Migration and the ecology of American Cutaneous Leishmaniasis in Madre de Dios, Peru, and Acre, Brazil: A mixed methods approach

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1. Background

The Southwestern Amazon is an area of internal and cross-border population movement and transit due to the newly paved Transoceanic Highway, connecting Atlantic ports in Brazil with Pacific Ports in Peru. The last part of the highway – crossing the Peruvian Amazon – was only opened 5 years ago. As the highway crosses through forested and deforested jungles endemic for vector-borne diseases (VBDs), there is increased concern about the (re)emergence and increased distribution of diseases to non-endemic areas. Environmental destruction due to increased agriculture, farming, and illegal gold mining operations has expanded deforested areas in the Southwestern Amazon in Brazil and Peru, a known risk factor for vector-borne diseases (VBDs). Increased residence in forested, but also deforested areas are environments, where vectors breed and sylvatic animals act as hosts for zoonotic VBDs such as American Cutaneous Leishmaniasis (ACL) and Bartonellosis. Both diseases have defined habitats, restricted to specific geographical areas due to natural geographical and physical barriers. However, with the opening of the Transoceanic Highway, these barriers are potentially lost and the diseases circulating in the border region [1].

Brazil and Peru share almost 3,000 km of a border in the Amazon rainforest that has one of the highest biodiversities in the world. The tri-national border region between Bolivia, Peru and Brazil, is home to over 26 Amazonian indigenous peoples who are dedicated to sustainable management of the natural resources, and are of fundamental importance in the conservation of tropical forests and their carbon stocks mitigating the effects emissions of greenhouse gases [1]. South-western Amazonia, where Acre and Madre de Dios come together, only began to be populated by non-indigenous people in the early 20th Century [2, 3]. For the last hundred years, regular short range circular migration helped maintain bounds between families dispersed on both sides of the border [4]. In the course of the 20th Century and the rise of global economy, the region became the economic link between Brazil and the growing Asian and Latin American markets. The creation of the *Mercosur* (Mercado Común del Sur / Southern Common Market) in the late 20th Century, and the new Transoceanic Highway facilitating the export of Brazilian agricultural production to Asian markets, literally paved the way for the increasing migration between the two countries. Both Acre, in Brazil, and Madre de Dios, in Peru, rank low in terms of development and health indicators [5-7]. At a time with general growing intra-regional migration in South America [8], investigating health service access among migrants is urgently needed.

American Cutaneous Leishmaniasis (ACL) is a disfiguring parasitic disease which causes ulcerative cutaneous lesions on the skin leading to secondary infections, while the mucosal form can cause complete destruction of the nasal cavity. There are more than 1.5 million new cases of ACL. Leishmaniasis globally [9], with New World *American* Cutaneous Leishmaniasis accounting for a third of all cases globally [10]. Only 7 countries account for 90% of all ACL cases. Among these countries are Brazil and Peru, where socio-environmental changes (new settlements, intrusion into primary forest, deforestation, growing human migration, road paving, dams building) increase the exposure to sandfly vectors[1].

Madre de Dios, Peru and Acre, Brazil are highly endemic areas for ACL, with incidence rates 34 (713/100,000) [11] and 12 times (144/100,000) [12] the national average of ACL, respectively. Non-immunity to ACL is considered to be a risk factor for migrants arriving from non-endemic to endemic areas [13] and length of time (>6 years) has been shown to increase immune response to the disease [14]. Thus recent migrants are considered at higher risk for ACL infection,

particularly when employment activities are in areas of high environmental exposure. Also, because migrants tend to work and live further away from urban centres, access to health centres and subsequent treatment may be more difficult for migrants than for residents. The clinical manifestation and the severity of the disease is a result of complex transmission cycles involving various sandfly vectors and protozoa agents, combined with different levels of immunological response from the host [15]. It should also be noted that there are several clinical and epidemiological forms of ACL. The introduction or re-introduction of different strains could lead to new host-parasite interactions and unpredictable therapeutic management.

Bartonellosis is a highly fatal bacterial disease, thus far confined to Peru, and transmitted by *Lutzomyia* spp. The disease causes haemolytic anaemia and can evolve to organ and systems failure in the first phase, the second phase is distinguished through the presence of chronic blood-filled nodules, often referred to as "Peruvian Warts". Bartonellosis is considered a possible emerging disease in the Southwest Amazon [16, 17]. An increase of Bartonellosis cases in Madre de Dios is a possibility through internal migration from endemic regions [18].

The geographic distribution of disease transmission may increase or decrease when vectors move into new areas with changed climatic conditions, or changing conditions that decrease vector reproduction and survival. Even small extensions of the range of a vector can result in the exposure of new populations [19]. To know which sandfly vectors and protozoa pathogens are involved in the disease cycle is fundamental for the success of the control measures. The finding of naturally infected sandflies gives us information about the risk to become infected in a given geographic area and at a given time.

Species of the sub-genus *Leishmania* (*Viannia*) (Kinetoplastida: Trypanosomatidae) are the causative parasitic agents of ACL in the Peruvian and Brazilian tropical areas of the Eastern side of the Andean Cordillera. The transmission of *Leishmania* parasites occurs through the bites of infected sandflies of the Genus *Lutzomyia* (Diptera: Psychodidae: Phlebotominae). The phlebotomine sandflies become infected after a blood meal on infected wild animals, and transmit the parasite to another animal or a human in the next blood meal. Humans become most often infected when they enter the forest after sunset.

The natural infection of sandflies with *Leishmania* has been traditionally determined by direct microscopic examination of Leishmania protozoans in dissected sandfly guts. Sometimes several thousands of sandflies were collected to be dissected. The parasites found were cultured in artificial mediums or inoculated in hamsters for isolation and subsequent identification with isoenzymes or monoclonal antibodies. The detection of *Leishmania* in sandflies more recently uses molecular methods such as the Polymerase Chain Reaction (PCR), which employs primers targeting the parasite nuclear or kinetoplast DNA [20, 21]. This approach has a higher sensitivity than conventional methods. Parasite species identification is performed by treating the PCR products of targeted genes with the appropriate restriction enzymes [22, 23].

This research sought to determine, through a mixed qualitative and quantitative method whether migrants are at higher risk for ACL than the non-migrating population. We also wanted to identify patterns of population movements between Brazil and Peru, and to document experiences migrants in relation to policies and health systems. The vectors for ACL transmission have been assessed both in Acre, Brazil and Madre de Dios, Peru.

2. Methodology

The research was conducted in two highly endemic States for ACL: Madre de Dios, Peru (population 130,876) [24] and Acre, Brazil (790,101 in 2014) [5]. We have used an interdisciplinary study design and mixed methods to address the objectives that cross social, biomedical, epidemiological, and ecology questions. Both quantitative and qualitative approaches enrich our knowledge.[25].

2.1 Qualitative Study

The qualitative study was designed to investigate links between ACL transmission and migration. We explored insights into drivers of migration, prevention behaviors and social and environmental risk as well as health seeking behaviors and barriers to healthcare access among Brazilian migrants in Madre de Dios, Peru and Peruvian migrants in Acre, Brazil. Data collection was conducted with purposive sampling among cross-border migrants with residency between 3 months and 5 years in April and June, 2013 among Brazilian migrants in Madre de Dios and among Peruvian migrants in April, July and September, 2013 in Acre, Brazil. Internal Peruvian migrants living in Madre de Dios were additionally interviewed in April and May 2014.

Key informant interviews with Ministries of Health and Departments of Immigration and Federal Police in Madre de Dios, Peru and Acre, Brazil identified areas with high population movement between the two countries. Additionally, we surveyed cities along the Transoceanic Highway in Acre and Madre de Dios for the presence of cross-border migrants. A semi-structured interview guide was used to conduct in-depth interviews among 25 Brazilian migrants in Madre de Dios, with 18 Peruvians living across the border in the Brazil border city Assis Brasil or Rio Branco, and 9 internal Peru migrants. Topics explored included past five year migration patterns and drivers of migration, border and documentation process and experience, access to health services, employment, knowledge of ACL and vector prevention activities, as well as regional policy surrounding migration and its application among cross-border migrants.

