PARAMETERS OF LEISHMANIA BRAZILIENSIS TRANSMISSION BY INDOOR LUTZOMYIA OVALLESI IN VENEZUELA

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Abstract. We developed a mathematical model of cutaneous leishmaniasis (CL) transmission predicting CL incidence based on field data of number of positive sand flies, new CL cases, and number of susceptible people. We estimated the following parameters: (1) the incubation period of one month, (2) the overall susceptibility (Phil. = 0.793), the serologic force of infection (λm = 0.108/person/year, SD = 0.014), the clinical force of infection (λc = 0.114/year), the proportion of infections that result in skin lesions (α = 1.556), and the instantaneous reversal rate of Montenegro skin test–positive (MST+) people to MST− (ρ = 0.124/year, SD = 0.021). We also provide the first field estimate of the transmission efficiency (ε = 0.0045, SD = 0.0009). The model predictions conform well with the observed new cases except for some small departures in the peaks and in some depressions (Dmax = 0.1494, P < 0.2). We discuss possible sources of error of our estimate of ε, and compare our parameter estimates with those obtained in Peru.

INTRODUCTION

Lutzomyia ovallesi (Diptera: Psychodidae) is the primary vector of cutaneous leishmaniasis (CL) in northcentral Venezuela, predominantly infected with Leishmania braziliensis, and occasionally with L. mexicana. The insect vector becomes infected after biting wild animals, mainly rodents. The anthropophilic habits and the vectorial importance of L. ovallesi have been recognized in Panama, Belize, Colombia, and Venezuela. Use of a polymerase chain reaction (PCR) and DNA probes has shown that in Venezuela Lu. ovallesi was infected by L. braziliensis.

Sand flies have a nocturnal feeding activity, and their indoor populations play an important role in the transmission of CL. This was shown by a significant correlation between mean intradomiciliary abundance of Lu. peruensis and cases of CL in Peru. In Venezuela, a significant relationship between indoor Lu. ovallesi abundance and annual CL incidence was determined.

Several epidemiologic parameters of the transmission of L. peruviana were evaluated in Peru: λn, the instantaneous rate at which individuals negative on the Montenegro test (MST−) show CL skin lesions (clinical force of infection); λm, the rate at which they become serologically positive (serologic force of infection); α, the proportion of infections that result in skin lesions (λl = α λm); π, the relative risk of skin lesions for MST+ individuals compared with MST− individuals (thus, MST+ people develop new skin lesions due to new inoculations at a rate of λl π); and ρ, the instantaneous reversal rate of MST+ people to MST−.

The clinical force of infection, λc, results from the product of 1) the mean number of sand fly bites received per person per unit time (β), 2) the proportion of positive sand flies (ρ), and 3) the transmission efficiency (ε), defined as the probability that after the bite of one infected sand fly a person will develop CL lesions. The product of the first two parameters (β and ρ) is the biting rate of positive sand flies, which we designate as m.

The incubation time between infection and cutaneous lesions produced by L. braziliensis has been poorly studied, and is quite variable, but is usually considered to be in the range between a few weeks to approximately three months. A significant cross-correlation between incidence of L. peruviana and the density of Lu. peruensis was reported with a lag of one month.

No estimate of ε was found in the literature on CL epidemiology. In this work, we develop a model that predicts incidence as a function of the biting rate of positive sand flies to estimate ε under field conditions of indoor transmission of L. braziliensis in Venezuela. Our model requires knowledge of the number of susceptible individuals and the CL incubation period, which we also estimated from field data. Our analysis also provides estimates for λm, λn, p, and α.

MATERIALS AND METHODS

Study area. The study area is El Ingenio, located in the Miranda State of Venezuela, 3.5 km from the city of Guatire and 30 km east of Caracas. The geographic area is known as the Cordillera de la Costa, and has been classified as premontane dry forest or basic tropomphile montane forest. The vegetation of the premontane dry forest at the study site has been extremely altered and no climax vegetation can be found. The secondary forest is composed mainly of leguminous trees, such as the roble (Platymiscium sp.), the drago (Pterocarpus podocarpus), the cuji (Prosopis juliflora), and the samán (Platycocolobium saman). It has an average annual precipitation of 853 mm (period 1990–1993) and an average temperature of 20°C. The climate is markedly seasonal, with five dry months (December–April). The village, with a population of 258 people, consisted of 55 houses, of which 10 were uninhabited or under construction at the time of this study.

People lived in four types of houses: 1) those made of zinc and pasteboard (22%), 2) those made of a mixture of mud and straw supported by a structure of sticks called locally bahareque in the colloquial language (25%), 3) those made of concrete blocks (27%), and 4) those made of wood (26%). Bahareque and cement houses had their internal and external walls plastered.

The main occupation of the inhabitants of this community is agriculture, with the growing of flowers the major economic activity. Some men have temporary or part-time jobs in the agriculture.
neighboring cities of Guatire, Guarenas, and Caracas, but they usually return home at night, which means that they are continuously at risk for CL transmission. Recreational activities consist of weekend riverside barbecues and watching television indoors mostly after 8:00 PM. The activities of the inhabitants and frequent CL cases in young children suggest that transmission probably occurs indoors at night. The study area has been considered to be a medium endemic region for CL with approximately 34% of the new cases of the State of Miranda occurring in the village of El Ingenio and more recently as a hyperendemic region with 42% of the local serologic prevalence.18

Field methodology. The taxonomic identification of *Lu. ovallesi* was carried out as previously reported.19 Between January 1991 and March 1995, a field survey was carried out to sample sand flies using a fluorescent illuminated Shannon trap with two collectors. Details of the parasitologic procedures and of the sand fly sampling have been previously described.1,9

Statistical methodology. We provide below a description of the estimation procedure for each parameter.

