pregnant Women. Indian dermatology online journal. 2017;8(5):310-8.
15. Terbinafine. Drugs and Lactation Database (LactMed). Bethesda (MD): National Library of Medicine (US); 2006.
16. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS medicine. 2009;6(7):e1000100.
17. Higgins JPT SJ, Savović J, Page MJ, Hróbjartsson A, Boutron I, Reeves B, Eldridge S. A. A revised tool for assessing risk of bias in randomized trials. Cochrane Methods Cochrane Database of Systematic Reviews 2016(10):(Suppl 1).
18. Sterne JAC, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomized studies of interventions. british journal of medicine. 2016;355(4919).
19. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC medical research methodology. 2014;14:43.
20. Roony A. Extending a Risk-of-Bias Approach to Address In Vitro Studies. In: OHAT, editor. Washington, USA: National Toxicology Program Office of Health Assessment and Translation; 2015.

21. Ukşal Ü, Şahin İ, Durgut S, Özcan H, Gürel MS. In vitro and in vivo effect of terbinafine in cutaneous leishmaniasis. Journal of the European Academy of Dermatology and Venereology. 1998;11(Suppl 2):s242.
22. Bonatto AF, Bonatto SF, Sampaio RNR, Vilela H, Vexenat A. Estudo da eficácia da terbinafina, in vitro. Resumos do IV Congresso de Iniciação Científica da Universidade de Brasília 1998. p. 128.
23. Lu-juan GAO, Jin YU, Yaguchi T, Wei C, Ruo-yu LI. A case of cutaneous leishmaniasis misdiagnosed as cutaneous aspergillosis and successfully treated with terbinafine. Chinese Journal of Dermatology. 2011;44(11):811-3.
24. Ebrahimian S, Asilian A, Faghihi G. Comparetaive Study on Glucantime and Oral Terbinafine along with Systemic Glucantime on Cutaneous Leishmaniasis. J Isfahan Med Sch. 2011;28(118):1246-52.
25. Farajzadeh S, Esfandiarpour I, Haghdoost AA, Mohammadi S, Mohebbi A, Mohebbi E, et al. Comparison between Combination Therapy of Oral Terbinafine and Cryotherapy versus Systemic Meglumine Antimoniate and Cryotherapy in Cutaneous Leishmaniasis: A Randomized Clinical Trial. Iranian J Parasitol. 2015;10(1):1-8.
26. Farajzadeh S, Heshmatkhah A, Vares B, Mohebbi E, Mohebbi A, Aflatoonian M, et al. Topical terbinafine in the treatment of cutaneous leishmaniasis: triple blind randomized clinical trial. J Parasit Dis. 2016;40(4):1159-64.
27. Bahamdan KA, Tallab TM, Johargi H, Nourad MM, Ibrahim K, el Sherbini AH, et al. Terbinafine in the treatment of cutaneous leishmaniasis: a pilot study. Int J Dermatol. 1997;36(1):59-60.
28. Zakai HA, Zimmo SK. Effects of itraconazole and terbinafine on Leishmania major lesions in BALB/c mice. Ann Trop Med Parasitol. 2000;94(8):787-91.
29. Sampaio RN, Takano GH, Malacarne AC, Pereira TR, de Magalhaes AV. [In vivo Terbinafine inefficacy on cutaneous leishmaniasis caused by Leishmania (Leishmania) amazonensis in C57BL/6 mice]. Rev Soc Bras Med Trop. 2003;36(4):531-3.
30. Andrade-Neto VV. Papel do colesterol exógeno nos mecanismos de adaptação de Leishmania spp a condições de estresse metabólico e farmacológico. Rio de Janeiro: Fundação Oswaldo Cruz. Instituto Oswaldo Cruz; 2013.
31. Andrade-Neto VV, Cicco NN, Cunha-Junior EF, Canto-Cavalheiro MM, Atella GC, Torres-Santos EC. The pharmacological inhibition of sterol biosynthesis in Leishmania is counteracted by enhancement of LDL endocytosis. Acta Trop. 2011;119(2-3):194-8.
32. Andrade-Neto VV. Avaliação da utilização de colesterol exógeno por Leishmania amazonensis como possível alvo farmacológico. Rio de Janeiro: Fundação Oswaldo Cruz. Instituto Oswaldo Cruz; 2009.
33. Beach DH, Goad LJ, Berman JD, Ellenberger TE. Effects of a squalene-2, 3-epoxidase inhibitor on propagation and sterol biosynthesis of Leishmania promastigotes and amastigotes. In: Hart DT, editor. Leishmaniasis. New York, USA: Plenum; 1989.
34. Berman JD, Gallalee JV. In vitro antileishmanial activity of inhibitors of steroid biosynthesis and combinations of antileishmanial agents. J Parasitol. 1987;73(3):671-3.
35. Bezerra-Souza A, Yamamoto ES, Laurenti MD, Ribeiro SP, Passero LF. The antifungal compound butenafine eliminates promastigote and amastigote forms of Leishmania (Leishmania) amazonensis and Leishmania (Viannia) braziliensis. Parasitol Int. 2016;65(6 Pt A):702-7.
36. Chance ML, Havercroft PR, Goad LJ. Observations on leucine incorporation into sterol by Leishmania, and its inhibition by terbinafine. Ann Trop Med Parasitol. 1999;93(2):185-8.
37. Rangel H, Dagger F, Hernandez A, Liendo A, Urbina JA. Naturally azole-resistant Leishmania braziliensis promastigotes are rendered susceptible in the presence of terbinafine: comparative study with azole-susceptible Leishmania mexicana promastigotes. Antimicrob Agents Chemother. 1996;40(12):2785-91.
38. Tariq P, Khan KA. Comparative study on susceptibility of Leishmania isolated from dermal leishmaniasis to antifungal drug terbinafine and classic antileishmanial drugs. Pakistan Journal of Pharmacology. 1994;11(1):9-17.
39. Vannier-Santos MA, Urbina JA, Martiny A, Neves A, de Souza W. Alterations induced by the antifungal compounds ketoconazole and terbinafine in Leishmania. J Eukaryot Microbiol. 1995;42(4):337-46.
40. Zakai HA, Zimmo S, Fouad MA. Effect of itraconazole and terbinafine on Leishmania promastigotes. J Egypt Soc Parasitol. 2003;33(1):97-107.

41. Scarisbrick JJ, Chiodini PL, Watson J, Moody A, Armstrong M, Lockwood D, et al. Clinical features and diagnosis of 42 travellers with cutaneous leishmaniasis. Travel Medicine and Infectious Disease. 2006;4(1):14-21.
42. Albanese G, Di Cintio R, Galbiati G. Cutaneous leishmaniasis in the north of Italy. Int J Dermatol. 1996;35:223-4.
43. Gonzalez-Ruperez J, Javaloyas de Morlius M, Moreno Carazo A. Remission of localized cutaneous leishmaniasis in a HIV-positive patient using systemic terbinafine. Dermatology. 1997;194(1):85-6.
44. Mawenzi RL. A case of cutaneous leishmaniasis successfully treated with oral terbinafine in Kenya. International Journal of Research in Dermatology. 2018;4(3):433-6.
45. Vellin JF, Russier M, Mougeot G, Kemeny JL, Gilain L. Leishmaniose nasale. Annales d'Otolaryngologie et de Chirurgie Cervico-faciale. 2005;122(2):100-4.
46. Faergemann J, Zehender H, Denouel J, Millerioux L. Levels of terbinafine in plasma, stratum corneum, dermis-epidermis (without stratum corneum), sebum, hair and nails during and after 250 mg terbinafine orally once per day for four weeks. Acta dermato-venereologica. 1993;73(4):305-9.
47. Jensen JC. Clinical pharmacokinetics of terbinafine (Lamisil). Clinical and experimental dermatology. 1989;14(2):110-3.
48. Jones TC. Overview of the use of terbinafine (Lamisil) in children. The British journal of dermatology. 1995;132(5):683-9.
49. Leyden J. Pharmacokinetics and pharmacology of terbinafine and itraconazole. Journal of the American Academy of Dermatology. 1998;38(5 Pt 3):S42-7.
50. van den Bogaart E, Schoone GJ, England P, Faber D, Orrling KM, Dujardin JC, et al. Simple colorimetric trypanothione reductase-based assay for high-throughput screening of drugs against Leishmania intracellular amastigotes. Antimicrob Agents Chemother. 2014;58(1):527-35.
51. De Muylder G, Ang KK, Chen S, Arkin MR, Engel JC, McKerrow JH. A screen against Leishmania intracellular amastigotes: comparison to a promastigote screen and identification of a host cell-specific hit. PLoS Negl Trop Dis. 2011;5(7):e1253.
52. Gupta AK, Daigle D, Paquet M. Therapies for onychomycosis a systematic review and network meta-analysis of mycological cure. Journal of the American Podiatric Medical Association. 2015;105(4):357-66.
53. Gupta AK, Versteeg SG, Shear NH. Common drug-drug interactions in antifungal treatments for superficial fungal infections. Expert opinion on drug metabolism & toxicology. 2018;14(4):387-98.

Supporting information

S1 Table. Characteristics of case reports
This file can be accessed with the following link:
https://doi.org/10.1371/journal.pone.0249628.s001

S1 File. Full electronic search
This file can be accessed with the following link:
https://doi.org/10.1371/journal.pone.0249628.s002

S2 File. PRISMA 2009 checklist
This file can be accessed with the following link:
https://doi.org/10.1371/journal.pone.0249628.s003

Chapter 6

Quality of life of cutaneous leishmaniasis suspected patients in the Ecuadorian Pacific and Amazon regions: a cross sectional study

Jacob M Bezemer, Manuel Calvopiña, Andrea E Corral Zabala, Fernando Ortega Pérez, Veronica C Vargas Román, Henk D F H Schallig, Henry J C de Vries

BMC Infectious Diseases. 2022; 22(1):748

Abstract

Background

Yearly, up to 1 million patients worldwide suffer from Cutaneous Leishmaniasis (CL). In Ecuador, CL affects an estimated 5000 patients annually. CL leads to reduced Health Related Quality of Life (HRQL) as a result of stigma in the Asian and Mediterranean contexts, but research is lacking for Ecuador. The objective of this study was to explore the influence of CL suspected lesions on the quality of life of patients in the Pacific and Amazon regions.

Methods

Patients for this study were included in the Amazonian Napo, Pastaza, and Morona Santiago provinces and the Pacific region of the Pichincha province. Participating centers offered free of charge CL treatment. All patients suspected of CL and referred for a cutaneous smear slide microscopy examination were eligible. This study applied the Skindex-29 questionnaire, a generic tool to measure HRQL in patients with skin diseases. All statistical analysis was done with SPSS Statistics version 28.

Results

The skindex-29 questionnaire was completed adequately by 279 patients who were included in this study. All patient groups from the Amazon scored significantly ($P < 0.01$) higher (indicating worse HRQL) on all the dimensions of the Skindex-29 questionnaire than Mestizo patients from the Pacific region. The percentage of patients with health seeking delay of less than a month was significantly ($P < 0.01$) lower in the Amazon region (38%) than in the Pacific (66%).

Conclusions

The present study revealed that the influence of suspected CL lesions on the HRQL of patients in the Ecuadorian Amazon and Pacific depends on the geographic region more than on patient characteristics such as gender, age, number of lesions, lesion type, location of lesions, health seeking delay, or posterior confirmation of the *Leishmania* parasite. The health seeking delay in the Amazon might result from a lack of health infrastructure or related stigma. Together, the impaired HRQL and prolonged health seeking delay in the Amazon lead to prolonged suffering and a worse health outcome. Determinants of health seeking delay should be clarified in future studies and CL case finding must be improved. Moreover, HRQL analysis in other CL endemic regions could improve local health management.

Keywords

Leishmaniasis, cutaneous; Quality of life; Time-to-treatment; Geographic locations; Ecuador

Background

Yearly, up to 1 million patients worldwide are affected by Cutaneous Leishmaniasis (CL), and almost a third of those cases occur in South America [1, 2]. CL is a vector-borne parasitic disease, mainly characterized by cutaneous ulcers, and is considered a Neglected Tropical Disease by the World Health Organization (WHO)[2]. CL affects between 3900 and 6400 patients yearly in Ecuador, with clusters in the subtropical Pacific and Amazon regions. Mucosal Leishmaniasis (ML) occurs in approximately 2.5% of the Ecuadorian cases, mainly in the Amazon region [3, 4]. CL leads to reduced Health Related Quality of Life (HRQL) as a result of social and self-stigma, as has been established in the Asian and Mediterranean contexts [5]. In contrast, the few studies that assessed HRQL of CL patients in northern South America found no evidence for stigmatization [6, 7]. CL patients reported reduced self-esteem in the Ecuadorian subtropical Pacific in 1994 but no follow up was done [8]. Ecuador's Amerindian population has been marginalized and discriminated against since colonial history, echoing in health access inequalities despite contemporary constitutional equality [9-11]. The Ecuadorian Amazon society is a mix of multiple cultures, and almost half of the population self-identifies as Amerindian, contrasting with the Pacific region, where more than 90% is Mestizo (of mixed Amerindian and European origin) [12, 13]. Amerindian patients are seldom included in HRQL studies of CL, although it is highly endemic in their Amazonian territory, and the presence of patients with destructive ML might affect the disease perception (see figure 1) [3, 14, 15]. Hence the need for HRQL studies that include Amerindian CL patients from the Amazon region.

Fig 1. A traditional Ecuadorian Amazon Kichwa figurine from the Pastaza province depicts a patient with concomitant ML and CL. *The Creative Commons Public Domain Dedication waiver Creative Commons CC0 1.0 applies to this image.*

Methods

Objectives

The main objective of this study was to explore the influence of CL suspected lesions on the quality of life of patients in the subtropical Pacific and Amazon regions with the hypothesis that there was self- and social stigmatization. As a secondary objective, we aimed to explore determinants of health-related quality of life.

Participants

This study was part of a cross sectional project on CL suspected patients that included a quantitative questionnaire for HRQL, assessment of diagnostic tests, description of geographic *Leishmania* species distribution, and a multisite short-term ethnography.

Patients were included in private and public primary health care centers and hospitals in the Amazonian Napo, Pastaza, and Morona Santiago provinces and in the Pacific region of the Pichincha province. A part of the patients was included during community visits. All participating centers offered free of charge outpatient treatment for CL according to the guidelines of the Ecuadorian Ministry of Health (once daily intra-muscular meglumine antimoniate for 20 consecutive days) [16].

All CL suspected patients referred for a cutaneous smear slide microscopy examination in the participating centers were eligible to participate in the study. Participants were approached by doctors, nurses or laboratory technicians during normal workflow before diagnostic sampling from CL suspected lesions. The patient or its legal representative filled out the HRQL questionnaire without knowledge of the test results. When needed, help from family members, a translator, or a health professional was allowed. The results were sent to a central data repository by mail service or delivered personally. Patients were included from January 2019 through June 2021. Patients who answered less than 75% of the questionnaire items were excluded from the study.

Questionnaire and variables

The Skindex-29 questionnaire is a generic tool to measure HRQL in patients with skin diseases. It contains 29 questions related to three dimensions: 10 questions on the emotional dimension (e.g., I am worried, angry, or ashamed by my skin condition.), seven questions on symptoms (e.g., My skin condition hurts, irritates, or burns.), and 12 questions on functioning (e.g., My skin condition affects my social life). Following a 5-point Likert scale for each question, patients can respond either: Never, rarely, sometimes, often, or all the time [17]. The Skindex-29 questionnaire has been translated and validated in more than ten languages in North and South American, European, Asian, and African cultures [18-23]. This study applied the Spanish version, which was previously validated in Colombia [24].

In addition, the following variables were recorded: Gender (male or female), age in years, ethnicity (as recognized by the Ecuadorian government [25]), perceived place of infection, number of lesions separated by healthy skin, type of lesion(s) (ulcer, nodular or other), lesion location (indicated on a person image), health seeking delay (in weeks, months or years), and the result of the smear slide microscopy and Polymerase Chain Reaction (PCR) (positive or negative).

Analysis

The Skindex-29 questionnaire results and the other variables were entered in the data management platform Castor EDC (https://data.castoredc.com). Data entry was done in duplicate by JB and AC and validated with calculation fields. The categorical responses were transformed into linear variables on a scale from 0 – 100, with 0 indicating no impairment and 100 indicating the worst HRQL, as described elsewhere [17]. Averages were calculated per dimension and for the total. Missing Skindex-29 answers were replaced by the average score of the corresponding dimension. If a second variable was missing, the patient was subsequently excluded from that specific comparison.

Confirmed (positive for either microscopy, PCR or both) versus non-confirmed CL patients were compared to assess the feasibility of generalizing Skindex-29 scores for the entire patient group. Pacific and Amazonian regions are divided by the Ecuadorian highlands, where leishmaniasis is rare [26]. The prevalent *Leishmania* species, vector-human interaction, and social structure in the two regions differ and were therefore analyzed separately and compared [4, 26, 27]. To allow comparisons, patients were divided into four linguistic groups: Spanish speaking Mestizos, Kichwa (Amazon Kichwa, Andwa, and Zapara), Chicham (Shuar, Achuar, and Shiwiar), and other (Waorani, white, and Afro-Ecuadorian). Patients with multiple lesion types (e.g. nodules and ulcers) were categorized as ulcer type if at least one ulcer was present because then we anticipated worse HRQL [28]. The body location of lesions was categorized as: `head and face', `upper limbs', `lower limbs', or `trunk' as in a former study in Surinam that found an association between body location of the lesions with Skindex-29 scores in CL patients [14].

The sample size was not calculated but based on convenience sampling. All statistical analysis was done with SPSS Statistics version 28 [29]. Mean Skindex-29 scores were assessed for statistical significance with the independent samples T test or Oneway Anova. Other variables were compared with the independent samples Proportions (Wald) or T test. Statistical significance was defined as (two-sided) P value <0.05.

Results

Participants

A total of 324 patients provided written informed consent. Four patients presented exclusively with mucosal lesions and were therefore excluded. Forty-one patients filled in less than 22 items of the questionnaire and were excluded. The skindex-29 questionnaire was completed adequately by 279 patients who were included in this study. Less than 2% of the responses were missing in the remaining questionnaires.

Baseline characteristics

The majority of the patients (58%) were male, and the mean age was 28 years, ranging from 0 to 88 years. 153 (55%) patients were included from the subtropical Pacific region and 126 (45%) from the Amazon region. 192 (69%) patients were Mestizos, and 205 (74%) had one lesion. Most patients (91%) presented with at least one ulcerative lesion and had lesions on the upper or lower limbs. Health seeking delay was less than a month in 149 (54%) patients (Table 1, Additional file 1).

Table 1. Characteristics of the study population (N = 279) of cutaneous leishmaniasis confirmed and non-confirmed patients in the Ecuadorian subtropical Pacific and Amazon regions from January 2019 through June 2021.

Characteristic (N missing for variable)	CL confirmed[a]	CL non-confirmed	Two-sided P[b]	All patients
Number (%)	**208 (75)**	**71 (25)**		**279 (100)**
Male gender (%)	118 (57)	43 (61)	0.57	161 (58)
Age in years (1)				
Mean ± SD	27.3 ± 19.4	30.6 ± 21.4	0.23	28.1 ± 19.9
Range	0.1 - 88	1.0 - 75		0.1-88.0
Ethnicity[c] (0)				
Pacific Mestizo (%)	121 (100)[d]	32 (100)[d]		153 (100)
Amazon Mestizo (%)	23 (26)	16 (41)	0.11	39 (31)
Amazon Kichwa (%)	26 (28)	11 (30)	0.85	37 (29)
Amazon Chicham (%)	34 (39)	10 (26)	0.12	44 (44)
Amazon Other (%)	4 (5)	2 (5)	0.90	6 (5)
Geographic region (0)				
Pacific (%)	121 (58)	32 (45)	0.06	153 (55)
Amazon (%)	87 (42)	39 (55)	0.06	126 (45)
Number of lesions (1)				
1 (%)	151 (73)	54 (77)	0.44	205 (74)
2 (%)	37 (18)	8 (11)	0.17	45 (16)
≥ 3 (%)	20 (10)	8 (11)	0.67	28 (10)
Lesion type (1)				
Ulcer (%)	135 (89)	117 (93)	0.25	252 (91)
Location of the lesions (1)				
Head and face (%)	41 (20)	12 (17)	0.63	53 (19)
Upper limbs (%)	84 (40)	15 (21)	<0.01[e]	99 (36)
Lower limbs (%)	56 (27)	30 (43)	0.02[e]	86 (31)
Trunk (%)	27 (13)	13 (19)	0.28	40 (14)
Health seeking delay (2)				
1-4 weeks (%)	114 (55)	35 (50)	0.46	149 (54)
1-2 months (%)	42 (20)	12 (17)	0.55	54 (19)
≥2 months (%)	51 (25)	23 (33)	0.20	74 (27)

[a] Either by microscopy, PCR or both

[b] Comparing CL confirmed and non-confirmed cases with the independent samples proportions (Wald) or T test

[c] Kichwa (Amazon Kichwa, Andwa, and Zapara) and Chicham (Shuar, Achuar, and Shiwiar) are linguistic groups

[d] All patients from the Pacific region were Mestizos

[e] Statistically significant difference

Confirmed CL cases

Leishmania parasites were identified with microscopy and/or PCR in the skin lesions of 208 (75%) patients. Of the confirmed cases 84 (40%) presented with lesions on the upper limbs and 56 (27%) on the lower limbs compared to 15 (21%) and 30 (43%) respectively of the non-confirmed cases. This difference was statistically significant (P <0.01 for upper limbs and P = 0.02 for lower limbs). Confirmed cases did not differ significantly in gender, age, ethnicity, region of infection, number of lesions, lesion type, and health seeking delay from non-confirmed cases (Table 1). Confirmed cases scored lower on the Skindex-29 questionnaire in both the Pacific and Amazon regions, but the difference was not statistically significant (Table 2).

Table 2. Mean Skindex-29 scores of patients (N=279) suspected of having cutaneous leishmaniasis in the Ecuadorian subtropical Pacific and Amazon regions from January 2019 through June 2021.

Skindex-29 dimension:	Emotions (SE)	Symptoms (SE)	Functioning (SE)	Total (SE)
Pacific				
Confirmed leishmaniasis (n = 121)	31.7 (2.0)	37.9 (2.3)	17.4 (2.0)	27.3 (1.8)
Non-confirmed leishmaniasis (n = 32)	33.0 (4.3)	41.2 (4.9)	19.1 (4.5)	29.2 (4.2)
Two-sided P[a]	0.78	0.52	0.71	0.64
Amazon				
Confirmed leishmaniasis (n = 87)	50.8 (2.5)	53.3 (2.3)	41.8 (2.8)	47.7 (2.3)
Non-confirmed leishmaniasis (n = 39)	58.5 (4.1)	61.9 (4.1)	50.7 (4.7)	56.1 (4.0)
Two-sided P[a]	0.10	0.06	0.09	0.06

SE = Standard Error

[a] Comparing mean Skindex-29 scores of confirmed and non-confirmed cases with the independent samples T test

HRQL of CL suspected patients in the Pacific and Amazon regions

The percentage of males (52%) included in the Pacific region was significantly lower than in the Amazon (64%). The percentage of patients with age 0-12 (31%) was significantly higher in the Pacific region than in the Amazon (19%) and the percentage from the age group ≥40 was significantly lower (18 and 33% respectively). In the Pacific region, 100% of the patients were Mestizo but in the Amazon, the majority of patients were either from the Kichwa (29%) or Chicham (35%) linguistic groups. The percentage of patients with lesions on the head or face was significantly less in the Amazon region (13%) than in the Pacific (24%). The percentage with health seeking delay of less than a month was significantly (P <0.01 on the independent proportions test) lower in the Amazon region (38%) than in the Pacific (66%), but significantly (P <0.01) higher than in the ≥2 months delay group (resp. 39% vs 16%). Patients from the Pacific and Amazon regions presented no significant differences in lesion type or number of lesions (Table 3).

Table 3. Characteristics of the study population (N = 279) of suspected cutaneous leishmaniasis patients in the Ecuadorian subtropical Pacific and Amazon regions from January 2019 through June 2021.

