

An analysis of reported cases of leishmaniasis in the southern Ecuadorian Amazon region, 1986–2012



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ABSTRACT

An analysis of reported cases of cutaneous leishmaniasis (CL) was performed using the data registered in the southern Ecuadorian Amazon region during 27 years from 1986 to 2012. The cases/subjects with both the suspected CL lesions and the amastigote-positive results were recruited for the analysis. The yearly occurrence of cases showed a markedly higher number during the six years, 1988 and 1993. After 1994 when the insecticide spraying campaign using helicopter in 1993–1994, the number dropped remarkably. Then, the yearly occurrence gradually fluctuated from 101 cases in 1996 to 11 in 2009, maintaining a low number of cases after the campaign. The monthly occurrence of cases showed a markedly high number during March and August, suggesting a correlation to the rainy season (months) in the areas. A statistical significance was found between the monthly average number of the CL case and the average precipitation ($p = 0.01474$). It was suggested that the time of transmission of CL would depend on the rainy seasons at each endemic area of Ecuador, which has a diverse climatic feature depending on the geographic regions. Such information at given leishmaniasis-endemic areas of Ecuador would be important for the future planning of the disease control. Molecular analysis and characterization of clinical samples revealed the presence of *Leishmania* (*Viannia*) *braziliensis*.

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1. Introduction

Leishmaniasis is caused by a protozoan parasite of the genus *Leishmania*, and transmitted by a sand fly of the genera *Phlebotomus* and *Lutzomyia* in the Old and New World, respectively. A total of about 20 species of *Leishmania* parasites in the world are responsible for a wide range of clinical manifestations. Cutaneous leishmaniasis (CL) ranges from simple types to those producing destructive mucocutaneous leishmaniasis (MCL) and non-healing diffuse cutaneous leishmaniasis (DCL). Visceral leishmaniasis (VL) is the severe

chronic infection, which is often fatal if left untreated. These different types of the disease are endemic in many tropical and subtropical regions in the world with two million annual incidence and 350 million people at risk (Desjeux, 2001; Alvar et al., 2012).

In the New World, leishmaniasis are endemic in Central and South American countries, including southern parts of the US (Grimaldi et al., 1989). Control of the disease in the New World is complicated by the variety of different *Leishmania* species and their diverse clinical forms, and also complicated by unique epidemiological patterns of each *Leishmania* species. Furthermore, in many regions of the New World, two or more *Leishmania* species are often sympatric. In Ecuador, seven species of the genus *Leishmania*, *Leishmania* (*Viannia*) *braziliensis*, *L. (V.) panamensis*, *L. (V.) guyanensis*, *L. (V.) naiffi*, *Leishmania* (*Leishmania*) *mexicana*, *L. (L.) amazonensis*, and *L. (L.) major*-like are reported (Mimori et al., 1989; Armijos et al., 1990; Hashiguchi and Gomez, 1991; Hashiguchi et al.,

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1991; Furuya et al., 1997; Calvopiña et al., 2004; Kato et al., 2008, 2013; Gomez et al., 2014a,b). The CL is the most frequent clinical feature of the disease in the country, but MCL cases are also seen (Hashiguchi and Gomez, 1991; Calvopiña et al., 2004). The first case of CL in Ecuador was reported by Valenzuela in 1920 in Esmeraldas province, close to the border of Colombia, and MCL case was first described in 1924 by Heinert (Rodriguez, 1974). Since then, many clinical cases of CL and MCL have been reported in the country. Most of the clinical cases of leishmaniasis in Ecuador were reported from the Pacific coastal region (Heinert, 1924; Leon, 1951; Carrera, 1953; Rodriguez and Aviles, 1953; Rodriguez, 1974; Calero and Coronel, 1981; Hashiguchi et al., 1984; Mimori et al., 1989; Armijos et al., 1990; Hashiguchi and Gomez, 1991; Hashiguchi et al., 1991; Furuya et al., 1997; Calvopiña et al., 2004), with very few numbers from the Amazonian regions (Amunarriz, 1982; Hashiguchi and Gomez, 1991; Calvopiña et al., 2004; Kato et al., 2008, 2013) where communications and the medical care system are generally inadequate. Cases of CL have also been reported from the Andean region (Hashiguchi et al., 1991; Gomez et al., 2014a,b). Still, the lack of a well structured information system has not allowed the elaboration of a good and representative epidemiological feature of the disease, requiring more information at given endemic areas of the country.