**Estimation of the proportion of susceptible people (Φ) and of the parameters λm and ρ.** The proportion of susceptible people was calculated from reports of new cases of CL among all MST− and MST+ people in El Ingenio during a vaccination trial carried out from August 1993 to September 1994.18 We assumed, as was done in Peru,10 that in the control (unvaccinated) group MST− are fully susceptible, while a fraction *f* of MST+ people are fully susceptible and the rest (1−*f*) is resistant (i.e., they will not develop lesions after being reinfected). If during the study period a fraction *i* of the susceptible people developed lesions, then *f* can be obtained solving the system of equations

\[
\begin{align*}
MST^−,i &= \text{new cases among MST}^− \\
MST^+,f,i &= \text{new cases among MST}^+
\end{align*}
\]

The susceptibility of the overall population, based on the serologic survey prior to the vaccination trial,18 can be calculated as

\[
\Phi = \frac{f \cdot MST^+ + MST^-}{MST^+ + MST^-}
\]

where now MST+ and MST− are different from the ones used to calculate *f* because they represent the total population that participated in the vaccination trial.

Parameters λm and ρ were estimated by fitting equation 1 to prevalence-age data of El Ingenio18 by maximum likelihood.20,21 Under steady state conditions, the proportion of people positive for Montenegro’s test as a function of age, MST+(a), is given as

\[
MST^+(a) = \frac{\lambda_m}{\lambda_m + \rho} (1^{\lambda_m + \rho} a)
\]

This equation assumes that cross reactivity with antigens different from those of *Le. braziliensis* is negligible.

**Evaluation of β and the incubation period.** Estimation of *ε* requires data on two simultaneous time series (in the same time units): the number of infected sand flies biting people outdoors, and new cases of CL lesions among susceptible people, and their application to a transmission model. Our entomologic and new cases data are for the period 1991–1992, while the susceptibility calculation for El Ingenio corresponded to the period 1993–1994. Since the number of susceptible people at a given time results from the previous history of exposure, rather than from the force of infection during a given year, we consider that susceptibility during 1993–1994 adequately represents that of the 1991–1992 period.

The entomologic data consisted of a 14-month series of *Lu. ovallesi* abundance estimations January 1991–February 19929 expressed as the number of sand flies collected by two people in a Shannon trap (C) and a simultaneous series of proportion of infected sand flies (ρ) determined by a PCR and DNA probes. The collections were carried out between 7:00 PM and 10:00 PM and with an average sampling effort of six hours in two days per month.9

If *h* is the sampling effort by two collectors in hours, dividing *C* by 2*h* gives an estimate of sand fly abundance per collector per hour. Multiplying this by three gives abundance per collector during the 7:00–10:00 PM three-hour period. Finally, dividing by *b*, the fraction of the overnight sand fly population that is active during the same period,22 we obtain the number of sand flies per collector per night outdoors (*A*), by

\[
A = \frac{3C}{2bh}
\]

Assuming that the daily biting rate is relatively constant over the entire month, we can calculate the number of sand flies/person/month biting people indoors (β) as

\[
\beta = \frac{A30}{V_{SE}V_{LP}R}
\]

where *R* is the ratio between the number of sand flies/person biting people indoors and outdoors captured by a Shannon trap, as reported for San Esteban,23,24 a Venezuelan village similar to El Ingenio. *V* <sub>SE</sub> and *V* <sub>LP</sub> are the average number of
people per house in San Esteban and El Ingenio, respectively. The value 30 is a conversion factor from night to month units. Two important assumptions were made for this conversion: 1) that the relationship between the number of sand flies/person captured by a Shannon trap located outdoors and the number of sand flies/person biting people indoors found for San Esteban also holds for El Ingenio, and 2) that the total number of sand flies biting people inside the house is independent of the number of people in the house.

The $\beta$ and $p$ series were smoothed by three-points moving averages to remove noisy information and called $\beta^\prime$ and $p^\prime$, respectively; their product $(m)$ represents the number of infected sand flies/person/month biting people indoors.

Data for new cases of CL lesions were provided by the Institute of Dermatology of the Ministry of Health and Social Assistance of Venezuela, and given as a monthly series for years 1991 and 1992. A cross-correlation analysis of the new cases series lagging behind the $m$ series was carried out to estimate the incubation period, both in monthly time units.

**Modeling.** Different aspects of the modeling are presented in this section under separate headings.

**New cases and the force of infection.** If the clinical force of infection ($\lambda_i$) is time invariant, then the proportion of cases among $N$ initial susceptible people as a function of time is given by $1 - e^{\lambda T}$. However, under field conditions the force of infection is variable according to changes in sand fly density and in the proportion of infected sand flies. To overcome this difficulty, we developed a model that predicts new cases when transmission is variable (for mathematical details see Appendix 1).