Region of contagion:	Pacific	Amazon	Two-sided P[a]
Characteristics (N missing for variable)			
N (%)	**153 (55)**	**126 (45)**	
Males (%)	80 (52)	81 (64)	0.04[b]
Age quartiles (1)			
0-12 (%)	47 (31)	24 (19)	0.02[b]
13-22 (%)	37 (24)	30 (24)	0.92
23-39 (%)	40 (26)	31 (25)	0.74
40-88 (%)	28 (18)	41 (33)	<0.01[b]
Ethnicity[c] (0)			
Mestizo (%)	153 (100)	39 (31)	0.00[b]
Kichwa (%)	0 (0)	37 (29)	<0.01[b]
Chicham (%)	0 (0)	44 (35)	<0.01[b]
Other (%)	0 (0)	6 (5)	0.01[b]
Clinical presentation (1)			
Mean number of lesions (range)	1.5 (1-8)	1.6 (1-10)	0.36
Lesion type: ulcer (%)	135 (89)	117 (93)	0.24
Location of lesions (1)			
Head and face (%)	37 (24)	16 (13)	0.01[b]
Upper limbs (%)	55 (36)	44 (35)	0.83
Lower limbs (%)	42 (28)	44 (35)	0.19
Trunk (%)	18 (12)	22 (18)	0.18
Health seeking delay (2)			
1-4 weeks (%)	101 (66)	48 (38)	<0.01[b]
1-2 months (%)	26 (17)	28 (22)	0.27
≥2 months (%)	25 (16)	49 (39)	<0.01[b]

[a] Comparing Pacific and Amazonian patients with the independent samples proportions (Wald) or T test
[b] Statistically significant difference
[c] Kichwa (Amazon Kichwa, Andwa, and Zapara) and Chicham (Shuar, Achuar, and Shiwiar) are linguistic groups

Mean Skindex-29 scores were not significantly different between males and females and, except for the functioning dimension, between age quartiles. All patient groups (Amerindian and Mestizo) from the Amazon scored significantly higher on all the dimensions of the Skindex-29 questionnaire than Mestizo patients from the Pacific region. Amazon Amerindian patient groups scored higher than Mestizos on all the dimensions, but the differences were not significant in the Post-Hoc tests. The mean difference between mean Skindex-29 scores in the Pacific and Amazon regions was highest on the functioning dimension, although not statistically significant. Mean Skindex-29 scores were not significantly different between patients with one lesion or more than one lesion and, except for the total score, between those with or without ulcers. The location of the lesion had no significant influence on the total patients' mean Skindex-29 score nor on the emotions or symptoms dimensions. Body location was significantly (P = 0.05 on Oneway ANOVA) associated with the mean functioning Skindex-29 score, although not significant on the Post-Hoc tests. Patients with health seeking delay of less than a month, scored significantly lower on the emotions and functioning dimensions but not on the symptoms dimension. Mean Skindex-29 scores are shown in Table 4.

Table 4. Mean Skindex-29 scores of the study population (N=279) of patients with suspected localized cutaneous leishmaniasis in the Ecuadorian subtropical Pacific and Amazon regions from January 2019 through June 2021.

Skindex-29 dimension: Characteristic (N missing for variable)	Emotions (SE)	Symptoms (SE)	Functioning (SE)	Total (SE)
Gender (0)				
Male (n = 161)	43.1 (2.1)	46.9 (2.0)	32.5 (2.3)	39.6 (2.0)
Female (n = 118)	39.5 (2.2)	45.9 (2.4)	26.4 (2.4)	35.6 (2.1)
P-value[a]	0.25	0.77	0.08	0.18
Age quartiles (1)				
0-12 (n = 71)[b]	40.2 (3.2)	45.7 (3.4)	24.5 (3.1)	35.0 (2.9)
13-22 (n = 67)	39.5 (2.9)	42.4 (2.7)	25.9 (3.0)	34.6 (2.5)
23-39 (n = 71)	41.2 (3.3)	45.4 (3.2)	30.9 (3.7)	37.9 (3.2)
40-88 (n = 71)	44.7 (2.8)	51.8 (2.9)	37.5 (3.4)[c]	43.5 (2.8)
P-value[a]	0.63	0.19	0.03[d]	0.12
Pacific vs Amazon[e] (0)				
Pacific Mestizo (n = 153)[b]	32.0 (1.8)	38.6 (2.1)	17.8 (1.8)	27.7 (1.7)
Amazon Mestizo (n = 39)	46.4 (4.0)[c]	52.8 (3.8)[c]	34.0 (4.4)[c]	42.8 (3.8)[c]
Amazon Chicham (n = 44)	55.0 (3.3)[c]	57.6 (3.7)[c]	47.8 (3.7)[c]	52.7 (3.2)[c]
Amazon Kichwa (n = 37)	58.3 (3.9)[c]	58.2 (3.6)[c]	50.8 (4.3)[c]	55.2 (3.5)[c]
Amazon Other (n = 6)	52.9 (13.7)	50.6 (9.6)[c]	51.0 (15.9)	51.6 (13.2)
P-value[a]	<0.01[d]	<0.01[d]	<0.01[d]	<0.01[d]
Mean difference Pacific vs Amazon	21.3 (2.8)	17.4 (2.9)	26.8 (3.0)	22.6 (2.6)
95% Confidence Interval[a] of difference	15.7 - 26.8	11.6 - 23.2	20.8 - 32.8	17.5 - 27.7
Number of lesions (0)				
1 lesion (n = 205)	40.5 (1.8)	45.5 (1.8)	29.0 (2.0)	37.0 (1.7)
>1 lesion (n = 74)	44.4 (2.9)	49.1 (3.0)	32.2 (3.2)	40.5 (2.8)
P-value[a]	0.27	0.31	0.41	0.29
Lesion type (1)				
Ulcer (n = 252)	42.2 (1.6)	47.3 (1.6)	30.7 (1.7)	38.7 (1.5)
Non-ulcer (n = 26)	33.5 (6.0)	37.1 (5.7)	20.3 (6.0)	28.9 (5.6)
P-value[a]	0.10	0.06	0.07	0.05[d]
Location of the lesion (1)				
Head and face (n = 53)	41.2 (3.8)	45.8 (4.0)	28.3 (4.2)	37.0 (3.7)
Upper limbs (n = 99)	40.6 (2.5)	42.2 (2.5)	25.9 (2.5)	34.9 (2.2)
Lower Limbs (n = 86)	41.4 (2.7)	48.7 (2.6)	30.7 (3.0)	38.7 (2.6)
Trunk (n = 40)	43.6 (3.9)	52.1 (4.4)	38.9 (4.9)	43.7 (4.0)
P-value[a]	0.57	0.11	0.05[d]	0.12
Health seeking delay (2)				

1-4 weeks (n = 149)[b]	37.4 (2.1)	43.6 (2.1)	25.5 (2.2)	34.0 (1.9)
1-2 months (n = 54)	46.9 (3.2)[c]	50.1 (3.7)	32.4 (3.6)	41.7 (3.2)
≥2 months (n= 74)	45.7 (2.9)	49.5 (3.0)	36.5 (3.5)[c]	42.8 (2.9)[c]
P-value[a]	0.02[d]	0.14	0.02[d]	0.02[d]

SE = Standard Error

[a] With Oneway ANOVA or independent samples T test
[b] Reference category
[c] Statistically significant difference on the Post-Hoc test compared to the reference category
[d] Statistically significant
[e] Kichwa (Amazon Kichwa, Andwa, and Zapara) and Chicham (Shuar, Achuar, and Shiwiar) are linguistic groups

Discussion

The present study revealed that the influence of suspected CL lesions on the HRQL of patients in the Ecuadorian subtropical Pacific and Amazon depends on the geographic region of infection more than on patient characteristics such as gender, age, number of lesions, lesion type, location of lesions, health seeking delay or posterior confirmation of the *Leishmania* parasite. Moreover, HRQL was worse on all three dimensions of the Skindex-29 questionnaire in the Mestizo, Kichwa, and Chicham patient groups from the Amazon region compared to the subtropical Pacific.

Younger age (0-12 years), location of lesions (category not specified), and short health seeking delay (1-4 weeks) were also associated with lower scores on the functioning dimension of the Skindex-29 questionnaire. This could be explained by confounding as the patients from the Amazon region differed significantly from Pacific patients in these categories, and the mean difference in Skindex-29 scores between the regions was the highest on the functioning dimension. The proportion of patients in the 1-4 weeks health seeking delay category was almost double in the Pacific group, with better HRQL on the emotions dimension, compared to the Amazon group. Therefore, the association of the 1-2 months health seeking delay category with lower HRQL on the emotions dimension could be explained by confounding.

The association of ulcerative lesions with higher total Skindex-29 scores could be explained by the additional influence of the interruption of skin continuity in ulcerative skin diseases, as seen in another study involving leg ulcers [28]. Nevertheless, the difference in mean Skindex-29 scores between patients with ulcerative and non-ulcerative lesions was not significant on the separate dimensions.

The worse HRQL of patients from the Amazon region might result from confounding variables that were not included in our data. These unknown variables could be elucidated through qualitative research. We performed structured interviews with 30 of our participants, and the data will be reported elsewhere [30]. Stigma expressions from the qualitative interviews seem to be the best explanation for the significantly worse HRQL in our CL suspected patients from the Amazon. Our results suggest that the expressions of social and self-stigma might have a significant and widespread influence on HRQL in all its dimensions.

A Canadian study of 51 hidradenitis suppurativa patients showed that the Skindex-29 questionnaire can detect stigma expressions evoked by non-visual disease characteristics. Patients with malodor scored significantly higher on all the dimensions of the Skindex-29 questionnaire, although there was no significant difference in the Dermatology Life Quality Index [23]. Malodor might be one of the causes of the impaired HRQL in our patient group.

Health seeking delay and subsequent time to treatment of Amazon patients were significantly longer. The health seeking delay might result from transportation difficulties from remote jungle communities, lack of recourses, discrimination, failing diagnostic tests, and/or stigmatization as occurs with leprosy patients [31]. During the qualitative interviews, patients with shorter health seeking delays indicated worse stigma expressions [30]. Therefore, our study might even underestimate the HRQL impairment in Amazonian patients.

The Amazon patient group had significantly fewer lesions on the head and face than in the Pacific region, probably because of different biting patterns of the *Lutzomyia* vector in older humans [26, 32]. Additionally, the percentage of males (probably going shirtless more often) in the Amazon was significantly higher than in the Pacific, a finding that is in agreement with other studies and should be explained by the predominance of hunters and farmers in the Amazon CL patient group contrasted to domestic transmission in the Pacific [4, 33, 34].

Our study has some limitations: The Skindex-29 questionnaire has not been validated for use by parents for their children, but our data show no significantly different scores between the 0-12, 13-22, and 23-39 age groups, suggesting that it was valid to include all patients in the analysis [17, 24]. Second, this study used the Spanish version of the Skindex-29 questionnaire as validated in Colombia [24]. Nevertheless, many of the included Amerindian patients were non-native Spanish speakers and questions were translated by the health professional or a translator. This might have influenced the Skindex-29 scores of Kichwa and Chicham patients. We recommend the validation of quantitative HRQL questionnaires such as the Skindex-29 in Amerindian populations. Lastly, additional information on the ulcers (e.g. ulcer smell, presence of liquid discharge, and diameter) would have been of value.

We consider that the results of this study might be fairly generalizable for the patient populations in the Ecuadorian Amazon and Pacific areas because patients were included both from the public and private health care system, including representative Amerindian groups. Therefore, health authorities should strengthen their efforts to improve CL case detection in the Amazon and secure prompt treatment initiation. Additionally, the causes of health seeking delay should be clarified in future studies of health seeking behaviors combining quantitative and qualitative methods.

The Kichwa and Chicham linguistic groups extend into the Peruvian Amazon, where similar HRQL impairment in CL patients might occur as in Ecuador [13]. On the other hand, patients with suspected CL lesions in other regions could also have different HRQL outcomes. Hence, we recommend that research including quantitative questionnaires, such as the Skindex-29, combined with qualitative interviews should be considered for CL endemic countries.

Conclusion

Suspected CL patients from the Ecuadorian Amazon report significantly impaired HRQL compared to their counterparts in the Pacific region. Additionally, Amazonian patients have significant health

seeking delay, leading to prolonged suffering and a worse health outcome. Determinants of health seeking delay should be clarified in future studies, including quantitative and qualitative methods, and CL case detection and management by health authorities must be improved. Moreover, HRQL analysis in other CL endemic regions could improve local health management.

List of abbreviations

CL: Cutaneous Leishmaniasis

HRQL: Health Related Quality of Life

ML: Mucosal Leishmaniasis

Declarations

Ethics approval and consent to participate

The study protocol was approved by the ethical committee of the `Universidad Internacional del Ecuador', permission number UIDE-FCM-EDM-COM-18-0069, and by the Ecuadorian Ministry of Health, permission number MSPCURI000284-3, prior to its initiation. All participants or their legal representative gave written informed consent prior to participation in the study.

Availability of data and materials

Al data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interest

The authors declare that they have no competing interests.

Funding

Foundation `Latin Link Nederland' provided funding for the current study. The funding body had no role in the study design, collection, analysis, and interpretation of data, nor in writing the manuscript.

Authors contributions

JB contributed to the conception and design of the work, data acquisition, analysis and interpretation, and drafted the work.

MC contributed to the conception and design of the work, data acquisition, analysis and interpretation.

AC contributed to the data analysis

FO contributed to the data interpretation

VV contributed to the data interpretation

HS contributed to the conception and design of the work, data analysis, interpretation, and substantial revision.

HdV contributed to the conception and design of the work, data analysis, interpretation, and substantial revision.

All authors approved the submitted version.

All authors agreed both to be personally accountable for their own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved and the resolution documented.

References

1. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PloS one. 2012;7(5):e35671.
2. Saeedi M. Leishmaniasis Geneva: World Health Organization; 2022. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis. Accessed: 25 January 2022.
3. Henríquez-Trujillo AR, Coral-Almeida M, Hinojosa MC. The burden of cutaneous and mucocutaneous leishmaniasis in Ecuador (2014-2018), a national registry-based study. bioRxiv. 2019:751446.
4. Calvopina M, Armijos RX, Hashiguchi Y. Epidemiology of leishmaniasis in Ecuador: current status of knowledge -- a review. Memorias do Instituto Oswaldo Cruz. 2004;99(7):663-72.
5. Bennis I, De Brouwere V, Belrhiti Z, Sahibi H, Boelaert M. Psychosocial burden of localised cutaneous Leishmaniasis: a scoping review. BMC Public Health. 2018;18(1):358.
6. Ramdas S, van der Geest S, Schallig HD. Nuancing stigma through ethnography: the case of cutaneous leishmaniasis in Suriname. Soc Sci Med. 2016;151:139-46.
7. van Wijk R, van Selm L, Barbosa MC, van Brakel WH, Waltz M, Philipp Puchner K. Psychosocial burden of neglected tropical diseases in eastern Colombia: an explorative qualitative study in persons affected by leprosy, cutaneous leishmaniasis and Chagas disease. Glob Ment Health (Camb). 2021;8:e21.
8. Weigel MM, Armijos RX, Racines R, Zurita C, Izurieta R, Herrera E, et al. Cutaneous leishmaniasis in subtropical Ecuador: popular perceptions, knowledge, and treatment. Bulletin of the Pan American Health Organization (PAHO); 28 (2), jun 1994. 1994.
9. Siguencia Ibadango SK. Discriminación salarial por etnia en el período 2010-2017 en el Ecuador: PUCE-Quito; 2018.
10. Whitten Jr NE. Symbolic inversion, the topology of El Mestizaje, and the spaces of Las Razas in Ecuador. Journal of Latin American Anthropology. 2003;8(1):52-85.
11. Bautista-Valarezo E, Duque V, Verhoeven V, Mejia Chicaiza J, Hendrickx K, Maldonado-Rengel R, et al. Perceptions of Ecuadorian indigenous healers on their relationship with the formal health care system: barriers and opportunities. BMC Complementary Medicine and Therapies. 2021;21(1):1-10.
12. Lopez G. POBLACIÓN QUE SE AUTOIDENTIFICÓ INDÍGENA, SEGÚN PROVINCIA DE EMPADRONAMIENTO, NACIONALIDAD O PUEBLO INDÍGENA AL QUE PERTENECE Y SEXO Quito: INEC; 2010 https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwi6per4kJv2AhVCNewKHUBlBewQFnoECBMQAQ&url=https%3A%2F%2Fwww.ecuadorencifras.gob.ec%2Fwp-content%2Fplugins%2Fdownload-monitor%2Fdownload.php%3Fid%3D339%26force%3D0&usg=AOvVaw1sZMlqB_kh_8O316Yy4T8I. Accessed: 25 February 2022.
13. CONFENIAE. Nacionalidades Puyo: CONFENIAE; 2022 https://confeniae.net/nacionalidades. Accessed: 24 February 2022.

14. Hu R, Ramdas S, Nieuwkerk P, Reis R, Lai AFRFM, de Vries HJC, et al. Body location of "New World" cutaneous leishmaniasis lesions and its impact on the quality of life of patients in Suriname. PLoS neglected tropical diseases. 2020;14(10):e0008759.
15. Calvopiña M, Angel G, Armijos R, Gomez E, Mimori T, Cooper P, et al. Clinical features of mucocutaneous leishmaniasis in the Amazonian region of Ecuador. Res Rep Ser. 2005;6:82-9.
16. Espin CJT, Procel MC. Leishmaniasis en el Ecuador: revisión bibliográfica. Mediciencias UTA. 2021;5(3):2-11.
17. Chren MM, Lasek RJ, Flocke SA, Zyzanski SJ. Improved discriminative and evaluative capability of a refined version of Skindex, a quality-of-life instrument for patients with skin diseases. Archives of dermatology. 1997;133(11):1433-40.
18. Chren MM. The Skindex instruments to measure the effects of skin disease on quality of life. Dermatol Clin. 2012;30(2):231-6, xiii.
19. Aksu AE, Urer MS, Sabuncu I, Saracoglu ZN, Chren MM. Turkish version of Skindex-29. International journal of dermatology. 2007;46(4):350-5.
20. He Z, Lu C, Chren MM, Zhang Z, Li Y, Ni X, et al. Development and psychometric validation of the Chinese version of Skindex-29 and Skindex-16. Health Qual Life Outcomes. 2014;12:190.
21. Zghal A, Zeglaoui F, Kallel L, Karmous R, Ben Ammar H, Labbane R, et al. [Quality of life in dermatology: Tunisian version of the Skindex-29]. Tunis Med. 2003;81(1):34-7.
22. Paula HR, Haddad A, Weiss MA, Dini GM, Ferreira LM. Translation, cultural adaptation, and validation of the American Skindex-29 quality of life index. An Bras Dermatol. 2014;89(4):600-7.
23. Alavi A, Farzanfar D, Lee RK, Almutairi D. The Contribution of Malodour in Quality of Life of Patients With Hidradenitis Suppurativa. Journal of cutaneous medicine and surgery. 2018;22(2):166-74.
24. Vasquez D, Aguirre DC, Sanclemente G. Construct validity and responsiveness of the Colombian version of Skindex-29. The British journal of dermatology. 2019;181(4):770-7.
25. CODENPE. Modulo 3: Interculturalidad. Quito: CODENPE; 2011.
26. Hashiguchi Y, Velez LN, Villegas NV, Mimori T, Gomez EAL, Kato H. Leishmaniases in Ecuador: Comprehensive review and current status. Acta tropica. 2017;166:299-315.
27. Kato H, Gomez EA, Martini-Robles L, Muzzio J, Velez L, Calvopiña M, et al. Geographic Distribution of Leishmania Species in Ecuador Based on the Cytochrome B Gene Sequence Analysis. PLoS neglected tropical diseases. 2016;10(7):e0004844.
28. Szewczyk MT, Mościcka P, Jawień A, Cwajda-Białasik J, Cierzniakowska K, Ślusarz R, et al. Quality of life in patients with leg ulcers or skin lesions - a pilot study. Postepy Dermatol Alergol. 2015;32(6):465-9.
29. IBM. SPSS Statistics for Windows. Version 28.0. Armonk. New York: IBM; 2021.
30. Vargas V, Bezemer J, Calvopina M, Schallig H, Ortega F, Salazar N, et al. Perceptions of American tegumentary leishmaniasis in Ecuador: questioning the visuality of aesthetic stigma. 2022.
31. Dharmawan Y, Fuady A, Korfage I, Richardus JH. Individual and community factors determining delayed leprosy case detection: A systematic review. PLoS neglected tropical diseases. 2021;15(8):e0009651.
32. Shaw J, Lainson R, Ward R. Leishmaniasis in Brazil: VII. Further observations on the feeding habitats of Lutzomyia flaviscutellata (Mangabeira) with particular reference to its biting habits at different heights. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1972;66(5):718-23.
33. Conteron E. Prevalencia de leishmaniasis en el area II de Pastaza, asociados a factores de riesgo que influyen en el desarrollo de la enfermedad. Ambato: Universidad Tecnica de Ambato; 2015.
34. Armijos R, Weigel M, Izurieta R, Racines J, Zurita C, Herrera W, et al. The epidemiology of cutaneous leishmaniasis in subtropical Ecuador. Tropical Medicine & International Health. 1997;2(2):140-52.

Supplementary information

Additional file 1. Excel spreadsheet.xlsx. Skindex-29 results. Individual patient variables and Skindex-29 scores.

This file can be accessed with the following link: https://ndownloader.figstatic.com/files/37595041

Chapter 7

Sensorial perceptions of risk: the aesthetics behind (muco)cutaneous leishmaniasis-related stigma in Ecuador

Veronica C Vargas Roman, Jacob M Bezemer, Manuel Calvopiña, Fernando Ortega, Noel B. Salazar, Henk D F H Schallig, Henry J C de Vries

Submitted to: **Anthropology & Medicine**

Abstract

Previous research on the stigma associated with cutaneous leishmaniasis, a vector-transmitted parasitic disease, focuses on aesthetic appearance affectation as the leading cause of stigmatization. However, indigenous populations in the hinterland Amazon of Ecuador trigger stigma expressions by recognizing (muco)cutaneous leishmaniasis, primarily through atypical smell, followed by the odd voice sound, appearance and taste. This empirical way of recognizing symptoms resorts to embodied forms of identifying disease approached in sensorial anthropology, contesting the western supremacy of visuality. Through ethnographic research and data retrieved from eighty-three semi structured interviews, and fifteen focus groups in seven Ecuadorian ethnic groups –including six indigenous groups in the Amazon region– this paper analyses how the sensorium is a thermometer of health. Findings reveal that the sensorium allows for differentiated cultural responses to a sense of peril, contagion and social (self)rejection, which are linked to the holistic approach to health shared by indigenous populations. In forest societies, well-being is explained through successful (non)human relationships, and disease permeates through bodies that lack balanced relations.

Keywords

Leishmaniasis, Stigma, Sensorial anthropology, Amazon, Ecuador

Introduction

Cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL) are clinical manifestations of infectious zoonotic diseases caused by protozoan parasites, *Leishmania* spp, which are transmitted to humans and other mammals through the bites of female *Phlebotomus* (genus: *Lutzomyia*) sandflies. Both diseases may occur concomitantly, separately, or subsequently [1, 2]. CL is characterised by chronic ulceration of the skin exposed to vector bites, leaving permanent scars. CL is endemic in the Ecuadorian Amazon and Coastal (Pacific) regions. MCL has been reported only in the Amazon Region of Ecuador and destroys the mucosal tissues of the nose, mouth and throat, resulting in obstruction, ulceration, disfiguration and eventually death [3, 4]. Leishmaniases affect approximately 5,000 new patients in Ecuador annually, causing between 212 and 464 cases per 10,000 inhabitants per year in endemic areas in the north-western and Amazon provinces of the country [5].

Evidence in the last years shows that CL can cause stigmatization in patients, who are prone to a higher risk of mental illness and low quality of life [6]. The need to attend to stigma meets the urgency of the WHO's call for action to prevent psychosocial impacts in neglected tropical diseases like leishmaniasis [7]. Despite this call, qualitative studies analysing the sociocultural perceptions of leishmaniasis in the tropical areas of Latin America are limited. A previous study informs that, in Ecuador, the geographical remoteness of the disease has hampered patients' access to appropriate health care, the actual incidence reporting, and qualitative social research on leishmaniasis [8]. A recent study on health-related quality of life with CL conducted by Bezemer and colleagues in Ecuador concluded that suspected patients from the Amazon region have the worst quality of life markers compared to those in the Pacific [9]. The study suggests stigma manifestations and health-seeking delay may cause prolonged suffering, acknowledging there is a need for further culturally disaggregated qualitative data to explain the reasons and social determinants.

This study is part of a broader research project aiming to improve the diagnosis and clinical management of (muco)cutaneous leishmaniasis in primary health care, including the study of the psychosocial effects in the central Amazon of Ecuador and the North-west cloud forest area of the country. The specific objective of this qualitative study is to understand the perceptions leading to CL and MCL leishmaniasis-related stigma expressions. Considering the evidence gap on leishmaniasis-related stigma in Ecuador, this paper analyses the possible causes leading to stigmatisation among seven ethnic groups of the Amazon and Northwest areas of Ecuador. By exploring how CL and MCL leishmaniasis and its risk are embodied by suspected patients, their families and community members, we aim to understand the sociocultural imaginaries related to the disease leading to stigmatisation.