Interviews were recorded and transcribed in their original language and translated into English entered into Microsoft Word. Data was imported separately into MaxQDA software version 11 (VERBI Software Consult, Berlin, Germany) for analysis. Text segments were coded using thematic analysis to identify multiple domains for migration and relationships between migration and biosocial and environmental risk for ACL, risk mitigation, health access, and policy influencing migration. Vulnerability was understood as the relationship between the perception of risk situations and the subjective decision-making in of individual or collective hazards [26]

2.2 Policy review

The policy review focused on identifying literature and documentation related to migration in the study zone. Specifically the review explored policies that influence migration and access to health services. At the international level, documents were sought from the International Organization for Migration (IOM), the Organization of American States and *Mercosur*. The national level search was on national policies and scientific articles. Local level investigations included interviews with State authorities. An analysis matrix was constructed, encompassing access to health services, housing, employment and education [27, 28].

2.3 Epidemiological Study

The epidemiological study was designed to determine if migrants are at higher risk for ACL in an endemic area for the disease, in the Southwestern Amazon of Madre de Dios Peru. The study explored biosocial, behavioral, clinical factors that may influence disease risk, including migration. The study compared those positively diagnosed with ACL by microscopy conducted through the health authorities in Acre, Brazil and Madre de Dios, Peru, compared to an uninfected control population matched by age, sex and geographic location of diagnosis. Migration was defined as living in another municipality at least 3 months or longer within the past five years. Finally, qualitative and epidemiological interviews and migration data was mapped to explore population flow between endemic and non-endemic areas for ACL.

Data collection for the epidemiological study was conducted between September, 2013 and April, 2015 in Madre de Dios. Data collection was coordinated with the health authorities through the local health centers in 7 sites identified in the qualitative study. Study participants were recruited through public health centers located in each site, and interviewed by trained health professionals at the local health care centers. Diagnosis was done clinically ACL cases were confirmed by microscopy through the health authority in Madre de Dios. ACL controls were matched to ACL case based on age, sex and geographic location. Information on demographics, socioeconomic status, access to health services, prior infection with ACL, vector prevention behavior, migration, biosocial environment and employment were collected through structure questionnaires. Clinical data were also collected through patient medical records. Samples were further shipped to Lima for confirmation by PCR.

Biological samples were also taken from people in Assis Brasil, from 1st December 2014 to 31st January 2015, after their written informed consent. At the Assis Brasil's health centre, 13 patients with active ACL were recruited and filled in a same questionnaire. In addition, 13 controls were included (minimal odds ratio to detect migration as risk factor for ACL was 7.5 at a 95% confidence level - 80% power - with an expected exposure rate of 75% among cases and 20% among controls). For the "Freedom from Bartonellosis study in Assis Brasil, 310 intravenous blood samples were provided by individuals randomly chosen. Assuming a diagnostic sensitivity of 90% for the forthcoming PCR tests and a 1% estimated Bartonellosis prevalence, if all individuals test negative, the prevalence of Bartonellosis is accepted to be below 1%, at a 95% confidence level. Data and samples (confirmation and typing of *Leishmania* spp. by PCR) are currently being processed.

Data were entered using Microsoft Access 2013. Data analysis was conducted using STATA version 12 (Stata Corporation, College Station, USA). We conducted univariate analyses comparing cases and controls using Fisher's exact test and logistic regression for explanatory variables to investigate associations with ACL infection and socioeconomic status, clinical factors (exposure to others, prior infection), healthcare access and health seeking behavior, prevention behavior, environmental and household risk, past 5 year migration patterns, and occupation.

2.4 Phlebotomine sandfly collection in Peru and in Brazil

Phlebotomine sandflies were collected in the surroundings of Mazuko, Department Madre de Dios, geographically at S 13° 04.504´, W 70° 21.812´ and 300 meters of altitude above sea level. The area is dominated by secondary forest, and was located around 400 meters from houses and the transoceanic highway. Large areas of gold extraction from the river sand were close to this area, where many people from the Andean highlands, mainly of Cuzco and Puno, go to work for several months. These people often return to the highlands with an episode of ACL, evinced by the development of skin lesions. Sandflies were collected using 7 miniature CDC light traps and a Shannon trap with protected human attractant, as described by Perez et al. (1988) [29]. The traps operated from 18:00 to 24:00, from November 19 to 24, 2014. The collected sandflies were killed exposing them to the evaporation of Cutex® within a plastic bag for 30 minutes, and were then dried with silica gel. Once in Lima the specimens were preserved at -20°C.

Assis Brasil-Acre-Brazil and Iñapari-Madre de Dios-Peru, separated by the Acre River, share a unique epidemiological situation regarding the sandfly transmission of Neglected Tropical Diseases (NTDs). Twelve monthly entomological captures were performed, in accordance with the Brazilian Health Ministry's guidance for phlebotomine monitoring. Twelve CDC-like [30] HP light-traps [31] were placed at about 1.5 meters above ground, from sunset until the next morning, at three different locations (intradomicile, peridomicile and forest) of three different sites (urban, periurban and rural) in Assis Brasil and at one rural site in Iñapari, during five consecutive nights of the New Moon from April 2013 to March 2014. The peridomicile traps were located near chicken-houses, cannels and stables; forest traps were located at least 50 meters inwards from the forest edge. In the same occasions, GPS coordinates and microclimate information (wind speed; minimum, average and maximum air temperatures; humidity) were recorded at the peridomicile and forest traps.

2.5 Species identification of sandflies

The sandflies collected were first segregated from other arthropods and then separated by sex. The males captured by the eight intra- and peridomicile traps (6 in Assis Brasil and 2 in Iñapari) were identified according to the key developed by Galati (2003) [32]. They were used in this study because they were considered to be more anthropophilic. The male phlebotomines collected by the four forest traps (3 in Assis Brasil and 1 in Iñapari) are being kept apart to be used in a parallel study, which focus on climate related influences. All female phlebotomines captured are being kept for the on-going DNA extraction and PCR that will allow the detection of the *Leishmania* spp (see below) and the existence, or not, of *Bartonella bacilliformis*, in the study region.

2.6 DNA extraction and kDNA PCR on *Lutzomyia* pools for *Leishmania* detection

Sandfly pools (containing 10 sandflies each) were subjected to DNA extraction. After being centrifuged at 8000 g for 2 minutes, tissue was disaggregated with a pestle for microcentrifuge tubes. Disaggregated tissue was subjected to overnight lysis with Proteinase K and processed for DNA isolation using a column-based method (High Pure PCR template preparation kit, Roche®), according to the manufacturer instructions.

Leishmania DNA detection was performed using a PCR assay optimized at the Laboratory of Cellular and Molecular Biology of Trypanosomatids of the IMTAvH. This employs the primers[33] that amplify a 70 bp fragment of the kinetoplast DNA (kDNA) of species from the subgenus *Leishmania (Viannia)*. A second set of primers was included in the same reaction and served as internal control primers [34].The reaction was performed in a final volume of 25 μL. Amplification products were visualized in a 3% agarose gel electrophoresis, using as reference the molecular weight marker "Low Range" from Fermentas® (50ng/μL), followed by staining with ethidium bromide (1 mg/mL) and use of an image Analyzer with UV trans-illuminator from Bio-Rad®. Each PCR experiment included: a control of DNA from an uninfected (*Leishmania*-negative) *Lutzomyia*, a positive control of DNA from a *Leishmania* culture, and a negative PCR control.

2.7 Ethics Statement

This research was formally approved by the Ethikkommission beider Basel and the Comite Institutional de Etica (CIE) de Universidad Peruana Cayetano Heredia. In Brazil, clearance was obtained for the qualitative and quantitative works in Assis Brasil (AC) (CEPE-UNIFRAN 112/08), following authorisations of the local authorities. Informed written consent to participate in the survey was obtained from study participants, who were also provided with information on ACL in the language of the participant.

3. Results

3.1 Legislation, health systems and migration

We have selected 23 documents from the human rights, working, health and migration domains to illustrate the fragmentation of migrant social service policies (**Figure 1, Annex**). Countries ratify international standards and these should be reflected in the national legal systems. In our classification we considered international guidelines as determinants although, per se, they do not include obligations or sanctions in case of non-compliance with the agreed terms. Few documents consider the dimensions of access to health more broadly and access to basic social services such as education and health.