In this study, we performed an analysis of leishmaniasis cases reported in the southern Ecuadorian Amazon region during 27 years from 1986 to 2012. The yearly and monthly occurrence of the CL cases was analyzed, in relation to the climate changes especially precipitation, insecticide spraying campaign and other factors, such as human life styles, deforestation and migration. Furthermore, to obtain information on the causative *Leishmania* species circulating in the areas, molecular analysis and characterization of clinical samples were made.

2. Materials and methods

2.1. Study sites

Zamora-Chinchiipe province is composed of nine cantons/regions in the southeast of Ecuador, and Palanda and Chinchiipe are located in the southernmost of the province. The present study sites (Fig. 1), canton Chinchiipe (4°51'46" S latitude,

79°07'58" W longitude; 1113–1200 m a.s.l., 1207 km², av. temp. 21–22 °C) with 9119 inhabitants in 2010 and canton Palanda (4°38'59" S latitude, 79°07'56" W longitude; 1020–1735 m a.s.l., 1986 km², av. temp. 20–22 °C) with 8089 inhabitants in 2010, belong to Zamora-Chinchiipe province (815–2800 m a.s.l.; precipitation, 2000 mm/year; av. temp. 17–22 °C; relative humidity, >99%; humidity index, 92%; Plan de Desarrollo y Ordenamiento Territorial, Unidad de Gestion Territorial Zamora-Chinchiipe, <http://www.zamora-chinchiipe.gob.ec>) at the southern Ecuadorian Amazon region. Fig. 2 shows panoramic features of study sites, Palanda and Chinchiipe (Ishimanchi), located in forested and mountainous areas. Inhabitants of the areas work mostly in agriculture at the field surrounded by primary or secondary forests, except those engaging in commercial activities in the urban areas of parish Zumba and Palanda. Population migration from rural areas to urban areas was found from the past to date and the construction of new roads has been progressed in the regions (see Fig. 2).

2.2. Subjects

The subjects recorded had come from different endemic foci within canton Chinchiipe and Palanda, Zamora-Chinchiipe province. All the subjects received differential diagnosis of leishmaniasis at the laboratories of outpatient facilities of the Zumba hospital (Chinchiipe) and Palanda health center. One of the main activities of the laboratories of these medical/health institutions is to provide differential diagnosis of various parasitological, bacterial, viral and fungal infections in the areas. Thus, the subjects with suspected CL lesions came from almost all the endemic areas of the Canton Chinchiipe and Palanda. Questionnaires were prepared to record the residence and occupation of the patients, the evolution and history of the disease, and information on the treatment and others. More than 95% of the study participants lived on farms located at dense forested and mountainous areas. In this study, only the clinical cases with both the suspected CL lesions and the amastigote (smear) positive results were recruited for the analysis, but those without demonstrating the parasites in the smears were excluded unless otherwise specified. Thus, a total of parasitologically confirmed 2323 CL cases registered at the laboratories between 1986 and 2012 were analyzed. Apart from these retrospective studies,

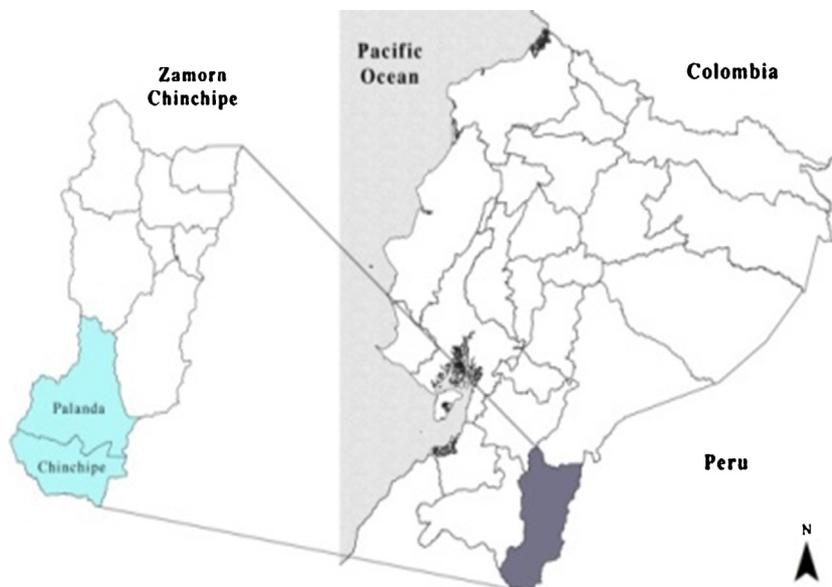


Fig. 1. Map of Ecuador, showing study sites, Canton Chinchiipe and Canton Palanda, in Zamora-Chinchiipe province (3°15'12" S latitude, 79°30'07" W longitude; <http://www.zamora-chinchiipe.gob.ec>).