Background

Approaches to stigma

The complex relationship between illness and stigma has been approached through the lens of different social science disciplines [6, 10]. Erving Goffman pioneered the concept of stigma by referring to it as a language of relationships –neither attributes nor sporadic attitudes– leading to discredit and undesired difference from social expectation, affecting integration [11, 12]. This approach highlighted the social construction of the phenomenon, as in stereotypes and the

modification of power relations [10]. Goffman's social theories were complemented by the psychological aspects of stigma focused on the mental health condition of the stigmatised [13]. Several studies confirm that social alienation and discredit affect the patient's (and their family's) well-being more than the disease itself [14]. From an anthropological perspective, considering the body as a sociocultural construction, imaginaries of the 'ideal' body affect individual and social perceptions and depend on cultural value systems determining what is unacceptable or morally low [15, 16]. Previous studies on attitudes and perceptions of leishmaniasis have exposed myriad positive and negative responses related to cultural value systems [17]. Positive attitudes related to acceptance, integration, and care are in contrast with manifestations of rejection, distancing, and isolation guided by fear and disgust.

The growing number of qualitative studies of the last decade indicate that having an awkward skin appearance is the leading cause of stigmatization [15]. While research on stigma caused by MCL is very rare, evidence suggests that CL-related stigma results in psychosocial suffering and mental ill-health linked to the perception of a distorted body image, exacerbated by face scars [6]. Ramdas refers to it as the aesthetic stigma type [18, 19]. This appraisal of disfigurement and being visibly different is reported to be a critical cause of stigma that tends to vary according to the place and size of sores [20]. Overall, the visuality of the disease highlights the apparent (cultural) predominance of the sense of sight in processes of CL stigmatisation. Despite the reduction of the term aesthetic to visual qualities, for the purpose of this study, we take the broader concept of aesthetics, which covers everything that can be perceived by the senses [21, 22].

The sensorial empirical appreciation of disease

Following Nichter's proposal for sensorial appreciation in medical anthropology, this study resorts to the philosophical approach of the phenomenology of embodiment to understand how health, illness and risk are mediated through the senses [23]. In phenomenology, the empirical experience with a disease can explain the worlds patients, family and community members inhabit, resulting in a perception that goes beyond the biological phenomenon. There is a difference between disease as a physiological process and as a lived experience as it affects people`s lives in specific social contexts [24].

Considering that the disease can only be experienced through the body, the sensorium is a medium through which the body registers aesthetic properties that inform the disease's presence and trigger cultural responses. The body is the instrument and result of a relationship between the person, environment and other (non)human beings as it is part of people's daily life experiences [25]. The sensorial aspects of an experience inform and explain how people respond in a particular sociocultural context. Humans form perceptions from the stimuli all five senses receive, resulting in important sources of information. But different senses play central roles according to the needs and contexts as senses are socially constructed [26]. It has been broadly argued that the senses are valued differently by different cultures [26-29].

Historically, visuality is attributed the highest sensorial value, especially among literate societies in the Western world [30, 31]. Social researchers working in the Amazon contest the importance of visuality and propose the supremacy of the whole sensorium in the personal experiences performed (and transmitted) routinely [26, 31-35]. As Glen Shepard points out, within Amazon societies, the sensorium stands as the culture-nature nexus, giving sense to health-related processes –including medicine and treatment– from a cultural and ecological perspective [35]. Sensual approaches to an illness are capable of raising alerts of peril based on sensorial perceptions that are culturally constructed [27, 28]. Amazonian groups tend to refer to smell to diagnose

disease and define healing treatments [35]. Their response to odours is vital in their everyday life experiences [28, 31, 33, 35].

Catherine Panter-Brick approaches the perception of risk evaluated through the senses and connects alerts to social and moral scopes [36]. The author states that odds and calamity may have logical reasonings, but explaining why a person goes through misfortune in a particular moment involves metaphysical or supernatural justifications linked to their potential misbehaviour. This makes the perceptions of risk highly nuanced according to social values [36]. Somatic sensations are transformed into (inter)subjective perceptions that give meaning to risk, which can manifest through emotional states like anxiety, uncertainty, or fear. The embodied response to risk initiated by the sensorial perception may involve references to daily life relations, like those with the environment, symbolic relations with (non) human beings, and believed ways of impeding harm [23].

Methodology

This qualitative research is based on a short-term and multisited ethnography. According to Pink and Morgan, short-term ethnography refers to intensive excursions into participants' lives in contexts of interventions, as in the case of health attention brigades [37]. Complementary, the fact that this study traces experiences with CL and MCL leishmaniasis, the ethnographic field became, to a certain extent, diffuse. For this reason, resorting to a multisited fieldwork helped connect different ethnographic experiences with the diseases [37, 38]. Likewise, considering the high mobility and temporary dwelling of social groups along the river basins in the Amazon area, a multisited fieldwork is in line with the constant 'placemaking' and geographical shifts of (muco)cutaneous leishmaniasis cases.

The research methods included semi-structured interviews, focus groups, informal conversations, observation, and visual methods. The interview guide included questions about how people make sense of the illness, including its origin (aetiology) and vernacular names, feelings (like fear or disgust) and the social relation between community and family members with suspected patients. The interviews were conducted in Spanish or local languages, with the support of translators, and transcribed. The English translations were made by the first author of this paper. While vernacular names have proved to relate to stigmatisation, Pires and colleagues also recommend exploring the aetiology and cultural imaginaries of the origin of the disease with stigma processes [15, 39].

Observation notes were recorded in fieldnotes during medical brigades and visits to the research sites, as researchers exchanged daily life activities and informal chats with community members. Visual methods included drawing and photography. Examples of photographic records comprised the reaction of community members towards leishmaniasis lesions. In addition, participants were invited to draw leishmaniasis sores on human bodies and differentiate which generate increased stigma. The principal author of this paper took pictures and made drawings to support interviews and observation notes during the medical consultations. All data were digitalised and analysed using NVIVO (release 1.6.1), a qualitative analysis software package that allows open coding and crossing of different variables, until reaching theoretical saturation.

Sites and participants were identified during medical consultation and through snowball sampling after reaching the sites. The selection criteria of patients was based on CL and MCL suspicion through private and public institutions in the Amazonian Napo and Pastaza provinces and in the Pacific region of the Pichincha province. To expand participation, suspected patients and key actors helped identify other persons affected by leishmaniasis, their families and community

members in endemic sites of MCL, granting a larger representativity. Data was gathered during site visits between January and April 2021. The suspected patients were included through Hospital Shell, a private health institution, and public health facilities from the Ministry of Health. Of ninety-eight participants, eighty-three were interviewed, and fifteen participated in focus groups; fifty-eight were suspected leishmaniasis patients. Other interviewees included eleven health workers and seven traditional healers. Family and community members participated through focus groups and semi-structured interviews.

This research included the participation of seven ethnic groups; six belonged to indigenous ethnicities of the Amazon Region (see Table 1). Figure 1 shows the map with the distribution of the research sites, ethnicities and their predominant language.

Table 1: Characteristics of the study populations

Linguistic group		**Spanish**	**Kichwa**			**Chicham**			**Total**
Ethnic group		**Mestizo**	**Amazon Kichwa**	**Andoa**	**Zapara**	**Shuar**	**Achuar**	**Shiwiar**	**All ethnicities**
Total		19	15	14	14	10	12	14	98
Urban exposure	Hinterland	0	12	13	14	2	11	14	66
	Urban areas	19	3	1	0	8	1	0	32
Suspected patients	Total	11	8	6	11	7	8	7	58

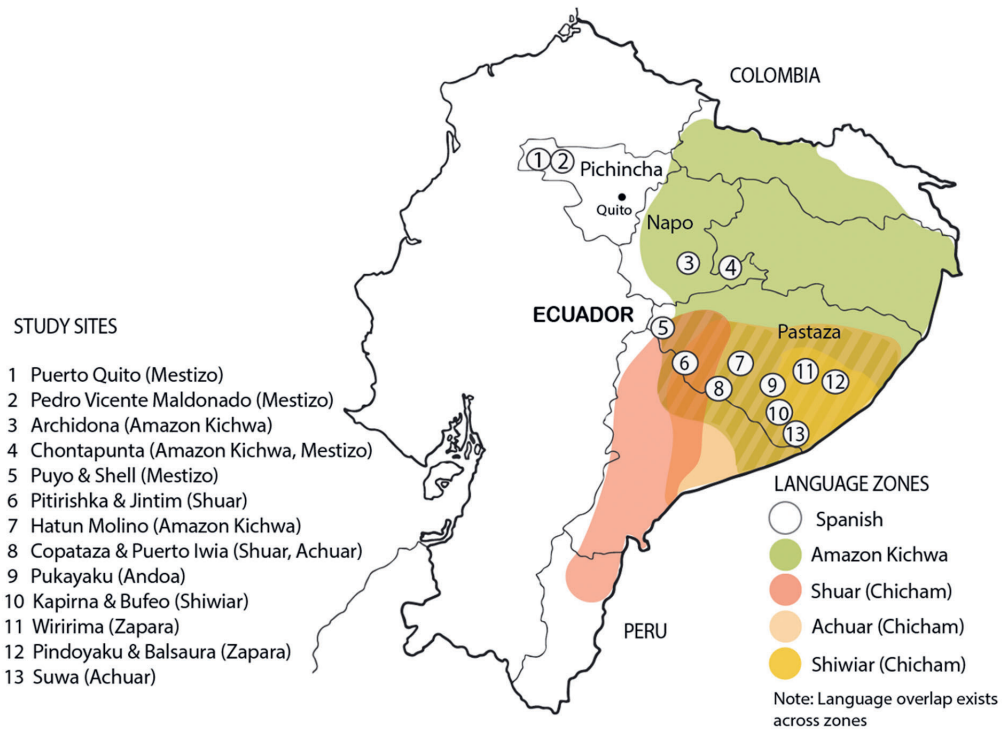


Figure 1: Map of Ecuador, language zones and study sites, adapted from Ortega Haboud 2008 [40]

Ethics

This study was approved by the ethical committee of the *Universidad Internacional del Ecuador* with permission number UIDE-FCM-EDM-COM-18-0069 and by the Ecuadorian Ministry of Health with permission number MSPCURI000284-3. Suspected patients provided written informed consent during inclusion in participating health centers or during medical brigades. Family and community members who participated less intensively in this study provided oral verbal consent. All patients, regardless of their participation, received free leishmaniasis diagnosis and treatment. All non-cases were given a free medical consultation, followed by a referral (if necessary) to the Ecuadorian Ministry of Health for free care. This paper does not disclose sensitive personal data to protect the participants' confidentiality.

Stigma expressions triggered by the senses

The concern about physical appearance among the Mestizos

When conducting this research, we were informed that there was a common concern about having visual marks among suspected mestizo patients in the North West area of Pichincha. We were searching for a patient in this area, but he was no longer living on his farmland. When reaching him via phone; this patient explained he had migrated to Quito, the capital city, to hide and be taken care of by his daughter. During the course of the call, he elaborated on the shame he felt for his

facial appearance: 'It started as a pimple, then it spread like papules. I had it on my forehead, temples, sideburns, lips and cheek […] The disease has spread; it's disgusting; I produce that, disgust'.

The health personnel in Northwest Pichincha –who have more experience diagnosing and treating CL than those in the Amazon– corroborated the patients' visual worries. The recurrent fear, they said, concerned the scars and incapacity of performing physical work. They also explained:

> They [patients] talk about shame, but at home, it [their appearance] doesn't matter as they continue to be loved and cared for […] It [skin sores] looks antiaesthetic. They put on overalls or cover themselves with Band-Aids or things like that, especially women. When lesions are on the face, they get more worried; the body is easier to cover.

The sanitary personnel thought CL was spreading, thus suspected patients were more acquainted with cutaneous lesions, trusted the health centres, and usually shared the same concerns. Spanish is the dominant language among mestizos, and because they are settled in more urbanised environments, they have good access to health services, basic services, formal education and the mainstream influence of Western thinking. The health workers mentioned that even when patients had social support from family and the community, they shared reactions of self-isolation, shame, desperation, and uncertainty, especially when medicine did not have immediate effects. Mestizos supported worldwide evidence that points out that appearance caused stigma expressions, as scar marks, disfiguration, and open wounds were connected to self-rejection and fear [6]. Previous research in the area supports the dominance of visuality as a stress factor in mestizo groups. This stress on appearance was reflected in the way CL was often named for the visual effect on the skin; *huequera* (hole maker), *relamida* (spreading of papules) [41, 42].

The disgust for rotting smell, snoring sounds and tasteless food in the Amazon

The Mestizo suspected patients seemed to have a different experience in recognising and fearing CL compared to their Amazonian Indigenous peers. It is worth mentioning that the North West of Pichincha has not reported cases of MCL as the Amazon has. The deeper we went in the hinterland, the more recurrent the mentions of smell, sound and taste to identify (muco)cutaneous leishmaniasis became. Table 2 disaggregates the leading aesthetic causes leading to stigma expressions per ethnicity participating in this study.

Table 2: Aesthetic causes and stigma expressions according to ethnicity

Linguistic group	**Spanish**	**Kichwa**		**Chicham**		
Ethnicity	**Mestizo**	**Kichwa**	**Zapara**	**Andoa**	**Achuar**	**Shuar**	**Shiwiar**
Aestetic causes triggering stigma expressions	Visual injuries and scars cause self-rejection and shame	Smell, appearance, sound, reflected in stigma expressions of light shame and fear of physical defects needed for self-subsistence.	Visual aspects leading to ugliness. Light stigma expressions of self-shame.	Smell leading to fear of social rejection linked to suspicion of witchcraft. Fear of name-calling and jokes.	Smell leads to fear of contagion and death. Uncertainty of the illness effects, physical dysfunction. Social distancing and isolation are natural responses towards patients and family.	Smell, sound, aspect, lead to fear of contagion and death, physical dysfunction, inappropriate behaviour/ adultery. Social distancing, segregation, isolation, relation with inappropriate behaviours	Smell and taste are linked to fear of contagion, inappropriate behaviour/ adultery and segregation of patients and families.

The reference to bad smell was common for CL and MCL. Suspected patients and community members expressed (self) disgust and fear of feeling an odd smell:

> Patient: When I was in the military barracks, I had a terrible smell. My mates said, "where does that ugly smell come from?". I stayed silent and far away […] they said that an ugly smell was coming out when I spoke. I was embarrassed and hid. […] I thought it was contagious, I didn't get very close to my wife, and she never caught it. I was afraid of infecting my children and grandchildren.
> Wife: When sitting down together I couldn't see him or talk; he smelled like garlic. He slept on one side further (of the bed). Yes, I was afraid of getting infected.

Many of the interviewees from all indigenous ethnicities compared the bad smell with rotting flesh, especially when the lesions are caused by MCL: 'When the rotten-smelling water came out, I stayed at home', 'I breathed and was rotten, ugh!, it smelled bad', 'Pus came out, like snot, and an ugly smell. It smelled rotten'. The reactions from most of the suspected patients were of shame and self-isolation when they were conscious of the awkward smell, even when other community members did not express disgust. Some of the vernacular names given to MCL in Kichwa are linked to the smell feature, like *ismu shimi* (rotten mouth) *or ismu sinka* (rotten nose). Name-calling using these expressions characterised physical dysfunctionality.

Other senses mentioned frequently to detect MCL were hearing sight and taste. Regarding hearing, participants mentioned snoring or a change in the voice tone. About the sight, the concern for the appearance of CL and MCL lesions was exacerbated by face disfiguration, where physical features were compared to animal or nature characteristics like 'pecari nose' or 'peperbell nose'. The change of taste was felt by MCL affected who mentioned that food had a different taste. Stigma expressions were linked to derision and comparison to animal features, being more exacerbated in Chicham-speaking groups. As this Achuar participant explained:

> People make fun; they do not talk to her; they say: "she has the disease, do not drink her chicha". Then, she doesn't prepare chicha. She makes porky sounds, they [MCL patients] always do that [noise], like peccary. […] if they snore, they are sick, the nose is being eaten from the inside, it is disgusting. My brother had it; I couldn't visit him.

Apparently, the mucosal form of the disease was the most feared and made evident stigma expressions, as many considered it could not be treated. Fear reached a point where sinus and allergies, with their symptoms of nasal congestion and halitosis, were frequently confused with mucocutaneous leishmaniasis.

Shame and self-disgust were shared by all ethnicities, but social isolation and segregation were more common among Chicham-speaking groups (Shuar, Achuar and Shiwiar). This social rejection had different manifestations, like eating from a separate dish, sleeping in different beds, or avoiding visiting the sick ones. Nested in a strong social tie tradition, the Chicham-speaking patients expressed going through pain when segregation happened. Social stigma seemed attenuated when living close to urban settlements as there is easier access to health centres. The Chicham ethnicities Shuar and Shiwiar linked the aetiology of the disease with shamanic witchcraft, which meant CL and MCL affected got sick because of sexual misbehaviour; thus they were punished. Demonstrations of stigma seemed harsher among the Shiwiar group, who exerted greater social rejection in CL and MCL affected and their families, awarding them stories that questioned their sexual reputation and good behaviour. In contrast, suspected patients from Kichwa-speaking groups (Runa, Andoa and Zapara) felt supported by community members, who wished for fast healing or advised them with remedies or medical treatment.

Sensorial perceptions helped evaluate aesthetic characteristics as markers of good health, physical strength and beauty. A body capable of performing activities like hunting, walking long periods, and carrying heavy loads equalled healthiness and also allowed for social functionality. The physician treating patients during medical brigades in the fieldwork of this research explained that he saw how other community members laughed about those sick with MCL. He said that even when the disease was not that critical or noticeable to sight, people could tell someone was sick by their bad smell and nasal voice sound, making the sick one the ugliest person in the community, thus affecting social relations. During an interview with a Shuar community member, he explained:

> Those with leishmaniasis feel despised by this disease because they cannot get close to other people. This shame is specifically on *kuchap* (skin leishmaniasis) because they think it will lead to death. Formerly in our culture, many had this disease and died. The illness starts and continues eating the leg until it causes death. They [the sick] will die alone from the bad smell that cannot be healed.

To summarise, our research findings revealed that there were stigma manifestations ranging from mild to severe among the different ethnicities. We identified behaviours of (self)discredit, (self)isolation and segregation balanced with social support in the case of Kichwa and Spanish linguistic groups. These perceptions and practices are a product of the imaginaries generated after a sensorial recognition of CL and MCL that are not constricted to the visual affectation of the skin.

Discussion

The aesthetics of sickness and death

Compared to the Mestizos in urbanised areas, indigenous peoples in the hinterland Amazon demonstrated the importance of sensorial perceptions other than the sole visual aspect to identify CL and MCL. This finding challenges previous studies that concluded visual aesthetics of scarring and deformities are the principal worldwide cause of stigmatisation, quality of life affection and psychological distress in CL [6, 39, 43]. The indigenous empirical recognition of a disease linked to the complete spectrum of sensorial capacities challenges western attempts to generalise visuality as the leading sense and refers to a particular appreciation of the aesthetics informed by forest relations.

Aesthetic qualities were points of reference to disclose imaginaries of risk that triggered stigma expressions. To explore this further, we depict Salazar's definition of imaginaries as 'culturally shared and socially transmitted representational assemblages that interact with the personal imagination and are used as meaning-making devices, mediating how people act, cognise and value the world' [44]. In this regard, this is how such imaginaries of risk helped contrast highly valued health and well-being with illness and death. The first sensorial imaginary is the link between the smell of rotting flesh and deceased corps. Christine Taylor noted among the Chicham that the perception of flesh decomposition, especially in the face, transmits imaginaries of death, and depersonalisation from a person's physical identity [45]. The second sense is sight, through visually non-healing sores noticeable on the skin surface related to a fear of disease contagion. The third is hearing as the sound of nasal voice was associated with the inability to taste food properly, pointing to a physical dysfunction inside the body. Particularly for the Chicham, MCL and CL were considered diseases that eat from the inside and kill. For Amazon societies, the connection of body and death is emphasised in the process of decomposing that marks the detachment of the self from the body [45, 46]. The persistent appreciation of being alive or dead comes from a forest environment where living beings seem to exist in a perpetual notion of being either hunter or prey [34, 47]. Amazon societies are aware that the risk of death is a quotidian threat and also realise death is intrinsic to life despite their ethnically differentiated responses and ways to cope with illness.

Stigma, a social protection response to unbalanced forest relations

'Being among kin, people say, is the essence of living a 'good life' and the essential criteria of happiness' (Uzendoski) [48].

One of the principles of sensorial anthropology is that sensorial perceptions explain peoples' (social) environments, which are often communicated through metaphors [26]. Senses are intertwined with cognitive activity, and embodied experiences –like an illness– can explain cultural value systems. In this case, practices and beliefs –including values– of Amazon ethnicities are tied to forest relations [48]. For Amazon societies, disease and death are metaphors for unbalanced (non)human relations. While the indivisible relation among (non)human beings is embedded in a holistic cosmovision of unity, highly praised well-being and healthiness are understood as the outcome of balanced network relations [32]. In this sense, the body is the materiality where relations are created and destroyed; thus life, illness and death are events connected to such relationships [49].

Our research unveiled that despite the fact that Kichwa and Chicham linguistic groups' responses towards the suspected patients with CL and MCL differed in stigma expressions, they were linked to social protection mechanisms. Such actions seemed paradoxical despite the fact that they shared the same rainforest environment. Practices of social support in the case of the Kichwa, contrasted with those of isolation and segregation –determining social stigma for suspected patients (and families)– in the case of the Chicham. These differences existed due to their conceptions of life, disease and death (see Table 3).

Table 3: Life, disease and death conceptions for Kichwa and Chicham linguistic groups

Analysis dimension	Amazon Kichwa	Chicham
Cosmovision	Holistic. Unity between human and non-human beings.	Holistic. Unity between human and non-human beings. The real world is invisible; it is revealed through visions and dreams.
Life	Given by the *samai*, the life spirit. Life is a state of constant transformation.	Depends on the attainment of the *arutam*, the life (with purpose) spirit. Life has stages of intensity of being more or less alive.
Health/wellbeing	Equilibrium of (non)human relations.	Equilibrium of (non)human relations.
Illness/Disease	Rupture of (non)human equilibrium. Is natural, we are all susceptible to (bodily) transformation.	Rupture of (non)human equilibrium. The weak (of spirit) fall ill as they lose their *arutam*.
Death	Death is part of the life continuum. Detachment of the self from the body.	Death is part of the life continuum. Detachment of the self from the body.
Life restoration strategies	Restoration of (non)human relations through social support.	Social segregation as the ill is already dead. The ill need to find ways to recover their *arutam*.

The Kichwa acknowledgement of the life-(illness)-death continuum means everything is in constant transformation, and strong (non)human networks help cope with disease processes [50]. This implies that the constant construction of identities is relational and therefore changing [51]. In other words, one person can (self)identify sick with MCL, and the social group would help change that identification by procuring healing and support so they can recuperate their life spirit called *samai* [33, 48]. A close (non)human relationality allows the incorporation of the qualities or 'vitalities' of other (nature) beings (including plants, animals and objects), resulting in the compound construction of selfhood through a permeable body [31, 33]. The fact that bodies alter and become vulnerable imprints empathic reasoning over illness susceptibility. The inevitability of everyone's (and everything) constant transformation may explain the low levels of social stigma and segregation despite a generalised fear of contagion –that explain self-isolation– as they align with the high value of (non)human relations maintenance.

In contrast, the Chicham understand illness-death as a process of being more or less alive and being ill/death signifies a rupture of (non)human connections, losing the purpose of life. This conception of 'intensity' of life means illness and death are not sharply differentiated as 'both are viewed as a process linked by a series of metamorphoses rather than as ontologically distinct conditions […] illness is a single phenomenon, whatever its symptoms, whether psychic or somatic;

there are no specific illnesses, just undifferentiated suffering' [34]. The ill are considered incapable of communicating with their bodies as they fail to acquire the purpose of life spirit called *arutam,* also referred to as 'life with a purpose' [45]. Certain practices aiming to reach equilibrium help acquire the *arutam*, like the intake of hallucinogens, shaman support, keeping good relations, behaving appropriately and dieting [34]. Likewise, illness is also justified through misbehaviour that causes a rupture of (non)human relations and interferes with reaching the *arutam*. In this context, feeling or being ill is a personal and social construction that involves an idea of malfunctioning in the self that derives in a negative (self-)image and (self-)perception [34, 49].

Kin relations are important for constructing the self-image, stressing the high-value social relations have for the Chicham societies. In this understanding, segregation is the expected outcome of an illness, explaining the radical behaviours of social and self-stigmatisation. Herein, stigma is not only justified by the sense of control and immunity from danger [52], but it also helps to distance from the weak (of spirit), who are seen as morally low [13]. Disease and death involve a process of disentanglement, where the ill distance from their kin and turn absent as beings, cutting off from social and affective relations. If a person's physical characteristics change due to disease – like having rotting skin/flesh– social ties will shift to hostility, eliminating all forms of communication with their kin and making the sick highly vulnerable [34, 45, 49].