International health documents were ratified by Brazil, among them the Constitution of the World Health Organization; the Declaration of Alma-Ata; the Millennium Declaration; the WHA Resolution 61.17, which recommends member states to establish an agenda for the treatment of migrant health; and the Rio Declaration on Social Determinants of Health. Brazil ratified human rights documents at the global and inter-American levels committing to grant the people in its territory economic, social and cultural rights, including the right to health. Article 12 of the UN Committee on Economic, Social and Cultural Rights (CESCR), ratified by Brazil in 1992, states that "… the present Covenant recognizes the right of everyone to the enjoyment of the highest attainable standard of physical and mental health" [28]. In the inter-American context, the American Declaration of the Rights and Duties of Man (1948) and the Protocol of San Salvador (OAS, 1988) complement each other and determine access to health for its States Parties, but admit the differences in development of different countries and hence their commitment "to the maximum of available resources and taking into account their degree of development." [35]. The

latter collides somehow with the Declaration of Alma-Ata advocating Primary Health Care for all [36]. Finally, in the South American context, the "Residence Agreement for Nationals of the *Mercosur* States, Bolivia and Chile", to which Brazil joined in 2009 and Peru in 2011, ensures equal rights and civil liberties, social, cultural and economic national of the receiving country for migrants (Art. 9). Indirectly it favours access to health care with equal treatment for all and aims to make the process less bureaucratic. Nevertheless, it expressively does not speak about the access to health care by the migrants, which provides space for interpretation in regards to the rights of migrants and health care. In this respect, Branco and Torronteguy (2013) studied health care seeking of citizens living at a border. They argued that even foreigners residing in the border region have guaranteed access to the Brazilian public health system [37]. On the other hand, a study in the tri-national border region of Argentina, Brazil and Paraguay showed that, in practice, difficulties in implementing public policies has denied access to cross-border citizens [38].

The Brazilian Constitution of 1988 determines access to health services to anyone in its territory, regardless of registration status, and guarantees access to employment and primary and secondary education to migrants and children of migrants. The national Health Law of 1990 is in line with the Constitution, although it does not explicitly outline respect for ethnic and cultural issues of migrant people. In contrast, the Brazilian Foreigners' Statute of 1981 is a more restrictive policy for foreign migrants than the Federal Constitution [8]. However, since the *Mercosur* Agreement between Brazil and Peru in 2011, the Peruvians living in Brazil are governed by a more flexible agreement and can benefit from right of free and universal access to health services that are foreseen in the Constitution. The Unified Health System (SUS) in Brazil is universal and free and uses the Family Health Strategy as is set out in National Policy of Primary Care [39].

In Peru, the Constitution of 1993 determines access to work and education of migrants, and promotes access to health services. The Peruvian General Health Law determines access to health services in all dimensions studied, which are also favored by the Foreigners Act and the *Mercosur* Agreement. The National Health System in Peru is fragmented between four different ministries (Ministries of Health, Labor, Defense and Internal Affairs) and service providers. Public sector health services are provided on a sliding scale based on household income and offered free of charge through the Integral Health Insurance for the very poor. The proportions of Peruvians without any insurance decreased from 67.7% in 2000 to 58% in 2008. The Ministry of Labour runs the EsSalud, a health insurance with its own network and professionals self-financed through workers and their families. In 2008, 20% of the Peruvian population was covered by EsSalud insurance. Since services are for a fee, more cross-border migrants tend to use them. Still, in Madre de Dios there were people who were completely outside of any governmental health systems due to undocumented status. Without documents, migrants were not able to register for insurance scheme and are often too poor to pay for the government provided insurance.

In conclusion, the international regulations for international migrants were ratified by Brazil and Peru and are incorporated in the national health sector legislations both in Brazil and Peru, and by regional agreements and – in theory - allow migrants' access to health services and also to education, housing and employment. Nevertheless, a study in a border region between Argentina, Brazil and Paraguay showed that the implementation of public policies has blocked the access of cross-border migrants [38]. Thus, the interest and the ability to apply the law by local institutions

and migrant empowerment to demand their rights in the host country will determine the achievement of the right to health care access, particularly of Brazilian migrants in the region [40].

3.2 Internal and external migration in the border region

Through our qualitative research we identified patterns of cross-border population movement in Madre de Dios that drew primarily from the border adjacent Brazilian state of Acre, Brazil, and secondarily from other non-contiguous Brazilian states, and lastly from inside Peru and from other international locations. There were important internal population movement primarily between Peruvian departments of Ucayali and Loreto, followed by Cusco, Ayecucho, Arequipa, Lima and Junin. (**Map 1, Annex**), which was unexpected. In the Peruvian department of Madre de Dios, seven sites in areas of high ACL transmission and high population movement in the gold mining cities of Mazuko, Huepetuhe, and Laberinto, and in the brazil nut harvesting cities of Iberia and Planchon were selected, as well as the commercial center, Puerto Maldonado and the border town Inapari. Both mining and brazil nut harvesting areas bring in circular internal migrants from other areas of Peru, while the commercial center of Puerto Maldonado is a primary destination of cross-border migrants from Brazil.

Brazilian migrants in Peru concentrated in the urban Madre de Dios capital of Puerto Maldonado (**Map 2**). Family relationships were among primary drivers for cross-border migration due to the proximity of the border between the two countries. Brazilians were from bi-national parents, were married to Peruvians and/or had children born in Peru, or had family members residing in Madre de Dios.

Peruvian migrants interviewed in Brazil lived in Assis Brasil, a border town in Acre, or Rio Branco (**Map 3**), the capital of Acre state. Interviewees confirmed that the preferred destination among Peruvians in Brazil is the State of Acre. The road paving between the borders and beyond allow nowadays easier access to the capital and from there to other destinations of Brazil. Interviewed Peruvian migrants in Brazil were in working age (mean 34.4 years) and they have migrated in search of work or more generally for better living conditions. Family reunion was also cited among motives for migration.

During the qualitative study, in-depth interviews were conducted among 25 Brazilian cross-border migrants (**Map 2**) with ages ranging from 19 to 56 years, 17 females and 13 males, and 9 internal Peru migrants, ages 20 to 44, 5 males and 4 females. There were three confirmed ACL cases and two suspected cases among Peruvian migrants, and one confirmed and one suspected among Brazilian migrants. In the epidemiological study, preliminary results are shown for 64 ACL cases and 27 controls. There were 49 males (77%) and 15 females (23%) among the cases, and 15 males (56%) and 12 females (44%) among the randomly selected the controls. The mean incomes were 1184 PEN and 1184PEN (~377 USD) for cases and controls respectively, and without statistical difference.

3.3 Migration and American Cutaneous Leishmaniasis in Madre de Dios, Peru

Drivers of migration for Brazilians (**Map 2**) included employment in Madre de Dios, with respondents citing benefits from niche markets for Brazilian foods and products – both informal

street vendors selling Brazilian "churrasco" or barbeque, Brazilian beauticians, and informal investment for the sales of Brazilian soft drinks, beer and food products..

"Here the people don't know this type of BBQ that we do in Brazil and they like to eat different things" (Brazilian street vendor, male)

Origin unemployment was also an incentive for migration from the border areas of Assis Brasil. Many of these respondents maintained cross-border relationships and were sometimes recruited to work in hotels, restaurants and as household help through family relationships or other contacts. Brazilians also arrived to Madre de Dios for ministry work supported through evangelical ministries in Brazilian Churches.

"in Peru there 's a lot of witchcraft, sorcery, terrible things…and we came to help and show them Jesus Christ" (Brazilian missionary, female, 32)

Internal Peruvian migrants were largely attracted to employment in gold mining and Brazil nut or "*castana"* harvesting, a seasonal activity with circular migration between cities in the Andes and other Departments in Peru. Both Brazilian and Peruvians migrated in search of a better life for themselves and family.

"*We came here because of the children, right? For them to study here" (Brazilian who migrated from Japan with Peruvian wife, male, 35)*

"I was looking for a better life, better opportunities for my family" (Peruvian harvester, male, 45),

While internal Peruvian movement was largely for temporary settlement, Brazilians sought stable residency despite inaccessibility to visas available through the South American *Mercosur* accord, a multi-country agreement promoting free movement of goods and people. Typically, Brazilians were issued 'tourist' visas for 90 days upon entry and with 30-day renewals thereafter, requiring most to undertake a 6-hour round trip to the Brazil-Peru border from Puerto Maldonado.

"they always want to give me [only] 30 days…it's a headache and expensive going back and forth" (Brazilian, cleans Brazil nuts, married to a Peruvian, 39)

Investors were issued 2 year residency visas, however benefits were often not extended to family members. Missionaries were required to be from a church established for at least 7 years and with >10,000 members.

Barriers to the documentation process for Brazilian migrants was unfamiliarity with the *Mercosur* policy, Federal police in Peru who were neither familiar with *Mercosur* policy nor guided migrants on available options, and costly documentation. A significant issue for Brazilians in Madre de Dios in accessing documentation was the absence of Brazilian consular services. Details of the *Mercosur* accord will be discussed in the section on Policy and Migration.