Fig. 2. Showing panoramic features of the study sites, Canton Palanda (A) and Chinchipe (Ishimanchi) (B), Zamora-Chinchipe province, located in mountainous areas. Partial deforestations, cultivation-field spaces, road constructions, etc. are visible, leaving patched mountainous and/or forested areas still enough to maintain vector sand fly breeding sites.

clinical samples from CL patients were collected, and molecular characterization was performed for the identification of parasite species of the genus *Leishmania* circulating in the areas. Informed consent was obtained from the subjects that participated in the study.

2.3. Microscopic examinations

Materials were taken from the margin of ulcerous (Fig. 3) and/or nodular lesions using a surgical knife by experienced technicians, and then smeared onto a slide glass to make a thin film. After



Fig. 3. (A) A patient, 26-year-old male, with two cutaneous leishmaniasis ulcer lesions on his arm from Valle Hermoso (04° 60' 53" S latitude, and 79° 16' 47" W longitude; 1712 m a.s.l.), Palanda, Zamora-Chinchipe province, Ecuador. (B) Magnified view of the two ulcer lesions.

drying the materials at room temperature, they were stained with Giemsa and then examined for the amastigotes under a microscope at magnifications of 400× and/or 1000×.

2.4. Meteorological data

In the present study sites (Chinchipe and Palanda), no meteorological station was available. Therefore, the meteorological data employed here were derived from the most closely situated station to the study sites: M503 San Francisco San Roman – INAMHI, Zamora-Chinchipe (Instituto Nacional de Meteorología e Hidrología; <http://www.inamhi.gob.ec>) located at 03°57'50" S latitude, and 79°04'19" W longitude; 1620 m a.s.l.). Still, however, in this station only the data of precipitation were available and no other data such as maximum and minimum temperatures and humidity were recorded. Furthermore, the complete data of monthly precipitation were available after 1990 onwards.

2.5. Clinical samples from patients for PCR analysis

Clinical samples for PCR analysis were taken by scraping the margin of active lesions of the patients, spotted onto a FTA Classic Card (Whatman, Newton Center, MA) and stored at room temperature. Disks 2 mm in diameter were punched out from each filter paper and washed three times with FTA purification Reagent (Whatman) and once with Tris–EDTA buffer. The disks were air-dried and directly subjected to PCR amplification. Nested PCR was performed to amplify the *Leishmania* cytochrome b (cyt b) gene from a patient specimen collected on the FTA Cards. Primers used for nested PCR were L.cyt-AS (5'-GCGGAGAGRARGAAAAGGC-3') and L.cyt-AR (5'-CCACTCATAAATATACTATA-3') for the first PCR, and L.cyt-S and L.cyt-R for the second PCR (Kato et al., 2008, 2013). Materials from smear specimens on the slide glass were also processed to the PCR analysis.

2.6. Phylogenetic analysis

Cyt b gene sequences were aligned with CLUSTAL W software (Thompson et al., 1994) and examined using the program MEGA (Molecular Evolutionary Genetics Analysis) version 5.1 (Tamura et al., 2011). Phylogenetic trees were constructed by the neighbor-joining method with the distance algorithms available in the MEGA package. Bootstrap values were determined with 1000 replicates of the data sets. The database for phylogenetic analyses consisted of cyt b gene sequences from Gen Banks of 12 *Leishmania* species, viz., *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, *L. (V.) shawi*, *L. (V.) naiffi*, *L. (V.) lainsoni*, *L. (L.) mexicana*, *L. (L.) amazonensis*, *L. (L.) major*, *L. (L.) tropica*, *L. (L.) donovani*, and *L. (L.) infantum*.

