

Research



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Data scarcity and ecological complexity: the cutaneous leishmaniasis dynamics in Ecuador

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Cutaneous leishmaniasis (CL) is a neglected tropical disease transmitted by species of Phlebotominae sand flies. CL is responsible for more than 1000 reported cases per year in Ecuador. Vector collection studies in Ecuador suggest that there is a strong association between the ecological diversity of an ecosystem, the presence of potential alternative or reservoir hosts and the abundance of sand fly species. Data collected from a coastal community in Ecuador showed that *Leishmania* parasites may be circulating in diverse hosts, including mammalian and potentially avian species, and these hosts may serve as potential hosts for the parasite. There has been limited reporting of CL cases in Ecuador because the disease is non-fatal and its surveillance system is passive. Hence, the actual incidence of CL is unknown. In this study, an epidemic model was developed and analysed to understand the complexity of CL transmission dynamics with potential non-human hosts in the coastal ecosystem and to estimate critical epidemiological quantities for Ecuador. The model is fitted to the 2010 CL outbreak in the town of Valle Hermoso in the Santo Domingo de los Tsachilas province of Ecuador and parameters such as CL transmission rates in different types of hosts (primary and alternative), and levels of case reporting in the town are estimated. The results suggest that the current surveillance in this region fails to capture 38% (with 95% CI (29%, 47%)) of the actual number of cases under the assumption that alternative hosts are dead-end hosts and that the mean CL reproduction number in the town is 3.9. This means that on the average 3.9 new human CL cases were generated by a single infectious human in the town during the initial period of the 2010 outbreak. Moreover, major outbreaks of CL in Ecuador in coastal settings are unavoidable until reporting through the surveillance system is improved and alternative hosts are managed properly. The estimated infection transmission probabilities from alternative hosts to sand flies, and sand flies to alternative hosts are 27% and 32%, respectively. The analysis highlights that vector control and alternative host management are two effective programmes for Ecuador but need to be implemented concurrently to avoid future major outbreaks.

1. Introduction

1.1. Cutaneous leishmaniasis background

Leishmaniasis is a family of diseases caused by an intracellular protozoan parasite (genus *Leishmania*) transmitted by the bite of a female phlebotomine sand

fly [1]. Leishmaniasis may be primarily categorized based on three main types of clinical symptoms (cutaneous, mucocutaneous and visceral). The type of the disease is a result of which species of sand flies, species of parasite, and hosts are present in a region. Cutaneous leishmaniasis (CL), common in Latin American countries, is transmitted by sand flies of the subfamily Phlebotominae of the genus *Lutzomyia* [2]. The sand flies are infected with various species of *Leishmania* parasite (e.g. two dominant species in Ecuador are *L. (V.) guyanensis* and *L. (V.) braziliensis*), when they bite a natural reservoir including humans [1,3]. Existence of different hosts complicates the transmission cycles as it could change the efficiency of transmission by less competent hosts in the transmission cycle [4,5]. The transmission occurs in natural sylvatic, rural and peri-urban regions, seldom emerging in urban zones because of human movements [6].

There are many *Leishmania* reservoirs including wild vertebrates like mammals, marsupials and potentially birds [6,7], and domestic animals such as dogs [8,9]. The sand flies also feed on hens as chicken coops are often their shelters but these poultry are not considered as reservoirs of *Leishmania* [7,10,11]. However, the birds *Anser anser* and *Phasianus colchicus* have been mentioned as putative hosts of *L. infantum* [7]. In the neotropics, the *Leishmania* spp. sand fly vectors belong to genus *Lutzomyia* and in a natural state these sand flies inhabit humid forests with soils rich in decaying organic matter, tree holes, fallen logs and burrows of wild mammals [12]. The population density of *Lutzomyia* species increases in the rainy season. Its flight activity is usually performed at dawn and dusk, which corresponds to the feeding period; it can fly a few metres from the ground and up to 200 metres away [12]. In Ecuador, out of 81 phlebotomine species reported [13] 15 species have been reported as anthropophilic and considered to be potential vectors of human leishmaniasis [14]. According to Kato *et al.* [3] and Hashiguchi *et al.* [13], *Leishmania guyanensis*, *L. braziliensis*, *L. naiffi*, *L. lainsoni*, *L. panamensis*, *L. amazonensis*, *L. mexicana* and *Leishmania major*-like have been isolated from human samples from tropical and subtropical areas of Ecuador.

1.2. Epidemiology and ecology of cutaneous leishmaniasis in Ecuador

Ecuador is a country located in northwest of South America that is extremely ecologically diverse and leishmaniasis in it is characterized by both diversity and complexity. Ecuador's ecology ranges from dry forest in the coast, cloudy forest in the Andes to tropical rain forest in the Amazon lowlands [15], which are typical environments for abundance of different species of sand flies. In Ecuador, the first case of CL was reported in 1920 from Esmeraldas province close to the border of Colombia [13]. Since then the country has reported many outbreaks of CL including the recent one in the small rural town of Valle Hermoso of Santo Domingo de los Tsachilas province in 2010 (figure 1). During the last decade (2001–2011), more than 20 000 human CL cases were recorded through surveillance in Ecuador, ranging yearly from around 800–2000 [13]. There are 22 provinces out of 24 that report leishmaniasis cases in Ecuador. The highest percentage of cases were reported in Pichincha, Esmeraldas and Santo Domingo de los Tsachilas [16]. The most affected province in this period was Pichincha (20% of the total in the country), followed by Esmeraldas (16.4%) and Santo Domingo de los

Tsachilas (12%) [13]. In 2014, a total of 1183 cases were reported to Ecuadorian Ministry of Public Health (MSP in Spanish), with 262 cases (22.1%) from Pichincha province, 148 cases (12.5%) from Santo Domingo de los Tsachilas and 136 cases (11.5%) from Esmeraldas (Departamento de Epidemiología, MSP, 2014). Currently, clinical diagnosis of CL in Ecuador remains the only method to confirm cases and to get officially reported. Hence, these reported numbers are likely to underestimate the incidence because the disease is principally found in the populations living in the remote, rural and forested areas of Ecuador, where transportation and medical care systems are very poor, making it difficult for patients to access limited health clinics, especially in the Amazonian and western, northernmost provinces. In this study, one of the objectives is to estimate case underreporting levels of CL using the 2010 outbreak in the town of Valle Hermoso, which lies in the Santo Domingo de los Tsachilas province of Ecuador.

CL remains an important public health problem in Ecuador with cases getting reported regularly from 23 out of the 24 provinces of the country (except in the Galapagos Islands) [13] in spite of enhanced control programmes. Limited studies have been conducted to determine possible reservoir hosts of CL. Some studies from Latin America have identified three new mammalian species with the parasite including the sloth *Choloepus hoffmanni didactylus*, the squirrel *Sciurus granatensis*, and the kinkajou *Potos flavus* [17–22]. Serological studies in the Pacific and Andean region determined CL infection in dogs with the same human strain isolated in the respective region [15,23,24]. Studies have used novel molecular techniques which allow estimation of the biting rate of vectors on various types of hosts, thereby making it possible to determine the host preferences of vectors that influence transmission of the parasite [25]. In CL hyper-endemic area of the coastal region of Ecuador, like Valle Hermoso (Santo Domingo de los Tsachilas province), birds have been found as the main blood meal source of sand flies via molecular technique [26,27]. However, the presence of alternative hosts (potentially birds) has also been shown as a critical risk factor of the CL in the region [28]. Hence, it seems that the presence of avian hosts may be linked with outbreaks in Ecuador. *Lutzomyia longipalpis/L. mexicana* model has shown that chickens may be able to host *Lu. longipalpis* parasite population; however, there is no conclusive information as to whether chicken blood is likely to support the development of transmissible *Leishmania* infections in *Lu. longipalpis* [29].

