

Cutaneous leishmaniasis in the Peruvian Andes: an epidemiological study of infection and immunity

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SUMMARY

A prospective longitudinal survey of cutaneous leishmaniasis (*Leishmania peruviana*) was carried out in Peru on a study population of 4716 persons living in 38 villages (Departments of Lima, Ancash and Piura). Demographic and clinical data were collected from all individuals, and a Montenegro skin test (MST) was carried out on 72% (3418) of the study population. Each household was revisited at 3-monthly intervals for up to 2 years to detect new leishmaniasis cases; 497 people received a second MST at the end of the study. Analysis of the epidemiological data indicated that (i) 17% (16/94) of all infections were subclinical, (ii) this percentage increased significantly with age, (iii) clinical infections led to 73.9% protective immunity (95% C.I. 53.0–85.5%) and relatively permanent MST responsiveness (recovery rate = 0.0098/year; 95% C.I. 0.000–0.020/year), (iv) sub-clinical infections led to protective immunity, which was positively correlated with their MST induration size (increasing by 17.9% per mm; $P < 0.0001$), and a mean MST recovery rate of 0.114/year (4/421 man-months), and (v) recurrent leishmaniasis was dominated by reactivations, not by reinfections.

INTRODUCTION

The clinical response to leishmania infection in humans depends on both parasite and host variation. Parasites demonstrate inter- and intra-specific genetic variation in virulence [1, 2], and clinical response is also influenced by parasite dose [3]. Similarly, human susceptibility is likely to have a genetic basis [4, 5], as well as non-genetic determinants, such as malnutrition [6].

Up to now, most studies on leishmania virulence have involved measurements of pathogenicity in laboratory animal models [e.g. 7, 8]. An alternative, but complementary, approach involves the epidemiological analysis of human populations at risk of leishmaniasis. Field surveys of cutaneous leishmaniasis invariably encounter people with no apparent history of disease, but who have a positive response to a Montenegro skin test (MST). The explanation usually

suggested for this apparent lack of specificity is that these people were exposed to sub-clinical leishmanial transmission, possibly because they were infected with avirulent (non-pathogenic) parasites [9–15].

Previous studies of the proportion of leishmania parasites which are avirulent have been largely qualitative, with the notable exception of Weigle and co-workers [14], who suggested that the 'relative pathogenicity' (r_p) of the parasite population in an endemic area can be estimated by 'the fraction of [MST] reactive individuals who manifest signs or a history of prior leishmanial lesions', as detected in a cross-sectional survey. The value of r_p calculated from previous cross-sectional studies varies considerably, for example: 0.02 [16], 0.03 [17], 0.07 [11], 0.11 [18], 0.22 [19], 0.25 [12], 0.67 [20], and 1.0 [21].

In addition to genetic variation in parasite virulence, r_p could be influenced by a number of factors [22]: (i) r_p will be negatively correlated with the extent of cross-protection that a sub-clinical infection confers against subsequent clinical infection; (ii) MST sensitivity will vary with dose, antigen type and storage conditions of the leishmanin [14]; (iii) a positive MST response could be induced by cross-reacting parasitic infections, such as glandular tuberculosis, lepromatous leprosy or lizard leishmania [23]; (iv) a positive MST response could be stimulated by a previous MST [24]; (v) the clinical response to leishmania infections will depend on human susceptibility; (vi) following human infection, there may be a latent period between MST conversion and clinical response; and (vii) cryptic infections could be due to mis-diagnosis [18]: scars could be 'lost' more quickly than MST positivity, due to a combination of skin ageing and patients' inability to remember their past infections with time. Thus cross-sectional epidemiological data are likely to be an unreliable indicator of either parasite virulence or host susceptibility.

The most direct evidence for sub-clinical infections comes from prospective longitudinal studies of leishmaniasis epidemiology, in which repeated MSTs are carried out at intervals on cohorts of initially asymptomatic and MST negative persons. For example, in a prospective study of cutaneous leishmaniasis in the municipality of Tumaco (on the Pacific coast of Colombia), where most parasites isolated from patients are *Leishmania panamensis*, the MST conversion rate was 6.6 per 100 man-years, of which 88.1% exhibited no symptoms [15].

Recurrent cutaneous leishmaniasis, i.e. repeated lesions in the same individuals, has been observed in most longitudinal surveys in Latin America [25]. However, the frequency of recurrences has rarely been measured quantitatively, and there have been few attempts to distinguish whether recurrences are due to reinfections (due to incomplete or temporary acquired immunity) or to reactivations of the primary infection. Perhaps the most successful attempt was made during a 3-year prospective survey of *L. panamensis* transmission in Tumaco [15], during which recurrences were significantly more frequent than primary infections. On the basis of the apparent genetic identity of parasites isolated from primary and secondary infections in the same patient, Saravia and co-workers [26] suggested that at least 50% of recurrent leishmaniasis in this endemic area were caused by relapses (i.e. the reactivation of persistent infections). However, the exact ratio of relapses: reinfections is difficult to estimate solely on the basis of genetic comparisons of parasite isolates, because there is a finite but unknown probability of re-infection

with the same parasite variant, and because primary infections may be genetically mixed infections [27].

In this paper, we take an epidemiological approach to measure variation in the course of leishmania infection in humans. In particular, we are concerned with the proportion of infections which induce a clinical response, the degree of acquired immunity following either clinical or sub-clinical infections, and the frequency of reactivations. The analysis derives from the results of a cross-sectional and prospective longitudinal field study on Andean cutaneous leishmaniasis (*uta*) in six Peruvian valleys, where the only parasite species isolated from patients with cutaneous lesions is *L. peruviana* [28, 29].

MATERIALS AND METHODS

Study sites

The study area consisted of 38 villages and hamlets located in six valleys, endemic for Andean cutaneous leishmaniasis, in the Departments of Lima (Provinces of Huarochiri [H] and Canta [C]), Ancash [A] (Province of Bolognesi) and Piura [P] (Province of Huancabamba). In this paper, study valleys are referred to as follows: H1, C1 (district, San Buenaventura), C2 (Arahuay), A1, P1 (Canchaque) and P2 (Sondor). All settlements are located between 1500 and 3000 m above sea-level. Houses are typically constructed of adobe bricks with a corrugated iron roof. The main activity is subsistence farming, supplemented with livestock. The valleys in Lima and Ancash are naturally unforested, with steep slopes and xerophytic vegetation; the land around the villages is irrigated to cultivate fruit trees, cereals, root vegetables and legumes. The valleys in Piura are flatter, and less arid: both have lost their primary vegetation (high jungle or paramo), and are now mostly cultivated with maize, legumes, root vegetables and (in P1 only) coffee and citrus fruits.