2.7. Statistical analysis

For the statistical analysis, a linear regression analysis was employed using the package Statplus of Microsoft Excel 2011 and RStudio software for Macintosh 10 OSX operating system. Correlation between the variables, monthly reported CL cases and monthly precipitation (in mm), was determined by the correlation coefficient (*R*) and the coefficient of determination (*R*²) considering a 95% of confidence for the statistical significance. For the normality assumption of the variables, the Shapiro–Wilk test was used. The analysis was performed within four years between 1990 and 1993 for the following two reasons: no data were available at the meteorological station (M503-INAMHI) before 1990; and the dropping of CL cases after 1994 following the insecticide spraying campaign; the low numbers of cases were not sufficiently enough for considering a further analysis thereafter. The total data were analyzed

and also the variables by individual year and the average of both variables per month.

3. Results

3.1. Yearly occurrence of reported cases

Between 1986 and 2012 (27 years), a total of 2323 *Leishmania* positive subjects with CL lesions were recorded monthly and arranged by year (Table 1). The total number of reported CL cases per year ranged remarkably from 11 (2009) to 405 (1992). The majority of reported cases were found in the six years from 1988 to 1993, showing a rate of 57.9% (1324) of the total in the period. A markedly low number of cases per year were recorded after the year 1994 through 2012, except 107 in 1996, 94 in 2004 and 86 in 2011.

3.2. Monthly occurrence of reported cases

A marked monthly fluctuation of reported CL cases was recognized during the period from 1986 to 2012, as shown in Table 1. Total monthly numbers in the table had a tendency to show higher rates during March and August (231 in July – 317 in March), suggesting a higher intensity of transmission and/or infection in the period. In the present study, climatic data (only precipitation) were available after 1990 through 2011 at the meteorological station (M503-INAMHI). The data were collaborated to the fluctuation of reported CL cases. For a further precise comparison, the CL case numbers reported during 1990 and 1993 were detected, in which 45.1% (1047/2323) of the total were reported. Again, the majority of reported cases were found in the period from March to August, suggesting a correlation to the rainy season in the present study areas.

3.2.1. Analysis of relationship between reported cases and precipitation

When the CL case data (1990–1993) were collaborated to the monthly precipitation (in mm), a relationship was suggested. In comparison with the precipitation variable, the CL cases did not follow a normal distribution. For this reason, the rate was corrected applying a logarithm to all the elements of the variable. After the correction, scatterplot graphic with the linear regression line tendency was used to obtain the *R* and *R*² values for statistical correlation. The values showed a relationship between the variables, but not significant (*p*=0.11822). Both the variables in the yearly datasets achieved a normal distribution in all cases. No significant statistical relationship was found between the variables in the four independent associations. Monthly average number of the CL cases and monthly average precipitation were depicted in Fig. 4. The average numbers of both variables obtained in each month from the values of each year were analyzed. The precipitation did not follow a normal distribution, and it was therefore corrected applying a logarithm to all the elements of the variable. A statistical significance between the variables was found, after obtaining the tendency line, the *R* and *R*² (*p*=0.01474).

3.3. Clinical forms and localization of the lesions

To obtain information on the clinical forms and localization of the lesions in reported cases, parasitologically confirmed 54 subjects, 30 males, 21.8 (6–89) ages; 24 females, 26.5 (1–71) ages, reported in 2010 were analyzed. The test revealed 94.4% cutaneous; 3.7% cutaneous and mucous (mixed); and 1.9% mucous lesions. No other disease forms of leishmaniasis lesions, such as MCL and DCL were observed. Furthermore, during the study period (1986–2012), no these two forms were found in the areas (Communication: the

Table 1

Reported cases of leishmaniasis registered at Zumba hospital and Palanda health center, Zamora Chinchipe province, Ecuador, during 1986 and 2012.