1.3. Studies on modelling dynamics of leishmaniasis

Mathematical models have been used to study various aspects of transmission dynamics of leishmaniasis [30]. In particular, models can be used to design control strategies, via analysing a critical threshold quantity, model's basic reproduction number (R_0), interpreted as the average number of secondary cases of infection as a result of the introduction of a primary infection into a completely susceptible population [31–33]. For example, Gorahava *et al.* [34] developed an optimization model for anthroponotic visceral leishmaniasis control in India and used it to identify an optimal allocation strategy of choosing and distributing insecticide based on the number of human and cattle populations in each district of the affected region. Studies have also shown that the culling of seropositive dogs, the use of

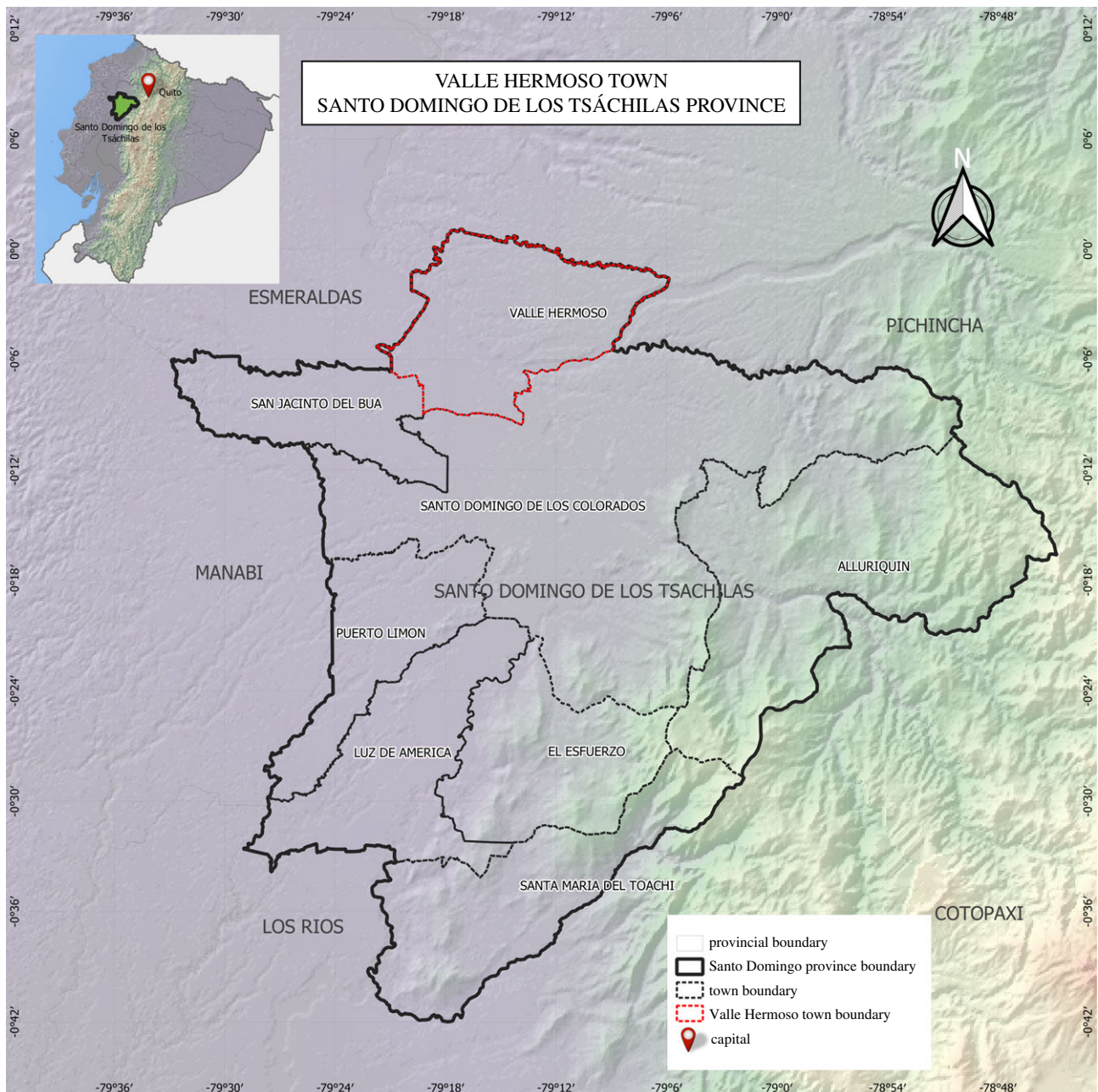


Figure 1. The study site, the town of Valle Hermoso, on the map of Santo Domingo de Los Tsachilas province of Ecuador. (Online version in colour.)

insecticide-impregnated dog collars, and the vaccination of dogs significantly contribute to reducing the prevalence of zoonotic visceral leishmaniasis infection in both canines and humans [35,36]. Besides, models have been used to quantify incidence underreporting levels and to study its impact on visceral leishmaniasis transmission dynamics [32]. This last study showed that reported data highly misinterpreted the true incidence levels in districts of Indian state of Bihar. Studies have linked population life cycle with CL transmission dynamics and have attempted to understand patterns of infection. They have used models incorporating vectors, humans, reservoirs, and/or environmental factors [31] to analyse the relationship between deforestation and movement of individuals [37], to illustrate understanding of the life cycle of the *Leishmania* parasite [38], to study the role of different types of hosts [39] and to estimate CL reproduction number (R_0) in ecologically different localities [40]. However, these studies have been either theoretical or have used data which highly underestimate true burden. This is

because of two reasons: (a) available data related to CL are limited and (b) reported data are underreported. In the present research, we link theoretically derived quantities with empirical information and quantify true incidence of CL in Ecuador.

1.4. Research focus of the study

The manual of procedures for disease control of the MSP-Ecuador recommends determining possible foci of the disease once a case of leishmaniasis has been reported from the region, spraying of dwellings of infected patients and identifying possible reservoirs and its management [41]. However, the basis of these controls need to be thoroughly and systematically analysed. On the other hand, MSP-Ecuador aims to understand the impact of ongoing interventions in the face of limited surveillance. The goal of this study is to assist and extend the ongoing government efforts in understanding complex cycles of leishmaniasis and estimating its

true burden. Specifically, *this study uses surveillance and entomological data together for the first time from Ecuador and (i) quantifies infection rates in potential alternative hosts, (ii) attempts to understand the impact of presence of alternative hosts and vector feeding preferences on the transmission dynamics of CL, (iii) estimates case-underreporting levels and (iv) suggests effective control policies for this resource-limited region.* The goal is achieved via the development and analysis of a mathematical model, which captures the transmission dynamics of CL infections in the presence of primary (humans) and alternative hosts and limited reporting of cases through surveillance. This study is expected to assist in providing specific actions on vector control and host management interventions as a response to control of CL in Ecuador.

2. Methods

2.1. Data sources

We conducted monitoring of sand flies in the town of Valle Hermoso which lies in the Santo Domingo de Los Tsachilas province of Ecuador. Valle Hermoso is a hyper-endemic area for leishmaniasis (figure 1). Phlebotomins were collected during the dry season, in July 2013 and during the rainy season, in March 2014. The samples were captured with the Centers for Disease Control and Prevention (CDC) miniature light traps (John W. Hock, Gainesville, FL). Four traps were set from 18.00 to 06.00 during two consecutive nights in the rainy and dry seasons. The nearest trap was placed 150 m from inhabited houses (peridomiliary area) and outward into the forest with a distance of 150 m between them, and the last light trap was placed 600 m from inhabited houses. Specimens collected were killed and stored at -20°C and transported to the laboratory. Specimens were identified, counted and classified into three groups: blood-fed, unfed and gravid females. Sand fly species taxa were morphologically identified using keys from Galati [42] and Young & Duncan [2].