Let us suppose that at time $t_0$ there are $N$ people susceptible to CL and let us assume a constant incubation period $z$ between inoculation and clinical symptoms. If $T$ is the random variable “time when a person among the $N$ people develops CL symptoms” it is shown (Appendix 1) that

$$P(t < T \leq t + k) = e^{-S(i,j) - e^{-S(i,j) - e^{\lambda z + k}} - e^{-S(i,j) + e^{\lambda z - k}}},$$

where $S(i,j)$ is the expected number of infected sand fly bites that a person receives during the time interval $(i, j)$ and $t > t_0$.

**Estimation of $\epsilon$ from field data.** The parameter $\epsilon$ and its variance due to the sampling error of new cases were estimated by maximum likelihood (equations 3 and 5, Appendix 2). In other words, we looked for the $\epsilon$ value that maximizes the probability of occurrence of the observed series of new cases, given the $m$ series and the incubation period. The time unit was one month and $S(i,j)$ was calculated from the $m$ series.

**Sensitivity analysis.** The abundance of positive sand flies series ($m$) was included in the model as an independent variable with no error, so the variance of $\hat{\epsilon}$ given by equation 5 in Appendix 2 only takes into account the variability of $\hat{\epsilon}$ due to the sampling error of the new cases series. A Monte Carlo approach was used to evaluate the additional variability introduced by the sampling error of sand fly abundance and proportion of infected sand flies. Two sets of data were randomly generated, one for the $m$ series and a second for the new cases series. To generate the former random data set, we considered the $C$ and $p$ series as if they were the expected (true) monthly values for sand flies captured in Shannon traps and the proportion of infected sand flies, respectively. Assuming that the number of sand flies captured in Shannon traps follows a Poisson distribution with a mean given by the $C$ series, we generated 200 random samples of $C$. For each of them we also generated a corresponding random series of the proportion of infected sand flies. For the latter we assumed that the number of positive sand flies for a given month follows a binomial distribution with a probability given by the true $p$ and the number of trials by the corresponding random value of the $C$ series for that month. Each pair formed by the corresponding $C$ and $p$ series was treated as showed in the previous section to produce the 200 random $m$ series.

To obtain random series of new CL cases, we first compute the expected cumulative distribution function of $X$ (equation 3 in Appendix 1) using the maximum likelihood $\hat{\epsilon}$, and the $m$ series. The expected cumulative distribution function of $X$ was used to randomly assign to each initially susceptible person the month number in which infection appears. A total of 200 series of new cases were generated in this manner.

Finally, each of the 200 random series of $m$ was combined with one of the 200 random series of new cases, generating a random set of 200 paired samples of $m$ and new cases series. The maximum likelihood $\hat{\epsilon}$ by solving equation 3 in Appendix 2 and its variance due only to sampling error of new cases (equation 5 in Appendix 2) was obtained for the 200 paired series.

The total variance of $\hat{\epsilon}$, that includes both sources of variability (abundance infected sand fly and new cases) was calculated as

$$\frac{\sum_{i=1}^{200} (\hat{\epsilon}_i - \bar{\epsilon})^2}{199}$$

where $\bar{\epsilon}$ is the average of the 200 maximum likelihood $\hat{\epsilon}$ values. The resulting value of equation 7 is compared with the variance due only to sampling error of new cases calculated as the average of the 200 maximum likelihood variance estimations (equation 5 in Appendix 2).

Our transmission model assumes that sand fly bites are allocated following a Poisson distribution. To evaluate how critical this assumption is, we analyzed the effect of non random allocation of infective bites on the expected number of new cases. We calculated predicted new cases for equal number of bites among people, and also for different degrees of clumping of bites among people assuming the negative binomial distribution. Data used for these calculations were the number of infective bites accumulated from January 1991 to February 1992, the maximum likelihood $\hat{\epsilon}$ value, and a fixed number of initially susceptible people.

To verify the seriousness of a departure from the Poisson distribution of sand fly bites allocation, we also evaluated $\epsilon$ assuming a uniform or clumped distribution. For that purpose, we used the actual number of annual new cases (21 cases) and the average number of positive sand fly bites per person per year in El Ingenio (25.3 cases), solving the following equations for $\epsilon$ numerically $N_c = N_s(1 - (1 - e^{-AB})^k)$ for the uniform distribution, $N_c = N_s \sum_{i=0}^{\infty} (1 - (1 - e^{-\epsilon AB}))^i NB(i;AB,k)$ for the clumped (negative binomial) distribution, where $N_c$ is the number of annual new cases, $N_s$ is the number of initially
suscetable people, $AB$ is the average number of positive sand fly bites per person per year, and $NB (i, AB, k)$ is the negative binomial probability of a person receiving $i$ infective bites when the mean number of bites per person is $AB$ and the degree of clumping is $k$. Two values of $k$ were used (3 and 0.85).