Conclusion

The aim of this study was to understand the perceptions leading to CL and MCL leishmaniasis-related stigma expressions. Even when stigma expressions varied among the different ethnicities, the perceptions leading to imaginaries of death were a commonality in Amazon ethnic groups. The use of the embodied senses of smell, sight, hearing and taste that gave rise to perceptions of risk and subsequent stigmatisation contested international evidence suggesting the predominance of visuality. The participation of Spanish-speaking Mestizos helped to back up the international evidence, and at the same time, contrast it with Amazon ethnic groups who prioritised other senses (but sight) in the identification of CL and MCL. The empirical experience with disease and death of forest societies makes sensorial perceptions a source of information that resorts to their environmental relations. The sensorial perceptions of Amazon ethnic groups also allowed disclosing a value system where well-being and healthiness conceived in a holistic approach are praised values linked to good (non)human relations.

That being said, the empirical ways of approaching illness by health practitioners and researchers in hinterland societies should incorporate a different view on well-being that would improve the beneficial results of health attention. Herein, there is a need for further qualitative research on diseases affecting Amazon societies. Despite the idea that an increased rational understanding of the illness –through health promotion– will improve well-being, health coverage aiming to treat and prevent psychosocial suffering can only be reached by considering empirical knowledge and local value systems. Disregarding sensorial embodied experiences with a disease in the Amazon would likely result in the population's low reliance on health practitioners. In this regard, treating (muco)cutaneous leishmaniasis and other diseases requires acknowledging a holistic cosmovision, for example, involving behaviour recommendations to maintain good (non)human relations to restore an individual's vital force.

Acknowledgements

This research was possible thanks to the participation of community leaders and health personnel who acted as gatekeepers supporting the researchers. The study was funded by *Fundación Mision*

Cristiana de Salud and the Amsterdam Institute for Infection and Immunology at Amsterdam University Medical Centers. This study was approved by the ethical committee of the *Universidad Internacional del Ecuador* with permission number UIDE-FCM-EDM-COM-18-0069 and by the Ecuadorian Ministry of Health with permission number MSPCURI000284-3. The authors declare that no potential competing interest was reported with respect to this study.

Data availability statement

The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to sensitive information that could compromise the privacy of research participants. Interview guides can be downloaded from: https://www.academia.edu/76844367/Interview_Guide_Perceptions_on_Cutaneous_Leishmaniasis_Ecuador?source=swp_share

References cited

1. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PloS one. 2012;7(5):e35671.
2. World Health Organization. Leishmaniasis: WHO; 2023 https://www.who.int/data/gho/data/themes/topics/gho-ntd-leishmaniasis#:~:text=Out%20of%20200%20countries%20and,are%20endemic%20for%20CL%20only. Accessed: 26 January 2023.
3. Calvopina M, Guevara AG, Armijos RX, Hashiguchi Y, Davidson RN, Cooper PJ. Itraconazole in the treatment of New World mucocutaneous leishmaniasis. International journal of dermatology. 2004;43(9):659-63.
4. Lessa HA, Lessa MM, Guimaraes LH, Lima CM, Arruda S, Machado PR, et al. A proposed new clinical staging system for patients with mucosal leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2012;106(6):376-81.
5. Henríquez-Trujillo AR, Coral-Almeida M, Hinojosa MC. The burden of cutaneous and mucocutaneous leishmaniasis in Ecuador (2014-2018), a national registry-based study. bioRxiv. 2019:751446.
6. Bennis I, De Brouwere V, Belrhiti Z, Sahibi H, Boelaert M. Psychosocial burden of localised cutaneous Leishmaniasis: a scoping review. BMC Public Health. 2018;18(1):358.
7. World Health Organization. Skin NTDs: prioritizing integrated approaches to reduce suffering, psychosocial impact and stigmatization Geneva: WHO; 2020 https://www.who.int/news/item/29-10-2020-skin-ntds-prioritizing-integrated-approaches-to-reduce-suffering-psychosocial-impact-and-stigmatization. Accessed: 23 December 2022.
8. Morales D, Paredes M, Morales-Butler EJ, Cruz-Aponte M, Arriola L, Cevallos V, et al. Data scarcity and ecological complexity: the cutaneous leishmaniasis dynamics in Ecuador. Journal of the Royal Society Interface. 2019;16(157):20190141.
9. Bezemer JM, Calvopiña Hinojosa M, Corral Zabala AE, Ortega Pérez F, Vargas Román VC, Schallig HDFH, et al. Quality of life of cutaneous leishmaniasis suspected patients in the Ecuadorian Pacific and Amazon regions: a cross sectional study. BMC infectious diseases. 2022;22(1):1-9.
10. Link BG, Phelan JC. Conceptualizing stigma. Annual review of Sociology. 2001;27(1):363-85.
11. Goffman E. Stigma: Notes on the Management of Spoiled Identity. Englewood Cliffs, NJ Prentice Hall. 1963:127-8.
12. Deacon H. Towards a sustainable theory of health-related stigma: lessons from the HIV/AIDS literature. Journal of community & applied social psychology. 2006;16(6):418-25.
13. Yang LH, Kleinman A, Link BG, Phelan JC, Lee S, Good B. Culture and stigma: Adding moral experience to stigma theory. Social science & medicine. 2007;64(7):1524-35.
14. Luka EE. Understanding the stigma of leprosy. South Sudan Medical Journal. 2010;3(3):45-8.
15. Pires M, Wright B, Kaye PM, da Conceição V, Churchill RC. The impact of leishmaniasis on mental health and psychosocial well-being: a systematic review. PloS one. 2019;14(10):e0223313.
16. Kleinman A, Ryan G. Between History and Anthropology: Stigma, the Subaltern and Leprosy in China: An essay review of Leprosy in China: A History, by Angela Ki Che Leung. New York: Columbia University Press, 2009. Culture, Medicine, and Psychiatry. 2010;34:548-52.
17. Holleman M. Introducing a novel approach to the cross-cultural measurement of stigma versus social integration using methods from the field of cognitive anthropology. Journal for the Theory of Social Behaviour. 2020;50(4):534-52.
18. Ramdas S, van der Geest S, Schallig HD. Nuancing stigma through ethnography: the case of cutaneous leishmaniasis in Suriname. Soc Sci Med. 2016;151:139-46.
19. Ramdas S. Perceptions and treatment of cutaneous leishmaniasis in Suriname: a medical-anthropological perspective: Universiteit van Amsterdam [Host]; 2015.
20. Thompson A, Kent G. Adjusting to disfigurement: processes involved in dealing with being visibly different. Clinical psychology review. 2001;21(5):663-82.
21. Elhaik T. Aesthetics and anthropology: Cogitations: Routledge; 2021.

22. Desjarlais R. Body and emotion: The aesthetics of illness and healing in the Nepal Himalayas: University of Pennsylvania Press; 1992.
23. Nichter M. Coming to our senses: Appreciating the sensorial in medical anthropology. Transcultural psychiatry. 2008;45(2):163-97.
24. Jackson J. " Camp pain": talking with chronic pain patients: University of Pennsylvania Press; 2000.
25. Desjarlais R, Jason Throop C. Phenomenological approaches in anthropology. Annual review of anthropology. 2011;40:87-102.
26. Howes D. Empire of the senses: The sensual culture reader: Routledge; 2021.
27. Howes D. Multisensory anthropology. Annual Review of Anthropology. 2019;48:17-28.
28. Classen C. Sweet colors, fragrant songs: sensory models of the Andes and the Amazon. American ethnologist. 1990;17(4):722-35.
29. Seremetakis CN. Sensing the everyday: dialogues from austerity Greece: Taylor & Francis; 2019.
30. Howes D. El creciente campo de los estudios sensoriales. Revista latinoamericana de estudios sobre cuerpos, emociones y sociedad. 2014(15):10-26.
31. Santos-Granero F. Sensual vitalities: non-corporeal modes of sensing and knowing in native Amazonia. In the world and about the world: Amerindian modes of knowledge Special issue in honor of professor Joanna Overing. 2006.
32. Kohn EO. Runa realism: Upper Amazonian attitudes to nature knowing. Ethnos. 2005;70(2):171-96.
33. Mezzenzana F. Encountering Supai: An Ecology of Spiritual Perception in the Ecuadorian Amazon. Ethos. 2018;46(2):275-95.
34. Taylor AC. The soul's body and its states: an Amazonian perspective on the nature of being human. Journal of the Royal Anthropological Institute. 1996:201-15.
35. Shepard Jr GH. A sensory ecology of medicinal plant therapy in two Amazonian societies. American Anthropologist. 2004:252-66.
36. Panter-Brick C. Health, risk, and resilience: Interdisciplinary concepts and applications. Annual Review of Anthropology. 2014;43:431-48.
37. Pink S, Morgan J. Short-term ethnography: Intense routes to knowing. Symbolic interaction. 2013;36(3):351-61.
38. Ingold T. Bindings against boundaries: entanglements of life in an open world. Environment and planning A. 2008;40(8):1796-810.
39. Al-Kamel MA. Stigmata in cutaneous leishmaniasis: Historical and new evidence-based concepts. Our Dermatology Online. 2017;8(1):81.
40. Haboud M. Pueblos y lenguas indígenas de la Amazonía Ecuatoriana. Atlas Sociolinguistico De Pueblos Y Lenguas Indígenas De América Latina. 2010:1-27.
41. Weigel MM, Armijos RX, Racines R, Zurita C, Izurieta R, Herrera E, et al. Cutaneous leishmaniasis in subtropical Ecuador: popular perceptions, knowledge, and treatment. Bulletin of the Pan American Health Organization (PAHO); 28 (2), jun 1994. 1994.
42. Calvopina M, Armijos RX, Hashiguchi Y. Epidemiology of leishmaniasis in Ecuador: current status of knowledge -- a review. Memorias do Instituto Oswaldo Cruz. 2004;99(7):663-72.
43. Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet (London, England). 2018;392(10151):951-70.
44. Salazar NB. On imagination and imaginaries, mobility and immobility: Seeing the forest for the trees. Culture & Psychology. 2020;26(4):768-77.
45. Taylor AC. Remembering to forget: identity, mourning and memory among the Jivaro. Man. 1993:653-78.
46. Cova V. Thinking the End: Desiring Death and the Undead in the Ecuadorian Upper Amazon. Ethnos. 2021:1-20.
47. Kohn E. How forests think: Toward an anthropology beyond the human: Univ of California Press; 2013.
48. Uzendoski MA. Manioc beer and meat: value, reproduction and cosmic substance among the Napo Runa of the Ecuadorian Amazon. Journal of the Royal Anthropological Institute. 2004;10(4):883-902.

49. Taylor A-C. Healing translations: Moving between worlds in Achuar shamanism. HAU: Journal of Ethnographic Theory. 2014;4(2):95-118.
50. Swanson TD, Reddekop J. Looking like the land: Beauty and aesthetics in Amazonian Quichua philosophy and practice. Journal of the American Academy of Religion. 2017;85(3):682-708.
51. Descola P. Beyond nature and culture: University of Chicago Press; 2013.
52. Deacon H. Understanding HIV/AIDS stigma: A theoretical and methodological analysis: HSRC press; 2005.

Chapter 8

General discussion

General discussion

Revealing the geographic distribution of *Leishmania* species and its implications

Protozoan parasites of the genus *Leishmania* can cause cutaneous (CL), mucosal (ML), and visceral (VL) human disease [1]. Researchers have discovered *Leishmania* in 4000-year-old Egyptian and 2800-year-old Peruvian mummies demonstrating humanity's millennial history with the parasite [2, 3]. The clinical image was described on Assyrian tablets dating back 2700 years and was depicted in human shapes called "huacos" by the Peruvian Moche people 1400 years ago [4, 5]. Historically, distinguishing leishmaniasis from other diseases, such as leprosy, was impossible because the causing agent was unknown [6]. This changed when the Scottish physician Cunningham used a light microscope to see the oval parasitic form for the first time in 1885, though he did not recognize it as *Leishmania* [4]. His fellow countryman, pathologist Leishman, reported the discovery of those bodies in *post-mortem* spleen smear preparations of a soldier who had fever and cachexia in 1903 [7]. A year later, European scientists generally accepted the genus name *Leishmania* [8]. *Leishmania* subgenera and species were further differentiated over the course of more than a century, with the final human pathogenic species being described in 2014 [9]. The geographic distribution of the *Leishmania* species is still being determined [10, 11].

We have extensively described the identification of *Leishmania* species in Ecuadorian patients with CL lesions in the Amazon and Pacific regions in **Chapter 2**. *L. guyanensis* was discovered in the Ecuadorian Amazon provinces of Napo, Pastaza, and Morona Santiago, *L. braziliensis* in the Pacific Imbabura province, and *L. lainsoni* in the Pacific Pichincha province and the Amazon provinces of Napo, Pastaza, and Morona Santiago. In addition, we report a sustained low prevalence of *L. braziliensis* in the Pacific region. Because *L. braziliensis* is the only species associated with ML in Ecuador, these findings have implications for the follow-up of CL patients [12, 13]. Only 46 species determinations in Amazon CL patients are reported in **Chapter 2**, with *L. braziliensis* accounting for nearly half of them. To further identify high-risk parishes, cantons, or regions (e.g. depending on the altitude), this number is insufficient. Further stratification of ML risk would be possible if the species of all CL cases found to be infected in the Amazon were known. Additionally, patients with *L. braziliensis* infections would be identified, enabling long-term surveillance to find any lesions of the nasal and oral early [14]. People from non-*L. braziliensis* endemic regions who become infected while working in the Amazon are more likely to receive the wrong diagnoses because they may not recognize the symptoms of ML and may consult medical specialists who are unfamiliar with it [15]. These groups include the military, oil company workers, loggers, gold diggers, and farmers [16]. According to Marsden, patients who have been infected with *L. braziliensis* should get yearly and ongoing clinical follow-up [17]. The American Society of Tropical Medicine and Hygiene and the Infectious Diseases Society of America both recommend two years of follow-up in their clinical practice guidelines. They recommend a clinical visit that includes nasal and oral mucosa revision [18]. Reference centers in Europe and North America provide at-risk patients with written information outlining the risk of ML [18, 19]. The Pan American Health Organization guidelines also recommend long-term ML follow-up for the growing number of *L. braziliensis*-infected patients who receive local treatment [20]. Early ML treatment may help to prevent therapy resistance and severe complications such as facial malformations, malnutrition, and laryngeal obstruction which could be fatal, so studies to determine the type, interval, and duration of follow-up are required [21, 22].

Sequencing issues

A limitation was that only half of the CL patients' species could be determined from DNA stored in filter paper (**Chapter 2).** This was due to a low DNA yield, which hampered cytochrome B sequencing in all of the samples. By enhancing the sample collection, handling, DNA extraction, and/or species determination processes, better results may be attained [11, 23]. Consequently, we propose developing an initial ML surveillance approach based on species assessments of each sample, followed by a risk stratification based on regions. **Chapter 2** demonstrates that this is possible with filter paper sampling in remote health centers without a cold chain or land transport, in tropical conditions, and with extended storage times due to the COVID pandemic.

Sequencing of the cytochrome B gene is an internationally validated method for determining *Leishmania* species [24]. When sequences are made publicly available in repositories such as GenBank, it allows for detailed international comparison of the results, which is an advantage of gene fragment sequencing over other methods such as restriction fragment length polymorphism analysis [25]. The cytochrome B sequences of *L. braziliensis* samples were indistinguishable from *L. peruviana* when compared to the NCBI database using the basic local alignment search tool and in a phylogenetic (**Chapter 2**)[26]. *L. braziliensis*-*L. peruviana* hybrids have been found in Peruvian samples but not in Ecuador [27, 28]. They have been reported in ML patient samples and should not be considered low-risk [29]. Sequencing of the mannose phosphate isomerase (MPI) gene has been proposed to finally differentiate the two species based on a single nucleotide polymorphism [30]. The majority of *L. braziliensis* samples whose MPI gene was sequenced revealed the absence of the 'G' allele at location 1082, confirming that these samples had to be classified as *L. braziliensis*. Interestingly, five samples revealed the presence of an 'A' allele in addition to the 'C' allele, which appears to be an Ecuadorian variant **(Chapter 2)**. These findings highlight the genetic diversity of the *Viannia* complex, which has previously been reported [27, 31]. For Ecuador, we recommend that the cytochrome B gene sequencing be supplemented with the sequencing of an additional gene, such as MPI. A phylogenetic tree constructed from the cytochrome B sequences of more than 100 *L. guyanensis* samples revealed a subclade with >95% Pacific samples and a subclade with >70% Amazon samples **(Chapter 2)**. This implies that mutations respect regional borders which may have consequences for diagnostic test accuracy, therapy resistance, and the risk of ML, even within the same species.

Management of leishmaniasis by region versus species

Current management of leishmaniasis in Ecuador is nationwide and species-specific at the level of the Americas [20, 32]. Our finding of an apparently Ecuadorian mutation of the MPI gene in *L. braziliensis* and region-specific subclades of *L. guyanensis* provides genetic evidence for comparative research of test accuracy, therapy efficacy, and ML risk between geographically delimited areas. It calls into question the applicability of current leishmaniasis management guidelines based on research from other regions or countries. Miltefosine, for example, was introduced by the Ministry of Health in Ecuador in 2008 but was discontinued after a few years, presumably due to a lack of efficacy [33, 34]. Even though no Ecuadorian trials with miltefosine have been reported, the new Pan American Health Organization (PAHO) guideline strongly recommends it [20]. This illustrates the importance of regional research, especially in a decade when new point-of-care tests, drugs, and vaccines for leishmaniasis are being developed and will most likely be used at the level of the Americas when safety and efficacy are proven in only a few clinical studies [35-37]. The finding of a persistently low prevalence of *L. braziliensis* in the Pacific region (5/89, 6%) is lower than Kato *et al.* reported (12/101, 12%) and does not confirm an increase

(**Chapter 2**)[11]. As a result, we do not recommend standard species determination for all Pacific CL patients because it would be a time-consuming and costly procedure with a minor impact on patient follow-up.

Health Seeking delay and implications for neighboring countries

Another reason why ML surveillance is not recommended in the Pacific is the relatively short health-seeking delay as described in **Chapter 2.** A short health-seeking delay allows for early treatment initiation and reduces the risk of ML development [38]. In the Amazon, on the contrary, the median health-seeking delay was twice as long as in the Pacific providing yet another reason for ML surveillance. In **Chapter 2** we describe that age, Amerindian ethnicity, infection at lower altitudes, lesions on the lower limbs, and non-ulcerative lesions are associated with prolonged health-seeking delays. All of these factors are associated with the Amazon region, except for the last. In the Amazon, transmission seems to be sylvatic involving adults who leave their homes and enter the rainforest for hunting, farming, logging, mining, oil extraction, or military purposes [16, 39]. Because the vector flies low, the proportion of bites on the lower limbs is higher in adults [40]. Roads in the Amazon begin in the highlands and descend into the tropical rainforest, where they end halfway, leaving vast areas of the Ecuadorian Amazon inaccessible by land [41]. Because the inaccessible lowlands are primarily inhabited by Amerindians, travel difficulties are linked to lower altitudes and ethnicity [42]. Even if they live in easily accessible areas, Amerindian patients may face longer health seeking delay due to cultural differences, language barriers, poverty, and discrimination [43, 44]. Stigma has been shown to delay health-seeking in leprosy patients [45]. The extent to which stigma caused health-seeking delay in suspected CL patients has not been studied yet. The variables associated with health-seeking delay should be interpreted with caution because the study excludes determinants such as occupation, educational level, income, marital status, distance to the health center, visit to a traditional healer, and possible doctors delay. A future research project should include all of these variables, as well as the potential impact of stigma, to develop a program that adequately addresses health-seeking delay [46]. The association between non-ulcerous lesions and health-seeking delay might be explained by a failure of healthcare professionals and/or patients to recognize atypical lesions. Only 16 (7%) of such patients are described in **Chapter 2,** so we recommend investigating health care professionals' knowledge of non-ulcerative manifestations of CL, followed by (if necessary) a training program in endemic areas.

Ecuador, Peru, and Colombia share geographic, ecological, and social characteristics at their border regions that have implications for infectious disease epidemiology [47, 48]. This includes potential animal reservoirs for leishmaniasis and *Lutzomyia* vectors found across borders [49, 50]. The prolonged health-seeking delay in southern Ecuador's Amazon region may also exist in neighboring Colombian and Peruvian provinces. As a result, we recommend studies of patient characteristics and determinants of health-seeking delay in suspected CL patients in Colombia and Peru to prevent prolonged suffering, therapy resistance, and the development of ML.

Challenges in the diagnosis of CL and ML

Despite the fact that the *Leishmania* parasite was discovered using a light microscope nearly 140 years ago, this method is still used to diagnose the disease and make treatment decisions in suspected Ecuadorian CL cases [4]. In **Chapter 3**, we assessed the diagnostic accuracy of a quantitative polymerase chain reaction (qPCR) on DNA extracted from lesion-imprinted filter paper and microscopic examination of Giemsa-stained smear slides for the diagnosis of CL. Healthcare professionals included over 300 participants based on clinical suspicion of CL. Unexpectedly, the

intended gold standard for CL confirmation, qPCR on DNA extracted from lesion-imprinted filter papers, generated false negative results in almost one-fifth of the samples. Therefore, Bayesian latent class analysis (LCA) was used to estimate true prevalence and test accuracy. The true prevalence in the study was estimated at approximately 80%. Both qPCR and microscopy sensitivity were lower than the predefined threshold of 80%. A comparison of microscopy sensitivity by region found statistically significant heterogeneity between the Pacific and the Amazon. Because an informative prior for the specificities of qPCR and microscopy was used (specificity for both was more than 97% according to previous studies), it was not a meaningful outcome in this study [51].

Both qPCR on DNA extracted from filter paper lesion imprints and microscopy on smear slides of lesion scrapings had poor sensitivity. This outcome might be explained in part by LCA's improved test accuracy estimations [52]. Previous studies on the accuracy of microscopy and PCR methods for CL diagnosis used imperfect or composite gold standards, which could have altered test accuracy estimates [51, 53]. As long as no perfect gold standard has been defined, it is recommended that LCA be used routinely to evaluate tests for CL diagnosis.

Both qPCR and microscopy identify the presence of *Leishmania* amastigotes in the sample. As a result, a low parasite load in the sample might explain the failure of both tests. Grading amastigote density in microscopy slides could have confirmed low parasite loads and should be done in future studies. In previous research, the distribution of amastigotes in cutaneous lesions caused by *L. (Viannia)* species was uneven and region-dependent [54, 55]. Uneven amastigote lesion distribution and regional differences might explain the heterogeneity between qPCR and microscopy as well as the Amazon and Pacific. Therefore, for each patient, a combination of sampling (e.g., scalpel scraping + filter paper imprint) and diagnostic (e.g., microscopy + qPCR) techniques is recommended. In samples infected with *L. braziliensis*, both the median *Leishmania* rDNA concentration and microscopy sensitivity were lower than in *L. guyanensis*. Because the number of confirmed *L. braziliensis* samples was small (less than 30), it cannot be concluded that parasite density varies by species. A future study on parasite density and test accuracy should include a larger patient group with *L. braziliensi*s.

Improvement in sample storage and analytic methods might also increase test accuracies. For microscopy, a future study should include data on a first (by the peripheral technician) and second (by a referral technician) revision of the slides to see whether improved training is required. For qPCR, future studies should avoid storing filter papers containing sample at ambient temperature for more than 12 months, and filter papers should be transferred by health personal while avoiding severe heat and humidity. Although the chelex-based method used for DNA extraction is inexpensive and non-time-consuming, it has only been used in a few CL studies [56]. The inclusion of a purification step, as described by Singh *et al.*, is recommended for future studies as contamination might cause false negative PCR results [57]. Because the qPCR used lacked a reverse transcriptase step, it was unable to detect *Leishmania* RNA, which may be more abundant in the lesions. Such a step is recommended for future studies as described by van der Meide *et al.* [58]. The low individual sensitivity of smear slide microscopy and qPCR on DNA from lesion filter paper imprints illustrate the difficulty in developing an accurate point-of-care confirmatory test for CL.

The microscopy test accuracy is particularly important because it is the current point-of-care test in all of Ecuador's endemic areas [59]. The low sensitivity reported in **Chapter 3** contradicts the Ministry of Health's 2013 guideline, which states that microscopy sensitivity ranges between 85 and 90% [59]. Ecuador reports approximately 1400 microscopy-confirmed CL cases each year [60]. Extrapolating the 33% (1-sensitivity) false negativity, to the national level leads to an estimate of

700 patients incorrectly classified as negative each year [60]. This number is concerning because microscopy-negative patients do not receive anti-*Leishmania* treatment [34]. Patients who are not treated will most likely resort to alternatives such as medicinal herbs, high temperatures, or toxic methods such as battery acid, lead, and mercury, which can cause severe scarring [61]. Moreover, prolonged patient suffering, waste of resources, and diagnostic failure will foster distrust in the involved health professionals and system [62]. Distrust in the healthcare system reduces the effectiveness of any preventive or curative program and widens health disparities [63]. Therefore, the microscopy-based test and treat program should be discontinued or modified.