Cross-sectional survey

All houses within the study area were visited by the field team in March 1991 (C1 and C2), April 1991 (A1), May 1991 (P1 and P2) or July 1991 (H1). The houses were mapped and given a unique code number, and the following details were recorded by questioning every adult resident in each house: number of individuals in each household; name, sex, and date of birth of all permanent residents; the year (and previous place of abode) when householders came to live in their present village (if they had immigrated); and which individuals had *uta*, and in which year. Past cases of *uta* were identifiable by characteristic scars, which were detected during a physical examination by trained field workers or nurses. Ambiguous scars were checked by a specialist in leishmaniasis. Scars caused by *uta* have a central depressed surface, covered by thin hyperpigmented skin, and rounded contours (meaning no sharp angles) with fine concentric ring-like traces. Suspected current (active) cases of *uta* were detected by questioning, and confirmed by clinical examination (and, with consent, by parasitological diagnosis). By this process, the study population (N) at the time of the cross-sectional survey was divided into two groups: L^+ (number of persons with a scar or lesion) and L^- (number of persons with no known history of *uta*) at the time of

the cross-sectional survey. Lower case letters, l^+ or l^- , are used to designate the proportions of a particular (specified) sub-population who belong to either L^+ or L^- , respectively. Patients were provided with free treatment (Glucantime®).

Montenegro skin tests

A single batch of the antigen for skin-testing ($30 \mu\text{g}/\text{ml}$ of protein nitrogen in saline solution, with 0.005% thimerosal) was prepared by ultrasonication from a reference strain of *L. peruviana* (MHOM/PE85/LP052) and stored in vials at -20°C at the Instituto de Medicina Tropical 'Alexander von Humboldt' [30]. Following preliminary tests (on healthy and infected student volunteers) of the sensitivity, specificity and safety of the leishmanin, procedures for inoculation (0.1 ml) and for the reading of skin-tests (at 48 and 72 h post-inoculation) were standardized between field workers, according to the technique recommended by the World Health Organisation [31], and skin tests were applied to all consenting individuals in the study population. When readings were taken at two time-points, the larger of the two induration sizes was used to decide MST status. The cut-off point to designate a positive MST response was chosen empirically (see below). By this process, the study population with a recorded MST response at the time of the cross-sectional survey (N_m) was divided into two groups: M^+ , the number who had a positive MST response, and M^- , the number with a negative MST response; $M^?$ defines the number who were untested, so that $N = N_m + M^?$. As for clinical status, proportions of specified sub-populations are designated by lower case letters: m^+ and m^- . When the study population is divided according to both clinical and MST status, the sub-populations are defined by a combination of parameters; for example, M^+L^- ('cryptic infections') is the sub-population who belong to both M^+ and L^- .

Prospective longitudinal study

Following the initial cross-sectional study, house-to-house surveys were carried out in each valley at 3-monthly intervals. During the second and third visits to each valley, MSTs were given to some previously untested residents. In each visit, all new cases of uta were recorded (as during the initial survey), and patients were followed up to check on their response to therapy. When permitted, new patients were given a second MST. Births, deaths, immigrants and emigrants were also recorded. Demographic and clinical details were repeatedly checked during subsequent visits to ensure reliability. The final visits of the prospective study for the whole study population were in October 1992 (P2), November 1992 (H1), December 1992 (C2), January 1993 (A1), February 1993 (P1) and March 1993 (C1). Additional visits were made to A1, C1, C2 and P1 up to December 1993, during which more than 50% of the initially L^-M^- people, plus a few of the initially L^-M^+ people, were checked for lesions and given a second MST.

Models for the epidemiology of cutaneous leishmaniasis

The parameters to be estimated in this study can be defined precisely in terms of the mathematical model depicted as a flow diagram in Fig. 1, which is a modification of that described by Dye [22]. At any point in time, each individual

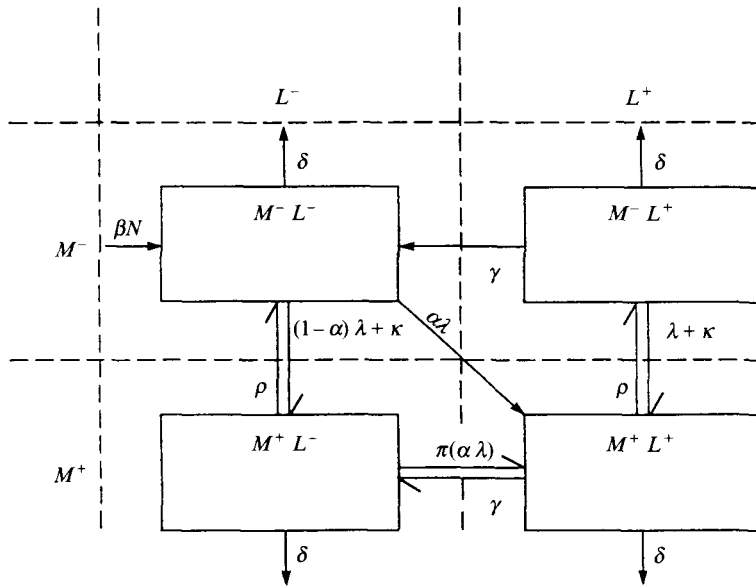


Fig. 1. Flow diagram depicting a model for *L. peruviana*. Since each person may be lesion/scar positive or negative, and MST positive or negative, there are four possible states: M^-L^- are susceptibles; M^+L^+ are those who have or have recently had uta; M^-L^+ have had uta but no longer respond to an MST; M^+L^- have been subclinically infected (by *L. peruviana* or some other cross-reacting parasite), or have been clinically infected but have lost their scar. Parameters describing the *per capita* rates of transition between states are β the human birth and δ the death rate, γ the rate of scar loss, ρ the rate of loss of MST positivity, λ the force of *L. peruviana* infection, κ the force of infection for a cross-reacting parasite, α the proportion of infectious bites by sandflies which produce a lesion followed by a scar, and π the relative risk of clinical infection for M^+L^- compared to M^-L^- .

in a population of size N is in one of four states: M^-L^- is the number of persons susceptible, M^+L^- is the number with cryptic infections, M^+L^+ is the number with a scar or lesion caused by uta, who have retained their positive MST response, and M^-L^+ is the number who, having had uta, have lost their MST responsiveness but have retained a detectable scar. Movement in and out of compartments depends on the relative values of λ , the force (instantaneous incidence) of infection for *L. peruviana*; α , the proportion of infections which lead to uta; π , the relative susceptibility to uta of people with cryptic infections; ρ , the rate of loss of a positive MST response; γ , the rate of scar loss; κ , the force of a cross-reacting infection; β and δ , the human birth and death rates.

Besides making clear definitions of parameters, the model also suggests how some of them may be estimated from age-prevalence data. Noting that the fraction of persons MST positive, $m^+ = [(M^+L^-) + (M^+L^+)]/N$, the partial differential equation describing the rate of change of m^+ with respect to age is

$$\frac{\partial m^+}{\partial a} = (\lambda_m + \kappa)(1 - m^+) - \rho m^+ \tag{1}$$

in which λ_m is the instantaneous rate at which individuals become MST positive

as a result of *L. peruviana* infection. The age-specific proportion MST positive, $m^+(a)$, is then

$$m^+(a) = \frac{\lambda_m + \kappa}{\lambda_m + \kappa + \rho} (1 - e^{-(\lambda_m + \kappa + \rho)a}). \quad (2)$$

Fitting equation (2) by maximum likelihood to the appropriate age-prevalence data, we can estimate $\lambda_m + \kappa$ and ρ , and their standard errors. A parallel expression for the age-specific fraction of persons with scars or lesions, $l^+(a)$ (where $l^+ = [M^+L^+ + M^-L^+]/N$), is not easily derived for any value of π . However, in the special case where $\pi = 1$,

$$l^+(a) = \frac{\lambda_1}{\lambda_1 + \gamma} (1 - e^{-(\lambda_1 + \gamma)a}). \quad (3)$$

Fitting equation (3) to the appropriate age-prevalence data gives an estimate of λ_1 , the force of infection for *uta*. It also provides an estimate of γ , but this will be misleading if π is substantially less than 1. In the case where $\kappa \approx 0$, equation (2) provides an estimate of λ_m . Then α is easily obtained from λ_1/λ_m .