*Estimation of $\lambda_i$ and $\alpha$.* Accumulating the monthly values of $m$ during a 12-month period yields the annual potentially infective biting rate in units of bites/person/year. If this value is multiplied by $\hat{e}$ (in units of person/infective bite), we obtain an estimate for $\lambda_i$ in year$^{-1}$ units. The fraction of successful infections that lead to skin lesions, $\alpha$, was estimated dividing $\lambda_i$ by $\lambda_m$.$^{10}$

**RESULTS**

*Proportion of susceptible people $\lambda_m$ and $\rho$.* The fraction of the MST$^+$ people who are fully susceptible ($f$) was 0.507 with an overall susceptibility ($\Phi$) of 0.793. Assuming that the number of MST$^+$ people in each age class follows a binomial distribution, the maximum likelihood fit of equation 3 to the data of Table 1 resulted in an estimated force of serologic infection of $\lambda_m = 0.108$ /person/year (SD = 0.014) and an MST$^+$ reversal rate of $\rho = 0.124$/year (SD = 0.021). The expected number of MST$^+$ using the estimated values of these parameters is also shown in Table 1 and age specific prevalence is given in Figure 2.

*Biting rate ($\beta$), incubation period, and transmission efficiency ($\epsilon$).* The average number of people per house in San Esteban and El Ingenio ($V_{SE}$ and $V_{LP}$) were 4.24 and 6.00 persons/house, respectively. The value of the conversion factor $R$ was 0.0468, resulting from the ratio between 0.31 sand flies/person/hour biting people indoors and 6.63 sand flies/person/hour captured outdoors by a Shannon trap. The estimation of the potentially infective bites/month/person ($m = \beta \rho \epsilon$), and the data used for its calculation are shown in Table 2.

The population of El Ingenio in January 1991 was 258 people, so the initial number of susceptible individuals ($N$), obtained by multiplying the El Ingenio population by the overall susceptibility ($\Phi = 0.793$), is 205 people.

The cross-correlation between the CL new cases series lagging behind the potentially infective bites/month/person series ($m$) was statistically significant for lags of one and two months ($r = 0.775$, $P = 0.001$, and $r = 0.708$, $P = 0.005$, respectively), while lags of zero and three months showed no statistically significant cross-correlations ($r = 0.279$, $P = 0.279$, and $r = 0.311$, $P = 0.279$, respectively). We assumed a one-month incubation period because of its larger correlation coefficient.

Columns $N_x$ and $m$ of Table 2 were used to estimate $\epsilon$ by means of the transmission model (equation 3 of Appendix 2). The maximum likelihood value of $\hat{\epsilon}$ is 0.0045 (SD = 0.0009). The observed and predicted CL new cases and the $m$ series are shown in Figure 3. The Kolmogorov-Smirnov test confirms a satisfactory fit between model and data ($D_{\text{max}} = 0.1494, N = 14; P < 0.2$).

*Sensitivity analysis.* Total variance of $\hat{\epsilon}$ due to the sampling error in sand fly number and new cases was $1.48 \times 10^{-6}$, while variance of $\hat{\epsilon}$ due to new cases sampling error alone was $8.66 \times 10^{-7}$, with the former 71% larger than the latter. Corresponding coefficients of variation were 26.6% and 20.0%, respectively.

The predicted number of new cases assuming an allocation of equal number of bites among people was 0.21% higher than when a Poisson allocation is assumed. Under the assumption of a clumped distribution, the predicted number of new cases decreased with increasing levels of clumping. For example, for clumping indices (variance/mean) of 58.8, 29.9, and 10.6, the number of new cases was 10.5%, 5.6%, and 2.0% lower than expected from a Poisson allocation, respectively. For a variance/mean < 6, the difference is less than 1%.

The estimates of $\epsilon$ for the Poisson uniform and negative binomial allocation of infective bites were 0.0045, 0.00427, and 0.00436 (with $k = 3$, variance/mean = 10), respectively. For the negative binomial, we tested an extremely clumped case with $k = 0.85$ (variance/mean = 30) obtaining $\epsilon = 0.00456$.

*Estimation of $\lambda_i$ and $\alpha$.* Since $\lambda_i$ is based on a 12-month period while the original sand fly data covered 14 months, we added the monthly values of the $m$ series for three 12-month periods: January 1991–December 1991 (22.16 infective bites/person/year), February 1991–January 1992 (27.81 infective bites/person/year), and March 1991–February 1992 (27.81 infective bites/person/year). We then averaged those three values of yearly accumulation of $m$ and obtained (25.25 infective bites/person/year); this multiplied by $\hat{\epsilon}$ resulted in a clinical

![Figure 2. Expected and observed number of Montenegro skin test-positive (MST+) individuals in El Ingenio, Venezuela. The expected values were calculated using the force of serologic infection ($\lambda_m = 0.108$/person/year) and the MST+ reversal rate ($\rho = 0.124$/year) estimated by maximum likelihood of equation 3 to the data of Table 1, and the assumption that the number of MST+ people in each age class follows a binomial distribution. The hump is suggestive of an increase of transmission in the last 10–20 years.](image)
force of infection ($\lambda_i$) of 0.114/year. Our estimate of the fraction of successful infections that lead to skin lesions (obtained dividing $\lambda_i$ by $\lambda_{\text{me}}$) is $\alpha = 1.056$.

**DISCUSSION**

There are few entomo-epidemiologic studies of CL that allow a comparison with the type of results here presented. A study carried out in Peru\cite{10} provides epidemiologic parameter estimations, and despite differences in climate, habitat, vector and parasite species, and methods, its results permit comparisons.