Females with blood meals were easily recognized by the presence of engorged abdomens. Female abdomens were dissected for DNA analyses. DNA was extracted, amplified and sequenced to identify the potential food source and identify parasitic infection in each sand fly. PCR amplifications were carried out first for vertebrate-specific primers (cytochrome B), and then positive samples were amplified for vertebrate prepronociceptin gene (PNOC) and avian DNA (cytochrome B). Positive samples for mammalian DNA were subjected to a primer specific multiplex PCR to identify cytochrome B DNA from humans, dogs, cows, and pigs. Positive samples for avian DNA were tested with PCR specific amplification for domestic chicken cytochrome B. Blood from chickens, humans, dogs, cows, and pigs was used as positive controls [26]. The data were collected from different chicken farms in the rural regions of our study site.

Epidemiological case surveillance data were also obtained from Valle Hermoso, a rural region of the province of Santo Domingo de los Tsachilas located in the northwest of Ecuador, which has approximately 10 000 inhabitants. The town is located at an altitude of 307 m.a.s.l. and the average temperature is 25°C . The data consist of incidence from 2009 to 2011 passively collected and registered at the MSP (shown in figure 2) and were used to estimate the model parameters. There were 318 total number of cases reported in these 3 years from Valle Hermoso. Since CL is non-fatal disease, the population from rural and distant areas never comes for treatment and hence CL in this region is highly under-diagnosed. In general, CL cases were reported by the government local healthcare unit. The epidemiological surveillance of CL and data collection were carried out by the Surveillance, Epidemiology Unit of the Ministry of Public

Health and the reports are made accessible through the technological data platform, Sistema Integrado de Vigilancia Epidemiologica (SIVE in Spanish) [43]. The detailed historical data were difficult to gather because of many reasons including the lack of notification of resources (both personnel and equipment), the tendency to report only the most serious cases and lack of information from private health centres among others [44]. In Panama, significant underreporting of CL was estimated and it was believed to be attributed to the lack of diagnostic methods and low levels of access to healthcare services [45]. In Ecuador, underreporting is considered between 2.8- and 4.6-fold, based on comparative data with Argentina [46].

2.2. Model description

A compartmental type epidemiological framework was proposed to model anthroponozoonotic parasite transmission in a community of two hosts (birds as potential host and humans as an alternative host) and a vector species (similar models are developed and analysed in [30,34,47,48]). The model includes an additional compartment that corresponds to the unreported cases, considering that in rural areas there is no access to conventional treatment, and lacks traditional and ancestral knowledge of the disease [15,32,49]. Such models are not only good to capture dynamics of vector-borne diseases (VBDs) but also have a capability of tracking missing information from empirical studies such as underreported cases. The human population was divided into susceptible (S_{hi}), infected unreported (I_{hi}), infected reported (P_{hi}) and recovered (R_{hi}) individuals, where total human population was $N_{hi} = S_{hi} + I_{hi} + P_{hi} + R_{hi}$. The vector and alternative host (potentially bird) populations were both divided into susceptible (S) and infected (I) subcategories with corresponding total population as $N = S + I$. The variables related to vector populations were indicated with subscript v and alternative hosts (birds) with subscript h_2 .

2.2.1. The modelling framework was constructed under the following assumptions

Human population: The sand flies bite humans at a constant rate b (defined as average number of mosquito bites received by a host in a unit time, which is assumed constant over seasons). Infected individuals can recover from CL and become susceptible at the *per capita* rate δ . Not all the infected individuals are identified and reported to the surveillance system. Infected people do not die from CL. Population is assumed to remain constant over time. Reported infected individuals receive effective treatment and recover faster than unreported infected individuals.

Vector population: Sand flies bite humans and alternative hosts (birds) at different rates based on the preference for the two hosts. Biting of hosts by infected sand fly might result in successful transmission of the *Leishmania* parasite. The birth and death rates are assumed to be equal. Susceptible sand flies can also get infected from infectious humans and alternative hosts (birds). Sand flies once infected remain infected throughout their life and also do not die from the infection.

Alternative host (potentially bird) population: The preference of sand flies for alternative hosts (birds) relative to humans is defined by parameter α_v . Alternative hosts (birds) can get infected due to bites from infected sand flies and infected alternative hosts (birds) can transmit the infection to susceptible sand flies. Additionally, we assume that there is no disease-induced deaths in infected alternative hosts (birds). The population of alternative host (bird) remains constant over time.

2.2.2. Models from modelling framework

In order to systematically study each of the components of the modelling framework and develop robust model-based estimates

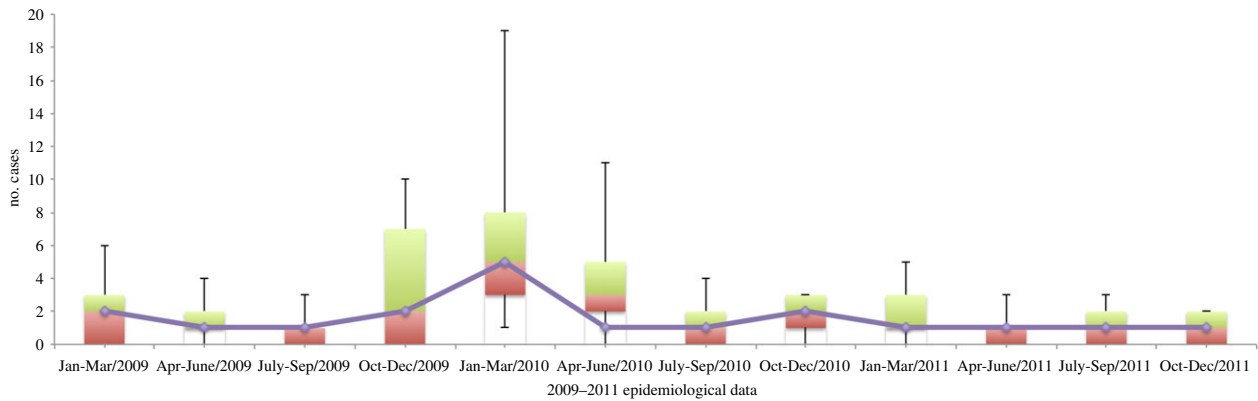


Figure 2. Cutaneous leishmaniasis incidence by epidemiological week in the town of Valle Hermoso of the Santo Domingo de Los Tsachilas province from 2009 to 2011. Data from Ecuador's Ministry of Public Health. (Online version in colour.)

and control implications, the framework was categorized into four different models (Models 1, 2, 3 and 4). Model 1 is described by the complete framework that included two hosts (alternative hosts and humans) and a vector species with underreporting explicitly incorporated in the model (figure 3). Underreporting was not included in Model 2 whereas population of alternative hosts (birds) was not considered in Model 3. Model 4 was the simplest model with neither alternative host (birds) population nor underreporting considered. All the four model systems are explicitly stated in the electronic supplementary material and the parameters are defined in table 1.

2.2.3. Interpreting interventions in the model

Control of CL depends currently on passive case detection and rapid treatment and in some locations vector management. The World Health Organization (WHO) [56] is now suggesting local public health departments to improve collective effectiveness of interventions while using existing resources for the reduction of morbidity. However, to improve existing control of disease, the evidence for the effectiveness of different prevention and intervention strategies is needed. The integrated ecosystem approach to human health is a more comprehensive and coherent approach to controlling CL. In this study, we use an integrated disease control model-based approach that includes improvement in social participation at different levels, vector and reservoir management programmes, and therapeutic interventions. The integrated strategy can allow us to understand the role of lack of knowledge of CL among communities which can be improved via educational programmes, increase participants' adherence to the intervention, and active participation and surveillance of local healthcare individuals. However, in order to have sustainable long-term interventions, systematic understanding of the prevention and therapeutic programmes is required. Here, we study the effectiveness of various interventions using a CL transmission dynamics mathematical model for the case of Ecuador. Educational programmes may be effective in controlling CL because such programmes can educate individuals when, how and where to seek medical assistance and consequently reduce further transmission of infection and even improve underreporting [57]. It is believed that underreporting for CL in Latin America may range from 2.8 to 4.6 fold of reported cases [46]; however, there is no systematic study from Ecuador that measures the level of underreporting in the region.