We also want to know if the loss of MST positivity, at rate ρ , corresponds with recovery of susceptibility to *uta*, at rate σ . By analogy with the above, we write the age-specific incidence, $i(a)$, of *uta* in terms of λ_1 and σ . Assuming $i(a) = \lambda_1(1 - m^+(a))$, and noting again that $\lambda_1 = \lambda_m \alpha$,

$$i(a) = \lambda_m \alpha \left(\frac{\sigma + \lambda_m e^{-(\lambda_m + \sigma)a}}{\sigma + \lambda_m} \right). \quad (4)$$

Fitting equation (4) to age-incidence data, for a given value of α , yields an estimate of σ which may be compared with the estimate of ρ above.

RESULTS AND ANALYSIS

Montenegro skin test responses

The initial study population comprised 4716 persons (Table 1), of whom 50.2% were male (Table 2), from 38 villages within the 6 valleys. A further 203 persons (138 births and 65 immigrants) were recruited during the study period, a birth rate of 0.0194/man-year. An MST was carried out on 72% (3418) of the initial study population. Of these, 2500 (73%) were tested at two time-points (48 and 72 h). Using the conventional cut-off point of 5 mm, amongst those people tested twice, there was no significant difference in the proportion of people with a positive MST response at the two time-points ($P_{48} = 45\%$, $P_{72} = 45\%$, McNemar's $\chi^2 = 0.15$, 1 D.F., $P = 0.7$): only 2.4% changed in either direction. Of those who had a positive MST response at both time-points, the geometric mean induration was 5.6% greater at 48 h than at 72 h (15.94 and 15.10 mm, respectively; t test on log-transformed data, $t = 7.52$, $n = 1097$, $P < 0.0001$). In the following analyses of MST induration sizes, we use the 48 h time-point value, if available ($n = 3179$) and the 72 h value otherwise ($n = 226$); amongst N_m , there are 13 missing values at both time-points. To standardize the values at the two time points, we include a term in all regression analyses which adjusts the 72 h values to have the same overall mean as those taken at 48 h.

Table 1. Study population for the cross-sectional survey

Valley*	N†	Nm‡	n (village)§
A1	875	577	17
P1	907	707	2
C1	646	461	7
C2	708	468	4
P2	1114	852	3
H1	465	353	5
Total	4716	3418	38

* A, Ancash; P, Piura; C, Canta; H, Huarochiri. Specific locations of each valley given in text.

† The number of people censused in each valley.

‡ The number of people skin tested, whose MST response was recorded.

§ The number of villages censused in each valley.

Table 2. Distribution of the study population by age and sex

Age (years)	Female	Male	Total
0-9	660 (28)*	666 (28)	1326 (28)
10-19	445 (19)	500 (21)	945 (20)
20-29	259 (11)	252 (11)	511 (11)
30-39	223 (10)	248 (10)	471 (10)
40-49	224 (10)	210 (9)	434 (9)
50-59	218 (9)	194 (8)	412 (9)
60-69	177 (8)	167 (7)	344 (7)
70-79	101 (4)	101 (4)	202 (4)
80+	40 (2)	31 (1)	71 (2)
Total	2347 (100)	2369 (100)	4716 (100)

* Number within the specified age-group at the time of the initial cross-sectional survey (percentage in parentheses).

The bi-modal distribution of induration sizes (Fig. 2) confirms the effectiveness of the MST to distinguish between (supposedly) infected and uninfected individuals. However, the results suggest that the most suitable cut-off point should be 2 mm (not 5 mm). With one exception (see below), this alteration has no qualitative effect on the conclusions drawn, as only 29/3418 people are affected. The estimated parameter values are largely insensitive to the changes in cut-off point, although the probability values are more variable. On empirical grounds we have therefore chosen to use 2 mm as the cut-off in all the analyses presented below. Thus, in this paper, a positive MST response indicates an induration size greater than or equal to 2 mm at either 48 or 72 h.

Fig. 2 illustrates the frequency distribution of induration sizes in persons with active lesions, scars or with no apparent symptoms. The geometric mean induration size for M^+L^+ people, 16.06 mm was significantly higher than that for M^+L^- people, 11.91 mm (t -test on log-transformed data: $t = 8.93$, 1658 D.F., $P < 0.0001$).

Transmission rates

Amongst the whole study population, m^+ was 0.494 (1690/3418). The valleys differed markedly with respect to the transmission rate of cutaneous leishmaniasis, as characterized by a variety of parameters, of which incidence amongst M^- people

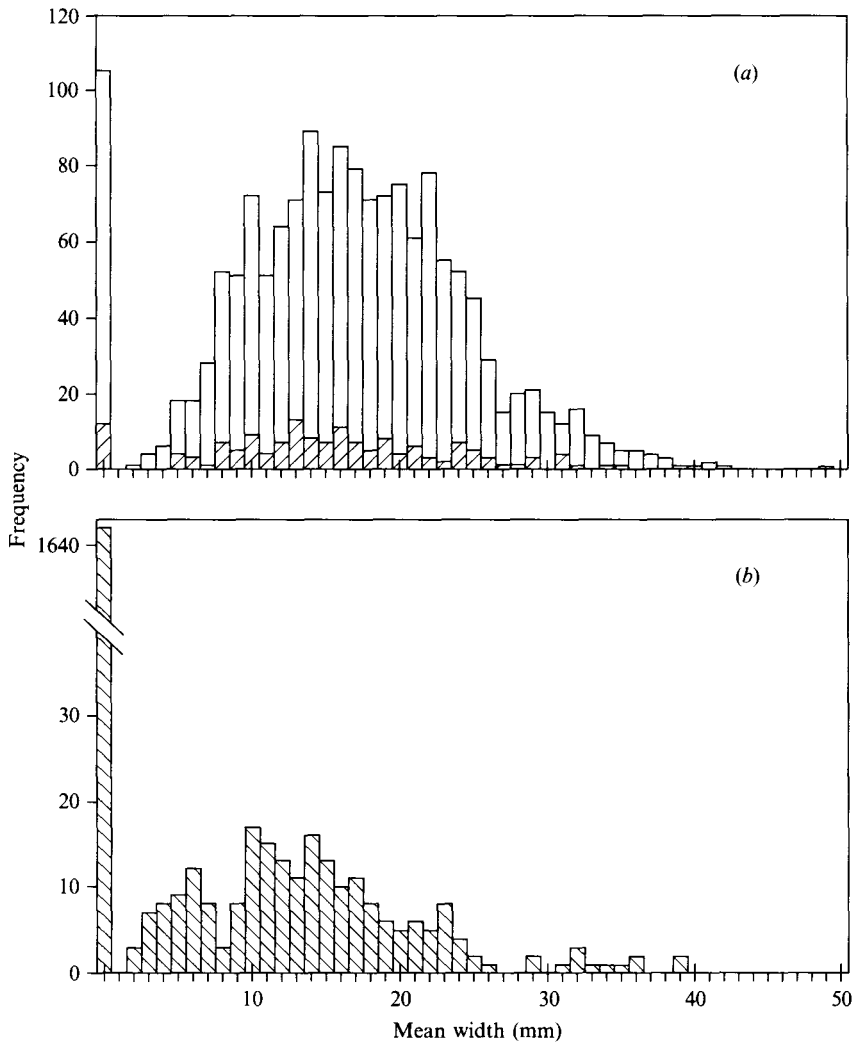


Fig. 2. The frequency distribution of MST induration sizes amongst the whole study population for (a) the L^+ population (hatched = active patients, open = scarred), and (b) the L^- population.

generated the greatest maximum: minimum ratio (Table 3), suggesting that it is the most sensitive measure of transmission rate. There was no significant difference between the transmission rates in males and females, whichever measure was used; for example, m^+ was 0.487 in males and 0.501 in females ($\chi^2 = 0.67$, 1 D.F., $P > 0.4$).