"it costs about 600 soles and it's not only that…there's the travel too" (Brazilian married to Peruvian minister, female, 36)

3.3.1 Knowledge of American Cutaneous Leishmaniasis, risk and prevention in Madre de Dios

Knowledge of ACL was explored in detail in the qualitative study among Brazilian and Peruvian internal migrants. Half of respondents were able to identify ACL from a photo of a lesion,

primarily using the local terminology "ferida brava" (bad sore) by Brazilian migrants and "Uta" by Peruvian migrants. Other respondents knew of ACL, primarily by the local term, when prompted by the interviewer, and 12% had never seen nor heard of ACL. When correctly identified, respondents described symptoms, *"it begins and grows";* treatment, "*strong injections, he couldn't be in the sun and couldn't' work";* the ACL vector, *"Uta I know is a lesion from a mosquito that puts larvae";* and risk *"I watched a news report that said it's transmitted by dogs"*. However, while identifiable by a photo or when prompted, the majority of those interviewed were unaware of the risks for the disease.

Misunderstanding included incorrect examples of disease etiology, *"I got it from my sister in law"*, vector misidentification such as the triatomine or 'kissing' bug for Chagas Disease and botfly larvae,*"this is UTA…no it's not a mosquito, it's a little animal that's like a sponge that walks on wood"*; and geographic risk "*I didn't know that the disease was common here….I've never heard anyone talk about it…I thought it was more common in Brazil than here"*. Additionally, if not from an endemic area, migrants may not be aware of ACL until arrival in the migration destination or may not have any knowledge of the disease.

"Before that, I hadn't heard about it. Over there (Ucayalli – another department in the Peruvian Amazon), it's rare someone would have that, unlike here, it's very popular. Over there I would hear about Dengue". (Peruvian logger, male, 39)

The epidemiological study showed that neither ACL cases nor controls used repellents consistently, with only 8 cases (13%) and 4 controls (15.4%) using repellents and only one in each group using repellents "always". There was no significant difference in repellent use comparing cases and controls.

"We don't use repellent…it doesn't work, we put (detergent) powder o our clothes and we're fine…that's the repellent when we're in the field" (Peruvian Brazil Nut Harvester, female, 42)

Protective clothing was more often used by respondents working in mining and harvesting areas of Peru, although there was no significant difference between ACL cases and controls. Bednets were regularly used in Peru by both ACL cases (93.4%) and controls (81.5%), citing daily use during nighttime hours or when sleeping, although it appeared from observation in the camps that these nets were untreated with insecticides. Other prevention measures included using household insecticides and smoke from fires or cigarettes. There were no Brazilian cases in Peru during the study period, however during qualitative interviews, Brazilians did not use bednets frequently, and cited instead the use of household insecticides, fans, and cleaning to reduce environmental exposure to vectors. Many Brazilians, however, didn't use any protective measures.

The primary risks identified by the epidemiological study in Peru was migration in the past five years (OR: 2.7; 95% CI 0.8-9.1 p value=0.01) and sleeping outside (OR: 2.5; CI 95% 1.1-6.3; p-value 0.06). As mentioned in the previous section, internal migration is to forested areas for mining, brazil nut harvesting and logging that are highly endemic for ACL. As such, internal migrants in the qualitative study cited sleeping in encampments in Madre de Dios for the purpose of these occupational activities, or visiting a rural "chacra" or forested residence used for weekends and holidays. Temporal periods of heightened outdoor sleeping were January through May, with February, March and May times most frequently cited. These months are also the harvest season for Brazil nut harvesting when more ACL cases were engaged in nut collections and living in forest camps for multiple months.

3.3.2 Access and Use of Health Services and ACL

Brazilian cross-border migrants in the qualitative study sought health services in Madre de Dios for illnesses such as prenatal and delivery care, dengue, accidents, surgery, oral health and pediatric care. Overall, these migrants were content with service delivery, and language generally was not problematic during consults. As public health services are free of charge in Brazil, a primary complaint was cost of services in the public health system in Peru, which was more expensive for non-residents, the majority of migrants in our study.

"to do your record you pay 3 soles, for a consult you pay 8 soles, and for an IV you pay 8…you pay for everything" (Brazilian food stall operator, Female, 36,)

"put it this way, it's public, but you also have to pay for the service" (Works with father, male, 21)

Despite non-residency, healthcare access in the public system was available to cross-border migrants, however many Brazilians also sought private healthcare, primarily due to expedience of service delivery.

"there's always a long line so I paid a private physician, because otherwise I wouldn't have time" (Brazilian manicurist, female, 25)

Specialist services, such as urology and allergy, etc., were not generally available in Madre de Dios, with the closest specialty services available in Cuzco in the Andes mountains. For these services, and more complicated health problems, many cross-border migrants returned to Brazil for medical care.

"there was no option…because of the surgery, so he had to go to Sao Paulo" (missionary, female, 32)

Additionally, Brazilians returned to Acre, Brazil for general checkups and vaccines, as these were already recorded in the electronic medical records through the national Brazilian health plan.

"The vaccine card is there…all of it is there then I can do the health schedule for vaccines for all of them there" (Brazilian mother of 3, female, 39)

One barrier was discomfort with facility hygiene and perceptions from narratives told by other migrant experiences.

"it was cluttered, right? Trash, trash seen in the hallway …very dirty, like really dirty, you know?" (Married to Peruvian, male, 35)

In the epidemiological study, ACL cases had significantly more difficulties reaching health care services than those without the disease (OR: 4.6; 95%CI: 1-21.8; p-value=0.05). Reasons for difficulty in accessing healthcare services primarily were related to distance and cost of transport, with the many cases (43%) walking to the healthcare center on the day of the interview.

More than three-fourths (78%) of cases took a month or more after symptom onset to seek diagnosis, the mean time to diagnosis was 56 days. Reasons for delays in diagnosis were often related to unfamiliarity with ACL (17.9%), thinking it would heal (14.3%), distance to health services for diagnosis (10.7%) in addition to other reasons such as mistaking the disease for fungus, work, time and using alternative or antibiotic self-treatment. Seeking diagnosis in non-

endemic areas also delayed diagnosis, reflecting likely that Peruvian doctors are not trained or conscious or time-limited to ask patients about past travelling.

> *"In Lima, they didn't diagnose me with anything… it started to heal after 3 yrs. I had the wound 4 years ago, but last year it barely started to heal"(Brazil nut harvester, male, 21)*

Many (42%) cases with ACL self-treated prior to seeking diagnosis with both pharmaceuticals and alternative folk remedies. In the epidemiological study, antibiotics were used by 41% of ACL cases who treated themselves prior to diagnosis. In fact, pharmacies were often a first attempt at treatment.

> *"A [pharmacy] in Puerto Maldonado told him it was not Leishmaniasis. They sold him an ointment. This ointment only helped to close the wound superficially, but the inside of the wound was not healing" (Brazil nut harvester, female, 42)*

Herbal and medicinal plant, creams, tobacco and battery acid were additionally sought as remedies.

> "*No I didn't get treatment….I usually cure it with Cocona leaves, lime and salt. Boil everything, and then use that water to rinse the wound (logger/Brazil nut harvester, male)*

Some migrants recounted stories of purchasing ACL treatment (Pentevalent Antimonial) injections through the local 'botica' or pharmacy. ACL treatment in Peru is dispensed only through the public healthcare system, and offered free of charge, with referrals required from private physicians as ACL is a reportable disease under surveillance by the national Ministry of Health. However, Brazilian *Glucotime* is considered more efficacious for treatment than that offered through the Peruvian public health system.

> *"but here everyone talks about vaccines, and they know it by the Peruvian injection. The hospitals have the Peruvian injection and the pharmacies carry the Brazilian injections for 35 soles (12 USD)" (Brazil nut harvester/logger, male, 39)*

ACL caused loss of days of work for cases in our study (38.9%) from 1 day to three months. Approximately one-third (27.8%) lost at least two weeks of employment.

We investigated several biosocial factors, such as household and employment contact with others with ACL. While there were no significant differences between ACL cases and controls, cases tended to have had someone at home or work infected with ACL compared to controls. It is unlikely that household presence of ACL can be explained by poor housing, as there were no significant differences in housing materials or flooring between ACL cases and controls., Having a need to go to a health centre during the past year, but could not was a highly significant prophylactic for ACL (OR: 0.2 95% CI: 0.1-0.5; p value=0.001), which can be explained possibly because ACL cases that were interviewed sought services for their diagnosis. This finding should be interpreted with caution given that the respondents were from a hospital-based sample.