The impact of interventions are captured in our model via various model parameters. For example, a parameter γ_2 (per capita rate of case reporting) can be altered to study the role of surveillance efforts via educational programmes (or active reporting) on patterns of CL. The impact of insecticide spraying and larvae management programmes can be studied through our model via

changing estimates of the parameters μ_v (mortality of the vector population) and N_v (total vector population), respectively. The increases in insecticide spraying rate in a community can result in higher death rates of sand flies (μ_v) whereas larvae control programmes can limit the development rates of sand flies from larvae to adult stage and thus can reduce the total population size of adult vectors (N_v), which are only modelled in the equations. On the other hand, the use of insecticide-treated bed nets by individuals can reduce average transmission rate of a VBD. This is because insecticide kills the vectors and hence the number of vector-bites received by those individuals becomes zero. In our model, the use of impregnated bed nets by population is captured via transmission efficiency parameter β_1 . Host management programmes (such as culling and treatment of alternative hosts and reservoirs) can also be used for controlling CL and the role of such programmes is incorporated in the model via the parameter N_{h_2} (total alternative host (bird) population).

2.3. Computations using model quantities

The models are evaluated here via thorough mathematical analysis, estimations of model parameters using empirical data, and parameter sensitivity and uncertainty analysis. The details of analyses are given in electronic supplementary material.

2.3.1. Mathematical analysis

Model 1 equilibrium analysis resulted in computation of two equilibria (disease free and endemic equilibrium) and a threshold quantity, basic reproduction number, R_0 . The disease free state and the basic reproduction number of the model (system of equations (A.1) in electronic supplementary material) are given, respectively, as: $E_0 = (N_{h_1}, 0, 0, 0, N_v, 0, N_{h_2}, 0)$ and

$$R_0^2 = \underbrace{\left(\frac{b\beta_1}{\mu_v} \right) \left(\frac{N_{h_1}}{\alpha_v N_{h_2} + N_{h_1}} \right)}_{\text{sandfly-human interaction}} \underbrace{\left(\frac{b\beta_2}{\gamma_1 + \gamma_2 + \mu_{h_1}} \right) \left(\frac{N_v}{(\alpha_v N_{h_2} + N_{h_1})} \right)}_{\text{human-sandfly interaction}} + \underbrace{\left(\frac{b\tilde{\beta}_1 \alpha_v}{\mu_v} \right) \left(\frac{N_{h_2}}{\alpha_v N_{h_2} + N_{h_1}} \right)}_{\text{sandfly-alternative host interaction}} \underbrace{\left(\frac{b\beta_2 \alpha_v}{\mu_{h_2}} \right) \left(\frac{N_v}{(\alpha_v N_{h_2} + N_{h_1})} \right)}_{\text{alternative host-sandfly interaction}} \quad (2.1)$$

Remark 2.1. The expression of R_0 indicates that it depends on parameters related to human interventions (through γ_2 and β_1), alternative host and reservoir management (through N_{h_2}), and vector control (through N_v and μ_v). See previous section for more details on implementation of interventions in the model.

Remark 2.2. The analysis suggests that if $R_0 > 1$ the CL will become endemic whereas if $R_0 < 1$, CL can be controlled. The

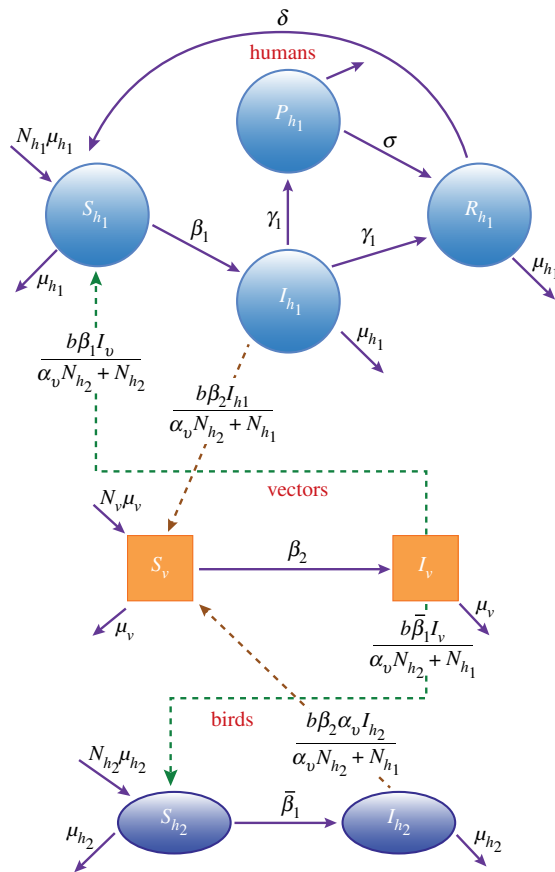


Figure 3. The flow chart of the modelling framework, that is, Model 1. (Online version in colour.)

electronic supplementary material provides some mathematical details related to the endemic states.

2.3.2. Model fitting and estimation of parameters

Some parameters of the model are taken directly from the literature. Estimates of the rest of the model parameters such as the probability of successful transmission of infection from vector to human host given a bite, β_1 , the probability of effective transmission from human host to a vector given a bite, β_2 , and the *per capita* reporting rate, γ_2 , were unknown for Ecuador and thus were estimated indirectly by fitting the four models separately (see table in figure 3) to the 2009–2011 CL cumulative incidence surveillance data (see data in figure 2) via the WLS procedure (WLS; see electronic supplementary material for details).

2.3.3. Sensitivity analysis on relevant quantities

Parameter sensitivity analysis (SA) is used to quantify the effects of variation in uncertain model input parameters on the model outputs [58,59]. SA allows for prioritization of the most influential parameters on the model output, to the least important parameters, and quantifies those intervention strategies that influence the system most. Here, we also quantify uncertainty generated in the output variables as a result of measurement errors in data via the parameter estimation procedure using observational data and the model.

3. Results

In order to analyse the behaviour of the model, we first estimated parameters and then used parameter estimates to simulate various scenarios of dynamics of CL infection.

compartment	definition
N_{h1}	total human population
S_{h1}	susceptible human population
I_{h1}	infectious human population
P_{h1}	number of reported cases
R_{h1}	recovered human population
N_v	density of sand flies
S_v	density of susceptible sand flies
I_v	density of infected sand flies
N_{h2}	total alternative host (bird) population
S_{h2}	susceptible alternative host (bird) population
I_{h2}	infectious alternative host (bird) population

model	explanation	fitted parameter
Model 1	model with both alternative hosts (birds) and reporting (figure 3)	β_1 , β_2 and γ_2 (but not $\tilde{\beta}_1$)
Model 2	with alternative hosts (birds) but no reporting ($\gamma_2 = 0$, $\sigma = 0$; figure b.1 in electronic supplementary material)	β_1 and β_2 (but not $\tilde{\beta}_1$)
Model 3	no alternative hosts (birds) but reporting included ($N_{h2} = 0$; figure C.1 in electronic supplementary material)	β_1 , β_2 and γ_2
Model 4	neither alternative hosts (birds) nor inclusion of reporting (figure D.1 in electronic supplementary material)	β_1 and β_2
Model 5	alternative hosts (birds) are dead-end host (figure E.1 in electronic supplementary material)	β_1 , β_2 and γ_2 (but not $\tilde{\beta}_1$)

3.1. Parameter estimation

The parameters are estimated using two different procedures: (1) finding estimates in the literature and fixing them to the obtained value for further model analysis and (2) parameters for which estimates cannot be found from the literature were estimated using WLS method and cumulative incidence data. The data used in the estimation include our collected entomological data and information gathered from similar studies from Ecuador and other leishmaniasis affected countries. Since most parameters were estimated using data from Ecuador, the model results were considered as a representative for the whole of Ecuador. The estimates of parameters are collected in table 1.