Proportion of infected persons with scars or lesions

From the cross-sectional survey, the fraction r_p was calculated for each village, where $r_p = (M^+L^+)/M^+$. A positive relationship was detected between the village values for m^+ and r_p (Fig. 3; Pearson correlation, weighted by N_m : $r = 0.582$, $n = 38$, $P < 0.0001$). Amongst the whole study population, r_p was 0.863 (1459/1690).

Table 3. Prevalence and incidence rates in each valley

Valley*	Cross-sectional			Prospective	
	P†	$l^+‡$	$m^+§$	I (cases/man-yr)	IM-¶
A1	0.076	0.737	0.780	0.041	0.111
P1	0.068	0.649	0.709	0.028	0.074
C1	0.061	0.453	0.505	0.046	0.054
C2	0.047	0.438	0.496	0.016	0.020
P2	0.012	0.134	0.163	0.006	0.006
H1	0.006	0.391	0.382	0.004	0.008

* See Table 1.
 † Proportion of the skin tested population ($n = 3418$) with a lesion during the cross-sectional survey (1991).
 ‡ Proportion of the skin tested population with a lesion or scar during the cross-sectional survey (1991).
 § Proportion of the skin tested population with a positive MST response during the cross-sectional survey (1991).
 || Crude incidence rate (cases/man-year) for the whole population ($n = 4919$) during the prospective survey (1991–3).
 ¶ Incidence rate (cases/man-year) during the prospective survey (1991–3) amongst the sub-population who were M^- , i.e. with a negative MST response ($n = 1728$), during the initial cross-sectional survey.

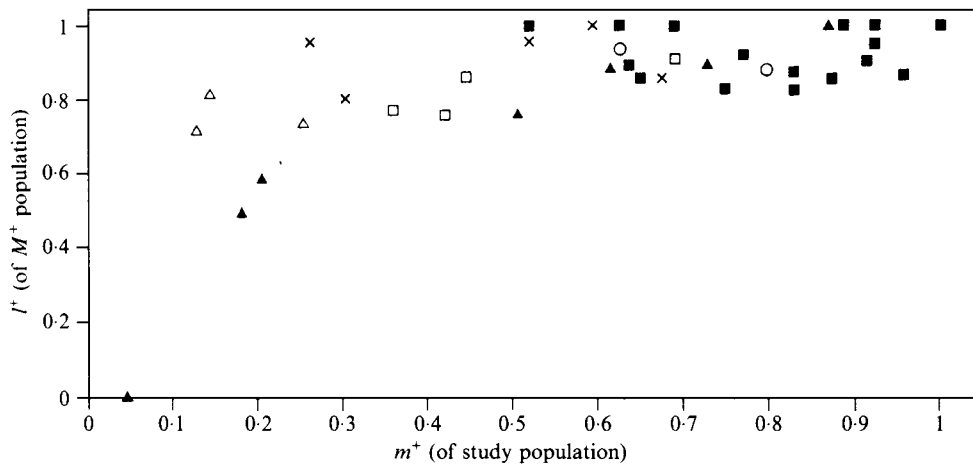


Fig. 3. The relationship between m^+ (the proportion of a village population who are M^+) and r_p (the proportion of the M^+ population who are L^+). Each point represents the value of r_p and m^+ for one of the 38 endemic villages in the study population. ■, A1; ○, P1; ▲, C1; □, C2; ×, H1; △, P2.

Subclinical infection rates

Longitudinal data

Of 877 people in C1, C2, A1 and P1, who were M^- when first tested, 480 (55%) were retested in 1992 or 1993 (i.e. after a mean period of 21 months). Of these 480,

3.3% (16) converted to M^+ with no associated disease symptoms, indicating a sub-clinical infection rate of 0.019/man-year; and 16.3% (78) converted to M^+ in association with the development of a cutaneous lesion, indicating a clinical infection rate of 0.093/man-year. Thus the proportion of conversions associated with lesions, $\alpha = 0.830$ (78/94). This proportion was the same in the northern (P1) and southern (A1, C1, C2) field sites: 0.85 (44/52) and 0.81 (34/42), respectively. However the overall conversion rate was significantly greater in the northern sites, 27.2% (52/191), than in the southern sites, 14.5% (42/289) ($\chi^2 = 11.76$, 1 D.F., $P < 0.001$).

A significant association was detected between age and the proportion of infections associated with lesions. The geometric mean ages of those converting were 15.01 (95% C.I. 9.83–22.91 years) and 7.66 years (95% C.I. 6.42–9.14 years), for subclinical and clinical infections, respectively (t test on log-transformed data: $t = 3.085$, 92 D.F., $P < 0.005$).

Of the 78 persons who demonstrated a clinical response in association with MST conversion, four developed a lesion after their positive MST response was detected (between 3 and 9 months afterwards). In the analysis presented above, these conversions have been counted as clinical infections, on the assumption of a relatively long incubation period prior to lesion development (although we cannot discount the possibility of subsequent clinical infection following a preliminary sub-clinical infection). It follows that some of the 16 people with apparent subclinical infections may represent clinical infections. These people were followed up for an average of 8 months (range: 1–15 months) after their positive MST response. By examining those people with asymptomatic conversions who have been followed up for at least 9 months, we estimate that the probability that a sub-clinical infection is followed by lesion development within the first 9 months is 33% (4/12). By excluding the possibility that latency can extend beyond 9 months (the maximum observed in this study), we predict that a third (2.7) of the eight sub-clinically infected people who have been followed up for less than 9 months may yet develop lesions. In this scenario, our best estimate for α would be 0.859 (80.7/94).

