The epidemiology and control of leishmaniasis in Andean countries

Epidemiologia e controle da leishmaniose nos países andinos

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Abstract *This paper reviews the current knowledge of leishmaniasis epidemiology in Venezuela, Colombia, Ecuador, Peru, and Bolivia. In all 5 countries leishmaniasis is endemic in both the Andean highlands and the Amazon basin. The sandfly vectors belong to subgenera Helcocyrtomyia, Nyssomiya, Lutzomyia, and Psychodopygus, and the Verrucarum group. Most human infections are caused by Leishmania in the Viannia subgenus. Human Leishmania infections cause cutaneous lesions, with a minority of L. (Viannia) infections leading to mucocutaneous leishmaniasis. Visceral leishmaniasis and diffuse cutaneous leishmaniasis are both rare. In each country a significant proportion of Leishmania transmission is in or around houses, often close to coffee or cacao plantations. Reservoir hosts for domestic transmission cycles are uncertain. The paper first addresses the burden of disease caused by leishmaniasis, focusing on both incidence rates and on the variability in symptoms. Such information should provide a rational basis for prioritizing control resources, and for selecting therapy regimes. Secondly, we describe the variation in transmission ecology, outlining those variables which might affect the prevention strategies. Finally, we look at the current control strategies and review the recent studies on control.*
Key words *Leishmaniasis; Andean Ecosystem; Endemic Diseases; Vector Control*

Resumo *Este trabalho revisa o conhecimento atual sobre a epidemiologia da leishmaniose na Venezuela, Colômbia, Equador, Peru e Bolívia, países nos quais a doença é endêmica, tanto nos Andes quanto na Amazônia. Os vetores flebótomos pertencem a vários subgêneros e ao grupo Verrucarum. A maioria dos casos de infecção humana é causada pelos parasitas Leishmania do subgênero Viannia. As infecções humanas por Leishmania provocam lesões cutâneas, com uma minoria de infecções por L. (Viannia) levando à leishmaniose mucocutânea. Tanto a leishmaniose visceral quanto a leishmaniose cutânea difusa são raras. Em cada país, parte significativa da transmissão de Leishmania ocorre no intra ou peridomicílio, muitas vezes próximo à lavoura de café ou cacau. Não se sabe ao certo quais são os hospedeiros reservatórios para os ciclos de transmissão doméstica. Discute-se a carga da doença provocada pela leishmaniose na região, chamando atenção para os coeficientes de incidência e para a variabilidade dos sintomas. Tal informação fornecerá uma base racional, visando priorizar os recursos voltados para o controle da doença e selecionar esquemas terapêuticos. Os autores também descrevem a variação na ecologia da transmissão, delineando as variáveis que poderiam afetar a definição de estratégias preventivas.*
Palavras-chave *Leishmaniose; Ecossistema Andino; Doenças Endêmicas; Controle de Vetores*

The incidence, distribution, and heterogeneity of the leishmaniases

Incidence rates

National data on the annual incidence of leishmaniasis are notoriously unreliable, and may underestimate true case numbers from 2-fold (Yadon et al., in press) to 40-fold (Copeland et al., 1990) in rural areas, depending on the effectiveness of the public health infrastructure. Hence, case notifications in endemic urban sites tend to be much more efficient, e.g. 80% (97/121) of cases detected by active search in Trujillo, Venezuela, had previously attended a Health Center for treatment (Scorza et al., 1985). While it is therefore not feasible to determine absolute estimates of case numbers, comparisons of national notification data between countries and between years provide a tool for identifying spatial and temporal trends, which can then be validated by smaller-scale epidemiological surveys (Davies et al., 1994).

From 1996-98, the average number of leishmaniasis cases notified annually in the Andean region was 14,082 (range: 12,659-16,396), with 6,155 from Colombia, 2,668 from Peru, 2,240 from Bolivia, 1,936 from Venezuela, and 1,084 from Ecuador (unpublished data from their respective Ministries of Health). Given the differences in population sizes, this suggests that the highest incidence rate is in Bolivia, which also has the lowest per capita Gross National Product and is therefore least equipped to deal with the burden of disease. Long-term notification data are not available from Bolivia, but the numbers in all the other four countries increased steadily during the 1980s (e.g. from just over 1,000 to about 5,000 in both Peru and Colombia: Restrepo, 1992; Rodriguez, 1992), reaching a plateau in the 1990s (for Colombia and Venezuela), or followed by a significant decline (Ecuador and Peru) (Figure 1). The increase in the 1980s is usually attributed to changes in land use and human activity patterns (e.g. new settlements) leading to increased exposure of humans to the zoonotic *Leishmania* life cycles. While some researchers claim that the recent decline in cases in Peru and Ecuador is due to the increasing frequency of the *El Niño* Southern Oscillation, which is particularly severe in those countries, we are unaware of any evidence to validate this.