In terms of crude incidence (new CL cases in the total population) our situation differed in some aspects from that of the Peruvian situation.\cite{10} In El Ingenio, there were 32 new CL cases in a period of 16 months (Table 2), that is, an average of 24 new CL cases/year, which for a total population of 258 people in the village produces an average of 0.093 new CL cases/person-year, which is about twice the crude incidence of 0.046 CL new cases/person-year obtained for the Peruvian valley with the maximum crude incidence value.\cite{10}

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* See Materials and Methods for different sources of data.

**FIGURE 3.** Time series of biting rate (potentially infective bites/month/person), and observed and expected new cases of cutaneous leishmaniasis per month in El Ingenio, Venezuela during the study period. The expected values were obtained from the transmission model (for details see Materials and Methods, equations 6 and 7, and Appendix 1).
The calculation of the incidence rate (new CL cases in the MST− subpopulation) for El Ingenio, based on the data of Table 2, is 0.117 CL new cases/person-year (24 new cases/year among 205 susceptible people, overall susceptibility [Φ = 0.793] times 258 people), which is similar to the highest incidence rate obtained in Peru (0.111).10 The serologic force of infection obtained for El Ingenio (λm = 0.108 /person/year, SD = 0.014) is also relatively similar to the ones obtained after a cross-sectional study in several valleys in Peru: 0.109, 0.118, and 0.202.10

The proportion of the MST− of the three first age groups in Figure 2 shows a hump and not a monotonically increasing curve as expected from a constant force of infection. Since these age groups correspond to the youngest age groups (1–18 years old), they suggest an increase in the force of infection in the last 10–20 years. This is confirmed by the gradual increase from 1–2 new cases/year in 1984 to 35 new cases/year in 1994.14 Entomologic data also shows a 40% increase between 1991–1992 and 1993–1994 of indoor Lu. ovallesi density per house per year,17 and this is reflected by an extremely high incidence rate among MST− in the period 1993–1994 (58.8%) as compared with the 11.7% of incidence from the new cases time series data the previous year (Table 3).

As a consequence of this increase in transmission, our analysis of cross-sectional data underestimates the force of infection (λm) for the period 1992–1993 and overestimates the reversal rate (ρ = 0.124/year compared with the Peruvian situation of ρ = 0.0291 and 0.0090/year for two valleys from a cross-sectional study). This leads to an asymptotic age-specific prevalence given by λm/(ρ + λm) (see equation 3), which is quite low (48%) when compared with the Peruvian situation (85%). The underestimation of the serologic force of infection (λm) of El Ingenio explains the α value slightly over unity obtained for our study site.

To obtain better estimates of the reversal rate and the force of infection previous to the increase of transmission in the last 10–20 years, we fitted the data of Figure 2 after eliminating the first three age groups, and obtained λm = 0.0187/person/year, ρ = 0.00459/year, and an asymptotic age-specific prevalence λm/(ρ + λm) = 80.3%. Thus, the reversal rate ρ and the asymptotic age-specific prevalence are of the same order of magnitude as those the Peruvian situation.10

There are no estimates of ε in the literature. However, the Peruvian study10 provides information that allows an approximate estimate of ε. The results show (see Appendix 3 for details and assumptions) that the average ε for five villages is 0.034 (range = 0.0088–0.1283). Additional data from Peru27 also permits an estimation of ε (see also Appendix 3 for details and assumptions) resulting in an estimated ε between 0.0044 and 0.0079 depending on the degree of acquired immunity of MST+ people.

The estimation of the efficiency of transmission (ε) required two basic pieces of information: the biting rate of positive sand flies (m) and the observed CL new cases per month. The series m results from the product of β (mean number of sand fly bites received per person per unit time) and p (proportion of positive sand flies), and thus is sensitive to all assumptions used in their calculation. For example, to calculate β we resorted to data from two different places (villages of San Esteban and El Ingenio), and assumed 1) that the relationship between outdoor and indoor numbers of sand flies/person captured by a Shannon trap found in San Esteban also holds for El Ingenio and 2) that the total number of sand flies biting people inside the house is independent of the number of people in the house (an average of 4.24 people/house for San Esteban and 6 people/house for El Ingenio). There are few reports in the literature for the indoor sand fly abundance and biting rate relationship. In El Ingenio, 0.01428 sand flies were collected in a Centers for Diseases Control (CDC) trap per sand fly collected by a Shannon trap 100 meters from a house;9 in San Esteban, 0.31 sand flies/person/hour were collected biting humans while 6.63 sand flies/person/hour were collected in a Shannon trap outside houses. Thus, the number of sand flies biting people for each sand fly collected in a CDC trap is 3.3 = 0.31/(6.63 × 0.01428), which is quite similar to the value obtained for Peru (3.2 in the same units).10 The data from San Esteban can be combined with that from El Ingenio with some confidence, since both villages are very similar in demographic composition, latitude, altitude, and type of ecologic habitat.