Cross-sectional data

Fig. 4 illustrates the age prevalence curves for m^+ and l^+ in A1, P1, C1 and C2. Given the similarity in the transmission rates (Table 3) of the two study sites in Canta, as well as their close geographic proximity, the data from C1 and C2 have been combined in Figure 4. In contrast, the data from A1 and P1 are treated separately, as the transmission rates in these valleys are sufficiently high for independent analysis. The low transmission rates in P2 and H1 (Table 3) do not permit us to fit either λ or ρ by maximum likelihood from the cross-sectional data. The age prevalence curves in A1 and P1 are characterized by a number of features. In particular, the data describe simple monotonic curves (at least in some valleys) with asymptotes less than one. This suggests either a relatively constant transmission rate during recent history, or that historical variation is masked by the high incidence rates. In contrast, the transmission rates in C1 and C2 were temporarily reduced during the period of the DDT campaign, and have since risen [32]; and this historical variation is reflected in the shape of the age prevalence

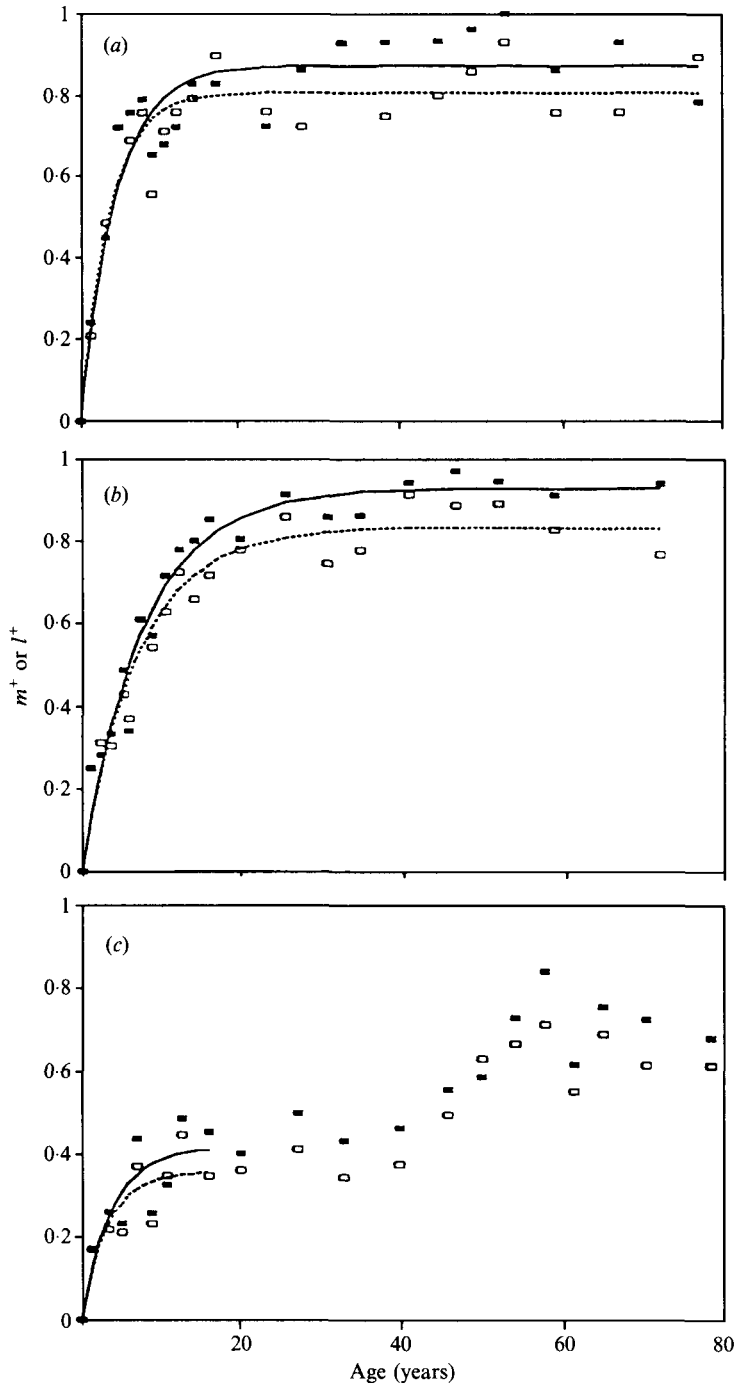


Fig. 4. The age prevalence curves for m^+ (solid line and filled squares) and l^+ (dotted line and clear squares) detected during the first cross-sectional survey in 1991 in (a) A1, (b) P1, (c) C1+C2. Each point represents the proportion positive for the sub-population within a particular age-range. Data sets were divided into 20 age groups, by equalizing the size of the sub-population in each age-range. The lines have been drawn by maximum likelihood fit to the infection-recovery model (see text).

Table 4. Force of infection estimated from cross-sectional data

Valley*	λ_m^\dagger	λ_1^\ddagger
A1	0.202 ± 0.035	0.228 ± 0.044
P1	0.118 ± 0.013	0.114 ± 0.015
C1 + C2	0.109 ± 0.060	0.107 ± 0.061

* See Table 1.

† Force of infection for skin test conversions (\pm s.e.) estimated by maximum-likelihood from the age prevalence data using equation 2 (see text).

‡ Force of infection for lesions (\pm s.e.) estimated by maximum-likelihood from the age prevalence data using equation 3 (see text).

curves. Thus, for fitting values for λ and ρ in C1 + C2 (Tables 4, 5), we have used data only for those aged up to 15 years, as the transmission rate during the last 15 years appears to have been relatively constant.

Table 4 illustrates the estimates for λ_1 and λ_m made by fitting the cross-sectional data to equations 2 and 3, respectively (assuming $\kappa = 0$). For each study site, the estimates of λ made from the prospective study (Table 3) are lower than those from the cross-sectional data, indicating that transmission rates were unusually low during our prospective survey. In all valleys, the ratio λ_1/λ_m is not significantly less than 1, indicating that α is close to 1. This result can be visualized by comparing the slopes at the origin of the paired age-prevalence curves within each graph (Fig. 4). However, the high standard errors (Table 4) show that the data are not inconsistent with the longitudinal estimate of λ_1/λ_m (0.859).

Evidence that the majority of cryptic infections detected in the cross-sectional study are not due to cross-reacting parasites arises from a comparison of l^+ , the cumulative prevalence of scars or lesions, in each village with M^+L^-/L^- : a Spearman correlation was used due to the non-normal distribution of the data (Fig. 5; $r = 0.408$, $n = 35$, $P < 0.02$). If most cryptic infections are due to cross-reactants, the null hypothesis is that there is no positive relationship between the transmission rate of uta and the transmission rate of cryptic infections. The significant positive relationship between the prevalence of clinical and cryptic infections indicates that cross-reactants only account for a small fraction of cryptic infections (i.e. κ is close to zero).

Acquired immunity: MST response

Longitudinal data

The rate of loss of MST positivity for M^+L^- people was estimated by a prospective longitudinal analysis of 17 people who were retested after an average of 25 months, during which they had not developed any clinical symptoms: four people reverted to M^- status, representing a recovery rate of 0.114/year (4/421 man-months).

For L^+ people, we use a retrospective longitudinal analysis to calculate changes with time since infection in (a) the fraction of L^+ persons who are M^+ , and (b) the mean induration size of a positive MST response.

(a) The rate of loss of MST responsiveness amongst M^+L^+ , ρ , estimated by logistic regression of m^+ against time since infection, was 0.0098/year (95% C.I.

Table 5. Rate of loss of MST positivity and rate of recovery of susceptibility

Valley*	ρ^\dagger	σ^\ddagger
A1	0.0291 \pm 0.051	0.0345 \pm 0.117
P1	0.0090 \pm 0.017	0.0053 \pm 0.050
C1 + C2	0.1526 \pm 0.128	0.0372 \pm 0.063

* See Table 1.

† The rate (/year) at which MST positivity is lost (\pm s.e.), estimated by maximum likelihood from the age prevalence data using equation 2 (see text).