Interestingly, the estimated true CL prevalence was relatively high among patients included on the base of clinical suspicion. Treatment of all suspected patients in this study with meglumine antimoniate would expose too many non-CL patients to its toxicity, would be costly, and time-consuming [64]. Nevertheless, the 2022 PAHO guideline recommends thermotherapy for CL [20]. Thermotherapy has few side effects, is inexpensive, rapid, and can cure more than 70% of CL patients [65]. Therefore, it is recommended that clinical studies be conducted to explore algorithms for the management of patients on the base of CL suspicion incorporating thermotherapy.

Clinical variables to detect cutaneous and mucosal leishmaniasis

The impact of sociodemographic or clinical variables such as age (cut-off 20 years), gender, altitude of infection (cut-off 500m), and body location of the lesion (head and neck versus other location) on the predictive values of microscopy or qPCR was examined in **Chapter 3**. The addition of one of those variables did not affect predictive values, particularly the negative predictive value (NPV). That's unfortunate because such variables are easily accessible in remote settings. A future study that includes an evaluation of professionals' arguments for clinical suspicion of CL could aid in the development of a CL management algorithm.

According to the literature, the diagnostic accuracy of microscopy for ML is even lower than for CL [23, 66]. In **Chapter 4,** a systematic literature review examines the detection rates of clinical variables for ML in patients reported in case series. The study includes ten papers that cover more than 190 ML patients. The detection rate of the male gender was the highest (88%), followed by nasal mucosa ulceration (77%), age>15 (69%), and duration of symptoms >4 months (63%). Microscopic examination of biopsies and direct smears yielded detection rates of 55 and 41% respectively. The cumulative detection rate of two or three positives from the last three variables was 84% in men and 79% in women, which is encouraging for clinical practice. Because the papers included in the study did not include non-ML patients, no specificity could be calculated. All of the included studies were found to have a high risk of bias. This study is a first, but small, step toward developing diagnostic criteria for ML. Future studies should include non-ML patients to define the most discriminative criteria and calculate the predictive values of combinations. Such studies should be carried out at the local level because the predictive values (both negative and positive) are affected by the prevalence of diseases included in the differential diagnosis.

Challenges in the treatment of leishmaniasis

Russell, an eighteenth-century physician, reported that the treatments for the Aleppo boil (later identified as CL) were both countless and useless [67]. Since the discovery of leishmaniasis in 1903, treatment efficacy has been compared globally. Within a decade, the metalloid antimony, an ancient remedy, was discovered to be effective against leishmaniasis [68, 69]. Its compounds tartar emetic and urea stibamine saved the lives of thousands of VL patients in India, but they had serious side effects [70]. The pentavalent antimonial meglumine antimoniate was introduced in 1946 and

quickly replaced other antimonials because it had fewer effects [71]. Despite its high cost, toxicity, parasite resistance, lack of oral formulations, and long treatment time, meglumine antimoniate remains a cornerstone in the treatment of leishmaniasis In the Americas more than 75 years after its introduction [20].

Allylamines, particularly terbinafine, are used to treat fungal infections of the skin. They are widely available at reasonable prices in topical and systemic formulations [72]. A systematic review aimed at assessing the efficacy and safety of allylamines for the treatment of CL and ML found only one well-designed randomized controlled trial in CL patients infected with *L. tropica* **(Chapter 5)**. In this trial, 28 days of oral terbinafine + cryotherapy (n=40) was compared to 28 days of meglumine antimoniate + cryotherapy (n=40), and cure rates were only 38% versus 53% in the control group [73]. Studies on cultured amastigotes revealed that the effective concentration was five times higher than skin concentrations achieved with common terbinafine doses [74, 75]. As a result, we conclude that further research on allylamine monotherapy is not warranted and that it should not be used in CL patients. In an *in vitro* study of terbinafine in combination with the triazole drug ketoconazole, the minimally inhibitory concentration was reported to be more than 1000 times lower than that reported for allylamine monotherapy [76]. This synergistic effect appears promising, but it must be replicated in other *Leishmania* species. Consequently, before considering human studies, animal studies should be conducted to define ideal combinations and concentrations [77]. When human studies confirm the safety of a combination of allylamines and triazoles, that is only the beginning of the process. Pharmacokinetic studies, efficacy studies, randomized controlled trials, implementation studies, and finally pilot introduction studies would be the next steps. Unless incredible collaborations are achieved, the timeline for this process will be decades-long [78]. Even so, given the already existing resistance of leishmaniasis to monotherapy with both allylamines and triazoles, resistance development would remain a major concern (**Chapter 5**) [79]. The potential repurposing of an allylamine-triazole combination for leishmaniasis can be compared to the history of miltefosine. Miltefosine was originally used in oncology and is the only oral drug used to treat leishmaniasis [20]. It took 34 years after the discovery of its anti-leishmaniasis activity for the Food and Drug Administration of the United States to approve it. Almost a decade later, it is discovered to have severe side effects, to be teratogenic, and resistance is reported [80, 81]. Also, because Knight Therapeutics, the pharmaceutical firm that holds the rights to it, is greedy, it is unavailable for the majority of patients [82]. The miltefosine case illustrates how challenging drug repurposing can be and why new medications should ultimately be developed [83]. The Drugs for Neglected Diseases initiative (DNDi) is currently a key player in the development of new leishmaniasis drugs. Their portfolio for leishmaniasis treatment includes five drugs in phase 1 trials and one in phase 2 trials [36]. This appears promising because phase 2 trials could result in a drug being available within 6 years, but it remains uncertain when we consider the considerable percentage of phase 1 and 2 drugs that do not reach the patient population [83]. Furthermore, new drugs may be effective only for one type of leishmaniasis (e.g. VL or CL) or one geographic region [37]. Given the number of new drugs being developed, the limitations of drug repurposing, and limited resources, research teams should carefully consider whether the combination of allylamines and triazoles is worthwhile [84]. Collaboration with international initiatives such as DNDi would be preferable to ensure funding and planning of follow-up studies until clinical implementation and beyond [85].

Leishmaniasis related Quality of Life

The first systematic study on the quality of life of CL patients originates from Turkey and was published in 2004. Patients with active lesions had a lower quality of life than scarred patients [86]. This Turkish study was published more than 25 years after the term 'quality of life' became a keyword in the medical subject headings of the US national library of medicine (MEDLINE) [7, 87]. Weigel *et al.* had already published the results of a short, non-validated, questionnaire on the psychosocial impact of CL among 27 patients with active lesions, 77 with CL scars, and 104 additional Ecuadorian Pacific respondents a decade before. He reported that the majority of subjects thought the disease was severe, hampered their ability to work, and had a negative impact on their self-esteem [88]. **Chapter 6** describes the first systematic assessment of the health-related quality of life of 279 suspected CL patients from Ecuador's Pacific and Amazon regions using the internationally validated Skindex-29 questionnaire. CL was confirmed in 208 (75%) of the participants who scored similarly as non-confirmed participants. Patients from the Amazon had higher scores on all three dimensions of the questionnaire (emotions, symptoms, and functioning) indicating lower quality of life. This difference was statistically significant for participants who spoke Spanish as their first language (Mestizos), Chicham speakers (Shuar, Achuar, and Shiwiar), and Kichwa speakers (Amazon Kichwa, Andwa, and Zapara). Ulcerative lesions were associated with a lower quality of life. Gender, age, the number of lesions, the body location of lesions, and health-seeking delay were not associated with a lower quality of life. The lack of validation of the Skindex-29 questionnaire in children limited this study, but their scores (partially reported by their representatives) did not differ from those of adults. Another limitation is the absence of data on the wetness, odor, and diameter of the lesions, which should be included in future studies as they may help to explain the determinants of quality of life. Suspected CL patients in the Ecuadorian Amazon experience increased suffering that should be addressed.

Smell, sound, and taste as stigmatizers

The 'hospital anxiety depression scale' and the 'body image satisfaction scale' were added to the evaluation in Turkey's first leishmaniasis-related quality of life study. They found that patients with CL scars had higher anxiety and depression scores as well as lower body image satisfaction, when compared to healthy controls and attributed this to social stigmatization of the patient's appearance [86]. In **Chapter 7**, the validity of appearance as a cause of social stigma in the population studied in **Chapter 6** is evaluated. During a multisite, short ethnography, more than fifty suspected leishmaniasis patients (CL and/or ML) and nearly as many family and community members, including health professionals and traditional healers, were interviewed or participated in focus groups. Mestizos, who predominate in the Pacific region, stated that the appearance of open wounds, disfigurement, and scar marks was associated with self-rejection and fear. This finding is in agreement with previous research from Africa, Asia, and South America [89]. Amerindians, who predominate in the Amazon hinterlands, stated that the smell of open wounds and the odd sounds produced by ML patients were the principal causes of (self) rejection. Less common causes included the patient's appearance and a different taste of food. The dominance of smell and sound over appearance as causes of leishmaniasis stigma is a new finding [90]. This difference originates probably from the Amazon forest peoples' cosmovision, which prioritizes unity with family, community, and nature for health and happiness [91]. The smell of decomposing flesh and the atypical sounds produced by leishmaniasis lesions may evoke images of danger, separation, and death [92]. Amerindian participants associated smell, sound, visual appearance, and taste with shame, fear of contagion, physical dysfunction, witchcraft, and death (**Chapter 7**). This resulted in

social isolation and distancing, as well as name-calling, jokes, and accusations of inappropriate behavior by society. Both external and internal stigma apply to these attitudes and experiences [93]. Stigma may be worse than physical suffering in CL or ML, as patients who survive, experience social death [94]. Despite the fact that leishmaniasis-related stigma has been reported for nearly two decades, no intervention studies have yet been published [95, 96]. Stigma is, in general, a complex social problem that requires interventions at the intrapersonal, interpersonal, community, organizational, and governmental levels. Interventions should be carefully designed, including continuous efficacy monitoring, because they may unintentionally worsen stigma [97]. The discovery that non-visual aesthetics (smell, sound, and taste) cause stigma expressions in the Ecuadorian Amazon population asks for a rethinking of the interventions currently promoted by the WHO to reduce the stigma associated with NTDs. Counseling, empowerment, cognitive therapy, education, social contact, care teams, advocacy, training of professionals, and legislation are some of the interventions available [98]. To begin, it should be carefully considered at what levels stigma reduction interventions are required and how they should be implemented. The Amerindian population has historically faced discrimination and education or communication interventions may exacerbate this, particularly if the results of **Chapter 7** are not carefully communicated [99]. Because the transmission of CL and ML is zoonotic in the Americas, this information may alleviate patients' and health professionals' fear of contagion without increasing discrimination [100]. Worldwide, the visible appearance of CL scars and ML deformations lead to long-term stigma [90]. It is unknown how long the stigma will persist in the Amazon population after the smell, atypical sound, and taste normalize during lesion healing. This would be clarified by a study comparing health-related quality of life, external stigma, and internal stigma in CL and ML patients before, during, and after cure [86]. Because the internal and external harmony of suspected leishmaniasis patients in the Amazon may be jeopardized, a holistic approach to restoration is recommended. This should include culturally appropriate dietary advice (for example, to optimize protein, caloric, and micronutrient intake) as well as behavioral advice (for example, to avoid trauma to lesions or vector bites) among others [101].

Conclusion

Almost 140 years after the first sighting of the *Leishmania* parasite, we discover that leishmaniasis-induced changes in smell, sound, and taste cause stigma expressions in the Amazon population (**Chapter 7**). They may result in decreased health-related quality of life (**Chapter 6**) and prolonged health-seeking delay (**Chapter 2**). This is exacerbated by diagnostic (**Chapter 3 and 4**) and therapeutic (**Chapter 5**) failures that may be dependent on parasite and host characteristics (**Chapter 2**). Together they cause prolonged suffering which may also exist among leishmaniasis patients in larger neighboring countries Colombia and Peru. This suffering should be alleviated through a multi-level approach that includes the collaboration of the local, national, and international communities, dedicated professionals, availability of resources, and future research initiatives.

References

1. World Health Organization. Leishmaniasis: WHO; 2023 https://www.who.int/data/gho/data/themes/topics/gho-ntd-leishmaniasis#:~:text=Out%20of%20200%20countries%20and,are%20endemic%20for%20CL%20only. Accessed: 26 January 2023.
2. Zink AR, Spigelman M, Schraut B, Greenblatt CL, Nerlich AG, Donoghue HD. Leishmaniasis in ancient Egypt and Upper nubia. Emerging infectious diseases. 2006;12(10):1616.
3. Frías L, Leles D, Araújo A. Studies on protozoa in ancient remains-A Review. Memórias do Instituto Oswaldo Cruz. 2013;108:1-12.
4. Steverding D. The history of leishmaniasis. Parasites & vectors. 2017;10(1):1-10.
5. Correa-Trigoso DE. Presencia de paleopatologías en las representaciones mochica: Un estudio de la colección cerámica del Museo Larco. Horizonte de la Ciencia. 2017;7(12):43-60.
6. Florentino P, Real F, Bonfim-Melo A, Orikaza C, Ferreira E, Pessoa C, et al. An historical perspective on how advances in microscopic imaging contributed to understanding the Leishmania Spp. and Trypanosoma cruzi host-parasite relationship. BioMed Research International. 2014;2014.
7. Leishman WB. On the possibility of the occurrence of trypanosomiasis in India. British medical journal. 1903;1(2213):1252.
8. Gibson ME. The identification of kala-azar and the discovery of Leishmania donovani. Medical history. 1983;27(2):203-13.
9. Desbois N, Pratlong F, Quist D, Dedet J-P. Leishmania (Leishmania) martiniquensis n. sp.(Kinetoplastida: Trypanosomatidae), description of the parasite responsible for cutaneous leishmaniasis in Martinique Island (French West Indies). Parasite (Paris, France). 2014;21.
10. Songumpai N, Promrangsee C, Noopetch P, Siriyasatien P, Preativatanyou K. First Evidence of Co-Circulation of Emerging Leishmania martiniquensis, Leishmania orientalis, and Crithidia sp. in Culicoides Biting Midges (Diptera: Ceratopogonidae), the Putative Vectors for Autochthonous Transmission in Southern Thailand. Tropical medicine and infectious disease. 2022;7(11):379.
11. Kato H, Gomez EA, Martini-Robles L, Muzzio J, Velez L, Calvopiña M, et al. Geographic Distribution of Leishmania Species in Ecuador Based on the Cytochrome B Gene Sequence Analysis. PLoS neglected tropical diseases. 2016;10(7):e0004844.
12. Ronquillo TEF, Almeida F RT. Reporte de lesiones mucosas en leishmaniosis tegumentaria americana, en el litoral (Costa) Ecuatoriano. Rev patol trop. 2012;41(3):356-66.
13. Calvopina M, Guevara AG, Armijos RX, Hashiguchi Y, Davidson RN, Cooper PJ. Itraconazole in the treatment of New World mucocutaneous leishmaniasis. International journal of dermatology. 2004;43(9):659-63.
14. Blum J, Lockwood DN, Visser L, Harms G, Bailey MS, Caumes E, et al. Local or systemic treatment for New World cutaneous leishmaniasis? Re-evaluating the evidence for the risk of mucosal leishmaniasis. International health. 2012;4(3):153-63.
15. Schwing A, Pomares C, Majoor A, Boyer L, Marty P, Michel G. Leishmania infection: Misdiagnosis as cancer and tumor-promoting potential. Acta tropica. 2019;197:104855.
16. Conteron E. Prevalencia de leishmaniasis en el area II de Pastaza, asociados a factores de riesgo que influyen en el desarrollo de la enfermedad. Ambato: Universidad Tecnica de Ambato; 2015.
17. Marsden PD. Mucosal leishmaniasis due to Leishmania (Viannia) braziliensis L (V) b in Três Braços, Bahia-Brazil. Revista da Sociedade Brasileira de Medicina Tropical. 1994;27:93-101.
18. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clinical infectious diseases. 2016;63(12):e202-e64.
19. Van der Auwera G, Davidsson L, Buffet P, Ruf M-T, Gramiccia M, Varani S, et al. Surveillance of leishmaniasis cases from 15 European centres, 2014 to 2019: a retrospective analysis. Eurosurveillance. 2022;27(4):2002028.

20. Pan American Health Organization. Guideline for the treatment of leishmaniasis in the Americas. Second ed. Washington, DC: PAHO; 2022.
21. Llanos-Cuentas A, Tulliano G, Araujo-Castillo R, Miranda-Verastegui C, Santamaria-Castrellon G, Ramirez L, et al. Clinical and parasite species risk factors for pentavalent antimonial treatment failure in cutaneous leishmaniasis in Peru. Clinical infectious diseases. 2008;46(2):223-31.
22. Calvopiña M, Angel G, Armijos R, Gomez E, Mimori T, Cooper P, et al. Clinical features of mucocutaneous leishmaniasis in the Amazonian region of Ecuador. Res Rep Ser. 2005;6:82-9.
23. Cantanhêde LM, Mattos CB, Cruz AK, Ikenohuchi YJ, Fernandes FG, Medeiros E, et al. Overcoming the Negligence in Laboratory Diagnosis of Mucosal Leishmaniasis. Pathogens. 2021;10(9).
24. Van der Auwera G, Dujardin J-C. Species typing in dermal leishmaniasis. Clinical microbiology reviews. 2015;28(2):265-94.
25. National Library of Medicine. GenBank. 2023 https://www.ncbi.nlm.nih.gov/genbank/. Accessed: 10 March 2023.
26. National Library of Medicine. Basic Local Alignment Search Tool. 2022 https://blast.ncbi.nlm.nih.gov/Blast.cgi. Accessed: 02 December 2022.
27. Calvopina M, Armijos RX, Marco JD, Uezato H, Kato H, Gomez EA, et al. Leishmania isoenzyme polymorphisms in Ecuador: relationships with geographic distribution and clinical presentation. BMC infectious diseases. 2006;6:139.
28. Kato H, Cáceres AG, Hashiguchi Y. First evidence of a hybrid of Leishmania (Viannia) braziliensis/L.(V.) peruviana DNA detected from the phlebotomine sand fly Lutzomyia tejadai in Peru. PLoS neglected tropical diseases. 2016;10(1):e0004336.
29. Nolder D, Roncal N, Davies CR, Llanos-Cuentas A, Miles MA. Multiple hybrid genotypes of Leishmania (viannia) in a focus of mucocutaneous Leishmaniasis. The American journal of tropical medicine and hygiene. 2007;76(3):573-8.
30. Tsukayama P, Lucas C, Bacon DJ. Typing of four genetic loci discriminates among closely related species of New World Leishmania. International journal for parasitology. 2009;39(3):355-62.
31. Kato H, Gomez EA, Seki C, Furumoto H, Martini-Robles L, Muzzio J, et al. PCR-RFLP analyses of Leishmania species causing cutaneous and mucocutaneous leishmaniasis revealed distribution of genetically complex strains with hybrid and mito-nuclear discordance in Ecuador. PLoS neglected tropical diseases. 2019;13(5):e0007403.
32. Ministerio de Salud Pública. Estrategia Nacional de Metaxénicas y Zoonóticas: MSP; 2019 https://www.salud.gob.ec/estrategia-nacional-de-metaxenicas-y-zoonoticas. Accessed: 23 December 2022.
33. Hashiguchi Y, Gomez EL, Kato H, Martini LR, Velez LN, Uezato H. Diffuse and disseminated cutaneous leishmaniasis: clinical cases experienced in Ecuador and a brief review. Tropical medicine and health. 2016;44:1-9.
34. Hashiguchi Y, Velez LN, Villegas NV, Mimori T, Gomez EAL, Kato H. Leishmaniases in Ecuador: Comprehensive review and current status. Acta tropica. 2017;166:299-315.
35. Cruz I, Albertini A, Barbeitas M, Arana B, Picado A, Ruiz-Postigo JA, et al. Target Product Profile for a point-of-care diagnostic test for dermal leishmaniases. Parasite epidemiology and control. 2019;5:e00103.
36. Drugs for Neglected Diseases initiative. Research and development Portfolio 2022 https://dndi.org/research-development/portfolio/. Accessed: 22 March 2023.
37. Pinheiro AC, de Souza MVN. Current leishmaniasis drug discovery. RSC Medicinal Chemistry. 2022.
38. Machado-Coelho GL, Caiaffa WT, Genaro O, Magalhaes PA, Mayrink W. Risk factors for mucosal manifestation of American cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2005;99(1):55-61.
39. Amunárriz M. Estudios sobre patologías tropicales en la Amazonia ecuatoriana: Cicame; 1991.
40. Gaglio G, Brianti E, Napoli E, Falsone L, Dantas-Torres F, Tarallo VD, et al. Effect of night time-intervals, height of traps and lunar phases on sand fly collection in a highly endemic area for canine leishmaniasis. Acta tropica. 2014;133:73-7.

41. Benabent Fernández de Córdoba M. El transporte público terrestre y la accesibilidad, instrumentos para el análisis funcional del sistema de asentamientos: el caso de Ecuador. Estoa Revista de la Facultad de Arquitectura y Urbanismo de la Universidad de Cuenca. 2017;6(11):99-122.
42. CONFENIAE. Nacionalidades Puyo: CONFENIAE; 2022 https://confeniae.net/nacionalidades. Accessed: 24 February 2022.
43. Carpio-Arias TV, Verdezoto N, Guijarro-Garvi M, Abril-Ulloa V, Mackintosh N, Eslambolchilar P, et al. Healthcare professionals' experiences and perceptions regarding health care of indigenous pregnant women in Ecuador. BMC Pregnancy and Childbirth. 2022;22(1):1-11.
44. Bautista-Valarezo E, Duque V, Verhoeven V, Mejia Chicaiza J, Hendrickx K, Maldonado-Rengel R, et al. Perceptions of Ecuadorian indigenous healers on their relationship with the formal health care system: barriers and opportunities. BMC Complementary Medicine and Therapies. 2021;21(1):1-10.
45. Ni'mah ZFF, Kusariana N, Ginandjar P, editors. Perceived Stigma as a Risk Factor for Delay in Seeking Treatment of Leprosy Patients: A Cross-Sectional Study in Tuban Regency. E3S Web of Conferences; 2020: EDP Sciences.
46. Brito Filho MF, de Santana ME, Mendes CP, de Jesus Costa D, Dos Santos CAAS, de Araújo MFM, et al. Cultural, social, and healthcare access factors associated with delays in gastric cancer presentation, diagnosis, and treatment: A cross-sectional study. Journal of Cancer Policy. 2021;28:100277.
47. Kroeger A, Mancheno M, Alarcon J, Pesse K. Insecticide-impregnated bed nets for malaria control: varying experiences from Ecuador, Colombia, and Peru concerning acceptability and effectiveness. The American journal of tropical medicine and hygiene. 1995;53(4):313-23.
48. Kumar R. Malaria Transmission in Border Regions of the Western Amazon: Incorporating watersheds into timeseries analysis to address disease reintroduction and spillover along the Ecuador-Peru border. 2021.
49. Maroli M, Feliciangeli M, Bichaud L, Charrel R, Gradoni L. Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. Medical and veterinary entomology. 2013;27(2):123-47.
50. Kato H, Cáceres AG, Gomez EA, Mimori T, Uezato H, Hashiguchi Y. Genetic divergence in populations of Lutzomyia ayacuchensis, a vector of Andean-type cutaneous leishmaniasis, in Ecuador and Peru. Acta tropica. 2015;141:79-87.
51. Pena HP, Belo VS, Xavier-Junior JCC, Teixeira-Neto RG, Melo SN, Pereira DA, et al. Accuracy of diagnostic tests for American tegumentary leishmaniasis: a systematic literature review with meta-analyses. Tropical medicine & international health : TM & IH. 2020;25(10):1168-81.
52. Dendukuri N, Schiller I, De Groot J, Libman M, Moons K, Reitsma J, et al. Concerns about composite reference standards in diagnostic research. bmj. 2018;360.
53. Gomes CM, Mazin SC, Santos ER, Cesetti MV, Bächtold GA, Cordeiro JH, et al. Accuracy of mucocutaneous leishmaniasis diagnosis using polymerase chain reaction: systematic literature review and meta-analysis. Memorias do Instituto Oswaldo Cruz. 2015;110(2):157-65.
54. Suárez M, Valencia BM, Jara M, Alba M, Boggild AK, Dujardin J-C, et al. Quantification of Leishmania (Viannia) kinetoplast DNA in ulcers of cutaneous leishmaniasis reveals inter-site and inter-sampling variability in parasite load. PLoS neglected tropical diseases. 2015;9(7):e0003936.
55. Romero GA, de Farias Guerra MV, Paes MG, Macêdo VdO. Comparison of cutaneous leishmaniasis due to Leishmania (Viannia) braziliensis and L.(V.) guyanensis in Brazil: clinical findings and diagnostic approach. Clinical Infectious Diseases. 2001;32(9):1304-12.
56. Miranda A, Saldaña A, González K, Paz H, Santamaría G, Samudio F, et al. Evaluation of PCR for cutaneous leishmaniasis diagnosis and species identification using filter paper samples in Panama, Central America. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2012;106(9):544-8.
57. Singh UA, Kumari M, Iyengar S. Method for improving the quality of genomic DNA obtained from minute quantities of tissue and blood samples using Chelex 100 resin. Biological procedures online. 2018;20(1):1-8.
58. van der Meide W, Guerra J, Schoone G, Farenhorst M, Coelho L, Faber W, et al. Comparison between quantitative nucleic acid sequence-based amplification, real-time reverse transcriptase PCR, and real-time PCR for quantification of Leishmania parasites. Journal of Clinical Microbiology. 2008;46(1):73-8.