Our values of p fluctuate monthly between zero and a maximum of 3.8%, with a mean of 1.19% (51 of 4,269).1 This mean is higher than the average value of 0.51% (25 of 4,864) obtained in Venezuela9 and Guatemala12 for Lu. ovallesi. However, our estimation of p is lower than the value of 3.65% (95 of 2,600) obtained for Lu. ayacuchensis infected by I. mexicana in the Ecuadorian Andes.28

The exact meaning of ε depends on how positive sand flies are defined. Only a fraction of the sand flies that are classified as positive by the PCR method will show parasites after dissection, and from the latter only those with parasites in the foregut will be capable of transmitting the parasite to the definitive host. Since the incidence is determined by the product of ε and the number of positive bites per person, the PCR criterion leads to the lower ε values, the parasites in the foregut criterion produces the highest values, while the dissection criterion produces an intermediate one. For estimating ε in El Ingenio, we considered that a sand fly was positive when parasites could be detected by dissection. This was not the case for the calculations of ε we made in Appendix 3, where the PCR criterion was used.27 This may in part explain the differences, and higher values of ε would have been obtained if the dissection had been used in the Peruvian study.27 Regardless of the definition of positivity, in all estimations the ε values were surprisingly low.

The model predictions conform well the observed new cases, except for some departures in the peaks and some valleys. We will now discuss possible sources of error that may
have affected the estimate of $\epsilon$, which are summarized in Table 4.

Our model does not take into account the possibility that avirulent infections (which produce an MST$^+$ reaction but no clinical symptoms) could cause partial immunity against virulent infections. In a longitudinal survey of CL caused by *L. peruviana* in Peru, it was found that approximately 17% of all infections were subclinical. However, the omission of avirulent infections would cause the number of susceptible people used in our model to be larger than the actual number of susceptible individuals, and the model would have to lower the value of $\hat{\epsilon}$ to account for the observed number of new cases. In that case, $\hat{\epsilon}$ would have been underestimated. The estimation of $\hat{\epsilon}$ is also affected by the possible sand fly sampling errors; however, most of the variability of $\hat{\epsilon}$ comes from sampling errors of new cases series, and the order of magnitude of $\hat{\epsilon}$ is not modified when including sand fly sampling variability.

To apply the model, we had to estimate the incubation time. This was inferred from the statistically significant cross-correlations between new cases of CL lagging behind the estimated potentially infective bites, which resulted between one and two months, while lags of zero and three months were not significant. This result is similar to the one obtained in Peru. However, had our data been available on a weekly basis, the real incubation time would probably turn out to be between two and six weeks.

The departure of sand fly bites allocation from randomness has a negligible effect on the predicted number of new cases for the El Ingenio situation, which is characterized by a low value of the transmission efficiency $\epsilon$. However, the departure from a Poisson allocation of sand fly bites may become more critical in situations with larger values of $\epsilon$. Thus, we expect that our estimation of $\epsilon$ using a model that assumes random allocation of bites is not seriously affected by relaxing the assumption of random sand fly bites allocation. This conclusion was verified by estimating $\epsilon$ assuming uniform or clumped sand fly bites allocation for the actual number of annual new cases and positive sand flies in El Ingenio.

Our estimate that in El Ingenio the fraction of MST$^+$ people who are fully susceptible is approximately 50% ($f = 0.507$); thus, approximately half of the MST$^+$ people will not develop new scars after reinfection. Davies and others found in Peru that people acquire 74% protection following MTS$^+$ conversion.

The probability that a person will develop CL lesions after being bitten by one infected sand fly ($\hat{\epsilon}$), as obtained by maximum likelihood, is 0.0045 (SD = 0.0009), that is, an average of approximately 222 bites from infected sand flies are necessary to produce lesions. This is the first estimation of the efficiency of transmission ($\hat{\epsilon}$) of CL obtained under field conditions, and it can be useful in designing and evaluating control campaigns based on vector population reduction.

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### Table 4

<table>
<thead>
<tr>
<th>Source</th>
<th>Effects on $\hat{\epsilon}$</th>
<th>Other effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model assumptions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bites allocation is clumped but a Poisson distribution is assumed</td>
<td>Underestimation</td>
<td>Negligible</td>
</tr>
<tr>
<td>Bites allocation is uniform but a Poisson distribution is assumed</td>
<td>Overestimation</td>
<td>Negligible</td>
</tr>
<tr>
<td>Incubation time variable but assumed constant in model</td>
<td>–</td>
<td>Almost negligible</td>
</tr>
<tr>
<td>Errors in sand fly sampling, data handling, and conversion factor estimations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Few sampling days per month</td>
<td>Any direction</td>
<td>Increases with sand fly variability</td>
</tr>
<tr>
<td>Smoothing of sand fly density and positivity series</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Weak correlation between indoor and outdoor sand flies in the San Esteban situation</td>
<td>Any direction</td>
<td>Increases error of $\hat{\epsilon}$</td>
</tr>
<tr>
<td>Outdoor Shannon-indoor human biting rate conversion differs by $x$ times the actual value</td>
<td>Any direction</td>
<td>$\hat{\epsilon}$ differs in approximately $1/x$ times with respect to no error</td>
</tr>
<tr>
<td>Errors in CL new cases sampling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Misrepresentation in survey by a factor of $x$</td>
<td>Any direction</td>
<td>$\hat{\epsilon}$ differs in approximately $x$ times with respect to no error</td>
</tr>
<tr>
<td>A proportion of new cases are in fact relapses</td>
<td>Increases $\hat{\epsilon}$</td>
<td>$\hat{\epsilon}$ increases in approximately $1/(1-x)$ times with respect to no error</td>
</tr>
<tr>
<td>Errors in the estimation of the initial number of susceptible individuals</td>
<td>Error by a factor $x$</td>
<td>$\hat{\epsilon}$ differs in approximately $1/x$ times with respect to no error</td>
</tr>
</tbody>
</table>