‡ The rate (/year) at which susceptibility to clinical infection is recovered (\pm s.e.), estimated by maximum likelihood from the age incidence data using equation 4 (see text).

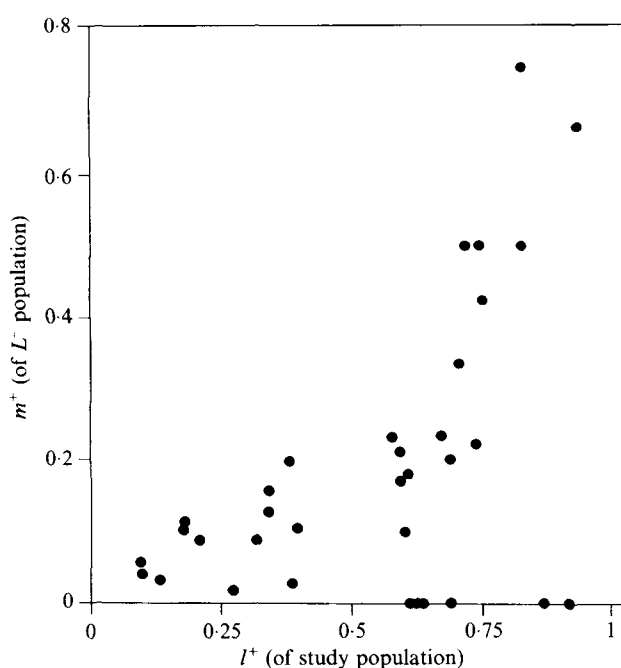


Fig. 5. The relationship between l^+ (the proportion of a village population who are L^+) and the proportion of the L^- population in a village who are M^+ . Each point represents the paired values for one of the 38 endemic villages in the study population.

0.000–0.020/year, $n = 1396$, $P = 0.06$). However, when the data were standardized for transmission rate (l^+) in each village, our best estimate for the reduction rate of MST responsiveness was 0.0089/year (95% C.I. –0.0013–0.0192/year, $n = 1396$, $P = 0.09$). In addition, m^+ amongst L^+ persons was significantly higher in villages with higher transmission rates, as measured by l^+ (logistic regression, $n = 1396$, $\chi^2 = 20.54$, $P < 0.0001$). Thus the longitudinal data provide evidence that MST responsiveness is not permanent, and that the rate of loss for M^+L^+ people is apparently less than that for M^+L^- people (as estimated from the prospective study). In addition, the data indicate that in areas of high transmission the rate of loss of MST responsiveness was balanced by the re-infection rate.

Alternatively, the data could be explained by mis-diagnosed lesions – which would account for a smaller proportion of all L^+ people in areas of high leishmania transmission.

(b) By regression analysis of the log transformed data, stratified by village, there is an indication that induration sizes in M^+L^+ persons are negatively correlated with time since infection, with a reduction rate of 0.00094/year (95% C.I. –0.00018–0.00205/year, $n = 1298$, $P = 0.1$). There was also a highly significant positive correlation between village transmission rate (l^+) and induration size (regression on log-transformed data, $n = 1298$, $t = 5.26$, $P < 0.0001$).

Cross-sectional data

By fitting curves to the age-prevalence data, we can estimate ρ from the infection-recovery model (Table 5, equation 2). These estimates represent the maximum possible values for ρ , due to the heterogeneity amongst the human population with respect to the risk of infection. The estimate of ρ fitted from the age prevalence data in C1 and C2 is unreliable, as it is significantly influenced by the apparent historical variation in transmission rate (Fig. 4). Nevertheless, given the large standard error in C1 and C2, none of the estimated values of ρ is significantly different from the rate calculated from the longitudinal analysis of the L^+ population.

Whilst it is clear that MST responsiveness is lost very slowly by M^+L^+ people, this is an indirect measure of protection status. To examine the extent and permanence of protection that ensues from *L. peruviana* infections, we need to analyse the risk of subsequent clinical infections.

Acquired immunity: clinical response

Longitudinal data

In the whole study population, the mean age at clinical infection observed during the prospective study (15.4 years) was considerably less than the mean age in the population (27.6 years). Furthermore, in village comparisons of recent cases (since January 1990), a significant negative relationship was detected between transmission rate (m^+) and the mean age when clinically infected (Fig. 6; linear regression: mean age = 25.25–16.62 (m^+) years. $F = 8.528$, $n = 32$, $P = 0.0066$). These results are most simply explained by acquired immunity. From the equation, we can see that in a completely susceptible population ($m^+ = 0$), we expect the mean age at clinical infection to be 25.25 years (95% C.I. 18.93–31.57), which is not significantly different from the mean age in the population (27.6 years).

Protection due to acquired immunity can be directly calculated from the prospective data. After adjusting for age, the incidence in M^+ people was on average only 0.261 (95% C.I. 0.145–0.470) of the rate in M^- people (Table 6; Cox proportional hazards regression, stratifying by village: $\chi^2 = 22.08$, $P < 0.0001$). Amongst M^- people, there was a highly significant reduction in incidence with age of 2.1%/year ($\chi^2 = 8.58$, $P < 0.01$); and amongst M^+ people, there was also an indication of a reduction in incidence with age of 1.2%/year ($\chi^2 = 2.93$, $P < 0.10$).

In theory, our most reliable measure of π should come from a comparison of incidence rates in M^+L^- versus M^-L^- people. A Cox proportional hazards

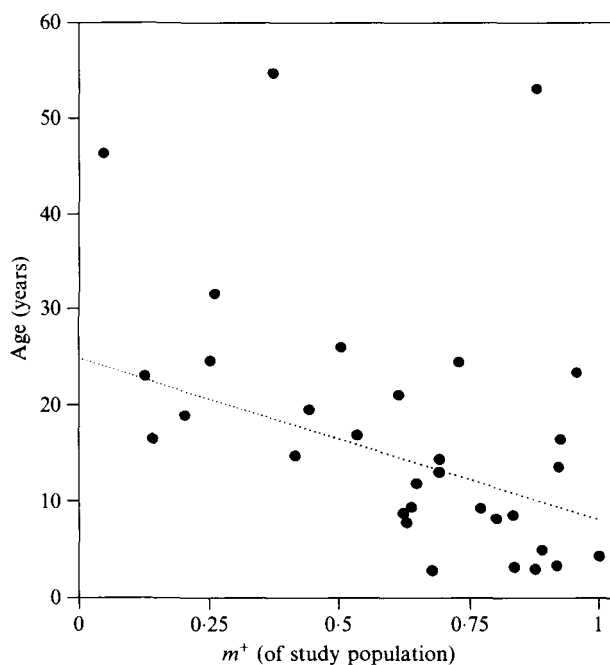


Fig. 6. The relationship between m^+ and the mean age when people were clinically infected (between 1990 and 1992). Each point represents the paired values for one of the 38 endemic villages in the study population. The line is drawn by linear regression (see text).

Table 6. Incidence rates in the M^- and M^+ populations

Valley*	M^- population† I (cases/man-yrs)§	M^+ population‡ I (cases/man-yrs)
A1	0.111 (21/189.2)	0.026 (18/704.3)
P1	0.074 (24/324.8)	0.010 (8/827.8)
C1	0.054 (20/370.1)	0.041 (15/362.6)
C2	0.020 (7/355.4)	0.005 (2/373.8)
P2	0.006 (6/938.8)	0.011 (2/176.9)
L1	0.008 (2/263.7)	0 (0/173.6)

* See Table 1.