3.4 Peruvian Migrants in Acre, Brazil

Peruvian migrants in Brazil said that they have a life plan, but have recognized that Peru offers few opportunities for its realization. Brazil with its economic growth, the geographical proximity, cultural identification and ease of understanding the language [8, 41] and fewer

bureaucratic procedures due to the *Mercosur* agreement [42] is a better alternative to residence in Peru. Interviewees had a high education level (above 11 years of schooling) and some came for higher education studies, particularly at technology universities, sometimes with scholarships from Brazil or Acre State. Previous studies have observed that Peruvian migrants in the Brazil Amazon had higher education levels than Bolivians, Colombians and Paraguayans [43]. However, once arrived, they perceived that Brazil has a more formal and structured social and labour legal framework than Peru. The employment situation of Peruvian residents in Acre, particularly in Assis Brazil and Rio Branco, showed that Peruvians were over-proportionally active in street trade and vending, which is widely practiced in Peru [44] or as informal employees in shops or services. Several were disappointed because they could not realize their life goals and some struggled to survive. But generally and particularly students planned to stay in Brazil, either in Acre State as final destination or to move on to other States.

All Peruvian interviewees had legal residency status in the country due the migration agreements within *Mercosur*. However, they did not know or misunderstood their rights, often owing to language barriers and their overall vulnerable situation. Still, no interviewee reported any restrictions, language barriers or hostility for the use of health care services. The majority was pleased with the services, particularly also that treatments and medications were free of charge. When there were complaints, these were the same voiced by Brazilians such as delays in treatment, inappropriate care and insufficient clinical examinations. Return to their home country to seek medical care was reported by a minority, due either to a lack of confidence in the Brazilian health system or due to treatment delays in the Brazilian health system.

Interviewees originated from different parts of Peru but many from the Sierra and Coast areas (**Map 3**) with quite different climatic and epidemiological environments. Access to health information, however, proved to be very difficult – also because no policy or actions existed to provide appropriate information to migrants. The migrants were uncertain about general aspects of prevention, illness and treatment of diseases such as ACL and Bartonellosis.

In the case of ACL in Brazil, access to services, from diagnosis to treatment, is free and is decentralized at the municipal level. In Peru, the initial diagnosis is paid by the patient, but is free of charge if ACL is diagnosed. Access to treatment, however, can be restrictive since the drug management is decentralized only to the regional level and is available at the local level only upon request according to diagnosed cases. Brazil uses N-methylglucamine antimonate for treatment and Peru sodium stibogluconate. The latter was perceived by interviewees as less efficacious and the perceived efficacy of the drugs used has influenced the overall perception of the health system and its access. It was also observed that Peruvians who sought treatment for ACL often go to Assis Brasil, Brazil from Madre de Dios, Peru. The municipal government of Assis Brasil with a low degree of institutional empowerment follows the Acre state government's recommendations. But they have different understanding of free treatment for all and can deny treatment for ACL to Peruvians coming to Brazil for treatment.

3.5 Phlebotomine sandfly identification at the border region

Table 1 shows that among the 22,334 phlebotomines captured in Assis Brasil (Acre-Brazil), 806 were from the urban, peri-urban and rural intradomicile traps; 3,495 from the three urban, peri-urban and rural peridomicile traps; and remaining 18,033 from the forest traps. In Iñapari (Madre de Dios,Peru), from the 560 phlebotomines captured, 51 were from the intradomicile trap; 396

from the peridomicile trap; and 113 from the trap located inside the forest found at the only site (rural) available for captures in Iñapari.

The difference between the number of individuals collected at each side of the borders is due different numbers of traps (9 in Assis Brasil and 3 in Iñapari) and because the forest environment at the rural site in Iñapari was partly destroyed during a fire blaze that also decreased the numbers captured of the nearby intra- and peridomicile traps. Among the phlebotomines captured in Assis Brasil, 10,733 were males, whereof 1,864 (17%) were captured by the intra- and peridomiciliary traps. In Iñapari, 249 were males, whereof 205 (82%) were captured by the intra- and peridomiciliary traps (**Table 1**).

Table 1 Phlebotomines collected in three different sites at each of the sides of the Brazil-Peru borders in South-western Amazonia, from April 2013 to March 2014, by site, location, sex and numbers of phlebotomines captured.

Site	Intradomicile				Peridomicile				Forest			
Location	Assis Brasil		Iñapari		Assis Brasil		Iñapari		Assis Brasil		Iñapari	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Apr/13	23	45	0	3	225	216	50	39	332	347	0	0
May/13	32	59	0	2	96	128	23	21	336	426	3	14
June/13	23	59	1	3	157	137	32	29	172	205	2	8
July/13	29	52	0	4	66	108	12	5	368	451	8	4
Aug/13	39	69	0	6	73	95	6	5	2688	1706	2	3
Sep/13	34	37	0	1	198	198	2	8	1001	1050	3	8
Oct/13	25	63	2	1	139	208	11	19	926	1030	16	17
Nov 13	40	54	0	6	232	264	29	26	787	1121	8	7
Dec/13	11	32	6	9	116	157	18	22	961	1296	2	3
Jan 14	16	24	0	2	79	102	4	10	270	395	0	2
Feb/13	5	16	0	1	187	251	5	9	677	679	0	1
Mrz 14	2	17	1	3	17	46	3	8	351	458	0	2
Total	279	527	10	41	1585	1910	195	201	8869	9164	44	69

The 1,864 from Assis Brasil and 249 males collected from Iñapari belonged to 47 and 17 species, respectively (**Table 2**). Among the 47 phlebotomine species identified from Assis Brasil, three of them represented 70% of all collected individuals: *Trichophoromyia auraensis* (33.35%), *Pressatia calcarata* (25.05%) and *Nyssomyia antunesi* (11.31%), while in Iñapari only one among the 17 phlebotomine species identified represented 70% of the collected individuals: *Trichophoromyia auraensis* (73.01%). Only three of the 17 species found in Iñapari were not found in Assis Brasil, each of them with one specimen. From the 7 *Trichophoromyia* individuals from Assis Brasil and the 2 from Iñapari that could not be identified to the species level, we argue that 1 from Assis Brasil and 1 from Iñapari might represent a new species of *Trichophoromyia (sp. NEW)*.

Table 2 Phlebotomine species identified among the male individuals collected in Assis Brasil, Acre, Brazil, and Inãpari, Madre de Dios, Peru between April 2013 and March 2014.

Assis Brasil				Iñapari			
Genus (*)	Species (*)	Total	%	Genus (*)	Species (*)	Total	%
Brumptomyia	*avellari*	8	0.51	*Brumptomyia*	*Avellari*	4	2.45
Brumptomyia	*brumpti*	2	0.13				
Brumptomyia	*cunhai*	1	0.06	*Brumptomyia*	*Cunhai*	1	0.61
Brumptomyia	*pentacantha*	72	4.6	*Brumptomyia*	*Pentacantha*	1	0.61
Brumptomyia	*sp.*	2	0.13				
Evandromyia	*andersoni*	1	0.06				
Evandromyia	*sallesi*	4	0.26	*Evandromyia*	*Sallesi*	5	3.07
Evandromyia	*termitophila*	1	0.06				
Lutzomyia	*flabellata*	2	0.13				
Lutzomyia	*marinkellei*	1	0.06				
Lutzomyia	*sherlocki*	8	0.51	*Lutzomyia*	*sherlocki*	1	0.61
				Martinsmyia	*oliveirai*	1	0.61
Micropygomyia	*longipennis*	7	0.45	*Migonemyia*	*longipennis*	3	1.84
Micropygomyia	*sp*	1	0.06				
Migonemyia	*migonei*	1	0.06				
Nyssomyia	*anduzei*	2	0.13				
Nyssomyia	*antunesi*	177	11.31	*Nyssomyia*	*antunesi*	6	3.68
Nyssomyia	*shawi*	2	0.13				
Nyssomyia	*sp.*	4	0.26				
Nyssomyia	*whitmani*	142	9.07	*Nyssomyia*	*whitmani*	10	6.13
				Pintomyia	*evansi*	1	0.61
Pintomyia	*nevesi*	5	0.32				
Pintomyia	*pessoai*	1	0.06				
Pintomyia	*serrana*	9	0.58	*Pintomyia*	*serrana*	1	0.61
Pintomyia	*sp*	1	0.06				
Pressatia	*triacantha*	1	0.06				
Pressatia	*calcarata*	392	25.05	*Pressatia*	*calcarata*	1	0.61
Pressatia	*choti*	44	2.81				
Psathyromyia	*aragaoi*	53	3.39	*Psathyromyia*	*aragaoi*	5	3.07
Psathyromyia	*campbelli*	2	0.13				
Psathyromyia	*dasymera*	1	0.06				
Psathyromyia	*dendrophyla*	6	0.38				
Psathyromyia	*digitata*	4	0.26				