3.1.1. Initial parameter estimates from the literature

Epidemiological parameters related to human host: It is assumed that all the populations (human, vectors and alternative hosts) are constant and were estimated using the census data and surveys. According to the survey carried out by the National Institute of Statistics and Census, INEC (for its acronym in Spanish), the estimated *human population size* of the town of Valle Hermoso in 2010 was around 10 000 inhabitants. Hence, N_h was taken as 10 000.

Since the data corresponding to CL from Ecuador were limited, we used a prospective longitudinal survey of CL from Peru to estimate *per capita* rate of loss in immunity (δ), and it was found to be as 0.0033 per day [54].

The manifestations of CL include the presence of lesions, which may later ulcerate. Lesions may appear in humans about 7 days after receiving a bite from an infectious sand fly and since time to access treatment was unknown, using expert

Table 1. Parameter definitions and point estimates.

parameter	definition	estimate	units	source
μ_{h_1}	per capita human natural mortality rate	$\frac{1}{26973.5}$	d ⁻¹	[50]
b	biting rate of the sand flies	0.2856	d ⁻¹	[51]
β_1	probability of successful transmission of infection from a infected vector to a susceptible alternative host (bird) given a bite	0.31399	unitless	[26]
α_v	level of feeding preference of sand flies for alternative hosts (birds) in comparison to human hosts	3.8182	unitless	estimated
μ_v	per capita natural mortality rate of sand flies	$\frac{1}{14}$	d ⁻¹	[52]
μ_{h_2}	per capita natural mortality rate of alternative hosts (birds)	$\frac{1}{90}$	d ⁻¹	assumed
δ	per capita rate of losing immunity	0.0033	d ⁻¹	[53,54]
$\frac{1}{\gamma_1}$	mean infection period of undiagnosed and unreported individuals	45	days	[55]
σ	per capita recovery rate with treatment	$\frac{1}{45}$	d ⁻¹	[55]
<i>following parameters were less precise in the literature, hence were indirectly estimated using surveillance data</i>				
β_1	probability of successful transmission of infection from a infected vector to a susceptible human host given a bite	0.0822	unitless	[26]
β_2	probability of effective transmission from a infected human host to a susceptible vector given a bite	0.25	unitless	[53,54]
$\frac{1}{\gamma_2}$	average time to get diagnosed and reported	15	days	[55]

opinion from the region, we assumed that the average time to access treatment after clinical manifestation was around 8 days. Hence, we used an average *time to reporting* among individuals receiving treatment as around 15 days (i.e. $1/\gamma_2 = 15$ days).

Often patients do not approach public healthcare facilities and take much longer time to recover via *natural spontaneous resolution of infection*. It is found that such patients recover in an average of 15 months, hence, $1/\gamma_1 = 15 \times 30 = 450$ days [55].

Pentavalent antimony derivatives such as metglumine antimoniate and antimony sodium stibogluconate, recommended by WHO, are used for treatment of CL in Ecuador. Treatment may be repeated three times at intervals of 15 days [55]. The doses applied to children and adults may vary and are based on reference to an individual's body weight. Hence, we estimated *recovery rate of individuals reporting and receiving treatment* as $\sigma = 1/(15 \times 3) = 1/45$ per day.

The *biting rate of sand flies* is assumed to be equal to the number of sand fly bites received by an individual per day and is estimated as 0.2856 per day [51].

Parameters related to alternative hosts (birds): The alternative hosts in our model are assumed to be chickens. According to the poultry breeding manuals [60,61], the time that a hen remains in hatcheries is from 60 to 70 days; poultry that are raised in country houses can live for 90 days on average. Since we collected data from different chicken farms in the rural regions of our study site, we assumed that the *life span of a chicken* ($1/\mu_{h_2}$) is 90 days.

Parameters related to sand flies: A study was conducted in the Valle Hermoso town of the Santo Domingo de Los Tsachilas province of Ecuador to determine the sources of blood meal for phlebotomine sand flies (figure 4a,b). A total of 442 female sand flies were collected and classified as non-engorged and engorged. The 106 engorged females were identified morphologically, and selected for blood

meal identification by PCR technique. A total of 84 sand flies of these were positive for blood meals from birds, primarily chickens. Since humans and chickens were the most preferred hosts for sand fly species in our samples, we assumed that these sand flies prefer to bite only humans and birds (chickens). Hence, we estimated the *feeding preference for the bird hosts* is $\alpha_v \approx (84/22) = 3.8182$.

In our dataset, we found that out of the 106 samples of engorged females, 42 were positive for leishmaniasis and 22 were positive for blood meals from mammals. Since there were no time-dependent data available for sand fly feeding and preference behaviours, we took initial estimate of *probability of transmission of parasite from a vector to a human host* as $\beta_1 \approx (22/106) \times (42/106) = 0.08223$. However, the parameter was also formally but indirectly estimated via fitting model to the incidence data as explained in §3.1.2.

Using the sand fly data, out of the 106 engorged females, 84 were positive for blood meals from birds and 33 of these (who fed on birds) were also positive for leishmaniasis. Hence, in the absence of detailed data from the region, we took estimate of the *probability of transmission of parasite from a vector to a bird host* as $\tilde{\beta}_1 \approx (33/106) = 0.3139$. Since some estimates were less precise, we also carried out parameter uncertainty and SA on some model outputs.

The entomological laboratory observations were used to estimate the *daily mortality rate of an adult sand fly* and it was taken to be around $1/14$ per day [52].

3.1.2. Parameter estimation via fitting of model to the reported incidence

We used WLS procedure to estimate parameters of the four different models (see figure F.1 for Model 1, F.2 for Model 5 and table 2). The four models are:

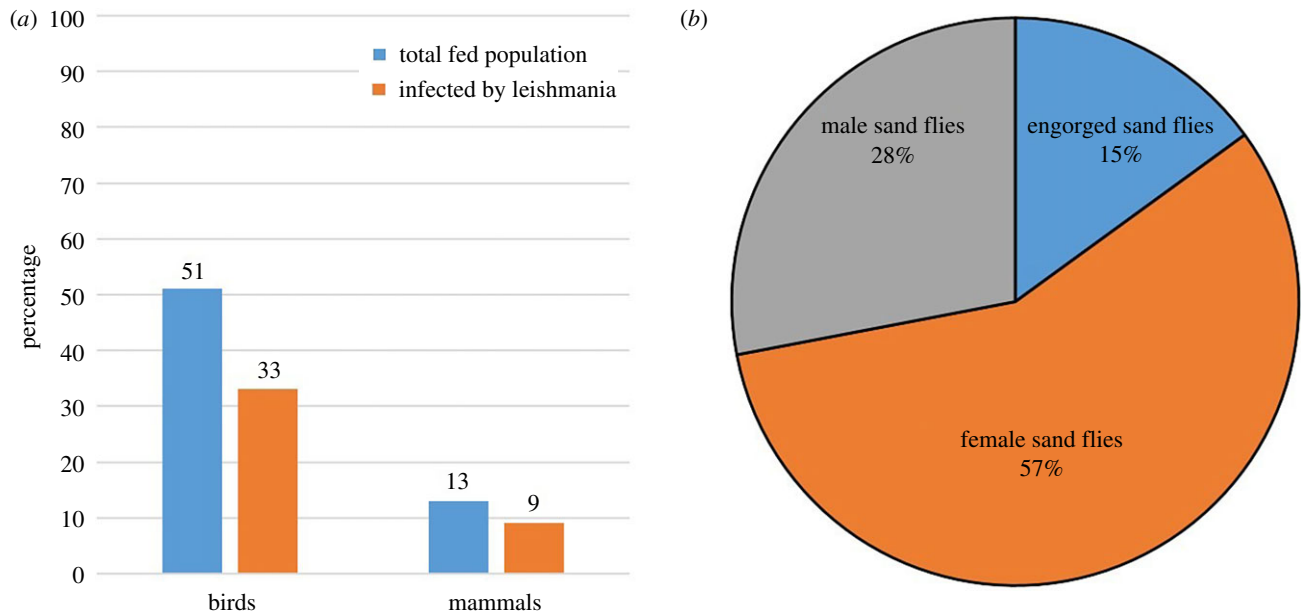


Figure 4. Percentage of fed sand fly by each host. (a) Sand flies collected in Valle Hermoso. (b) Type of sand fly blood meals. (Online version in colour.)