† People who had a negative MST response during the initial cross-sectional survey.

‡ People who had a positive MST response during the initial cross-sectional survey.

§ Incidence rates (number of new cases/number of man-years) during the prospective survey (1991–3).

regression, stratified by valley, found no evidence that this risk ratio, 0.864 (95% C.I. 0.50–1.49), was significantly less than 1 ($\chi^2 = 0.283$, $P = 0.6$). However, amongst M^+L^- people, there was a significant negative correlation between incidence and MST induration size, with incidence decreasing by 17.9%/mm. (Cox proportional hazards regression, stratified by valley: $\chi^2 = 15.57$, $P < 0.0001$); and the geometric mean induration size amongst those M^+L^- people infected during the prospective study was 6.54 mm (95% C.I. 4.62–9.25 mm), as compared to 11.91 mm for the whole M^+L^- population (see above).

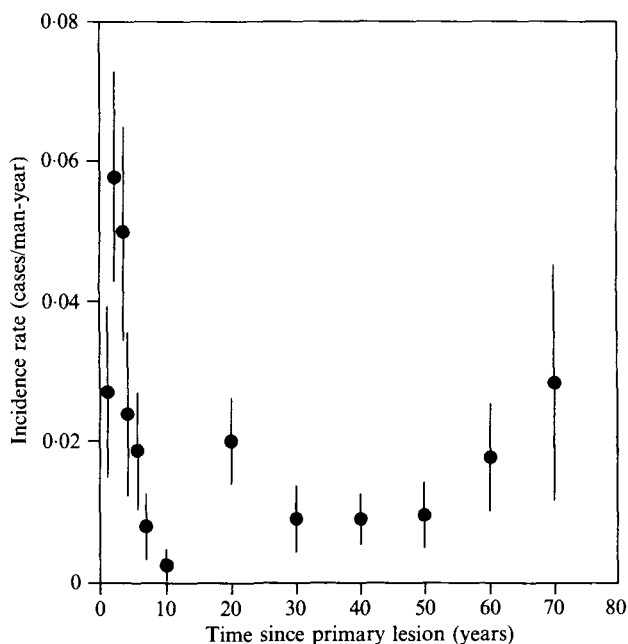


Fig. 7. Annual incidence rates of secondary lesions, $\pm 95\%$ confidence intervals, between 1990 and 1993 according to time since the primary lesion (estimated retrospectively). The x axis refers to the first year of the time periods during which each mean incidence rate was calculated.

The recovery rate of susceptibility (σ) can be deduced in those sites where incidence was sufficiently high to analyse (A1, P1, and the combined Canta valleys: C1 + C2), by fitting the age-incidence data to equation 4. In each site, there was no significant difference between our estimates for σ and ρ (Table 5), indicating that π is not significantly different from zero. But the standard deviations are large, so that this is not a very sensitive test, and the data are not inconsistent with our estimates of π from the prospective analysis.

Recurrent leishmaniasis: reactivation or reinfection?

From the longitudinal data, we can see that the majority of cases of recurrent cutaneous leishmaniasis occur within a few years of the primary infection (Fig. 7). This result is highly indicative of relapses. For comparison, the mean incidence rate in the unscarred population, weighted by valley, during the same period was 0.0731/year (95% C.I. 0.0638–0.0824/year).

Another way to distinguish relapses from reinfections is to compare the seasonal variation in incidence rates: reinfections should have the same seasonal patterns as primary infections, whilst relapses should have no particular seasonal pattern. For Andean cutaneous leishmaniasis, incidence rates are highest towards the end of the rainy season, when sandfly abundance is greatest [33]. This pattern was repeated for primary infections during our prospective study, but no seasonality was observed for recurrent leishmaniasis cases during the same study period (Fig. 8).

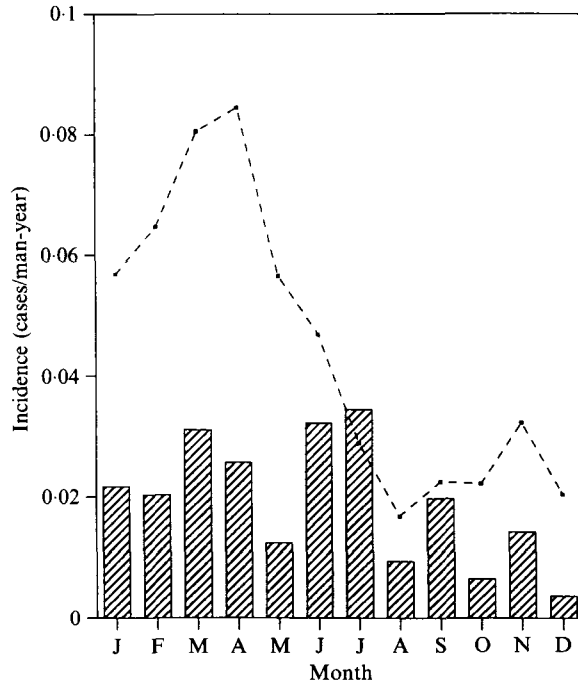


Fig. 8. The mean monthly incidence rates in the whole study population during the prospective study (1991-2) for primary lesions (dashed line) and recurrent leishmaniasis (hatched bars).

DISCUSSION

Subclinical infection rates

Our best estimate for the percentage of infections which are subclinical, from the prospective data, is 14-17%, and this is consistent with values from cross-sectional data, which give less precise estimates of α . The positive correlation detected between m^+ and r_p across villages is most simply explained by a variable force of infection and a constant rate of scar loss in each village [22]: variation in r_p does not necessarily imply variation in the parasite populations circulating in each village.

The possibility that all apparent subclinical infections detected in the prospective survey represent clinical infections with a long incubation period cannot be discounted; however, no incubation periods greater than 9 months have yet been detected. Furthermore, it is difficult to distinguish long incubation periods from new clinical infections. We reject the hypothesis that sub-clinical infections are mainly due to cross-reactions, on two grounds. Firstly, the correlation between the subclinical and clinical infection rates (detected in both the cross-sectional village comparison and the prospective regional comparison) is evidence that both agents of infection have the same transmission route; and secondly, the evidence that M^+L^- people have protection against subsequent clinical infections (proportional to their MST induration size). However, we cannot distinguish from these data whether subclinical infections are due to low parasite virulence, low parasite dose, or low human susceptibility.

Why the estimate of α (0.12) in the study of *L. panamensis* epidemiology in Tumaco [15] is much less than in our study of *L. peruviana* is unclear. Comparisons of epidemiological studies are hampered by a series of confounding variables. For example, *L. panamensis* populations could be intrinsically more phenotypically heterogeneous than *L. peruviana* populations; alternatively, the MST used in Tumaco may have been less specific than in our study, with a higher probability of detecting cross-reacting infections, which induce temporary MST responses. This would explain the relatively low cumulative MST prevalence amongst all age groups in Tumaco. However, the same pattern could also be due to a recent increase in transmission rate. Future studies of asymptomatic leishmania infections may be aided by the development of molecular tools, which are more sensitive and specific than the MST. For example, the polymerase chain reaction can detect *L. braziliensis* DNA in the blood of patients whose lesions have self-healed many years earlier [34].