Psathyromyia	*lutziana*	7	0.45				
Psychodopygus	*amazonensis*	1	0.06				
Psychodopygus	*carrerai carrerai*	2	0.13				
Psychodopygus	*claustrei*	6	0.38				
Psychodopygus	*davisi*	41	2.62				
Psychodopygus	*geniculatus*	1	0.06				
Psychodopygus	*hirsutus hirsutus*	2	0.13				
Psychodopygus	*lainsoni*	1	0.06				
Sciopemyia	*sordelli*	1	0.06				
Trichophoromyia	*auraensis*	522	33.35	*Trichophoromyia*	*auraensis*	119	73.01
				Thichophoromyia	*napoensis*	1	0.61
Trichophoromyia	*octavioi*	5	0.32	*Trichophoromyia*	*octavioi*	1	0.61
Trichophoromyia	*reburra*	1	0.06				
Trichophoromyia	*sp.*	6	0.38	*Trichophoromyia*	*sp*	1	0.61
Trichophoromyia	*sp. NEW*	1	0.06	*Trichophoromyia*	*sp. NEW*	1	0.61
Trichophoromyia	*ubiquitalis*	10	0.64				

Reference: Galati EA. Classificacao de Phlebotominae. In: Rangel EF, Lainson R, editors. Flebotomineos do Brasil. Rio de Janeiro: Fiocruz; 2003. p. 23 - 52. [32]
(*) Those shaded correspond to species already accredited as vectors of American Cutaneous Leishmaniasis

3.6 Detection of *Leishmania* in *Lutzomyia* sandflies in Mazuko, Peru

A total of 1126 female *Lutzomyia* sandflies were collected in Mazuko, Department Madre de Dios, Peru. The most frequent species were identified and separated in 62 pools of 10 specimens of the same species and same collection; the pools were then processed for the detection of *Leishmania* DNA (**Table 3**).

Table 3. Number of *Lutzomyia* spp. specimens processed by PCR for the detection of *Leishmania* (*Viannia*) spp.

	Collecting Method				Total	
***Lutzomyia* spp.**	**SH**	**(+)**	**CDC**	**(+)**	**T**	**T(+)**
L. carrerai	10	(1)	40	(1)	50	(2)
L. davisi	20		130	(3)	150	(3)
L. llanosmartinsi	20		70		70	
L. yuilli	90	(1)	230	(4)	320	(5)
L. (*Trichophoromyia)*			10		10	
Total	140	(2, 1.4%)	480	(8) 1.66%	620	(10, 1.6%)

SH= Shannon traps; CDC= CDC light traps; T= Total, (+)= Positive pools for *Leishmania* (*Viannia*)

We assumed that a single infected sandfly was present in each of the *Leishmania* positive pools, The 10 positive pools thus represented 1.6% of the total processed sandflies (in the CDC light traps).

Three sandfly species were found to be infected with *Leishmania* (*Viannia*) spp, These were *Lutzomyia carrerai* (2), *L. davisi* (3), and *L. yuilli* (5). These were very highly anthropophilic and were reported infected with *Leishmania* in the past. *Lutzomyia carrerai* (0.16 %) was found infected with *Leishmania (Viannia) braziliensis* in Alto Beni, Bolivia [45, 46] and the area in Bolivia was very similar to our site. The other isolate is from La Convención in Peru. Many sandfly species are common for both places [47]. *Lutzomyia davisi* was previously found infected with *Leishmania (Viannia) braziliensis* in Rondonia, Brazil [48]. Finally, *Lutzomyia yuilli* was previously reported infected with *Leishmania* (*Viannia*) sp. in La Convencion (Cuzco, Peru), in Aguas Calientes (1.27 %) [47] and in Alto Ivochote (18.35 %) [49].

Leishmania (Viannia) braziliensis is the main species identified in patients with Cutaneous and Mucocutaneous Leishmaniasis from the area studied (data not shown).

4. Discussion

Neglected Tropical Diseases have long been associated with poverty in the global south, and ACL has been associated with poverty and poor environmental conditions [50]. In our study, while income levels were low, we found no significant difference between socioeconomic status comparing those infected and those not infected with ACL in Madre de Dios, nor was there any significant difference between migrants and residents. Other studies on migration and health have also found similar results where neglected disease is endemic and communities exhibit high levels of social homogeneity [51]. While poverty is an overarching theme in tropical disease

transmission, migrant health presents a complex scenario when various biosocial factors converge that collectively increase risk for disease.

Past 5-year migration into Madre de Dios was associated with ACL infection when comparing ACL cases with uninfected controls. In this region, more than one third of the population are recent migrants, mainly from areas in the Peruvian Andes and northern Peruvian Amazon [52]. In our study, migrants originated primarily from the state of Acre, Brazil among Brazilians, and internally from the Peruvian departments of Ucayali, Loreto, Cusco, Ayecucho, Arequipa, Lima and Junin (**Map 1**). While there is active transmission in the majority of these areas, only Acre, Brazil and Madre de Dios, Peru are hyper-endemic areas for ACL. Other research on vector-borne disease has also found an association with migration for HAT [53-55], schistosomiasis [56], Dengue [57], and ACL [58], and increased risk for lymphatic filariasis [59], schistosomiasis [60], and visceral leishmaniasis [61] among migrants even when they originate in an endemic area for disease.

Risk factors associated with vector-borne diseases (VBDs) surround increased exposure and settlement in areas that put migrants directly in contact with vectors and reservoirs for infection [54, 62]. While these are also factors that contribute to VBD infections in residents non-immunity to disease is considered an increased risk when migrating from a non-endemic to endemic area. Earlier research in Peru showed that immunological protection against ACL parasite infection requires a 6-year residence for protection [14].

Throughout the world, seasonal labor migration often supplements income for vulnerable populations, and in Madre de Dios, there is significant circular population movement between the Andes and Madre de Dios for brazil nut harvesting and gold mining in areas with the highest incidence of ACL in Madre de Dios. We found that sleeping outdoors was associated with ACL infection, although specific employment in Brazil nut harvesting and mining was not associated with infection. Brazil nut harvesters, or castañeiros, live in camps deep in the forested jungles of the Southwestern Amazon where they are in close contact with the *Lutzomyia* vectors that carry ACL. Artisanal gold miners are encamped in heavily deforested areas with open water pits and no sanitation. Outdoor exposure, such as this has also been associated with Visceral Leishmaniasis, also transmitted by Phlebotomine sandflies, among migrants [63]. As residents are more likely to live in closer proximity to their homes, they may have an increased opportunity for protection against invading vectors as well as a level of immunological protection. We did not find that cross-border migration was associated with ACL infection. As Brazilians were more likely to live and work in the urban area in Puerto Maldonado, protective factors such as adequate housing, and use of fans and household repellents may have provided protection against ACL infection, and most Brazilians originated from endemic areas in Brazil which may provide immunological protection against infection.

While more than half of respondents in our qualitative research were aware of ACL, there was nonetheless misinformation about protective measures and transmission risk. Repellents were not used consistently and thought to be ineffective against vectors. While bednets were regularly used in forested and deforested encampments, it was not clear whether these were treated with insecticides and were appropriate to reduce risk of transmitting lutzomyia vectors. Internal migrants were often not as familiar with ACL, as Brazilian migrants who primarily originated from endemic areas. For internal migrants originating from outside of Madre de Dios, information on ACL may have only been conveyed anecdotally given long distances to health

and urban centers where campaigns are centered in Madre de Dios. Unfamiliarity with ACL was also a reason cited for delay in seeking health services, and subsequent late diagnosis.

Difficulty in reaching health services in Madre de Dios delayed diagnosis and treatment for ACL, given the long distances between mining and harvesting camps to health centers. For ACL, delayed diagnosis and treatment increases the risk for secondary infection and disfiguration due to scarring of ACL lesions and nasal destruction. Given the self-treatment measures, with both home remedies and black market pharmaceuticals, taken by migrants in our research due to inaccessibility to health services while in Madre de Dios, it is likely that disease incidence is underreported, or otherwise reported in other departments in Peru when migrants return from forested areas to their homes outside of Madre de Dios. In fact, migration from endemic rural areas can impact incidence of disease in areas where there is little risk of transmission [64], but subsequently may be underreported in endemic areas. While migrant populations may be overlooked when they are outside of zones that are the focus of control campaigns [65], efforts to restructure health services to accommodate migrants can be beneficial in increasing healthcare access.