Table 2. Estimated parameters of models using WLS.

				$\alpha(\theta)$
parameter	estimates	s.e.	95% CI	selection score
Model 1				
β_1	0.0179	6.8483×10^{-5}	[0.0177–0.0180]	0.2479
β_2	0.5163	0.0347	[0.4468–0.5858]	
γ_2	0.0689	0.1645	[0.3601–1.0189]	
Model 2				
β_1	0.0291	0.0001	[0.0288–0.0293]	0.0204
β_2	0.0707	0.0014	[0.0679–0.0735]	
Model 3				
β_1	0.0118	5.4029×10^{-6}	[0.0117–0.0118]	0.1491
β_2	4.9958	0.4496	[4.0955–5.8962]	
γ_2	1.3296	0.1580	[1.0132–1.6461]	
Model 4				
β_1	0.0305	7.3020×10^{-5}	[0.0303–0.0306]	0.0128
β_2	0.0764	0.0009	[0.0744–0.0832]	
Model 5				
β_1	0.0254	3.3095×10^{-5}	[0.0253–0.0255]	0.1852
β_2	0.1500	0.0013	[0.1473–0.1527]	
γ_2	0.0371	0.0069	[0.0234–0.0508]	

- (i) Model 1: assuming vector preference for alternative host (bird) and human hosts and reporting of only few cases in humans.
- (ii) Model 2: assuming vector preference for alternative host (bird) and human hosts with 100% reporting of human cases.
- (iii) Model 3: assuming vector bites only human hosts but reporting occurs only for some human cases.
- (iv) Model 4: assuming vector bites only human hosts and reporting occurs only in few human cases.

- (v) Model 5: assuming alternative hosts (birds) are dead end hosts and reporting in human population occurs.

The model (out of these four models) that best fitted the surveillance incidence data was identified via a metric, *selection score*, defined as the Euclidean norm of a vector whose entries are the coefficient of variation of parameters that are estimated [62]. The selection scores for the models were computed and the best fit model was identified based on the lowest selection score value (see last column of table 2).

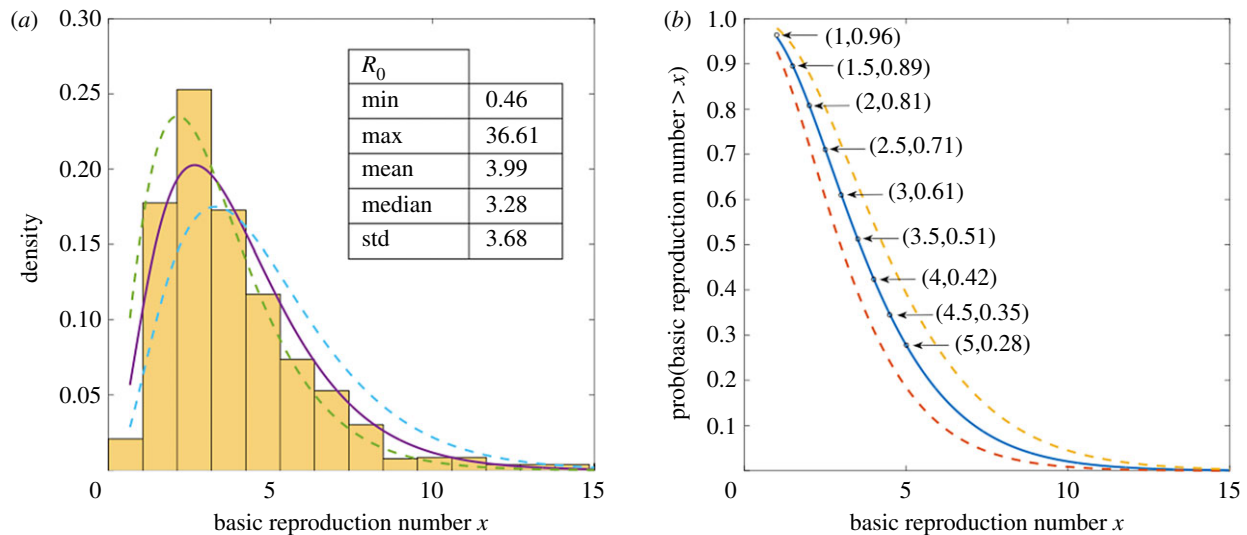


Figure 5. Distribution of R_0 from uncertainty analysis for Model 1. The estimated R_0 value is 3.9. (Online version in colour.)

Model 4 followed by Model 2 were found to be the best models using the reported incidence data from Valle Hermoso. In the models, we obtained point estimates, standard error, and confidence interval of γ_2 (only for Models 1–3), β_1 , and β_2 (table 2). The distribution of R_0 was also estimated using uncertainty quantification and fitting procedure (figure 5). Since Model 1 was the most comprehensive among the four models, it was used to report the mean estimates of R_0 for CL in Ecuador and we estimated mean (R_0) = 3.9. The uncertainty of R_0 was obtained via parameter uncertainty analysis. Each parameter in the analysis was sampled 10 000 times from its respective distribution and R_0 values were computed (figure 5a). This procedure was repeated for 1000 iterations to estimate robustness in the probability that R_0 is greater than certain value (figure 5b). We also found that it takes on average 14.5 days (i.e. $1/\gamma_2 = 1/0.0689$) for a symptomatic case to be reported in Ecuador presently and approximately 75% ($\approx \gamma_2/(\gamma_1 + \gamma_2) = 0.0689/(0.0689 + 0.0222)$) of the symptomatic cases are eventually reported. Using Model 4 (the best fit model), the transmission probabilities from alternative hosts (birds) to sand flies, humans to sand flies, sand flies to birds and sand flies to humans are estimated as $\beta_2\alpha_v = 0.07 \times 3.8 = 0.27$, $\beta_2 = 0.07$, $\tilde{\beta}_1 = 0.3$, and $\beta_1 = 0.03$, respectively (table 2). Reporting level using Model 5, i.e. under assumption that alternative hosts are dead end hosts, we estimated its value as approximately 62% ($\approx \gamma_2/(\gamma_1 + \gamma_2) = 0.0371/(0.0371 + 0.0222)$); hence, expected underreporting is around 38% with 95% CI=(29%, 47%); figure 6).

3.2. Impact of interventions on cutaneous leishmaniasis patterns and potential to control future outbreaks

In this section, we provide implications from model-based interventions (details are given in §2.2.3). Simulations are carried out to study the role of feeding preference (α_v) in patterns of CL in humans for low, medium and high regions of transmission (that is, for low, medium and high values of probability of transmission from a vector to a human host, β_1 , or to an alternative host (bird), $\tilde{\beta}_1$). Under certain conditions, when preference for alternative host (bird) relative to human host (α_v) is increased, the equilibrium prevalence of CL in humans decreases; however, the rate of decrease depends on the

level of endemicity of the region and transmission probability to host (that is, decrease is different for β_1 and $\tilde{\beta}_1$ with much larger variations for $\tilde{\beta}_1$; figures A.3(a) and A.3(b) in the electronic supplementary material). As expected, increasing the value of β_1 increases the number of human cases overall. However, increasing the transmission probability to alternative hosts (that is, increasing $\tilde{\beta}_1$) decreases the number of human cases overall because of dilution effect (electronic supplementary material, figure A.3(b)). Moreover, in low transmission areas, increases in sand fly preference for alternative hosts (birds) result in slower rate of decrease in human CL prevalence but faster rate of decrease for prevalence in alternative hosts (birds). As expected, prevalence in humans drastically decreases as reporting of cases improves (that is, $(\gamma_2/\gamma_2 + \gamma_1)$ increases); however, this decrease in prevalence could be significantly enhanced with the implementation of vector control programmes (through increases in μ_v) up to a critical value of increase in the reporting (electronic supplementary material, figure A.1).