Year	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
1986	0	6	11	10	7	12	9	12	4	3	2	5	81
1987	2	2	5	21	5	7	2	1	1	1	0	0	47
1988	2	0	43	8	30	11	17	12	6	4	3	0	136
1989	6	13	19	16	11	21	10	9	7	12	6	11	141
1990	8	16	19	22	7	12	15	22	10	6	8	10	155
1991	19	9	19	20	27	28	20	43	17	13	9	19	243
1992	12	19	29	85	45	38	68	51	8	21	18	12	406
1993	16	22	48	31	26	33	15	21	9	10	10	3	244
1994	9	6	10	17	7	5	4	1	9	4	1	4	77
1995	6	3	4	6	6	2	5	12	2	4	5	10	65
1996	28	2	19	12	8	14	9	4	2	2	4	3	107
1998	9	6	5	3	0	3	0	1	1	0	1	1	30
1999	1	3	4	4	4	4	1	3	3	0	0	2	29
2000	1	2	1	10	4	3	5	3	1	1	2	0	33
2001	1	3	10	4	3	0	5	3	0	1	0	0	30
2002	9	4	1	0	0	5	3	8	3	2	4	8	47
2003	24	8	5	6	6	2	0	0	0	0	0	0	51
2004	4	8	13	7	10	8	16	15	6	4	3	0	94
2005	2	3	1	2	5	7	3	8	2	1	2	9	45
2006	5	3	5	9	3	5	8	4	6	3	1	0	52
2007	0	2	5	4	1	2	1	0	2	1	2	0	20
2008	3	6	3	1	2	3	0	1	0	0	0	0	19
2009	1	0	1	2	1	0	3	1	0	0	1	1	11
2010	2	3	2	4	2	4	3	4	9	10	6	5	54
2011	6	5	9	6	12	12	8	6	11	1	5	5	86
2012	1	1	5	7	0	1	1	1	1	2	0	1	21
Total	177	155	296	317	232	242	231	246	120	106	93	109	2323

Note: No data are available in 1997, because of logistic, administrative and political reasons of the regional medical institutions in the areas.

first author H.R.O. epidemiologist in the area). The lesions recorded were localized on the face (17.9%), nose (5.4%), ear (1.7%), neck (3.6%), shoulder (1.7%), trunk (3.6%), buttock (3.6%), upper extremities (42.9%), and lower extremities (19.6%). The upper parts of the body surface, face, nose, ear, neck and upper extremities, revealed 71.5% of the total lesions, suggesting the preferred biting sites of sand fly species of the genus *Lutzomyia* spp., circulating in the areas.

3.4. Identification and characterization of *Leishmania* species

FTA card samples were obtained from five patients, three from Zumba and two from Palanda, showed positive PCR tests, and one

smear specimen (slide-glass) from Zumba revealed positive for the PCR test. All the parasites tested were identified as *L. (V.) braziliensis*, as shown in Fig. 5.

4. Discussion

Between 1986 and 2012, a total of 2323 subjects, who visited Zumba hospital and Palanda health center, revealed a positive diagnosis for *Leishmania* amastigotes in smear specimens. The total numbers shown here are considered to be rather lower than the actual rates of infection, since impression smears alone had been employed in this study as a diagnostic tool. The microscopic

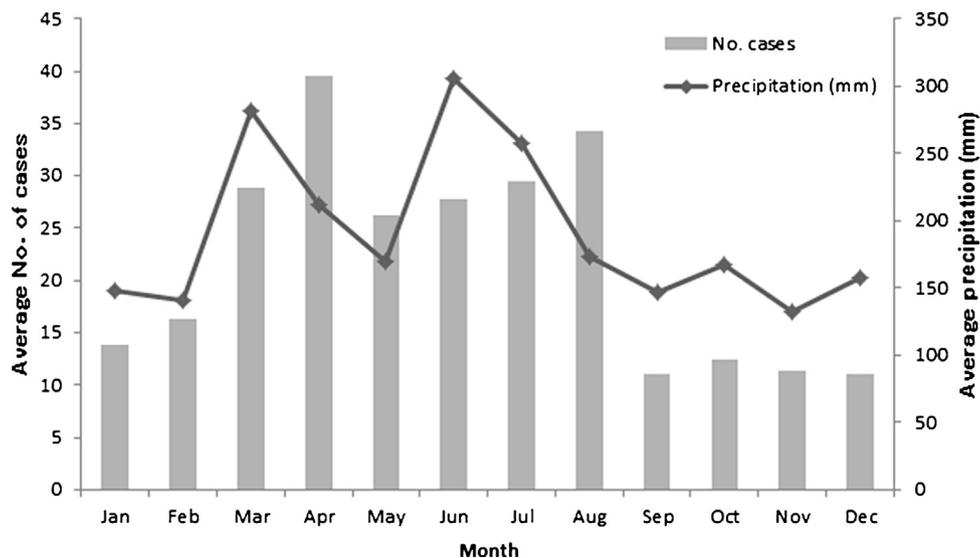


Fig. 4. Relationship between the monthly average number of CL cases and the monthly average precipitation (in mm) during the period of 4 years from 1990 to 1993.