In Brazil, international migrants have guaranteed access to health care services. However, our fieldwork in Brazil showed that the actual access is proportional to the legal enforcement capacity of the local institutions as well as to the migrant's empowerment conditions to know and demand their rights in the destination country. There was a partial lack of knowledge among migrants in Brazil about the benefits and rights available to them after the accession of Peru to *Mercosur* Agreement. This fact compromises their ability to take strategic decision-making about their life project and renders Peruvian migrants in Acre less empowered than Brazilians living in the same area. Any or more information on their rights could empower them importantly – and this could be done at low budget such as distribution of information leaflets, for example distributed by taxi drivers bringing them to a first destination in the foreign county. Finally, they should define their own demands that then could, in return, be translated into adapted public health policies.

In conclusion, we find that poor health literacy, housing conditions and prevention behavior in camps in forested and deforested areas for mining and harvesting where migration is associated with ACL, has contributed to the high incidence of ACL in Madre de Dios, Peru. As ACL is naturally occurring in the environment, and has little effect on sylvatic reservoirs, implementing prevention education campaigns that focus on migrant health can assist in reducing ACL incidence. Preventive behavior change education for ACL can be combined with other campaigns, for example those against dengue and malaria, to increase ITN use and decrease other biosocial risks for VBDs such as standing water sources. Health literacy in the forms of simple descriptive posters posted in outlets where migrants frequent when outside of encampments, such as stores, bars and in communal taxi services can provide an opportunity to increase ACL prevention and transmission knowledge in this vulnerable population. Due to the difficulty in accessing isolated mining and harvesting areas, peer education models with brazil nut concession owners and leaders in the artisanal mining community could facilitate improved early diagnosis. Increasing health service hours of operation on weekends, would provide resources for ease of access for both ACL diagnosis and treatment.

For the whole of the Brazilian State of Acre, 65 species were known: 52 species were reported in 2008 [66] and 13 new registries were added in 2013 [67]. Therefore, the 50 species found in the area encompassing the municipalities of Assis Brasil-Acre-Brazil and Iñapari-Madre de Dios-

Peru – that is a much smaller geographical region than the State of Acre, demonstrates the high concentrated variability of the sandfly fauna in this border region Considering the vector density, we argue that mainly two *Nyssomyia* species - in Assis Brasil, *Nyssomyia antunesi* (11.3%) and *Nyssomyia whitmani* (9.1%), while in Iñapari, *Nyssomyia whitmani* (6.1%) and *Nyssomyia antunesi* (3.7%) - are responsible for the ACL hyper-endemicity at the South-western Amazonian Brazil-Peru borders.

On the Peruvian side, the finding of infected sandflies with *Leishmania* implies the presence of infected animal reservoirs in the area, with rodents and other small animals or domestic dogs potentially involved in the transmission cycle of *Leishmania*. [68] did not find infected rodents in Mazuko and other areas along the Transoceanic Highway; therefore, the sources of infection should be other animals. This awaits further studies.

Indeed, since this project was about a complex zone of movement of populations, vectors, new risk factors of ACL and other neglected zoonoses – the work on vectors responsible for transmission and giving better estimates on potential exposures not yet described – was highly complementary. It provided the essential biomedical basis to the epidemiological and social science studies. This work represents also a first-time-ever sandfly vectors' monitoring, according to the parameters established by the Brazilian Ministry of Health at both sides of the Assis Brasil-Iñapari hyper endemic international frontier. Also, it shows for the first time the proportion of *Leishmania* spp. infected vectors in the region with high movements of people also in very remote rainforest regions. Work is ongoing to screen the collected and characterised vectors for infection with *Leishmania* and *Bartonella*. The latter will provide more information regarding potential spread across political borders. There is actually no reason why this should not happen given the same ecological zone on both sides. We have approached such complex research questions with mixed methods within a One Health concept.

Since the late 1990s, One Health has become a unifying concept of concerned with human and animal health, wildlife conservation and environmental sustainability at the human-animal-ecosystem interface. One Health has become the lead concept in interdisciplinary and integrated health research, capacity building and translational consortia such as ours. Added value in terms of better health, financial savings and environmental services comes from closer cooperation of human and animal health disciplines together with other concerned disciplines such as anthropology, which could not be achieved by working alone. One Health should translate into policy and practice for the betterment of health of communities and the integrity of their environment [69].

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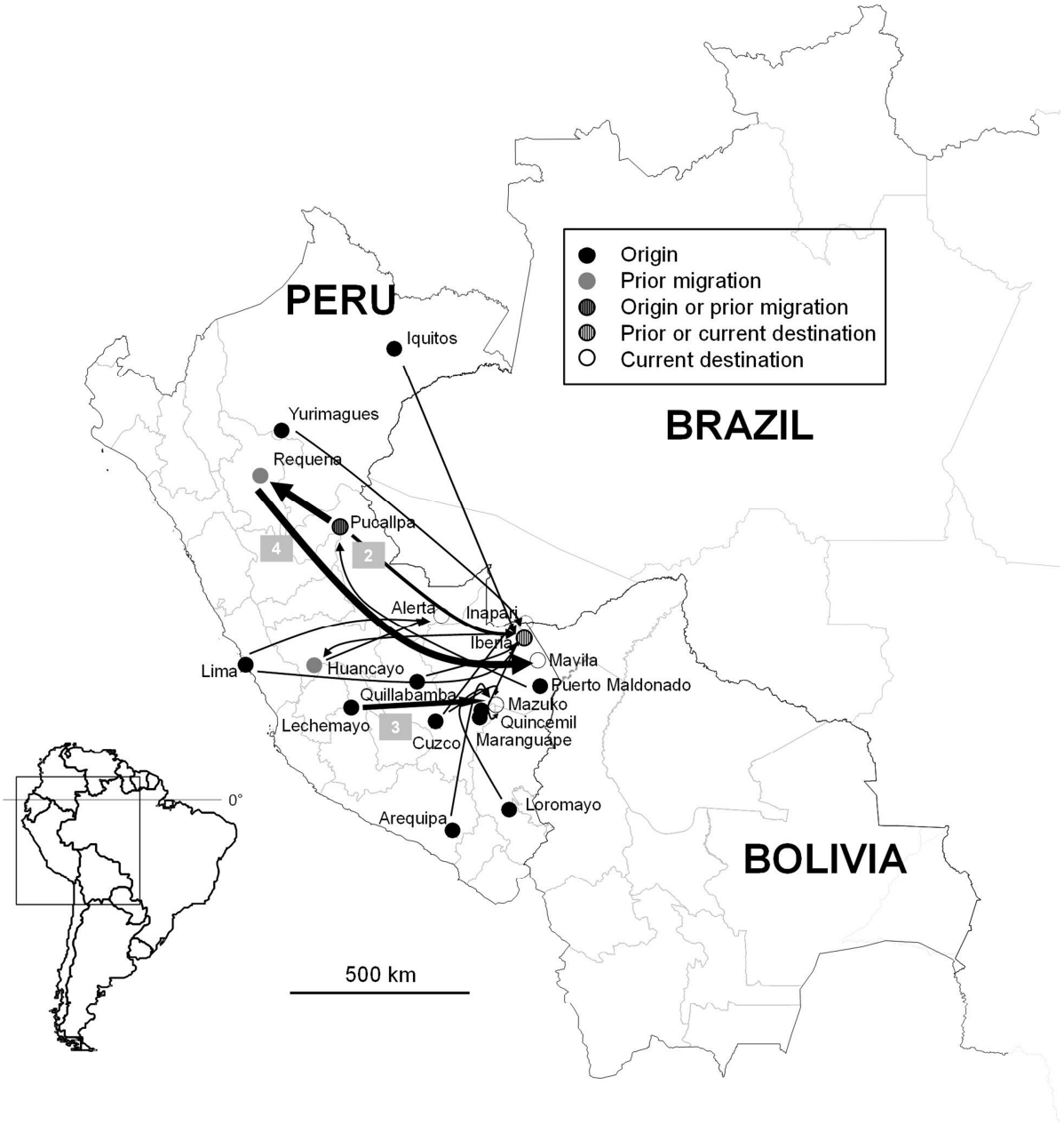
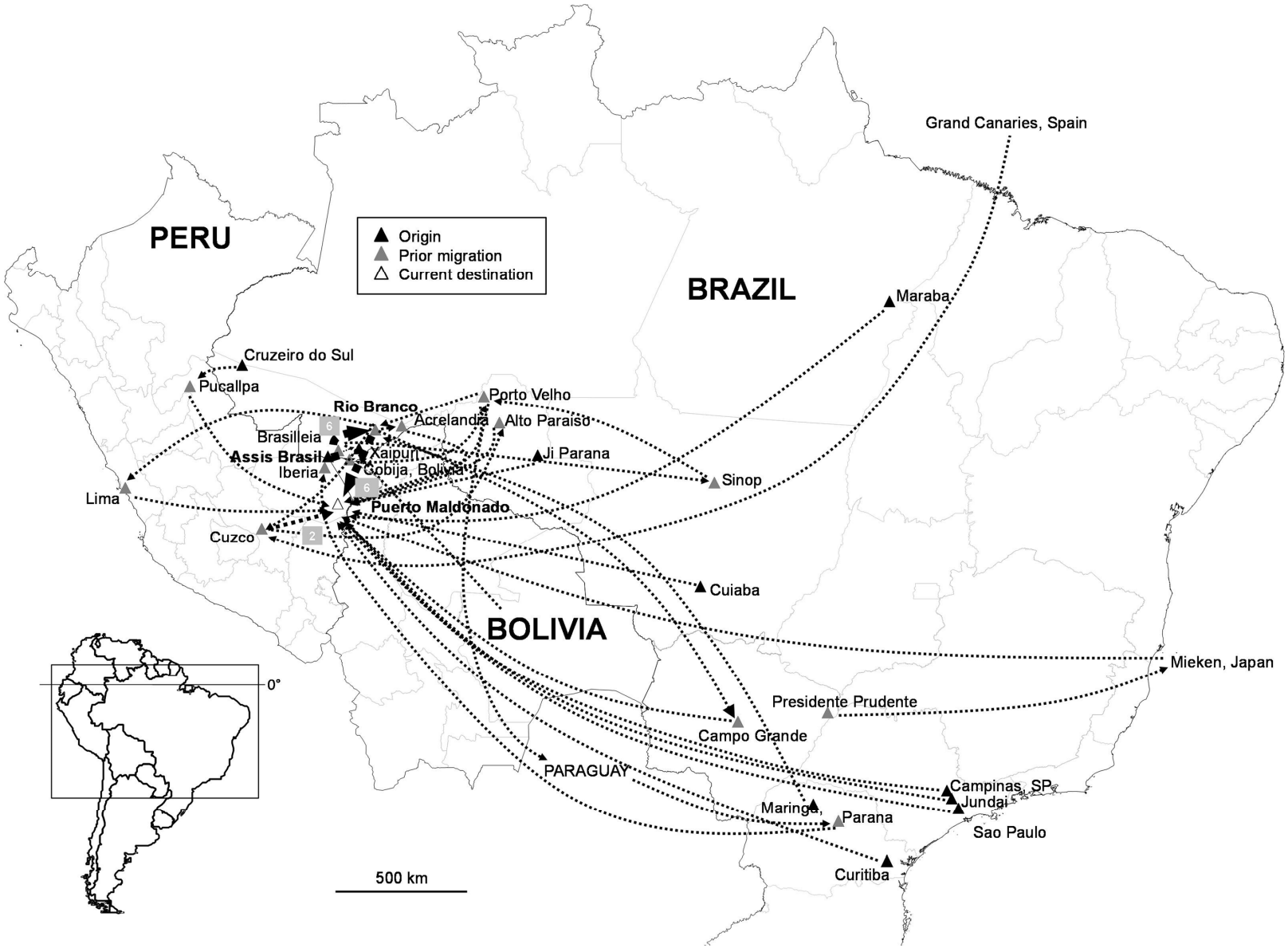
Annex