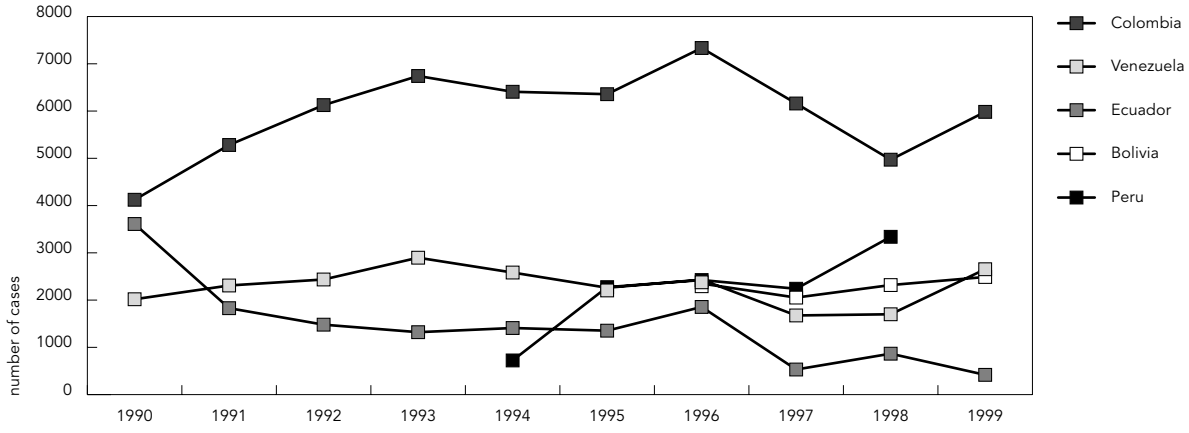
The burden of disease is traditionally measured not only by the number of cases but also by the disability-adjusted life years lost as a result of a disease. The costs of disease control are also influenced by clinical symptoms, as drug therapy and the requirement for hospitalization vary with symptoms. On these grounds, localised cutaneous leishmaniasis (LCL) is considered a relatively minor public health problem as compared with mucocutaneous leishmaniasis (MCL). However, calculations of burden of disease currently fail to take into account any of the social consequences of facial disfigurement by leishmaniasis scarring. Social costs are hard to measure, but a number of studies have now begun to explicitly address the attitudes of the local populations to leishmaniasis (Weigel et al., 1994; Isaza et al., 1999).

Parasite distributions

Within the whole region, an average of 13,080 of the notified leishmaniasis cases/year (from 1996-98) were LCL and 1,002 were MCL (i.e. 7.1%). As visceral leishmaniasis (VL) is rare and only found in Colombia (mean number of cases notified/year during the 1990s = 58, range: 22 – 79) and Venezuela (ca. 50 cases/year: Zulueta et al., 1999), except for very occasional cases reported in Ecuador (Hashiguchi & Gomez, 1991) and Bolivia (Dimier-David et al., 1991b), this review will focus mostly on the epidemiology of LCL and MCL (i.e. cutaneous leishmaniasis). The reported ratio of MCL:LCL is especially high in Bolivia (1:4) (David et al., 1993), Peru (1:7), low in Ecuador (1:13) (Hashiguchi & Gomez, 1991), and least in Colombia (1:44) and Venezuela (1:264) (unpublished data from respective Ministries of Health, unless otherwise stated). The principal reason for this variation is geographic variation in the distribution of the different parasite species, as there is clear interspecific variation in the probability of parasite dissemination, i.e. metastasis (Santrich et al., 1990; Osorio et al., 1998a; Saravia et al., 1998 – see below). However, as only a small proportion of all leishmaniasis cases are ever diagnosed to species, data on the relative importance of different parasites are not readily available. Published records of identified parasites tend to be highly biased towards the study region. The most objective estimate of parasite distributions is gained by listing parasite identifications at the departmental level and then weighting the results according to the mean number of cases notified by department. Such an analysis was carried out for this review, using all accessible published species identifications, combined with the average notification data available from the 1990s (Table 1). Ninety-four of the 106 departments in the Andean Pact countries have reported cases of LCL or MCL, and we were able to collect identification data

Figure 1

Annual number of leishmaniasis cases reported by the Ministries of Health (MOH) in each of the Andean countries during the 1990's. Data from Bolivia and Peru are incomplete. Note that the Peruvian data, which were provided by the *Oficina de Estadística y Informatica* in the MOH <www.minsa.gob.pe/estadisticas>, are much lower than those collated by the *Programa de Control de Malaria y Otras Enfermedades Metaxenicas* (also in the MOH), which reported 7,343 and 7,756 leishmaniasis cases in Peru in 1995 and 1996, respectively.