59. Ministerio De Salud Pública. Manual de procedimientos del Subsistema alerta acción SIVE – ALERTA. Second ed. Quito: MSP; 2013.
60. Henríquez-Trujillo AR, Coral-Almeida M, Hinojosa MC. The burden of cutaneous and mucocutaneous leishmaniasis in Ecuador (2014-2018), a national registry-based study. bioRxiv. 2019:751446.
61. Weigel MM, Armijos RX. The traditional and conventional medical treatment of cutaneous leishmaniasis in rural Ecuador. Revista Panamericana de Salud Pública. 2001;10(6):395-404.
62. Eckhardt M, Carlfjord S, Faresjö T, Crespo-Burgos A, Forsberg BC, Falk M. Universal health coverage in marginalized populations: a qualitative evaluation of a health reform implementation in rural Ecuador. INQUIRY: The Journal of Health Care Organization, Provision, and Financing. 2019;56:0046958019880699.
63. Wesson DE, Lucey CR, Cooper LA. Building trust in health systems to eliminate health disparities. Jama. 2019;322(2):111-2.
64. Fernandes HJ, da Silva RE, Ramalho DB, Aguiar MG, Silveira JN, Cota G. Safety profile of meglumine antimoniate intralesional infiltration for cutaneous leishmaniasis. Expert review of anti-infective therapy. 2020;18(4):381-7.
65. Cardona-Arias JA, Vélez ID, Lopez-Carvajal L. Efficacy of thermotherapy to treat cutaneous leishmaniasis: a meta-analysis of controlled clinical trials. PloS one. 2015;10(5):e0122569.
66. Boggild AK, Valencia BM, Veland N, Ramos AP, Calderon F, Arevalo J, et al. Non-invasive cytology brush pcr diagnostic testing in mucosal leishmaniasis: Superior performance to conventional biopsy with histopathology. PloS one. 2011;6 (10) (no pagination)(e26395).
67. Russell A. The natural history of Aleppo, and parts adjacent: A. Millar London; 1756.
68. Marsden PD. Pentavalent antimonials: old drugs for new diseases. Revista da Sociedade Brasileira de Medicina Tropical. 1985;18:187-98.
69. Debus AG. Antimony in medical history: an account of the medical uses of antimony and its compounds since early times to the present. Johns Hopkins University Press; 2000.
70. Marsden PD. The discovery of urea stibamine. Revista da Sociedade Brasileira de Medicina Tropical. 1986;19:115-.
71. Sneader W. Drug discovery: a history: John Wiley & Sons; 2005.
72. Kreijkamp-Kaspers S, Hawke K, Guo L, Kerin G, Bell-Syer SE, Magin P, et al. Oral antifungal medication for toenail onychomycosis. Cochrane Database of Systematic Reviews. 2017(7).
73. Farajzadeh S, Esfandiarpour I, Haghdoost AA, Mohammadi S, Mohebbi A, Mohebbi E, et al. Comparison between combination therapy of oral terbinafine and cryotherapy versus systemic meglumine antimoniate and cryotherapy in cutaneous leishmaniasis: a randomized clinical trial. Iranian Journal of Parasitology. 2015;10(1):1.
74. Faergemann J, Zehender H, Denouel J, Millerioux L. Levels of terbinafine in plasma, stratum corneum, dermis-epidermis (without stratum corneum), sebum, hair and nails during and after 250 mg terbinafine orally once per day for four weeks. Acta dermato-venereologica. 1993;73(4):305-9.
75. Andrade Neto VVd. Papel do colesterol exógeno nos mecanismos de adaptação de Leishmania spp a condições de estresse metabólico e farmacológico 2013.
76. Vannier-Santos MA, Urbina JA, Martiny A, Neves A, de Souza W. Alterations induced by the antifungal compounds ketoconazole and terbinafine in Leishmania. Journal of eukaryotic microbiology. 1995;42(4):337-46.
77. Ferreira LL, de Moraes J, Andricopulo AD. Approaches to advance drug discovery for neglected tropical diseases. Drug Discovery Today. 2022.
78. Jacobson J. Compressing the timelines for development and delivery: accelerating access to triple-drug therapy to eliminate lymphatic filariasis. The American journal of tropical medicine and hygiene. 2022;106(5 Suppl):4.
79. Galvão EL, Rabello A, Cota GF. Efficacy of azole therapy for tegumentary leishmaniasis: A systematic review and meta-analysis. PloS one. 2017;12(10):e0186117.
80. Ponte-Sucre A, Gamarro F, Dujardin J-C, Barrett MP, López-Vélez R, García-Hernández R, et al. Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. PLoS neglected tropical diseases. 2017;11(12):e0006052.

81. Pijpers J, den Boer ML, Essink DR, Ritmeijer K. The safety and efficacy of miltefosine in the long-term treatment of post-kala-azar dermal leishmaniasis in South Asia–a review and meta-analysis. PLoS neglected tropical diseases. 2019;13(2):e0007173.
82. Sunyoto T, Potet J, Boelaert M. Why miltefosine-a life-saving drug for leishmaniasis-is unavailable to people who need it the most. BMJ global health. 2018;3(3):e000709.
83. Ciociola AA, Cohen LB, Kulkarni P, Kefalas C, Buchman A, Burke C, et al. How drugs are developed and approved by the FDA: current process and future directions. Official journal of the American College of Gastroenterology| ACG. 2014;109(5):620-3.
84. Hotez PJ, Aksoy S, Brindley PJ, Kamhawi S. What constitutes a neglected tropical disease? : Public Library of Science San Francisco, CA USA; 2020. p. e0008001.
85. Abecassis P, Alesandrini J-F, Coriat B, Coutinet N, Leyronas S. DNDi, a distinctive illustration of commons in the area of public health. AFD Research Papers Series. 2019(2019-93).
86. Yanik M, Gurel MS, Simsek Z, Kati M. The psychological impact of cutaneous leishmaniasis. Clinical and experimental dermatology. 2004;29(5):464-7.
87. Wood-Dauphinee S. Assessing quality of life in clinical research: from where have we come and where are we going? Journal of clinical epidemiology. 1999;52(4):355-63.
88. Weigel MM, Armijos RX, Racines R, Zurita C, Izurieta R, Herrera E, et al. Cutaneous leishmaniasis in subtropical Ecuador: popular perceptions, knowledge, and treatment. Bulletin of the Pan American Health Organization (PAHO); 28 (2), jun 1994. 1994.
89. Bennis I, De Brouwere V, Belrhiti Z, Sahibi H, Boelaert M. Psychosocial burden of localised cutaneous Leishmaniasis: a scoping review. BMC Public Health. 2018;18(1):358.
90. Al-Kamel MA. Stigmata in cutaneous leishmaniasis: Historical and new evidence-based concepts. Our Dermatology Online. 2017;8(1):81.
91. Uzendoski MA. Manioc beer and meat: value, reproduction and cosmic substance among the Napo Runa of the Ecuadorian Amazon. Journal of the Royal Anthropological Institute. 2004;10(4):883-902.
92. Taylor AC. Remembering to forget: identity, mourning and memory among the Jivaro. Man. 1993:653-78.
93. Weiss MG. Stigma and the social burden of neglected tropical diseases. PLoS neglected tropical diseases. 2008;2(5):e237.
94. Muela Ribera J, Peeters Grietens K, Toomer E, Hausmann-Muela S. A word of caution against the stigma trend in neglected tropical disease research and control. PLoS neglected tropical diseases. 2009;3(10):e445.
95. Reithinger R, Aadil K, Kolaczinski J, Mohsen M, Hami S. Social impact of leishmaniasis, Afghanistan. 2005.
96. Grifferty G, Shirley H, McGloin J, Kahn J, Orriols A, Wamai R. Vulnerabilities to and the Socioeconomic and Psychosocial Impacts of the Leishmaniases: A Review. Research and Reports in Tropical Medicine. 2021:135-51.
97. Rao D, Elshafei A, Nguyen M, Hatzenbuehler ML, Frey S, Go VF. A systematic review of multi-level stigma interventions: state of the science and future directions. BMC medicine. 2019;17(1):1-11.
98. World Health Organization. Mental health of people with neglected tropical diseases: towards a person-centred approach. 2020.
99. Whitten Jr NE. Symbolic inversion, the topology of El Mestizaje, and the spaces of Las Razas in Ecuador. Journal of Latin American Anthropology. 2003;8(1):52-85.
100. Alemayehu B, Alemayehu M. Leishmaniasis: a review on parasite, vector and reservoir host. Health Science Journal. 2017;11(4):1.
101. Molnar JA, Underdown MJ, Clark WA. Nutrition and chronic wounds. Advances in wound care. 2014;3(11):663-81.

Chapter 9

Summary
Samenvatting
Resumen

Summary

Improved understanding, diagnosis, and treatment of cutaneous and mucosal leishmaniasis in rural Ecuador

Around the world, up to one million new people are infected with *Leishmania* parasites each year. These protozoans infects humans through the bite of female sandflies of the genus *Lutzomyia* or *Phlebotomus*. In most regions, *Leishmania* transmission is zoonotic (from animal to human). The World Health Organization classifies leishmaniasis as a Neglected Tropical Disease due to a lack of knowledge regarding the epidemiology and disease characteristics, as well as a lack of adequate diagnostics, drugs, vaccines, and vector control strategies. The global burden of leishmaniasis is rising as a result of poverty, socioeconomic vulnerability, and climate change.

In Ecuador, leishmaniasis affects an estimated 5000 people annually, causing two major diseases: cutaneous leishmaniasis which causes skin ulcers and nodules that heal spontaneously in months or years, leaving scars, and mucosal leishmaniasis, which causes mucosal inflammation and ulceration of the nose, mouth, and throat that does not heal spontaneously. Cutaneous leishmaniasis is caused primarily by the species *Leishmania guyanensis* and *L. braziliensis* and occurs throughout the tropical Amazon and the subtropical northern Pacific region. Mucosal leishmaniasis occurs only in the Amazon and is caused by *L. braziliensis*. The knowledge of the *Leishmania* species distribution and the clinical presentation of cutaneous leishmaniasis patients in Ecuador's Pacific and Amazon regions is outdated and incomplete.

A cross-sectional study in **Chapter 2,** describes the identification of *Leishmania* species in 135 patients from the Pacific and Amazon regions, as well as the clinical presentation with determinants of health-seeking delay in 245 cutaneous leishmaniasis patients. This study finds a sustained low prevalence of *L. braziliensis* in the Pacific region. It also reveals the presence of *L. guyanensis* in the provinces of Napo, Pastaza, and Morona Santiago, *L. braziliensis* in the province of Imbabura, and *L. lainsoni* in the provinces of Pichincha, Napo, Pastaza, and Morona Santiago. A phylogenetic tree constructed from the cytochrome B sequences of 102 *L. guyanensis* samples reveals that mutations respect regional borders. This may have consequences for diagnostic test accuracy, therapy resistance, and the risk of mucosal leishmaniasis even within the same species, emphasizing the importance of region-specific research. Amazon patients have a longer median health-seeking delay (2.0, interquartile range 3.0) than Pacific patients (1.0, interquartile range 1.5). Prolonged health-seeking delay is associated with older age, Amerindian ethnicity, infection at lower altitudes, non-ulcerative lesions, and lesions on the lower limbs. This could be due to limited access to health care as well as stigma. Routine species determination in Amazon cutaneous leishmaniasis cases is recommended, as are regional studies of determinants of health-seeking delay in Ecuador and regional studies of patient characteristics in neighboring countries Peru and Colombia.

Each year, the microscopy-based test and treat management of Ecuadorian cutaneous leishmaniasis-suspected patients potentially leaves thousands without treatment; thus, the accuracy of smear slide microscopy and a qPCR on DNA extracted from filter paper is analyzed in **Chapter 3**. The study uses statistical models to estimate test sensitivity, specificity, likelihood ratios, and predictive values with their respective 95% credible intervals (95%CrI). As a secondary objective, sociodemographic and clinical characteristics are assessed for their predictive value. Paired test results are available for 129 Amazon participants and 185 Pacific participants. In the Amazon and Pacific regions, qPCR sensitivity is estimated at 68% (95%CrI 49% to 82%) and 73% (95%CrI 73% to 83%), respectively, and microscopy sensitivity is estimated at 51% (95%CrI 36% to

66%) and 76% (95%CrI 65% to 86%). The regional difference in microscopy sensitivity cannot be explained by health-seeking delay. With an estimated disease prevalence of 73% among participants in the Amazon region, negative predictive value for qPCR is 54% (95% CrI 5% to 77%) and 44% (95% CrI 4% to 65%) for microscopy. With an estimated disease prevalence of 88% in the Pacific, the negative predictive value is 34% (95%CrI 25% to 57%) and 37% (95%CrI 27% to 63%). In the Amazon, the addition of qPCR parallel to microscopy increases the observed prevalence from 38% to 64% (+26 (95% CrI 19 to 34) percentage points)). The individual failure of both tests could be due to parasite, human host, or diagnostic method related factors, which should be clarified in future studies. It now leads to prolonged suffering for untreated patients. The inclusion of socio-demographic and clinical characteristics in the diagnostic pathway does not improve the posterior probability of excluding cutaneous leishmaniasis. The use of suspicion-based thermotherapy, a brief, safe, and low-cost alternative treatment for cutaneous leishmaniasis, recommended by the Pan American Health Organization, should be researched.

According to the literature, the diagnostic accuracy of microscopy for mucosal leishmaniasis is even lower than for cutaneous leishmaniasis. If not treated promptly, the parasite may develop treatment resistance or cause progressive facial deformities and death. Because clinical criteria are available in any setting, they can be combined to form a syndromic algorithm, which can then be used as a diagnostic tool. Through a systematic review of the literature, **Chapter 4** explores potential clinical criteria for a syndromic diagnostic algorithm for mucosal leishmaniasis. Ten studies describing a total of 192 mucosal leishmaniasis patients are included. Male gender has the highest detection rate (88%), followed by ulcer of the nasal mucosa (77%), age >15 (69%), and symptom duration >4 months (63%). No specificity can be calculated because the papers did not include non-mucosal leishmaniasis patients. All of the included studies have a high risk of bias. This study is a first, but small, step toward developing diagnostic criteria for mucosal leishmaniasis. Future studies should include non-mucosal leishmaniasis patients to define the most discriminative criteria and calculate the predictive values of combinations.

Current cutaneous and mucosal leishmaniasis treatments have severe side effects and must be administered via injection. Allylamine drugs, such as terbinafine, are safe and can be taken orally or topically, even during pregnancy. A systematic review of the literature in **Chapter 5** assesses the efficacy and safety of allylamine drugs as an alternative treatment for cutaneous and mucosal leishmaniasis. The study includes one uncontrolled trial, two randomized controlled trials, two animal studies, and 12 *in vitro* amastigote and/or promastigote studies. In the only well-designed randomized controlled trial that compares the treatment efficacy of oral terbinafine versus intramuscular meglumine antimoniate in 80 *L. tropica* infected patients, terbinafine has a non-significant lower cure rate (38% vs 53%). A meta-analysis is not performed due to the small number of studies, their heterogeneity, and low quality. According to this systematic review, there is no evidence that allylamine monotherapy is effective against cutaneous or mucosal leishmaniasis. Further trials of allylamines should be carefully considered because the *in vitro* studies showed minimal effective concentrations that are not clinically relevant. However, the synergistic effects of allylamines combined with triazole drugs *in vitro* warrant further exploration.

Cutaneous leishmaniasis reduces health-related quality of life due to stigma in the Asian and Mediterranean contexts, but research for Ecuador is lacking. The study described in **Chapter 6** assesses the influence of cutaneous leishmaniasis suspected lesions on the quality of life of 279 patients in the Pacific and Amazon regions. This study applies the Spanish version of the Skindex-29 questionnaire, a generic tool for measuring health-related quality of life in patients with skin diseases. Patients from all participating linguistic groups (Mestizo, Kichwa, or Chicham) in the

Amazon score significantly (P <0.01) higher (indicating worse health-related quality of life) on all the dimensions of the Skindex-29 questionnaire than Mestizo patients from the Pacific region. Gender, age, the number of lesions, the body location of lesions, health-seeking delay, and posterior confirmation of the parasite, are not associated with a lower quality of life. Future studies that include additional patient and lesion details should clarify whether the impaired health-related quality of life is related to stigma.

Previous research has focused on aesthetic appearance affectation as the primary source of stigma caused by cutaneous leishmaniasis. This aspect has been studied in the 90s in the Ecuadorian Pacific region but never in the Amazon. **Chapter 7** describes ethnographic research and data retrieved from 83 semi-structured interviews, and 15 focus groups conducted in seven Ecuadorian ethnic groups. Mestizos, who predominate in the Pacific region, state that the appearance of open wounds, disfigurement, and scar marks are associated with self-rejection and fear, which is consistent with previous studies. On the other hand, Amerindian populations in the Amazon's hinterland trigger internal and external stigma expressions by recognizing cutaneous and mucosal leishmaniasis through atypical smell, followed by the odd voice sound, appearance, and appearance taste. This reveals that the sensorium enables differentiated cultural responses to a sense of peril, contagion, and social (self)rejection, which are linked to Amerindian populations' holistic approach to health. These findings should be communicated carefully to avoid exacerbating already-existing discrimination against Amerindians. It is unknown whether stigma will persist in the Amazon population after the smell, atypical sound, and taste normalize during lesion healing. A study comparing health-related quality of life, external stigma, and internal stigma in cutaneous and mucosal leishmaniasis patients before, during, and after cure would clarify this. A focus on the fact that leishmaniasis does not spread from person to person may alleviate public fears and rejection of patients. A holistic approach to suspected leishmaniasis patients is recommended, including dietary and behavioral advice. Any intervention must be carefully planned with all actors and continuously monitored for effectiveness.

Almost 140 years after the first sighting of the *Leishmania* parasite, we discover that leishmaniasis-induced changes in smell, sound, and taste cause stigma expressions in the Amazon population (**Chapter 7**). They may result in decreased health-related quality of life (**Chapter 6**) and prolonged health-seeking delay (**Chapter 2**). This is exacerbated by diagnostic (**Chapter 3 and 4**) and therapeutic (**Chapter 5**) failures, which may be dependent on parasite and host characteristics (**Chapter 2**). Overall, leishmaniasis causes prolonged suffering which may also exist among leishmaniasis patients in larger neighboring countries Colombia and Peru. This suffering should be alleviated through a multi-level approach involving local, national, and international communities, dedicated professionals, resources, and future research initiatives.

Samenvatting

Verbeterd begrip, diagnose en behandeling van cutane en mucosale leishmaniasis op het platteland van Ecuador

Over de hele wereld worden elk jaar tot een miljoen mensen besmet met *Leishmania* parasieten. Deze protozoa infecteren mensen via de beet van vrouwelijke zandvliegen van het geslacht *Lutzomyia* of *Phlebotomus*. In de meeste regio's is de overdracht *van Leishmania* zoönotisch (van dier op mens). De Wereldgezondheidsorganisatie classificeert leishmaniasis als een verwaarloosde tropische ziekte vanwege een gebrek aan kennis over de epidemiologie en ziektekenmerken, evenals een gebrek aan adequate diagnostiek, medicijnen, vaccins en vectorbestrijdingsstrategieën. De wereldwijde last van leishmaniasis neemt toe als gevolg van armoede, sociaaleconomische kwetsbaarheid en klimaatverandering.

In Ecuador treft leishmaniasis jaarlijks naar schatting vijfduizend mensen en veroorzaakt twee belangrijke ziektebeelden: cutane leishmaniasis, die huidzweren en knobbeltjes veroorzaakt die binnen maanden of jaren spontaan met littekenvorming kunnen genezen, en mucosale leishmaniasis, die slijmvliesontsteking en ulceratie van de neus, mond en keel veroorzaakt en niet spontaan geneest. Cutane leishmaniasis wordt in Ecuador voornamelijk veroorzaakt door de soorten *Leishmania guyanensis* en *L. braziliensis* en is endemisch in het tropische Amazonegebied en de subtropische noordelijke Pacifische regio. Mucosale leishmaniasis komt alleen voor in de Amazone en wordt veroorzaakt door *L. braziliensis*. De kennis van de verspreiding van *Leishmania* soorten en de klinische presentatie van patiënten met cutane leishmaniasis in de Pacifische en de Amazoneregio van Ecuador is achterhaald en onvolledig.

Een cross-sectioneel onderzoek in **hoofdstuk 2** beschrijft de identificatie van *Leishmania*- soorten bij 135 patiënten uit het Pacifische en het Amazonegebied, evenals de klinische presentatie en determinanten van zorgmijding bij 245 patiënten met cutane leishmaniasis. Deze studie vindt een aanhoudend lage prevalentie van *L. braziliensis* in de Pacifische regio. Deze onthult ook de aanwezigheid van *L. guyanensis* in de provincies Napo, Pastaza en Morona Santiago, *L. braziliensis* in de provincie Imbabura en *L. lainsoni* in de provincies Pichincha, Napo, Pastaza en Morona Santiago. Een fylogenetische indeling, opgebouwd uit de cytochroom B-sequenties van 102 *L. guyanensis* monsters, onthult dat mutaties niet over regionale grenzen gaan. Dit kan gevolgen hebben voor de nauwkeurigheid van diagnostische testen, therapieresistentie en het risico op mucosale leishmaniasis, zelfs binnen dezelfde soort, wat het belang van regio specifiek onderzoek benadrukt. Patiënten uit de Amazone hebben een langere mediane zorgmijding in maanden (2.0, interkwartielbereik 3.0) dan Pacifische patiënten (1.0, interkwartielbereik 1.5). Langdurige zorgmijding wordt in verband gebracht met hogere leeftijd, Inheemse etniciteit, infectie op lagere hoogten, niet-ulceratieve laesies en laesies op de onderste ledematen. Dit kan te wijten zijn aan beperkte toegang tot gezondheidszorg en stigmatisering. Routinematige soortbepaling in patiënten met cutane leishmaniasis uit de Amazone wordt aanbevolen, evenals regionale studies naar determinanten van zorgmijding in Ecuador en regionale studies naar patiëntkenmerken in de buurlanden Peru en Colombia.

Elk jaar laat de op microscopie gebaseerde test- en behandelingsstrategie wellicht duizenden van de patiënten met mogelijke cutane leishmaniasis achter zonder behandeling. Daarom analyseert **Hoofdstuk 3** de nauwkeurigheid van microscopie op wonduitstrijkjes en van qPCR op DNA geëxtraheerd uit filtreerpapier. De studie maakt gebruik van statistische modellen om testgevoeligheid, specificiteit, waarschijnlijkheidsratio's en voorspellende waarden te schatten met

hun achtereenvolgende 95% geloofwaardigheidsintervallen (95% CrI). Als secundaire doelstelling worden demografische en klinische kenmerken beoordeeld op hun voorspellende waarde. Gepaarde testresultaten zijn beschikbaar voor 129 deelnemers uit de Amazone- en 185 uit de Pacifische regio. In de Amazone- en Pacifische regio wordt de qPCR-gevoeligheid geschat op respectievelijk 68% (95% CrI 49% tot 82%) en 73% (95% CrI 73% tot 83%), en de gevoeligheid voor microscopie op 51% (95 %CrI 36% tot 66%) en 76% (95%CrI 65% tot 86%). Het verschil in de gevoeligheid van microscopie tussen de regio's kan niet worden verklaard door vertraging bij het zoeken naar zorg. Met een geschatte ziekteprevalentie van 73% onder deelnemers in het Amazonegebied, is de negatieve voorspellende waarde voor qPCR 54% (95% CrI 5% tot 77%) en 44% (95% CrI 4% tot 65%) voor microscopie. Met een geschatte ziekteprevalentie van 88% in de Pacifische regio, is de negatieve voorspellende waarde 34% (95% CrI 25% tot 57%) en 37% (95% CrI 27% tot 63%). In de Amazone verhoogde de toevoeging van qPCR parallel aan microscopie de waargenomen prevalentie van 38% naar 64% (+26 (95% CrI 19 tot 34) procentpunten). Het individuele falen van beide tests kan te wijten zijn aan de parasiet, menselijke gastheer of diagnostische methode, wat in toekomstige studies moet worden opgehelderd. Nu veroorzaakt dit falen langdurig lijden van onbehandelde patiënten. Het opnemen van sociaal-demografische en klinische kenmerken in het diagnostische pad verbeterde de doeltreffendheid om cutane leishmaniasis uit te sluiten niet. Het gebruik van thermotherapie (die wordt aanbevolen door de Pan-Amerikaanse gezondheidsorganisatie) bij patiënten met mogelijke cutane leishmaniasis is een snelle, veilige, goedkope en redelijk effectieve alternatieve behandeling die moet worden onderzocht.