Increases in reporting (that is, increases in γ_2 ; because of improvement in diagnosis, treatment, or surveillance) and decreases in alternative host density (N_{h_v} ; via host and reservoir management programmes) result in decreases in reproduction number, R_0 (figure 7). In other words, improved reporting can eliminate the disease locally (via R_0 reduction); however, the rate of decrease depends on alternative host (potentially bird) density in the region. Similar trends are observed when β_1 (probability of transmission from a sand fly to human) and N_v (density of vectors) are both varied to see their impact on R_0 . That is, control programmes that reduce effective contacts of sand flies with humans such as distribution of impregnated bed nets, can reduce R_0 significantly; however, rate of reduction depends on the density of the sand flies in the region (electronic supplementary material, figure A.2).

3.3. Parameter sensitivity analysis of R_0

A global SA was performed to identify the parameters with the greatest influence on the model output, R_0 . In this study, we use partial rank correlation coefficient (PRCC) as a standard measure of global sensitivity [63]. We first verified assumptions of PRCC SA including confirming that R_0 varies monotonically with respect to each of the model parameters. A PRCC was obtained for 13

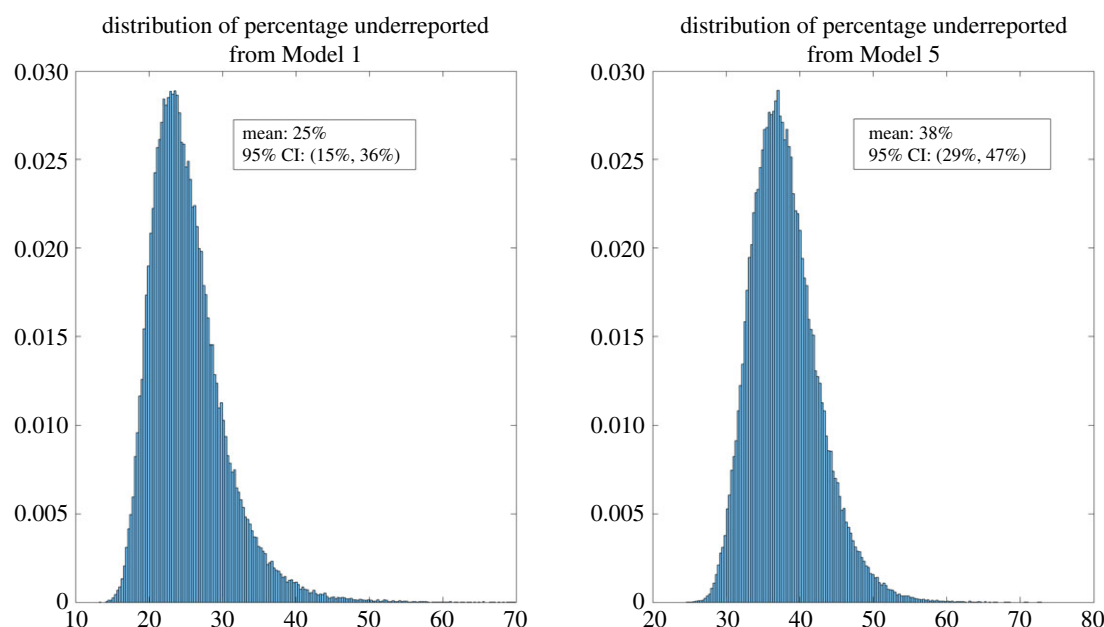


Figure 6. Distribution of percentage of underreporting of cases using Model 1 and Model 5. (Online version in colour.)

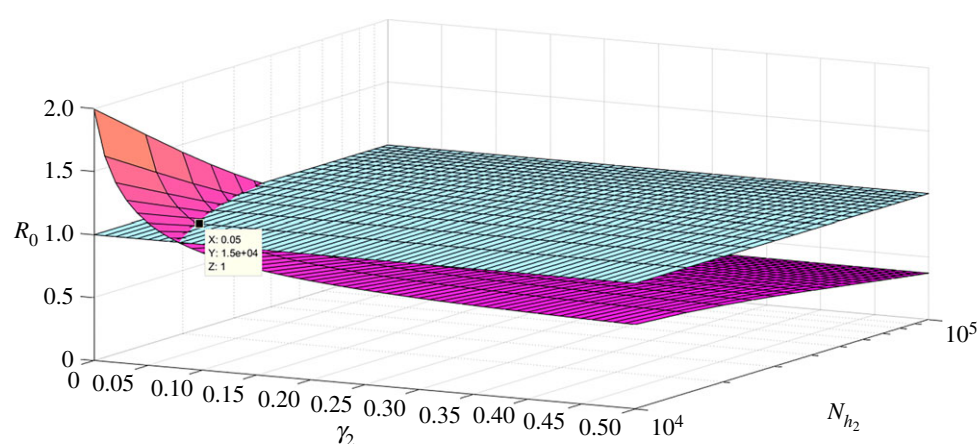


Figure 7. Variation in the mean value of R_0 (for Model 1) when γ_2 and N_v are varied. (Online version in colour.)

model parameters to understand the sensitivity of each of the parameters on R_0 . The SA showed that the model parameters such as human mortality rate (μ_{h_1}), infectious period ($1/\gamma_1$), rate of reporting (γ_2), alternative host (bird) mortality rate (μ_{h_2}) and sand fly mortality rate (μ_v) statistically have a significant influence on estimating R_0 with each of them being negatively correlated (negative PRCC between them) (see figure 8). Model parameters such as μ_{h_1} , σ , δ , μ_{h_1} and γ_1 are statistically insignificant to the estimation of R_0 . Moreover, parameters b , β_1 , α_v , $\tilde{\beta}_1$, β_2 are also statistically significant and are found to have a positive correlation with R_0 . Sand fly related parameters are most influential in estimating R_0 , followed by parameters for alternative hosts and parameters related to humans. In order to eliminate CL, these results strongly support the need to improve the monitoring and controlling of the sand flies, and the alternative host (potentially birds) population. In other words, this SA provides a specific intervention/monitoring strategy for controlling leishmaniasis in endemic areas in Ecuador. The analysis showed a strong negative correlation between μ_v and R_0 and a strong positive correlation of β_1 , β_2 and $\tilde{\beta}_1$ with R_0 (figure 8). The local analytical computation of sensitivity indexes (collected in the electronic supplementary material) further validates numerical global sensitivity results.

4. Discussion

Phlebotomine sand flies transmit *Leishmania* that affects humans, animals, and potentially bird alternative hosts worldwide in many tropical countries including in Latin America. In this study, we developed a data-driven modelling framework to study the role of sand fly feeding behaviours and host preferences in the transmission dynamics of leishmaniasis in Ecuador, when existing surveillance system is passive. Historically, cases of CL in Ecuador have fluctuated over years, with some years showing major epidemics in different regions of the country. However, some of the epidemics never came to light in time probably in part due to considerable underreporting. Moreover, the *Leishmania* infection has neither been systematically monitored in humans nor in the other hosts across Ecuador. Recently, some researchers in Latin America have suggested a rising trend in the number of CL cases in Ecuador, and attributed this to a lack of access to medical treatment, increased human migration into leishmaniasis endemic areas, and/or to ecological changes triggering vector adaptation to different alternative hosts.