on parasite isolates from all but 20 of these (most of which have very low numbers of notified cases). To deal with these missing data, we used the average results from those neighboring departments with similar ecological conditions. The clear findings are that the ratio of *Leishmania (Viannia)* to *L. (Leishmania)* infections is about 10:1, and that *L. braziliensis* is responsible for the greatest number of notified cases (49% of all), with a further 27% due to *L. panamensis*, and the remaining 23% shared between 8 relatively rare species. In geographical terms, *L. braziliensis* is also the most widespread, having been isolated from LCL patients in 50 of the 74 departments with data, followed by *L. panamensis* (27), *L. guyanensis* (20), *L. amazonensis* (16), *L. mexicana* (14), *L. peruviana* (7), and *L. lainsoni* (6). The remaining 3 species have apparently very limited distributions. Of course, there are a number of caveats to these interpretations, which include: (a) interspecific variation in isolation rates; (b) geographic variation in under-reporting rates; (c) some ambiguities in taxonomic identifications of parasites, e.g. *L. garnhami* (Guevara et al., 1992), *L. venezuelensis* (Bonfante-Garrido et al., 1992), and *L. colombiensis* (Delgado et al., 1993); and (d) the detection of apparent hybrids or "intermediate variants" between parasites in the *L. (Viannia)* subgenus in all 5 countries, using isoenzymes (Saravia et al., 1998) or (less frequently) RAPDs (Bañuls et al., 1999), microsatellites (Russell et al., 1999), and molecular karyotyping (Dujardin et al., 1995). Hybrids have been reported between *L. braziliensis* and either *L. peruviana* (Dujardin et al., 1995; Russell et al., 1999; Nolder, 2000), *L. guyanensis* (Bonfante-Garrido et al., 1992; Delgado et al., 1997), or *L. panamensis* (Bañuls et al., 1997; Bañuls et al., 1999), and between *L. panamensis* and *L. guyanensis* (Armijos et al., 1995; Saravia et al., 1998; Bañuls et al., 1999; Russell et al., 1999). Indeed the separate taxonomic status of the latter two species has recently been doubted by some (Bañuls et al., 1999) but not all (Armijos et al., 1997; Saravia et al., 1998) research groups. Differing conclusions may be in part due to whether electrophoresis was carried out on cellulose acetate or thin-layer starch gels (Nolder, 2000).

Variability in clinical symptoms

The principal clinical features that distinguish cutaneous *Leishmania* infections are (a) the proportion of infections leading to lesions; (b) the size, number, and physical characteristics of the lesions; (c) the frequency of self-healing and the time to self-healing; (d) the frequency of relapse; (e) the extent of protective immuni-

Table 1

Distribution of *Leishmania* parasites responsible for american cutaneous leishmaniasis within the Andean countries. Numbers estimated from mean departmental case notification data during the 1990s combined with published reports of parasite identifications from specified Departments (as described in the text).

Parasites species	**Countries**					**Total**	**%**
	Colombia	Peru	Bolivia	Venezuela	Ecuador		
L. braziliensis	2,375	939	2,108	1,425	299	7,145	49.4
L. panamensis	3,122	0	0	0	796	3,918	27.1
L. peruviana	0	922	0	0	0	922	6.4
L. guyanensis	139	358	0	25	112	634	4.4
L. colombiensis	55	0	0	0	0	55	0.4
L. lainsoni	0	45	0*	0	0	45	0.3
Hybrids[1]	3	58	0	23	108	193	1.3
L. amazonensis	92	130	196	173	149	740	5.1
L. garnhami	0	0	0	276	0	276	1.9
L. mexicana	176	0	0	69	30	275	1.9
L. venezuelensis	0	0	0	259	0	259	1.8
Total	5,961	2,452	2,303	2,250	1,493	14,459	100.0

* *L. lainsoni* has now been reported in Bolivia (A. L. Bañuls, personal communication)
[1] Various hybrids or "intermediates" between L. (*Viannia*) species (see text).
Parasite identification references: Weigle et al., 1986; Velez et al., 1987; Corredor et al., 1990; Montoya et al., 1990; Kreutzer et al., 1991; Weigle et al., 1993b; Saravia et al., 1998; Montoya-Lerma et al., 1999; Russell et al., 1999 (Colombia); Arana et al., 1990; Franke et al., 1990; Revollo et al., 1992; Dujardin et al., 1993; Lucas et al., 1998; Victoir et al., 1998; Russell et al., 1999; Nolder et al., 2000 (Peru); Desjeux et al., 1986; La Fuente et al., 1986; Urjel et al., 1987; Desjeux et al., 1987; Torres Espejo et al., 1989b; Revollo et al., 1992; Martinez et al., 1998 (Bolivia); Scorza et al., 1979; Aguilar et al., 1984; Grimaldi Jr. et al., 1989; Bonfante-Garrido et al., 1992; Rodriguez et al., 1994; Maingon et al., 1994; Delgado et al., 1997 (Venezuela); Mimori et al., 1989; Armijos et al., 1990; Hashiguchi et al., 1991; Dereure et al., 1994; Eresh et al., 1994; Gomez et al., 1994; Katakura et al., 1994; Le Pont et al., 1994a; Reyna et al., 1994; Armijos et al., 1995; Armijos et al., 1997; Furuya et al., 1997; Bañuls et al., 1999 (Ecuador).