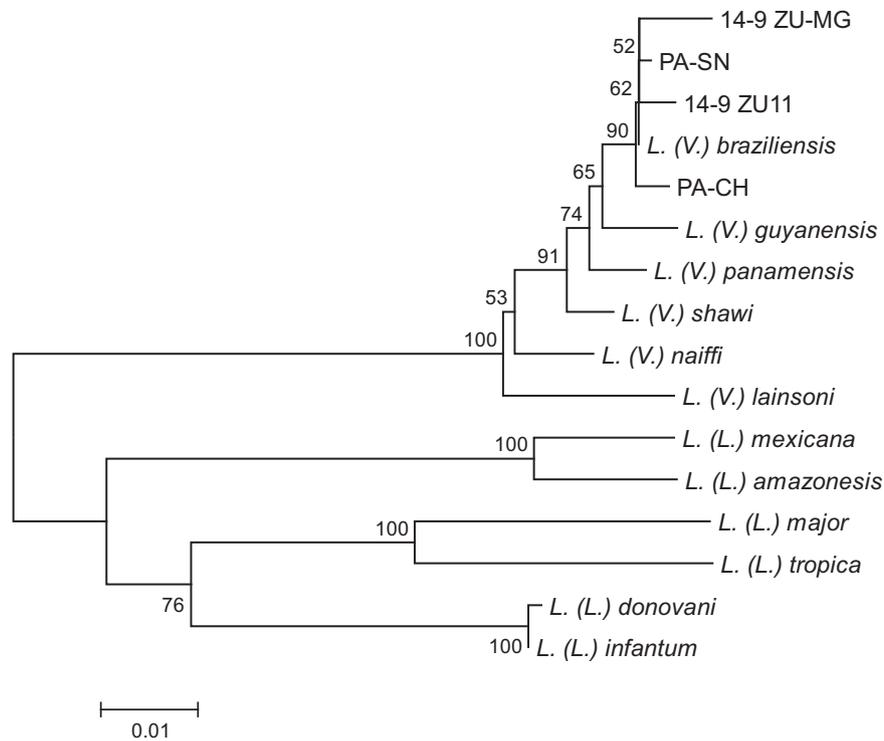


Fig. 5. Phylogenetic tree of *cyt b* gene sequences among species. *Cyt b* genes of the *Leishmania* parasites were amplified and sequenced from patients with cutaneous leishmaniasis (CL). A phylogenetic analysis of *cyt b* gene sequences was performed by the neighbor-joining method together with sequences from 12 *Leishmania* species. The scale bar represents 0.01% divergence. Bootstrap values are shown above or below branches. Here, two each of samples from Zumba (14-9 ZU-MG and 14-9 ZU11) and Palanda (PA-SN, and PA-CH: see Fig. 2) are depicted.

method is not so sensitive enough to detect all the positive leishmaniasis cases, showing a false negative result. The detection rate could therefore be enhanced if more sophisticated diagnostic tools, such as molecular and immunological techniques, are employed. However, no such a sophisticated diagnostic method is available in the present rural medical institutions. The aim/idea of the present study was to know the yearly and/or monthly fluctuation of registered smear-positive CL cases, in relation to the transmission-related factors, such as climatic, environmental and human behavioral changes in the study areas. In spite of such a long-term retrospective analysis, however, the numbers of reported cases are not enough for the precise analysis, especially because of a wide range (inter-country: Ecuador/Peru) of the insecticide spraying campaign using helicopter in the areas, which was conducted in 1993–1994 for the combat of dengue and leishmaniasis (Communication: epidemiologist H.R.O.). For this reason, only the data before the campaign was adopted for further detailed analysis.

In a revision of the registered CL cases in the two medical institutions, 57.9% of the total had occurred during six years from 1988 to 1993. The precise reason for such a high number of reported cases in the period is not clear. Therefore, suffice it to say that it was caused by some ecological, climatic and human behavioral factors in the endemic regions, favoring increases in vector sand fly and reservoir animal populations. Furthermore, a marked migration of inhabitants was found during these years (especially before and after 1990) from mountainsides to the suburban area of the study sites (Plan de Desarrollo y Ordenamiento Territorial, Unidad de Gestion Territorial Zamora-Chinchipe, <http://www.zamora-chinchipe.gob.ec>). Thereby the CL cases were notified more correctly at the present medical institutions. Thus, the change of life styles and activities of the inhabitants were discerned during the period. The high numbers

of CL cases in 1988–1993 were followed by a marked decrease after the insecticide spraying campaign in 1993–1994, with a relatively low and irregular number. The deforestation around the housing and work-field areas, as seen in Fig. 3, might be contributing to such a low number of CL cases from 1994 through 2012. Deforestation rate was 13.3% during 2000 and 2008 in the province (Unidad de Gestion Territorial Zamora-Chinchipe, <http://www.zamora-chinchipe.gob.ec>).