Volgens de literatuur is de diagnostische nauwkeurigheid van microscopie voor mucosale leishmaniasis zelfs lager dan voor cutane leishmaniasis. Bij uitstel van behandeling ontwikkelt de parasiet mogelijk resistentie tegen de behandeling en veroorzaakt progressieve gezichtsmisvormingen en/of de dood. Omdat klinische criteria in elke setting beschikbaar zijn, kunnen ze worden gecombineerd om een syndromaal algoritme te vormen, dat vervolgens als diagnostisch hulpmiddel kan worden gebruikt. Door middel van een systematische review van de literatuur onderzoekt **Hoofdstuk 4** mogelijke klinische criteria voor een syndromaal diagnostisch algoritme voor mucosale leishmaniasis. Tien onderzoeken, die in totaal 192 patiënten met mucosale leishmaniasis beschrijven, worden opgenomen. Het mannelijke geslacht heeft het hoogste detectiepercentage (88%), gevolgd door zweren van het neusslijmvlies (77%), leeftijd >15 jaar (69%) en symptoomduur >4 maanden (63%). Er kan geen specificiteit worden berekend, omdat de artikelen geen patiënten zonder mucosale leishmaniasis beschrijven. Alle geïncludeerde studies blijken een hoog risico op bias te hebben. Deze studie is een eerste en kleine stap in de richting van het ontwikkelen van diagnostische criteria voor mucosale leishmaniasis. Toekomstige studies zouden patiënten moeten includeren zonder mucosale leishmaniasis om de meest onderscheidende criteria vast te stellen en de voorspellende waarden van combinaties te berekenen.

De huidige behandelingen voor cutane en mucosale leishmaniasis hebben ernstige bijwerkingen en moeten via injecties worden toegediend. Allylamine geneesmiddelen, zoals terbinafine, zijn veilig en kunnen oraal of op de huid worden gebruikt, zelfs tijdens de zwangerschap. Een systematisch overzicht van de literatuur in **Hoofdstuk 5** beoordeelt de werkzaamheid en veiligheid van allylamine geneesmiddelen als een alternatieve behandeling voor cutane en mucosale leishmaniasis. De studie omvatte één ongecontroleerde studie, twee gerandomiseerde gecontroleerde studies, twee dierstudies en 12 *in vitro* studies met amastigoten en of promastigoten. In de enige goed opgezette gerandomiseerde gecontroleerde studie, die de werkzaamheid van de behandeling van oraal terbinafine versus intramusculair meglumine-antimoniaat vergelijkt onder 80 patiënten die met *L.*

tropica zijn geïnfecteerd, heeft terbinafine een niet-significant lager genezingspercentage (38% versus 53%). Een meta-analyse kan niet worden uitgevoerd vanwege het kleine aantal studies, hun heterogeniteit en lage kwaliteit. Volgens deze systematische review is er geen bewijs dat monotherapie met allylamine geneesmiddelen effectief is tegen cutane of mucosale leishmaniasis. Verder onderzoek met allylamine geneesmiddelen moet zorgvuldig worden overwogen, omdat de *in vitro* onderzoeken minimale effectieve concentraties aantonen die niet klinisch relevant zijn. De synergetische effecten van allylamine geneesmiddelen in combinatie met triazool geneesmiddelen *in vitro* rechtvaardigen echter verder onderzoek.

In de Aziatische en Mediterrane context vermindert cutane leishmaniasis de gezondheid gerelateerde kwaliteit van leven als gevolg van stigmatisering, maar onderzoek in Ecuador ontbreekt. De studie beschreven in **Hoofdstuk 6** beoordeelt de invloed van laesies, die mogelijk door cutane leishmaniasis worden veroorzaakt, op de kwaliteit van leven van 279 patiënten in het Pacifische en Amazonegebied. Deze studie paste de Spaanse versie van de Skindex-29-vragenlijst toe, een generiek instrument voor het meten van gezondheid gerelateerde kwaliteit van leven bij patiënten met huidziekten. Patiënten uit alle deelnemende taalgroepen (Mesties, Kichwa of Chicham) in het Amazonegebied scoorden significant (P <0,01) hoger (wat duidt op een verminderde gezondheid gerelateerde kwaliteit van leven) op alle dimensies van de Skindex-29-vragenlijst dan Mestiezenpatiënten uit de Pacifische regio. Geslacht, leeftijd, het aantal laesies, de locatie van de laesies op het lichaam, zorgmijding en latere bevestiging van de diagnose zijn niet geassocieerd met een verminderde kwaliteit van leven. Toekomstige studies, die aanvullende patiënt- en laesiedetails bevatten, zouden moeten verduidelijken of de verminderde gezondheid gerelateerde kwaliteit van leven verband houdt met stigmatisering.

Eerder onderzoek toonde aan dat het zichtbare aspect van leishmaniasis laesies stigma veroorzaakte. Dit is in de jaren 90 bestudeerd in de Ecuadoraanse Pacifische regio, maar nooit in de Amazone. **Hoofdstuk 7** beschrijft etnografisch onderzoek en gegevens die uit 83 semigestructureerde interviews en 15 focusgroepen verkregen zijn. Deze zijn uitgevoerd in zeven Ecuadoraanse etnische groepen. Mestiezen, die de meerderheid vormen in de Pacifische regio, verklaren dat het verschijnen van open wonden, misvormingen en littekens geassocieerd worden met zelfverwerping en angst, wat consistent is met eerdere studies. Onder de Inheemse bevolkingsgroepen in de Amazone daarentegen, roepen de atypische geur, gevolgd door het vervormde stemgeluid, uiterlijk en smaak, waaraan cutane en mucosale leishmaniasis herkend worden, interne en externe stigma-expressies op. Dit laat zien dat de zintuigen gedifferentieerde culturele reacties mogelijk maken bij een gevoel van gevaar, besmetting en sociale (zelf)verwerping, die verband houden met de holistische benadering van gezondheid door Inheemse bevolkingsgroepen. Deze bevindingen moeten zorgvuldig worden gecommuniceerd, om te voorkomen dat de reeds bestaande discriminatie van Inheemse bevolkingsgroepen verergert. Het is niet bekend of het stigma onder de Amazonebevolking zal voortduren nadat de wondgeur, het stemgeluid en de smaak zijn genormaliseerd tijdens de genezing van laesies. Een studie, waarin gezondheid gerelateerde kwaliteit van leven, extern en intern stigma bij cutane en mucosale leishmaniasis patiënten vóór, tijdens en na genezing worden vergeleken, zou dit kunnen verduidelijken. Een focus op het feit dat leishmaniasis niet besmettelijk is, kan de angst van het publiek en de afwijzing van patiënten verminderen. Een holistische benadering van patiënten met verdenking op leishmaniasis wordt aanbevolen, inclusief voedings- en gedragsadviezen. Elke interventie moet zorgvuldig worden gepland met alle actoren en voortdurend worden gecontroleerd op effectiviteit.

Bijna honderdveertig jaar na de eerste microscopische waarneming van de *Leishmania* parasiet, ontdekken we dat door leishmaniasis veroorzaakte veranderingen in geur, stemgeluid en smaak stigma-uitlatingen veroorzaken bij de Amazonebevolking (**Hoofdstuk 7**). Ze kunnen leiden tot een lagere gezondheid gerelateerde kwaliteit van leven (**Hoofdstuk 6**) en meer zorgmijding (**Hoofdstuk 2**). Dit wordt verergerd door diagnostisch (**Hoofdstuk 3 en 4**) en therapeutisch (**Hoofdstuk 5**) falen, dat afhankelijk kan zijn van parasiet- en gastheerkenmerken (**Hoofdstuk 2**). Concluderend zorgt leishmaniasis voor langdurig leed, dat mogelijk ook bestaat in de grotere buurlanden Colombia en Peru. Dit lijden behoeft verlichting door middel van een benadering op meerdere niveaus, die de samenwerking omvat van de lokale, nationale en internationale gemeenschappen, toegewijde professionals, middelen en toekomstige onderzoeksinitiatieven.

Resumen

Mejor comprensión, diagnóstico y tratamiento de la leishmaniasis cutánea y mucosa en zonas rurales del Ecuador

En todo el mundo, el parásito *Leishmania* puede afectar a un millón de personas nuevas cada año. Este protozoario infecta a los humanos a través de la picadura de flebótomos hembra del género *Lutzomyia* o *Phlebotomus*. La transmisión *de Leishmania* es zoonótica (de animal a humano) en la mayoría de las regiones. La Organización Mundial de la Salud clasifica a la leishmaniasis como una Enfermedad Tropical Desatendida debido a la falta de conocimiento sobre la epidemiología y las características de la enfermedad, así como a la falta de diagnóstico, medicamentos, vacunas y estrategias de control de vectores adecuados. La carga global de leishmaniasis está aumentando como resultado de la pobreza, la vulnerabilidad socioeconómica y el cambio climático.

En el Ecuador, la leishmaniasis afecta a un estimado de 5.000 personas anualmente, causando dos enfermedades principales: la leishmaniasis cutánea y mucosa. La primera causa úlceras y nódulos en la piel que curan espontáneamente en meses o años, dejando cicatrices. Es causada principalmente por las especies *Leishmania guyanensis* y *L. braziliensis* y se presenta en toda la Amazonía tropical y la región subtropical del Pacífico norte. Por otra parte, la leishmaniasis mucosa causa inflamación de las mucosas y ulceración de la nariz, boca y garganta que no sana espontáneamente. Se presenta únicamente en la Amazonía y es causada por *L. braziliensis*. No obstante, el conocimiento de la distribución de las especies de *Leishmania* y la presentación clínica de los pacientes con leishmaniasis cutánea en las regiones del Pacífico y la Amazonía del Ecuador está al momento desactualizado e incompleto.

Un estudio transversal, en el **Capítulo 2,** describe la identificación de especies de *Leishmania* en 135 pacientes de las regiones del Pacífico y Amazonía, así como la presentación clínica y los factores que determinan la demora de la búsqueda de atención médica en 245 pacientes con leishmaniasis cutánea. Este estudio encuentra una baja prevalencia sostenida de *L. braziliensis* en la región del Pacífico. También revela la presencia de *L. guyanensis* en las provincias de Napo, Pastaza y Morona Santiago; *L. braziliensis* en la provincia de Imbabura y *L. lainsoni* en las provincias de Pichincha, Napo, Pastaza y Morona Santiago. Un árbol filogenético construido a partir de las secuencias del citocromo B de 102 muestras de *L. guyanensis* revela que las mutaciones respetan los límites regionales. Esto puede tener consecuencias para la precisión de las pruebas de diagnóstico, la resistencia a la terapia y el riesgo de leishmaniasis mucosa incluso dentro de la misma especie, lo que enfatiza la importancia de la investigación regional. Los pacientes de la Amazonía tuvieron una demora promedia más larga de la búsqueda de atención médica en meses (2.0, rango intercuartil 3.0) que los pacientes del Pacífico (1.0, rango intercuartil 1.5). La demora prolongada de la búsqueda de atención médica se asocia con la edad avanzada, la etnia indígena, la infección en altitudes más bajas, las lesiones no ulcerosas y las lesiones en las extremidades inferiores. Esto podría deberse al acceso limitado a la atención médica, así como al estigma. Se recomienda la determinación rutinaria de especies en casos de leishmaniasis cutánea amazónica, así como estudios regionales de determinantes de la demora de la búsqueda de atención médica en Ecuador y estudios regionales de características de pacientes en los países vecinos Perú y Colombia.

En Ecuador, cada año, el tratamiento de pacientes sospechosos de leishmaniasis cutánea basado en la prueba de microscopía deja potencialmente a miles sin tratamiento. Por lo tanto, en **el Capítulo 3,** se analiza la precisión de la microscopía de frotis y una qPCR en el ADN extraído de papel de filtro. El estudio utiliza modelos estadísticos para estimar la sensibilidad, la especificidad, los

cocientes de probabilidad y los valores predictivos de la prueba con sus respectivos intervalos de credibilidad del 95% (95% CrI). Como objetivo secundario se valoran las características sociodemográficas y clínicas para su valor predictivo. Los resultados de las pruebas emparejadas están disponibles para 129 participantes de la Amazonía y 185 del Pacífico. La sensibilidad de qPCR en la región Amazónica y del Pacífico, se estima en 68% (95%CrI 49% a 82%) y 73% (95%CrI 73% a 83%), respectivamente. La sensibilidad microscópica se estima en 51% (95%CrI 36% a 66%) y 76% (95%CrI 65% a 86%). La diferencia regional en la sensibilidad de la microscopía no puede explicarse por la demora de la búsqueda de atención médica. La prevalencia estimada de la enfermedad es del 73% entre los participantes de la región amazónica, el valor predictivo negativo para qPCR es del 54% (95%CrI del 5% al 77%) y para microscopía del 44% (95% CrI del 4% al 65%). Mientras que la prevalencia estimada de la enfermedad es del 88% en el Pacífico, el valor predictivo negativo es del 34% (95%CrI 25% a 57%) y 37% (95%CrI 27% a 63%). En la Amazonía, la adición de qPCR paralela a la microscopía aumentó la prevalencia observada del 38% al 64% (+26 (95% CrI 19 a 34) puntos porcentuales). El fracaso individual de ambas pruebas podría deberse a factores relacionados con el parásito, el huésped humano o el método de diagnóstico, lo que debería aclararse en futuros estudios. Ahora causa un sufrimiento prolongado en los pacientes no tratados. La inclusión de características sociodemográficas y clínicas en la vía diagnóstica no mejora la probabilidad posterior de excluir leishmaniasis cutánea. Se debe investigar el uso de la termoterapia basada en la sospecha clínica, una alternativa de tratamiento breve, segura y de bajo costo para la leishmaniasis cutánea, recomendada por la Organización Panamericana de la Salud.

Según la literatura, la precisión diagnóstica de la microscopía para la leishmaniasis mucosa es incluso menor que para la leishmaniasis cutánea. Si no se trata a tiempo, el parásito puede desarrollar resistencia al tratamiento o causar deformidades faciales progresivas y la muerte. Dado que los criterios clínicos están disponibles en cualquier entorno, se pueden combinar para formar un algoritmo sindrómico, que luego se puede utilizar como herramienta de diagnóstico. A través de una revisión sistemática de la literatura, el **Capítulo 4** explora los posibles criterios clínicos para un algoritmo de diagnóstico sindrómico para la leishmaniasis mucosa. Se incluyen diez estudios que describen un total de 192 pacientes con leishmaniasis mucosa. El sexo masculino tiene la tasa de detección más alta (88%), seguido de úlcera de la mucosa nasal (77%), edad >15 (69%) y duración de los síntomas >4 meses (63%). No se puede calcular la especificidad porque los artículos no incluyeron pacientes con leishmaniasis no mucosa. Además, todos los estudios incluidos tienen un alto riesgo de sesgo. Este estudio es un primer paso, aunque pequeño, hacia el desarrollo de criterios diagnósticos para la leishmaniasis mucosa. Futuros estudios deberían incluir pacientes con leishmaniasis no mucosa para definir los criterios más discriminativos y calcular los valores predictivos de las combinaciones.

Por otro lado, los tratamientos actuales para la leishmaniasis cutánea y mucosa tienen efectos secundarios graves y deben administrarse mediante inyección. Los medicamentos de alilamina, como la terbinafina, son seguros y se pueden tomar por vía oral o tópica, incluso durante el embarazo. Una revisión sistemática de la literatura en el **Capítulo 5** evalúa la eficacia y seguridad de los medicamentos de alilamina como tratamiento alternativo para la leishmaniasis cutánea y mucosa. El estudio incluye un ensayo no controlado, dos ensayos controlados aleatorios, dos estudios en animales y 12 estudios *in vitro* de amastigotes y/o promastigotes. En el único ensayo controlado aleatorio bien diseñado que compara la eficacia del tratamiento de terbinafina oral versus antimoniato de meglumina intramuscular en 80 pacientes infectados con *L. tropica*, la terbinafina tiene una tasa de curación más baja no significativa (38% frente a 53%). No se realiza un metaanálisis debido al pequeño número de estudios, su heterogeneidad y baja calidad. No

obstante, de acuerdo con esta revisión sistemática, no hay evidencia de que la monoterapia con alilaminas sea efectiva contra la leishmaniasis cutánea o mucosa. Se deben considerar cuidadosamente los ensayos adicionales de alilaminas porque los estudios *in vitro* mostraron concentraciones efectivas mínimas que no son clínicamente relevantes. Sin embargo, los efectos sinérgicos de las alilaminas combinadas con fármacos triazólicos *in vitro* justifican una mayor exploración.

La leishmaniasis cutánea reduce la calidad de vida relacionada con la salud debido al estigma en los contextos asiático y mediterráneo, pero falta investigación para Ecuador. El estudio descrito en el **Capítulo 6** evalúa la influencia de las lesiones sospechosas de leishmaniasis cutánea en la calidad de vida de 279 pacientes en las regiones del Pacífico y Amazonía. Este estudio aplica la versión española del cuestionario Skindex-29, una herramienta genérica para medir la calidad de vida relacionada con la salud en pacientes con enfermedades de la piel. Los pacientes de todos los grupos lingüísticos participantes (mestizo, kichwa o chicham) en la Amazonía obtienen una puntuación significativamente (P <0,01) más alta (lo que indica una peor calidad de vida relacionada con la salud) en todas las dimensiones del cuestionario Skindex-29 comparado con los pacientes mestizos del Pacífico. El sexo, la edad, el número de lesiones, la localización corporal de las lesiones, la demora de la búsqueda de salud y la posterior confirmación del parásito, no se asocian con una menor calidad de vida. Los estudios futuros que incluyan detalles adicionales de pacientes y lesiones deberían aclarar si la calidad de vida relacionada con la salud deteriorada está relacionada con el estigma.

Las investigaciones previas sobre el estigma de la leishmaniasis cutánea se han centrado en la afectación del aspecto estético como fuente primaria. Este aspecto ha sido estudiado en la década de los 90 en la región del Pacífico ecuatoriano pero nunca en la Amazonía. El **capítulo 7** describe la investigación etnográfica y los datos obtenidos de 83 entrevistas semiestructuradas y 15 grupos focales realizados en siete grupos étnicos ecuatorianos. Los mestizos, que predominan en la región del Pacífico, afirman que la aparición de heridas abiertas, desfiguración y marcas de cicatrices están asociadas al auto rechazo y al miedo, lo cual concuerda con estudios previos. Las poblaciones indígenas del interior de la Amazonía, por otro lado, desencadenan expresiones de estigma interno y externo al reconocer la leishmaniasis cutánea y mucosa a través del olor atípico, seguido del sonido de la voz, la apariencia y el sabor extraños. Esto revela que los sentidos permiten respuestas culturales diferenciadas a una sensación de peligro, contagio y (auto)rechazo social, que están vinculados al enfoque holístico de la salud de las poblaciones indígenas. Estos hallazgos deben comunicarse con cuidado para evitar exacerbar la discriminación ya existente contra los indígenas. Se desconoce si el estigma persistirá en la población amazónica después de que el olor, el sonido atípico y el gusto se normalicen durante la cicatrización de la lesión. Un estudio que compare la calidad de vida relacionada con la salud, el estigma externo y el estigma interno en pacientes con leishmaniasis cutánea y mucosa antes, durante y después de la curación aclararía esto. Un enfoque en el hecho de que la leishmaniasis no se transmite de persona a persona podría aliviar los temores públicos y el rechazo de los pacientes. Se recomienda un enfoque holístico para los pacientes con sospecha de leishmaniasis que incluya asesoramiento dietético y conductual. Cualquier intervención debe planificarse cuidadosamente con todos los actores y monitorearse continuamente para verificar su eficacia.

Casi 140 años después del primer avistamiento del parásito *Leishmania*, descubrimos que los cambios en el olfato, el oído y el gusto inducidos por la leishmaniasis provocan expresiones de estigma en la población amazónica (**Capítulo 7**). Pueden resultar en una disminución de la calidad de vida relacionada con la salud (**Capítulo 6**) y una demora prolongada de la búsqueda de atención

médica (**Capítulo 2**) esto se exacerba por fallas diagnósticas (**Capítulo 3 y 4**) y terapéuticas (**Capítulo 5**), que pueden depender de las características del parásito y del huésped (**Capítulo 2**). En general, la leishmaniasis causa un sufrimiento prolongado que también puede existir en los países vecinos más grandes, Colombia y Perú. Este sufrimiento debe aliviarse a través de un enfoque de múltiples niveles que incluya la colaboración de las comunidades locales, nacionales e internacionales, profesionales dedicados, recursos e iniciativas de investigación futuras.

Addendum

Authors affiliations and contributions
PhD Portfolio
Acknowledgements
About the author

Authors affiliations and contributions

Chapter 2: *Leishmania* species and clinical characteristics of Pacific and Amazon cutaneous leishmaniasis in Ecuador and determinants of health-seeking delay: a cross-sectional study:

Jacob M. Bezemer, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Fundación Misión Cristiana de Salud, Hospital Shell, Shell, Pastaza, Ecuador. Contributed to the conception and design of the work, data acquisition, analysis, and interpretation, and drafted the work.

Byron P. Freire-Paspuel, Universidad de las Américas, Laboratorios de Investigación, Quito, Ecuador, Hospital Universitari Vall d'Hebron, Vall d'Hebron Research Institute, Barcelona, Spain. Contributed to the data acquisition, analysis, interpretation, and substantial revision.

Henk D. F. H. Schallig, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands. Contributed to the conception and design of the work, data analysis, interpretation, and substantial revision.

Henry J.C. de Vries, Amsterdam UMC location University of Amsterdam, Department of dermatology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Public Health Service, Center for Sexual Health, Department of Infectious Diseases, Amsterdam, the Netherlands. Contributed to the conception and design of the work, data analysis, interpretation, and substantial revision.

Manuel Calvopiña, Universidad de las Américas, Facultad de Medicina, OneHealth Research Group, Quito, Ecuador. Contributed to the conception and design of the work, data acquisition, data analysis, interpretation, and substantial revision.

Chapter 3: Diagnostic accuracy of qPCR and microscopy for cutaneous leishmaniasis in rural Ecuador: A Bayesian latent class analysis:

Jacob M. Bezemer, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Fundación Misión Cristiana de Salud, Hospital Shell, Shell, Pastaza, Ecuador. Contributed to the conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, visualization, and writing – original draft preparation.

Joanna Merckx, McGill University, Department of Epidemiology, Biostatistics, and Occupational Health, Montreal, Canada, Amsterdam UMC location University of Amsterdam, Department of

Epidemiology and Data Science, Amsterdam, the Netherlands. Contributed to the conceptualization, formal analysis, methodology, project administration, resources, visualization, and writing – original draft preparation.

Byron P Freire Paspuel, Universidad de las Américas, Laboratorios de Investigación, Quito, Ecuador, Hospital Universitari Vall d'Hebron, Vall d'Hebron Research Institute, Barcelona, Spain. Contributed to the investigation, resources, validation, and writing – review and editing.

Manuel Calvopiña, Universidad de las Américas, Facultad de Medicina, OneHealth Research Group, Quito, Ecuador. Contributed to the conceptualization, investigation, resources, supervision, and writing – review and editing.

Henry J.C. de Vries, Amsterdam UMC location University of Amsterdam, Department of dermatology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Public Health Service, Center for Sexual Health, Department of Infectious Diseases, Amsterdam, the Netherlands. Contributed to the conceptualization, resources, supervision, and writing – review and editing.

Henk D. F. H. Schallig, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands. Contributed to the conceptualization, resources, supervision, and writing – review and editing.

Mariska M G Leeflang, Amsterdam UMC location University of Amsterdam, Department of Epidemiology and Data Science, Amsterdam, the Netherlands. Contributed to the conceptualization, resources, supervision, and writing – review and editing.

Nandini Dendukuri, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada. Contributed to the conceptualization, resources, supervision data analysis, validation, writing – review and editing.

Chapter 4: Clinical criteria for Mucosal Leishmaniasis diagnosis in rural South America: a systematic literature review:

Jacob M. Bezemer, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Fundación Misión Cristiana de Salud, Hospital Shell, Shell, Pastaza, Ecuador. Contributed to the conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, visualization, and writing – original draft.