There are many *Leishmania* natural hosts and reservoirs all over the world. In particular, it is known that the transmission of parasites of the genus *Leishmania* involves a

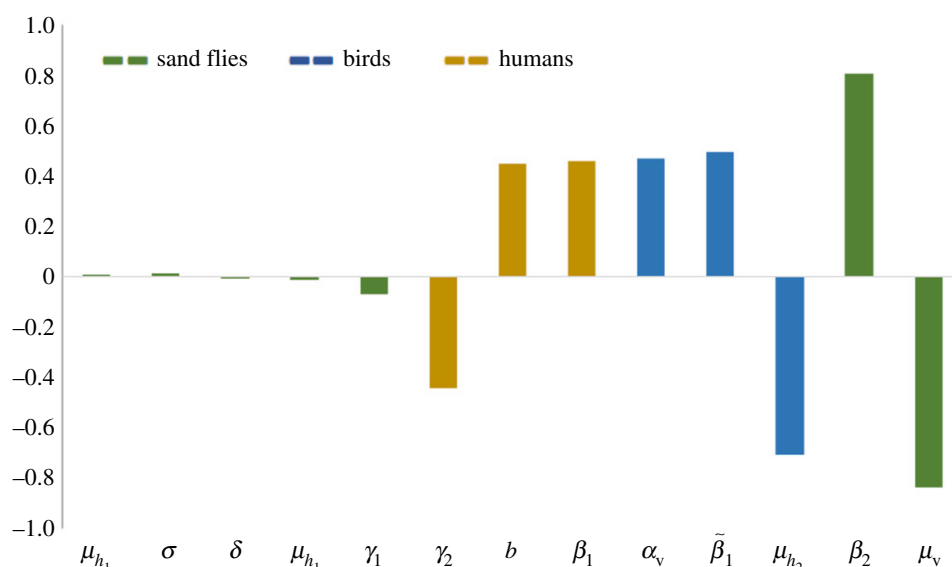


Figure 8. Partial rank correlation coefficient (PRCC) indexes from parameter sensitivity analysis of R_0 (for Model 1). (Online version in colour.)

large diversity of mammalian hosts. The identification of *Leishmania* reservoirs and hosts is usually carried out by blood meal analysis in the insect vectors. In Latin America, Brazil leads the effort in conducting studies on blood meal preferences among sand fly species and found that *Lutzomyia longipalpis* (the vector for transmission of leishmaniasis) also has a preference for birds [64]. We carried out a study in a coastal region of Ecuador that showed that birds, primarily chickens, are the preferred blood source for several species of sand flies [26]. The study by Anaguano *et al.* [26] further serves as an evidence of feeding preference of sand flies on birds during the dry and wet seasons.

A modelling framework is therefore developed to understand the dynamics of CL transmission between local sand fly species and its natural and preferred hosts [31]. The research goals of this study are to understand the role of alternative hosts (potentially birds), existing case surveillance system, and the current control programmes in the transmission dynamics of CL using epidemiological and entomological data from Ecuador. The modelling framework was classified into five different models in order to systematically study the research goals. The models presented in this study describe the interactions between the sand fly species and its two main hosts: humans and alternative hosts (birds). We collected two types of datasets for carrying out model parametrization: (i) incidence data from 2009 to 2011 outbreak in the town of Valle Hermoso obtained through the Ecuador public health surveillance system and (ii) feeding behaviours of sand flies to estimate host preference of vectors, via sand fly data collected by our research group.

Our results suggest that there is a wide gap between the reported and the total infected cases (around 40% of cases were unreported), confirming our hypothesis that there is huge underreporting of the disease in the region. Underreporting is a result of multiple factors including difficult accessibility to endemic areas, registration system failures, and ineffective diagnosis and treatment of patients in private medical centres [65]. The mathematical analysis resulted in computation of the threshold quantity, the basic reproduction number (R_0), a useful number for understanding the transmissibility of CL and designing of various intervention strategies. This threshold quantity is often used to describe the condition for the existence of an outbreak. If R_0 is less

than 1, the disease will decline and eventually die out, and if R_0 is more than 1, there may be an outbreak or epidemic. For our models, it is found that estimates of R_0 depend on multiple parameters including density of alternative hosts (birds), feeding preference of sand flies, mortality rate of sand flies, density of sand flies, and coverage of surveillance system. Using data from Valle Hermoso, we estimated R_0 to be around 3.9, suggesting potential for regular outbreaks in future and need for significant improvements in existing intervention programmes. Our estimate of R_0 is higher than estimate reported from neighbouring countries Colombia ($R_0 = 1.3$ [48]) and Peru ($R_0 = 1.9$, if domestic dogs are primary reservoirs [66]), which could be due to a difference in the reporting system and/or ecology of the subregions. Nevertheless, the results suggest that improved reporting and early treatment of cases can control the disease drastically; however, the rates of decrease in cases will depend on intensity to control alternative host (potentially bird) density in the region.

We observed a direct relationship between the increase in feeding frequency for potential alternative hosts (birds) (or the decrease in time to reporting of a case to surveillance system) and decreases in the CL prevalence in human population. The parameter SA of R_0 showed that vector control programmes are the most effective interventions for CL elimination when compared with the control programmes that focus on alternative host management or are directly related to humans. The analysis on equilibrium prevalence levels was also performed. It showed that the alternative hosts (birds) can play a significant role in the dynamics of the transmission of CL in Ecuador. These results further confirm the necessity of improving the monitoring and controlling of the sand flies as well as the alternative hosts.

Our modelling and data analysis provide a novel approach for investigating the transmission and control of CL in Latin America. We have (a) estimated for the first time transmission rates between different hosts (preferred and alternative) and vector species in the presence and absence of alternative reservoirs, (b) estimated underreporting levels in the presence and absence of alternative reservoirs (estimates comparable to those reported in the literature [67]), (c) estimated CL reproduction in Ecuador, and (d)

suggested that control of alternative reservoir hosts is critical to achieving optimal results of the vector control programme. The study also provides new dimensions to understanding the dynamics of VBDs in general: (a) it gives a method to estimate underreporting levels of a VBDs based on current incidence from surveillance, and data on vector feeding preferences and host competence, (b) it provides a procedure to link host-related empirical information to a dynamical model, and (c) it suggests how qualitatively characteristics of VBD prevalence can be studied when data are scarce and how uncertainty in prevalence can be quantified.

In conclusion, we present the first model-based estimates of CL underreporting and infection rates for potential alternative hosts (birds) in Ecuador. We collected some entomological data and used novel data-driven approach to parametrize the model. Our estimates constitute useful procedure for decision making and prioritization of CL control interventions in the endemic areas of Ecuador. Our research clearly shows that there is a need for improvement in data collection on different avian species and for implementation of active surveillance system to thoroughly evaluate long-term CL patterns in Ecuador. Vector density is critical to the establishment of CL in new susceptible regions. We anticipate that in near future surveillance programmes will integrate these methods and results in their systems. The current methodology should be further developed to address its limitations and provide more accurate estimates but this is dependent on the collection of detailed data. In the future, we would like to collect and use surveillance data from other provinces of Ecuador in order to comprehensively validate model results for the

whole country, identify and suggest effective sampling techniques to collect fine-grained sand fly related data in an effort to determine feeding preferences, and develop mechanisms for sampling birds to obtain data for understanding CL transmission efficiencies among alternative host species.

Ethics. An approval for conducting the sampling study for collecting blood meal analysis on sand flies was obtained from Institutional Review Board (IRB) at the Instituto Nacional de Investigación en Salud Pública (INSPI) of Ecuador in 2013. The case surveillance data was obtained from Ministry of Public Health, Ecuador.

Data accessibility. The details of the data are presented within the study. Additional materials are collected in electronic supplementary material.

Authors' contributions. All authors contributed in the development of study idea and writing of the initial manuscript. D.M.V., M.P., E.J.M.-B., M.C. and A.M. developed and analysed the mathematical models. D.M.V., V.C., P.P. and A.M. collected the data and verified it. M.P., E.J.M.-B., M.C. and A.M. fitted the data to the model. L.A. and A.M. performed sensitivity analysis on model outputs. M.P., A.M. verified the empirical and analytical methods. A.M. supervised and finalized the findings of this work. All authors discussed the results and contributed to the final manuscript.

Competing interests. We declare we have no competing interests.

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