*CL = cutaneous leishmaniasis.
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REFERENCES


APPENDIX 1

The transmission model

If ε is the transmission efficiency, defined as the probability that a susceptible person develops a cutaneous leishmaniasis (CL) lesion after being bitten by one infected sand fly, the probability of not developing a lesion with n potentially infective bites is

\[ 1 - (1 - \epsilon)^n \]  \hspace{1cm} (A1.1)

If we assume that allocation of infected sand fly bites among people is a random process described by a Poisson distribution with mean \( S \), the probability that a person receives \( n \) bites is given by \( S^n e^{-S}/n! \). Then the probability of a susceptible person become infected after receiving \( n \) potentially infective bites is

\[ \sum_{n=1}^{\infty} \frac{S^n e^{-S}}{n!} (1 - (1 - \epsilon)^n) = 1 - e^{-Se^\epsilon} \]  \hspace{1cm} (A1.2)

Let suppose that at time \( t_0 \) there are \( N \) people susceptible to
CL. If we assume a constant incubation period \( z \) between inoculation and clinical symptoms, it follows from equation A1.2 that the expected proportion of \( N \) that develop new CL lesions during the time interval \((t_i, t_f]\) will be

\[
\Psi(t_{i,f}) = 1 - e^{-xS(t)}
\]

(A1.3)

where \( S(t) \) is the expected number of infected sand fly bites that a person receives during the time interval \((i, j)\) and \( t > t_0 \)

If \( T \) is the random variable “time when a person among the \( N \) people develop symptoms of CL,” it follows from equation A1.3 that

\[
P(t < T \leq t + k) = \Psi(t_0, t + k) - \Psi(t_0, t)
\]

(A1.4)

The number of new cases expected for the period \((t, t + k]\) can be obtained multiplying this expression by \( N \).

**APPENDIX 2**

Estimation of \( \varepsilon \)

If \( N \) and \( z \) are known, and if a series of infected sand fly bites for \( K \) consecutive periods of time is available, the expression A1.4 of Appendix 1 can be used to predict expected new cases for each of the \( K \) periods as a function of \( \varepsilon \). An estimate of \( \varepsilon \) can be obtained comparing predicted new cases with observed new cases along the \( K \) periods.

If the numbers of observed new cases of infection during the \( K \) intervals \((t_0, t_f) \ldots (t_{K-1}, t_f) \ldots (t_K, + \infty) \), are denoted by \( NC = (N_1 \ldots N_K) \), then \( N_K \) is a random sample taken from the distribution of \( T \) (see equation A1.4), where \( N_s \) is the observed frequency for \( t_{s-1} < T \leq t_s \).

Note that for \( T > K \) the observed new cases must be \( N = N_K \), since according to equation A1.3, all of the \( N \) people in an endemic area will become infected when time of exposure is large. This setting allows one to estimate \( \varepsilon \) by the maximum likelihood method, as follows.

We compute the probability of the \( NC \) sample conditional on \( \varepsilon \) given \( N, z \), and the sand fly bites series (S, see Appendix 1):

\[
L(N_1\ldots N_K; \varepsilon) = \prod_{s=1}^{K} P(t_{s-1} < T \leq t_s|\varepsilon)^{N_s} \cdot P(T > K|\varepsilon)^{N - NK}
\]

(A2.1)

The \( \varepsilon \) value that maximizes \( L \), denoted by \( \hat{\varepsilon} \), is the maximum likelihood estimate for the transmission efficiency.

To find the value of \( \varepsilon \) that maximizes \( L \), instead of solving the equation

\[
\frac{\partial L}{\partial \varepsilon} = 0
\]

(A2.2)

for computational purposes we prefer to maximize \( \ln L \) with respect to \( \varepsilon \):

\[
\frac{\partial \ln L}{\partial \varepsilon} = \sum_{s=1}^{K} \left[ S(t_{s-1}) - S(t_{s-1} - z) \right] e^{-xS(t)} (N - NK)
\]

(A2.3)

from which \( \hat{\varepsilon} \) is obtained by numerical methods.

The variance of \( \hat{\varepsilon} \) is computed as

\[
s^2(\hat{\varepsilon}) = \left[ \frac{\partial^2 \ln L(\hat{\varepsilon})}{\partial \varepsilon^2} \right]^{-1}
\]

(A2.4)

which results in

\[
s^2(\varepsilon) = \left[ \sum_{s=1}^{K} N_s e^{xS(t_{s-1} - z)} \left( S(t_{s-1} - z) - xS(t_{s-1} - z) - e^{xS(t_{s-1} - z)} \right) \right]^{-1}
\]

(A2.5)