Acquired immunity

Clinical infections with *L. peruviana* lead to acquired immunity, as demonstrated by the negative correlation between the transmission rate and the mean age of infection in a village. Although this type of association has been alluded to in previous spatial [35] or temporal [36] comparisons of cutaneous leishmaniasis, we believe that our study provides the first quantitative measures of the relationship. Our finding that the predicted mean age at clinical infection in a fully susceptible (M^-) village population was the same as the mean age of the study population provides strong evidence that the transmission rate of uta is equal for all ages (as suggested by the age-prevalence curves of MST status). It follows that the significant decrease detected in the incidence of uta in M^- people with age is probably an artifact from combining data from a heterogeneous population. The presence of a human sub-population at low risk would generate an apparent decrease in transmission rate with age for the whole population.

From the prospective data, we estimate that people acquire 74% protection following MST conversion. However, if some secondary lesions were due to relapses, the actual protection against reinfections may be greater. A very slow rate of reduction in MST responsiveness (0.0089/year) was detected from the retrospective longitudinal data. This could be an underestimate due to the continuous risk of reinfection, which is indicated by our finding that the proportion of the scarred population in a given village who are M^- was negatively correlated with the village transmission rate. Our estimate of MST recovery contrasts with several previous attempts to measure the recovery rate of MST positivity from retrospective data. For example, M^+ amongst (presumed) *L. braziliensis* patients decreased from 0.67, during the first year after treatment, to 0.37 between years 2 and 7 [37]; in another study of *L. braziliensis* patients, M^+ decreased from 0.96 (within the first 3 years post-infection) to 0.70 after 20 years [10]. These results must be interpreted with caution, given the uncertainty of retrospective data with respect to the original infection event. Prospective surveys, in which MST recovery rates are measured by sequential MSTs, are more reliable. In two such studies of American cutaneous leishmaniasis, high recovery rates were again observed: 53% in 4 years [9]; and 15% in 6–48 months [14].

However, in both studies it is unclear whether the recovery rates of clinically and subclinically infected people were the same.

The rate of loss of MST responsiveness calculated from the cross-sectional data (in P1, A1 and C1+C2) is less reliable, as the model assumes (i) equal risk of infection for the whole population, and (ii) constant λ with time and age. The first assumption is unrealistic: there will be heterogeneity in risk between individuals, households and settlements. This heterogeneity leads to an overestimate of the rate of loss of MST responsiveness. The second assumption could also lead to an overestimate of ρ , if the transmission rate has significantly increased during recent years (as in Canta), or if there is a reduction in transmission rate with age. Despite these caveats, the data consistently demonstrate a low rate of loss of MST responsiveness, which is not significantly different from the recovery rate of susceptibility, as estimated from the age-incidence curves.

Immune status following subclinical infections

The lower mean induration size in M^+L^- versus M^+L^+ people could be due either to a lower immune response generated by subclinical infections, or to a reduction in immune response with time since infection (M^+L^- people who have been infected a relatively long time in the past may have lost their scars). Our failure to detect a significant difference between the incidence rates of uta in M^+L^- and M^-L^- people is probably because the comparison was made between populations that are incompletely matched with respect to variation in transmission rate. Eliminating the source of this error, by analysing only the M^+L^- population, demonstrated a highly significant negative correlation between MST induration size and incidence, confirming that $\pi < 1$. However, protection amongst M^+L^- people (as for all M^+ people) is certainly not complete, and the relatively high rate with which M^+L^- people lose their MST responsiveness suggests that the immune response to sub-clinical and clinical infections is not the same.

Our finding that the MST response in M^+L^+ people was significantly higher than in M^+L^- people mirrors several previous studies of American cutaneous leishmaniasis which have detected significant associations between MST induration size and clinical response. For example, MST induration size was negatively correlated with both the presence of granulomas and the lymphocyte transformation response [38], and positively correlated with fibrinoid necrosis and severity of disease [39]. In contrast, Saravia [40] found no correlation between MST response and lesion size, although there was an association between MST response and *Leishmania* species.

Clearly, we cannot distinguish whether the low susceptibility status of M^+L^- people is acquired or innate. Indeed, we cannot discount the possibility that a significant fraction of the M^+L^- people detected in the cross-sectional survey could be due to scar loss, and that the rate of scar loss (γ) is greater than the rate of loss of MST positivity (ρ). This hypothesis is impossible to test directly, as estimates of γ fitted from the age prevalence curves (equation 3) are unreliable, given that the model assumes $\pi = 1$. It follows that the degree of protection acquired from sub-clinical infections can only be estimated satisfactorily from long-term prospective studies in which the source of M^+L^- status is known.

Evidence that host factors must play some role in determining protection status

emerges from the age-related trend in α , as there is no difference in the parasite populations infecting the different age groups. Indeed, the results are consistent with a mixed human population: in one (relatively susceptible) sub-population, infections tending to produce a relatively permanent MST response and a lesion, and in the other (relatively resistant) sub-population, infections tending to produce a temporary MST response and no lesion. Thus, amongst the M^- population in endemic areas, the older age groups would be increasingly dominated by the resistant sub-population, so accounting for the reduction in α with age. Alternatively, the age-dependency of α may reflect a real increase in the effectiveness of the immune system with ageing. A similar negative correlation between age and α was detected in Tumaco [41], leading the authors to conclude that *L. panamensis* infections are more pathogenic in younger people.

Reactivation rates

The retrospective data are consistent with a reactivation rate, which decreases towards zero during the first 10 years post-infection, combined with a reinfection rate, which slowly increases with time post-infection (presumably due to a gradual loss of immunity). Even 70 years after the first lesion, the mean annual rate of recurrent leishmaniasis for the study population (0.029/man-year) is only 39.7% of the mean annual incidence (standardized by valley) in the unscarred population (0.073/man-year). Most secondary cases of leishmaniasis occur within a few years of the primary lesion, indicating that recurrent leishmaniasis is largely due to relapses. Further evidence for a high relapse: reinfection ratio is provided by the lack of seasonality in the incidence of secondary lesions, in contrast to the strong seasonality in the incidence of primary lesions.

In one of the few prospective studies to examine the risk of recurrent cutaneous leishmaniasis following infection [15], the presence of a previous scar was found to increase significantly the risk of acquiring a new lesion (an 11-fold increase amongst M^+ people). The most likely explanation is that recurrent leishmaniasis during the Colombian study was dominated by relapses, rather than reinfections, and that the relapse rate was significantly greater than the transmission rate. This contrasts with the relatively low ratio of recurrent leishmaniasis: primary lesions detected in our study – even in areas of low transmission. Despite anecdotal evidence indicating significant interspecific variation for different *Leishmania* species, the factors determining the relapse rate are little understood [25]. The relapse rate for *L. peruviana* appears to be closer to the low levels suggested for *L. major*, than to the relatively high levels observed amongst other members of the *L. braziliensis* complex or *L. tropica*. However, where leishmania parasites persist after an initial infection has cured, the risk of relapses will be enhanced by immunosuppression [42]. Thus, variation in the relapse rate will be influenced by the status of the human population, as well as by intrinsic features of the parasite population.

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