Kevin Meesters, University of British Columbia, Division of Infectious Diseases, Department of Pediatrics, Vancouver, Canada. Contributed to the conceptualization, formal analysis, investigation, methodology, and writing – review and editing.

Cristhian L Naveda, Fundación Misión Cristiana de Salud, Shell, Pastaza, Ecuador. Contributed to the formal analysis, and investigation.

Paulo R. L. Machado, Universidade Federal da Bahia, Hospital Universitário Prof. Edgard Santos, Salvador, Serviço de Imunologia, Bahia, Brazil. Contributed to the conceptualization, and writing – review and editing.

Manuel Calvopiña, Universidad de las Américas, Facultad de Medicina, OneHealth Research Group, Quito, Ecuador. Contributed to the conceptualization and writing – review and editing.

Mariska M G Leeflang, Amsterdam UMC location University of Amsterdam, Department of Epidemiology and Data Science, Amsterdam, the Netherlands. Contributed to the methodology and writing – review and editing.

Henk D. F. H. Schallig, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands. Contributed to the conceptualization, supervision, validation, and writing – review and editing.

Henry J.C. de Vries, Amsterdam UMC location University of Amsterdam, Department of dermatology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Public Health Service, Center for Sexual Health, Department of Infectious Diseases, Amsterdam, the Netherlands. Contributed to the conceptualization, resources, supervision, validation, and writing – review and editing.

Chapter 5: Safety and efficacy of allylamines in the treatment of cutaneous and mucocutaneous leishmaniasis: a systematic review:

Jacob M. Bezemer, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Fundación Misión Cristiana de Salud, Hospital Shell, Shell, Pastaza, Ecuador. Contributed to the conceptualization, formal analysis, investigation, methodology, project administration, and writing – original draft.

Jacob van der Ende, Fundación Quina Care Ecuador, Puerto el Carmen de Putumayo, Sucumbíos, Ecuador. Contributed to the conceptualization, formal analysis, investigation, methodology, and writing – review and editing.

Jacqueline Limpens, Amsterdam UMC location University of Amsterdam, Medical Library, Amsterdam, The Netherlands, Contributed to the conceptualization, formal analysis, investigation, methodology, software, validation, and writing – review and editing.

Henry J. C. de Vries, Amsterdam UMC location University of Amsterdam, Department of dermatology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Public Health Service, Center for Sexual Health, Department of Infectious Diseases, Amsterdam, the Netherlands. Contributed to the conceptualization, resources, supervision, and writing – review and editing.

Henk D. F. H. Schallig, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands. Contributed to the conceptualization, resources, supervision, validation, and writing – review and editing.

Chapter 6: Quality of life of cutaneous leishmaniasis suspected patients in the Ecuadorian Pacific and Amazon regions: a cross sectional study:

Jacob M Bezemer, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Fundación Misión Cristiana de Salud, Hospital Shell, Shell, Pastaza, Ecuador. Contributed to the conception and design of the work, data acquisition, analysis and interpretation, and drafted the work.

Manuel Calvopiña, Universidad de las Américas, Facultad de Medicina, OneHealth Research Group, Quito, Ecuador. Contributed to the conception and design of the work, data acquisition, analysis and interpretation.

Andrea E Corral Zabala, Fundación Misión Cristiana de Salud, Hospital Shell, Shell, Ecuador. Contributed to the data analysis.

Fernando Ortega Pérez, Universidad San Francisco de Quito, School of Public Health, College of Health Sciences, Quito, Ecuador. Contributed to the data interpretation.

Veronica C Vargas Román, Katholieke universiteit Leuven, Department of Social and Cultural Anthropology, Leuven, Belgium. Contributed to the data interpretation and substantial revision.

Henk D F H Schallig, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands. Contributed to the conception and design of the work, data analysis, interpretation, and substantial revision.

Henry J C de Vries, Amsterdam UMC location University of Amsterdam, Department of dermatology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Public Health Service, Center for Sexual Health, Department of Infectious Diseases, Amsterdam, the Netherlands. Contributed to the conception and design of the work, data analysis, interpretation, and substantial revision.

Chapter 7: Sensorial perceptions of risk: the aesthetics behind (muco)cutaneous leishmaniasis-related stigma in Ecuador:

Veronica C Vargas Roman, Katholieke universiteit Leuven, Department of Social and Cultural Anthropology, Leuven, Belgium. Contributed to the software, validation, formal analysis, investigation, resources, data curation, and writing – original draft, visualization.

Jacob M Bezemer, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Fundación Misión Cristiana de Salud, Hospital Shell, Shell, Pastaza, Ecuador. Contributed to the conceptualization, methodology, investigation, resources, writing – review and editing, project administration, and funding acquisition.

Manuel Calvopiña, Universidad de las Américas, Facultad de Medicina, OneHealth Research Group, Quito, Ecuador. Contributed to the conceptualization, methodology, investigation, resources, writing – review and editing, and supervision.

Fernando Ortega, Universidad San Francisco de Quito, School of Public Health, College of Health Sciences, Quito, Ecuador. Contributed to the methodology, writing – review and editing, and supervision.

Noel B. Salazar, Katholieke universiteit Leuven, Department of Social and Cultural Anthropology, Leuven, Belgium. Contributed to the writing – review and editing.

Henk D F H Schallig, Amsterdam UMC location University of Amsterdam, Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands. Contributed to the conceptualization, methodology, writing – review and editing, and supervision.

Henry J C de Vries, Amsterdam UMC location University of Amsterdam, Department of dermatology, Amsterdam, the Netherlands, Amsterdam Institute for infection and Immunity (AII), Infectious Diseases Program, Amsterdam, the Netherlands, Public Health Service, Center for Sexual Health, Department of Infectious Diseases, Amsterdam, the Netherlands. Contributed to the conceptualization, methodology, writing – review and editing, supervision, and funding acquisition.

PhD Portfolio

Name PhD student:	J.M. Bezemer
PhD period:	September 2017 – September 2023
Name PhD supervisor:	Prof. dr. H.J.C. de Vries
Names PhD co-supervisors:	Dr. H.D.F.H. Schallig and Dr. M. Calvopiña Hinojosa

1. PhD training

	Year	ECTS
General courses		
- Advanced Paediatric Life Support (APLS), by the Universidad de San Francisco de Quito	2020	1,0
- Maternal infections by The Global Health Network	2020	0,3
- To Prescribe or Not To Prescribe? Antibiotics and Outpatient Infections, by the Stanford Center for Continuing Medical Education	2020	0,1
- Novel Coronavirus (COVID-19) Update, by the Stanford Center for Continuing Medical Education	2020	0,1
- Decolonising Global Health, by the Nederlandse Vereniging voor Tropische Geneeskunde en Internationale Gezondheiszorg	2022	0,3
Specific courses		
- Writing in the Sciences, by the Stanford University	2018	1,0
- Best Practices For Biomedical Research Data Management, by the Harvard Medical School	2019	1,0
- Research ethics, by The Global Health Network	2019	0,2
- Good Clinical Practice, by NIDA Clinical Trials Network	2019	0,3
- Statistics in Medicine, by the Stanford University	2020	1,5
- Getting started, Study building and management, and Data entry in Castor EDC, by Castor EDC	2020	0,1

Seminars, workshops and master classes		
- Weekly seminars at the department of Family Medicine of Shell Hospital, Shell, Ecuador	2018-2023	5,0
Presentations		
- Improved diagnosis and treatment of (muco-) cutaneous leishmaniasis in resource limited settings in Ecuador. Presentation during the online work meeting of the Department of Medical Microbiology and Infection Prevention, Laboratory for Experimental Parasitology, Amsterdam UMC location University of Amsterdam, 06 April 2021	2021	0,5
- Leishmaniasis research in the Ecuadorian Amazon. Online presentation during peer evaluation meeting organized by the Nederlandse Vereniging voor Tropische Geneeskunde en Internationale Gezondheidszorg, 26 January 2022	2022	0,5
- Leishmaniasis in the Ecuadorian Amazon: a tale of neglect, clinical challenges, and stigma. Online presentation at the meeting of the infectious diseases division of the department of pediatrics of the University of British Columbia, 07 December 2022	2022	0,5
- Calidad de vida de pacientes sospechosos de leishmaniasis cutánea en la region amazónica e pacífica del Ecuador: un estudio transversal. Oral presentation at the meeting of the division of parasitology and leishmaniasis of the Ministry of Health, Quito, Ecuador, 29 September 2022	2022	0,5
- Quality of life of cutaneous leishmaniasis suspected patients in the Ecuadorian Amazon and Pacific regions: a cross-sectional study. Oral presentation at the WORLDLEISH7 congress, Cartagena, Colombia, 03 August 2022	2022	0,5
- A cross-sectional study of regional *Leishmania* species variation in Ecuador: consequences for clinical practice and research. Poster presentation at the WORLDLEISH7 congress, Cartagena, Colombia, 03 August 2022	2022	0,5

(Inter)national conferences		
- International Conference on Public Health, Health Iniquities, and Research and Fifth International Meeting on Research, Infectious Diseases and Tropical Diseases, 10-12 October 2018, Quito, Ecuador (participant)	2018	1,0
- WORLDLEISH7 congress, 01-06 August 2022, Cartagena, Colombia (including oral and poster presentation)	2022	1,4
Other		
- Evaluation of individual functioning organized by the Nederlandse Vereniging voor Tropische Geneeskunde en Internationale Gezondheidszorg	2019	0,5
- Peer evaluations organized by the Nederlandse Vereniging voor Tropische Geneeskunde en Internationale Gezondheidszorg	2019 - 2022	0,7

2. Teaching

	Year	ECTS
Lecturing		
- Lecture on cutaneous and mucosal leishmaniasis during the 13th national course on actualization in Internal Medicine, Intensive Care and Emergencies from the Universidad Internacional del Ecuador	2018	0,5
- Lecture on actual developments in the field of malaria during the 13th national course on actualization in Internal Medicine, Intensive Care and Emergencies from the Universidad Internacional del Ecuador	2018	0,5
- Seminars on parasitic infections at Shell Hospital, Shell, Ecuador	2018-2022	1,5
Tutoring, Mentoring		
- Tutoring of medical students at Shell Hospital, Shell, Ecuador	2018-2023	7,0
- Mentoring of medical residents at Shell Hospital, Shell, Ecuador	2021-2023	4,0
Supervising		

- Cristhian Naveda, Diagnosis of mucosal leishmaniasis, Facultad Ciencias de la Salud, Universidad Nacional de Chimborazo, Riobamba, Ecuador	2020-2022	2,0
- Andrea Corral, Quality of life of patients with cutaneous leishmaniasis, Fundación Misión Cristiana de Salud, Shell, Ecuador	2020-2022	2,0
- Gabriela Muñoz, Enfoque médico y sociocultural de leishmanaisis mucocutánea en dos pacientes de etnia indígena del oriente Ecuatoriano, Hospital Vozandes, Quito, Ecuador	2019-2020	1,0
Total of Training and Teaching	**2018-2023**	**36,0**

3. Publications

	Year
Peer reviewed publications in Thesis	
- **Bezemer JM**, van der Ende J, Limpens J, de Vries HJC, Schallig H. Safety and efficacy of allylamines in the treatment of cutaneous and mucocutaneous leishmaniasis: A systematic review. PloS one. 2021;16(4):e0249628.	2021
- **Bezemer JM**, Meesters K, Naveda CL, Machado PR, Calvopiña M, Leeflang MM, et al. Clinical criteria for Mucosal Leishmaniasis diagnosis in rural South America: A systematic literature review. PLoS neglected tropical diseases. 2022;16(8):e0010621.	2022
- **Bezemer JM**, Calvopiña Hinojosa M, Corral Zabala AE, Ortega Pérez F, Vargas Román VC, Schallig HDFH, et al. Quality of life of cutaneous leishmaniasis suspected patients in the Ecuadorian Pacific and Amazon regions: a cross sectional study. BMC infectious diseases. 2022;22(1):1-9.	2022
- **Bezemer JM**, Freire-Paspuel BP, Schallig HDFH, de Vries HJC Calvopiña M. Leishmania species and clinical characteristics of Pacific and Amazon cutaneous leishmaniasis in Ecuador and determinants of health-seeking delay: a cross-sectional study. BMC infectious diseases. 2023;23(1):395.	2023
Other peer reviewed publications	
- van Genderen PJ, Hesselink DA, **Bezemer JM**. Imported malaria is falling in Netherlands and Europe. BMJ. 2008;337.	2008
- Van Genderen PJ, Hesselink DA, **Bezemer JM**, Wismans PJ, Overbosch D. Efficacy and safety of exchange transfusion as an adjunct therapy for severe Plasmodium falciparum malaria in nonimmune travelers: a 10-year single-center experience with a standardized treatment protocol. Transfusion. 2010;50(4):787-94.	2010

- Calvopiña M, **Bezemer J**. Case Report: Evidence of Tungiasis in the Amazon Rain Forest of Ecuador. The American journal of tropical medicine and hygiene. 2021;105(3):698.	2021

Acknowledgements

Above all, I thank God for the opportunity to conduct research and pursue a PhD degree after having burned my bridges in the Netherlands in response to His calling.

Many people have contributed to the journey that culminates with the completion of my PhD. I thank you everybody and beg your pardon if you are not mentioned individually or as much as you deserve.

First and foremost, I would want to thank my promotor, Henry de Vries, for his guidance over the past six years. Henry, you've taught me lessons I didn't expect and you have motivated me as nobody else could have done. I am enormously grateful for the opportunity to continue and complete this PhD. I'm impressed by your vision, your management skills, and steadiness. I would really like to proceed with fresh study projects with your assistance.

I would want to express my immense gratitude to my co-promotor, Henk Schallig, for his coaching during this trajectory. Henk, the idea to write this thesis was born in your office and I am so grateful that it has come this far. You guided me all the way through the research cycle. We went from funding acquisition to ethical clearance, from scientific courses to research protocols, and from drafts to publications. I admire your perseverance, friendliness, knowledge, and thorough manuscript revisions. It would be wonderful to continue working on our shared goal of improving health care for those who suffer from neglected diseases.

My heartfelt thanks go to my co-promotor, Manuel Calvopiña, for being my scientific mentor in Ecuador. You have taught me very much about leishmaniasis and your energy, hard work, and knowledge are very inspirational. Thank you for accepting me with usual Ecuadorian warmth, sociability, and openness. Your assistance with the fieldwork has made this thesis possible and I much hope that the new research projects we are planning will become as fruitful as those included in this thesis.

I would also like to thank the members of my PhD committee. I am honored that you agreed to serve on my defense committee and have given your time and expertise to evaluate my thesis.

I would like to thank Fidel Dahua and Tsawant Tentets for their thrust in me and for convincing me of the unspoken suffering of leishmaniasis patients in the Amazon.

I thank all the participants in the studies included in this thesis. I thank you for thrusting the team, sharing your experiences and samples, teaching, and welcoming us. My heartfelt gratitude goes to Fanny Gualinga, who elaborated the figurine displayed on the title page. She used her ancestral knowledge and skills to express what words could not describe. I hope and pray that the findings of this thesis may help to alleviate the suffering of future leishmaniasis patients and their families.

I would like to thank the entire staff at Hospital del día Shell (day care hospital Shell) for their contributions to this research endeavor, as well as their support and encouragement. Eckehart and Klaudia Wolff for allowing me to devote an increasing portion of my time to research and providing the necessary physical infrastructure. Laura Sagñay, Amparito López, Michele Escobar, and Sandra

Andrade for their invaluable assistance with the laboratory work. Indira Cando and Shehawee Santamaría for their assistance and dedication during the fieldwork. I would like to thank Andrea Corral for her effort in processing patient data, as well as Cristhian Naveda for his motivation and assistance with the systematic review of literature on mucosal leishmaniasis. Menkaye Yeti and Gilberto Suarez thanks for counseling and guiding us both in the hospital and on fieldvisits to the hinterland.

I thank Franc Pots from the Ziekenhuis Groep Twente and his daughter Annika for their marvelous invention that enabled us to do nasal fiberscopies in remote settings. This allowed us to diagnose and treat patients that otherwise would have stayed under the radar.

I thank my neighbors, friends, and brothers from Sacha Runa, Shell, Pastaza, and from the Amazon in general. My heartfelt gratitude goes to apayaya Atanasio Mayancha for his unending joy, and for teaching me the Kichwa language. Apamama Magdalena Canelos, thank you for your regular visits and daily counsel. Klever Mayancha, president of my Sacha Runa community, for his friendliness and camaraderie. I am grateful to my friend Tiyua Uyunkar, Prefect of Morona Santiago, for welcoming me and for teaching me Achuar language and culture.

My heartfelt gratitude goes to all the professionals from the Ministerio Público del Ecuador (Ecuadorian Ministry of Health) who collaborated with participant inclusions and laboratory trainings.

I would like to thank everyone at the Universidad de las Americas Quito who helped with the molecular analysis of the material mentioned in this thesis. Byron Freire, your work has been outstanding and I hope we can collaborate on future projects to continue with *Leishmania* detection and species determination.

My heartfelt gratitude goes to Veronica Vargas, Noel Salazar, and Fernando Ortega for their tremendous work on the anthropological component of this thesis. Sahienshadebie Ramdas, I thank you so much for your efforts and the great time we shared during the preparation of the anthropological fieldwork.

I would like to thank everyone at the University of Amsterdam who collaborated during this PhD journey. Kirsten Douma from the Graduateschool, for your essential guiding. Sandra Menting for her assistance during the interpretation of the laboratory results. Mariska Leeflang for providing essential comments on the mucosal leishmaniasis systematic review and for organizing the Latent Class Analysis of our results. Marije Wolvers for your excellent help with the statistical analysis of our results. Jacqueline Limpens for guiding us during the systematic literature review on allylamines for leishmaniasis.

My heartfelt gratitude goes to Nandini Dendukuri and Joanna Merckx from the McGill University in Canada, for their excellent assistance with the Latent Class Analysis of our results. Joanna, it was an enormous pleasure to work together on our manuscript. It would be fantastic to continue working together on future research projects.

I would like to thank Vita Jongen from the GGD Amsterdam for her assistance with the statistical analysis of our results. Paulo Machado from the Universidade Federal da Bahia in Brazil for your

help reviewing the systematic literature review on mucosal leishmaniasis diagnosis.

I have countless happy memories with my classmates from `Studiegroep 25' at the Erasmus University. Gretchen, Kevin, Sylvia, and Thomas, a tremendous thanks for your unwavering friendship. Kevin, it was your advice that led to the publication of two systematic reviews. I am proud to have you as co-author of one of the publications in this thesis.

I would like to thank all the participants of my 'toetsgroep' for specialists in Tropical Medicine and International Health. You are very inspiring. A special thanks goes to my friends and colleagues Jacob and Carolien van de Ende. It's wonderful to share experiences in Ecuador. I really admire your dedication and the effectiveness in your work. Jacob, it was great to publish together!

I am grateful to Latin Link Ecuador for providing the context and encouragement I needed to keep my family healthy and stable. Katy, you are an excellent example of someone who is both humble and capable. Anna Claire, many thanks for reviewing one of our manuscripts. Latin Link Netherlands for supervising our mission, your outstanding fundraising efforts, and for channeling our resources. William, your purpose of providing practical assistance and proclaiming the good news of Jesus Christ has been a tremendous gift during the years we worked together. Eveline, thank you so much for continuing this amazing work. We expect to continue working with Latin Link in Ecuador for many years to come.

I would like to express my enormous gratitude to Hans, Karolyne, Toby, Marinus and Jacco of the foundation Supportunity for your generous support of this project. Without your commitment, this project could not have started. I admire your humility in giving and your dedication to God.

I would like to thank Gert Bekebrede and Jan Willem den Bok of EO Metterdaad for their fantastic effort on a television program on the preparatory work for this thesis which resulted in significant funding support. We would like to thank everyone who donated to EO Metterdaad in order to support this work. It has been of tremendous assistance.

I would like to thank Anke Daling for her friendship and support during a crucial part of the research that led to this thesis. GerJan Ekenhorst for accompanying us during the fieldwork and writing an amazing article about it in the newspaper Nederlands Dagblad.

Our enormous gratitude extends to the former and current members of our home front committee. Bert-Jan and Annemieke, Johanneke and Theo, Michel, Mirjam and Rick, Christiaan, Johan and Renske, Gea, Bertine, Erik, and Peter. You are our best friends and (like) family. May God reward your long standing and selfless efforts to make our ministry possible.

I thank my grandparents in law, Henk and Lia Harinck and Jan and Nellie van Dooijeweert, for their continuous interest in the project, their advice, and example of honest and righteous lives.

I thank my brothers and sisters in law, Susanne and Carl, Michel and Marina, Carina and Gerbert, Daniëlle, and Yvonne, for having accepted me as a full member of the family. Your fellowship during more than 15 years is highly appreciated.

My father and mother in law, Jacco and Nelleke Harinck, deserve my heartfelt gratitude. You've

always treated me like one of your own children, teaching me innumerable life lessons. Even more significant is your living example, which enhances my life. Above all, I want to thank you for your daughter, Linda, who has made my life complete.

I thank my brothers and sisters, Johanneke and Theo, Jan and Jannie, Loes and Joël, Elise, and Mirjam and Rick for every moment we have shared during our lives and for your unwavering love. Mirjam, your help with the elaboration of the cover pages of this thesis is very much appreciated. May God grant us eternal unity and prosperity.

Mom and dad, you took care of me from being a baby until adulthood. Your example of enduring love, care, mission, and faith has formed me forever and your lessons continue enlightening me. I am so happy and proud to present you this thesis today. Forever thanks.

My beloved children Corné, Helen, Arianne, Jacoline, Richard, and Erik. You have seen this project as yours because you know how much the patients suffer from leishmaniasis. You accompanied us during the fieldwork and enjoy living in the jungle. I am so grateful for your patience, your support, your loving care and happiness during many, many hours at home or elsewhere.

Linda, my lovely wife. We chose to embark on this years-long adventure together. It was difficult and beautiful, appreciative and hard all at the same time, and with each turn of reality, I got to know you even better. This thesis is jointly owned by you and me. Congratulations.

Thank you, Bedankt, Gracias, Pagarachu, Maketai, Yuminsajmai, Wakebi, Danke, Idike

About the author

Jaap (Jacob) Bezemer was born on the 28th of November 1985 in the fishing village Yerseke, in the province of Zeeland, in the Netherlands. His parents moved to southern Nigeria when he was five years old to work as missionaries among the Izzi people. Jaap suffered with malaria several times, most likely contracted cutaneous leishmaniasis, and witnessed the aftermath of a cholera epidemic. He pledged his life to serving Jesus when he was 11 years old, and shortly after that resolved to become a doctor for the unreached. He studied medicine at Erasmus University in Rotterdam, where he completed his doctoral (equivalent to masters) thesis on malaria. During the evenings, he studied theology at the CGO in Gouda, where he completed his bachelor thesis on European and African interpretations of biblical genealogies. Jaap is married to Linda for over 15 years, and they have six children: Corné, Helen, Arianne, Jacoline, Richard, and Erik. Jaap completed a three-year residency for the specialty of International Health and Tropical Medicine from the Netherlands Society for Tropical Medicine and International Health. Following that, he and Linda felt called to serve the indigenous population of the Ecuadorian Amazon and moved to Ecuador. The Ecuadorian government employed Jaap to work in a remote jungle clinic among the Achuar people. Visiting a patient at home, he was asked to inject an ampul of Meglumine Antimoniate (a drug used to treat leishmaniasis). Jaap recognized that, despite his studies, he knew almost nothing about leishmaniasis and began reading medical literature on the disease. Despite the fact that the test result was negative, Jaap concluded that his patient had mucosal leishmaniasis and started treatment. The patient's recovery exceeded all expectations covering physical, emotional, psychological, social, and spiritual dimensions. This thesis is the result of the route token to relieve such suffering. Jaap continues to work at the day care hospital of the Christian Health Mission Foundation. He has a guest appointment at the Department of Medical Microbiology and Infection Prevention at Amsterdam UMC location University of Amsterdam and the Amsterdam Institute for infection and Immunity. Jaap is one of the leaders of the Diospa Ñampi church in his Kichwa village Sacha Runa. He plans to continue providing holistic care for forgotten indigenous populations in the Ecuadorian Amazon as long as his health allows.

In this thesis, almost 140 years after the first sighting of the *Leishmania* parasite, we discover that leishmaniasis-induced changes in smell, sound, and taste cause stigma expressions in the Amazon population of Ecuador (Chapter 7). They may result in decreased health-related quality of life (Chapter 6) and prolonged health-seeking delay (Chapter 2). This is exacerbated by diagnostic (Chapter 3 and 4) and therapeutic (Chapter 5) failures, which may be dependent on parasite and host characteristics (Chapter 2). Overall, leishmaniasis causes prolonged suffering which may also exist among leishmaniasis patients in larger neighboring countries Colombia and Peru. This suffering should be alleviated through a multi-level approach involving local, national, and international communities, dedicated professionals, resources, and future research initiatives.