**APPENDIX 3**

Transmission efficiency in Peru

A) Transmission efficiency at the village of Chaute

A transmission study was carried out at the village of Chaute, Huarochiri province, Peru.\(^{27} \) It provided data of Shannon (outdoors) and Centers for Disease Control (CDC) (indoors) sand fly collections performed five times per month from April 1990 to May 1991, and new cases of cutaneous leishmaniasis (CL) occurred from June 1990 to May 1991. One Shannon trap with one human bait was operated from 5:00 pm to midnight 13 meters from the village boundary. Tree CDC traps were operated in different houses from 6:00 pm to 10:30 pm. The proportion of sand flies infected by *Leishmania peruviana* was determined by the a polymerase chain reaction in pools of more than 10 sand flies. *Lutzomyia verrucarum* (6,429 specimens) was the dominant species in the collections, followed by *Lu. peruensis* (1,499); the remainder consisted of two specimens of *Lu. noguchii* and one specimen of *Warileya phlebotomanaica*. Only two pools of sand flies were positive in the indoor collections from April 1990 to May 1991; these positive pools correspond to November and April 1999. To estimate the transmission efficiency, we made the following assumptions: 1) each positive pool contains one infected sand fly, 2) transmission occurs indoors, 3) the fraction (0.37) of the overnight sand fly population that is active during the 7:00 pm to 10:00 pm period obtained for *L. ovallesi*\(^{22} \) also holds for the present situation, and 4) the relationship between biting rate and CDC catches can be considered a fixed proportion, and has been estimated in a CDC catch-laying rate conversion factor of 0.580, after comparing biting rates and CDC catches over a wide range of sand fly abundances.\(^{29} \)

Under these assumptions, the number of positive sand flies that a CDC trap would have captured from April 1990 to May 1991 if it were operated all night and every day is calculated as

\[
\frac{2 \text{ positive sandflies} \times 30 \text{ days}}{5 \text{ days}} \times \frac{3 \text{ hours}}{4.5 \text{ hours}} \times 0.37 \times 0.58 = 21.622 \text{ positive sandflies}
\]

If bite allocation is random the number of new cases expected from June 1990 to May 1991 will be given by the expression

\[
\text{New cases} = \text{Susceptibles} \times (1 - e^{-21.622})
\]
from which \( \varepsilon \) can be easily obtained. A total of 14 new cases occurred between June 1990 and May 1991. The village of Chaute had at the time of the study 262 inhabitants, and 86.5% of 111 people had a positive reaction to the Montenegro skin test (MST). A transmission efficiency of 0.0044 is obtained if all of the 262 inhabitants are assumed to be fully susceptible, which increases to 0.0079 if we suppose that 50% of the MST+ people are protected against new lesions.

B) Transmission efficiency from five valleys in Peru

Sand fly abundance and sampling effort were given in a survey carried out in six valleys of Peru between March and July 1991.\(^{10}\) Sand flies were collected by CDC light traps left overnight in the bedrooms of randomly selected houses. Sand fly abundance in each house was monitored on two nights per visit; each house was visited at least in four consecutive occasions up to a maximum of seven. The catches were counted discriminating by species and sex. We assumed that *Lu. verrucarum* and *Lu. peruensis* are the CL main vector species.\(^8,29\)

For each valley, the annual number of infective bites (\( Ba \)) per person per year are calculated by the expression

\[
Ba = 365 \cdot \frac{Np \cdot Pp + Nv \cdot Pv}{Np + Nv} \cdot f \left( \frac{(Np + Nv)}{Se} \right)
\]

where \( Np \) and \( Nv \) are the total number of *Lu. peruensis* and *Lu. verrucarum* caught in CDC traps, \( Se \) is the sampling effort in house-nights, and \( Pp \) and \( Pv \) are the proportions of positive sand flies of each species. The function \( f(x) = 0.738 + 0.0903x^2 \) converts CDC sand fly catches in house-night units to sand flies biting people in person-night units.\(^{11}\) The proportion of positive *Lu. verrucarum* and *Lu. peruensis* were estimated from Table 3\(^{27}\) after pooling data of indoors and outdoors collections, and resulted in 0.0013 (6 of 4,645) and 0.0016 (2 of 1,214), respectively.

The transmission efficiency is calculated for each valley by

\[
\varepsilon = \frac{\ln(1 - \lambda)}{Ba}
\]

where \( \lambda \) is the annual force of clinical infection in each village from the longitudinal survey.\(^{10}\) The following table displays the landing rates (\( Ba \)), the forces of infection (\( \lambda \)), and the estimated \( \varepsilon \) for each of the 6 valleys in Peru.

<table>
<thead>
<tr>
<th>Valley</th>
<th>Ba</th>
<th>( \lambda )</th>
<th>( \varepsilon )</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>0.5992</td>
<td>0.0740</td>
<td>0.1283</td>
</tr>
<tr>
<td>P2</td>
<td>0.6878</td>
<td>0.0060</td>
<td>0.0088</td>
</tr>
<tr>
<td>C1</td>
<td>1.7955</td>
<td>0.0540</td>
<td>0.0309</td>
</tr>
<tr>
<td>C2</td>
<td>1.2747</td>
<td>0.0200</td>
<td>0.0158</td>
</tr>
<tr>
<td>H1</td>
<td>0.8273</td>
<td>0.0080</td>
<td>0.0097</td>
</tr>
<tr>
<td>A1</td>
<td>2.6822</td>
<td>0.1110</td>
<td>0.0439</td>
</tr>
</tbody>
</table>