Monthly average numbers of reported CL cases in the study period from 1986 to 2012, revealed a higher occurrence of cases during March and August. In the present study, meteorological data (only precipitation) at the station M503-INAMHI are available from 1990 to 2011. Monthly numbers of reported cases (45.1%; 1047/2323 of the total) from 1990 to 1993 were detected and used for the analysis in relation to the precipitation. As shown in Fig. 4, it has a tendency to show a higher average number of the occurrence of CL cases during March and August, the rainy season in the areas. A statistical significance was found between the monthly average number of CL cases and the monthly precipitation ($p = 0.01474$). As with other vector-borne diseases such as malaria, vector sand fly density is correlated to seasonal patterns and also correlated with number of CL cases (Gomez and Hashiguchi, 1991; Feliciangeli and Rabinovich, 1998; Rabinovich and Feliciangeli, 2004; Chaves and Pascual, 2006).

Frank et al. (2002) studied the impact of the El Niño/Southern Oscillation on VL in Brazil, and pointed out that the rainy season is followed by an increase in vector density (around May there), which triggers a severe increase in infection rate in high-risk populations. During the present study period, “strong” El Niño occurred in 1987–1988 and 1997–1998, and “moderate” one, in 1986–1987, 1991–1992, 1994–1995, 2002–2003, and 2009–2010; a “strong” El Niño has not occurred since 1997–1998 to present (El Niño and La Niña Years and Intensities, Jan Null, CCM 07-Feb-2015). No

clear-cut relationship between the “strong” or “moderate” El Niño and the reported CL cases was recognized, though higher numbers (243–406; 38.4% of the total) of the cases were recorded in 1991–1993.

In the Ecuadorian CL endemic areas (Pedro Vicente Maldonado, Los Bancos and Puerto Quito, Pichincha province), the northwest Andean slope (cordillera), about 100–650 m a.s.l., a relatively high number of the clinical cases (2007–2011) was found in the period from October to January, the rainy season in the area, demonstrating 45.4% of the total cases (196/432); the lowest number in May, 2.8% of the total (Calvopiña et al., 2012). In the Andean CL endemic area, Paute, Azuay province, 2300–2500 m a.s.l., the sand fly population density and the natural infection with the *Leishmania* promastigote were examined throughout the year, and both the density and the infection revealed higher rates during the period from February to July, suggesting the transmission time of CL in the rainy season in the area (Gomez and Hashiguchi, 1991). In the current study, a higher number of monthly CL cases was recorded between March and August of almost all of the years during 1986–2012, the rainy seasons and the beginning of dry seasons in the present study areas. It is noticeable to mention that the time of transmission of leishmaniasis would depend on the rainy seasons at each geographic region of Ecuador, which has diverse climatic features at each geographic region. Such information would be important for future planning of control of the disease, leishmaniasis in the country. Naturally, other factors should be taken into consideration, especially on the different and diverse human activities such as environmental change/modification, urbanization, migration and settlement of non-immune populations, development of agro-industrial projects, road constructions, etc.

To know the circulating *Leishmania* species in the area, patient's lesion materials of FTA card and smears were analyzed by PCR, and identified as *L. (V.) braziliensis*. In this area, Le Pont et al. (1994) identified the parasites as intermediate form between *L. (V.) panamensis* and *L. (V.) braziliensis* by isoenzyme electrophoresis. We characterized the clinical isolates from El Chorro and La Chonta, Canton Chinchipe, Zamora-Chinchipe province, and identified *L. (V.) braziliensis*, by serodeme analysis using species-specific monoclonal antibodies (Furuya et al., 1997). This time, we also identified *L. (V.) braziliensis* by PCR analysis of materials from FTA card and smear specimens from Zumba-Chinchipe and Palanda, Zamora-Chinchipe. The species is most wide-spread and responsible for severe CL cases in the New World.

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Conflict of interest

The authors have no conflicts of interest to declare.

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