Map 1 Internal migration flows in Peru

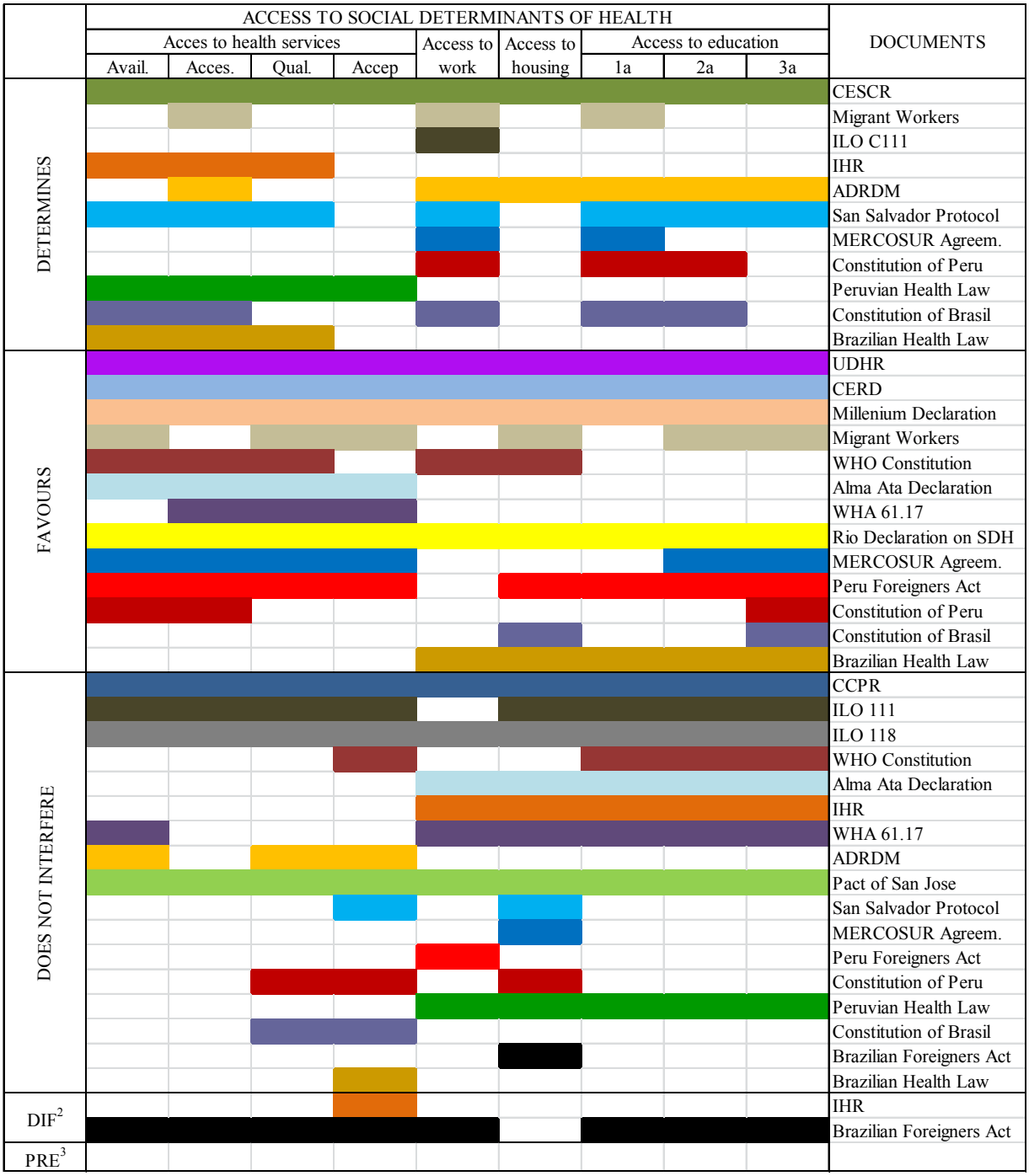


Map 2 Migration flows of Brazilians in Peru



Map 3 Migration flows of Peruvians in Brazil

Figure 1 Analysis matrix of relevant legislation documents



[1]Avaiability; Accessibility; Quality; Acceptability [2] Renders difficult [3] Prevents

Acronyms of Figure 1

CESCR	International Covenant on Economics, Social and Cultural Rights
Migrant workers	International Convention on the Protection of the Rights of All Migrant Workers and Members of their Families
ILO 111	International Labour Organization - Discrimination Convention
IHR	International Health Organization
ADRDM	American Declaration on Rights and Duties of Man
UDHR	Universal Declaration on Human Rights
CERD	International Convention on the Elimination of All Forms of Racial Discrimination
CCPR	International Covenant on Civil and Political Rights