ty to reinfection; (f) the frequency and severity of either mucosal or diffuse cutaneous leishmaniasis; and (g) the responsiveness to drug treatment. Unfortunately, comparative studies of any of these features following different parasite infections are rare in the literature (Saravia et al., 1998), and comparing the results of independent clinical studies of different parasites must be done with caution as there have been few attempts to standardize definitions or methodology. In addition, the clinical outcome of a *Leishmania* infection depends not only on parasite factors, but also on host susceptibility which may vary with age (Weigle et al., 1993b; Davies et al., 1995b; Muñoz, 1998), genetics (Cabrera et al., 1995; Shaw et al., 1995; Alcais et al., 1997a), or nutritional status (Weigel et al., 1995). Further variability in symptoms may be introduced by chemicals in sandfly saliva, which are not only vasodilatory (Warburg et al., 1994), but may also influence macrophage function at the site of inoculation, e.g. down-regulating nitric oxide synthesis and the TH1 response, and up-regulating the TH2 response (Mbow et al., 1998; Katz et al., 2000). There is now evidence from experimental animal models that pre-exposure to sandfly bites can also influence the severity of cutaneous disease caused by subsequent *Leishmania* infections (Belkaid et al., 1998). If this phenomenon acts under natural conditions, it may play a role in explaining variation in host susceptibility with age.

Despite these confounding variables, there appears to be overwhelming evidence that metastasis is essentially limited to infections with *L. braziliensis*, and to a lesser extent *L. panamensis* (Saravia et al., 1998; Osorio et al., 1998a), with only very occasional reports of MCL due to *L. guyanensis* (Santrich et al., 1990). The precise metastasis rate following either *L. braziliensis* or *L. panamensis* infection has rarely been satisfactorily measured, and cannot be reliably estimated from passive case detection data which are biased towards the most severe clinical cases (i.e. in favor of MCL). Cross-sectional active search surveys for both LCL and MCL should provide more reliable estimates. For example, in a cross-sectional survey in a *L. panamensis* focus in Santander Department, Colombia (Muñoz, 1998), 27 out of 938 (2.9%) LCL patients (past and current) had perforated nasal septa, and a further 69 (7.3%) had

nasal ulcers, but none had severe mucosal symptoms. Cross-sectional clinical surveys in foci of *L. braziliensis* tend to detect a similar percentage of mucosal complications amongst LCL patients, e.g. a weighted mean of 7.5% (112/1,503) from the 8 published surveys in Bolivia (De Muynck et al., 1978; Recacoechea et al., 1987; Torres-Espejo et al., 1989a; Bermudez et al., 1993; Alcais et al., 1997b), but these include significant numbers with extremely severe MCL pathology. None of these calculations take into account either age or the time since the primary lesion, and consequently underestimate the metastasis rate (i.e. because of the potentially long incubation period between LCL and MCL). In Huánuco Department, Peru (a *L. braziliensis* focus), using retrospective estimates of time since the LCL episodes within an actively surveyed population of 3,020, we recently calculated that LCL patients have a life-time risk of metastasis of 12.8% (C. R. Davies, unpublished). While there are some promising results from genetic studies of both hosts (Lara et al., 1991) and parasites (Kebede et al., 1999), the factors determining which LCL patients go on to develop MCL are essentially unknown, and remain a key research question.

The percentage of infections leading to cutaneous lesions (i.e. "pathogenicity") can only be reliably estimated by prospective studies, taking Montenegro skin test (MST) conversions as evidence for infections. The reported measurements of pathogenicity from the few such studies that have been carried out do not indicate any particular interspecific trends and are inconsistent (probably in part due to variations in the sensitivity of the MSTs): for *L. peruviana* 83% (78/94: Davies et al., 1995b), for *L. panamensis* 12% (24/227) in Nariño, Colombia (Weigle et al., 1993b), but 69% (36/52) in Santander, Colombia (Muñoz, 1998), and for *L. braziliensis* 21% (8/39) in Huánuco, Peru, but 50% (23/46) in Cochabamba, Bolivia (C. R. Davies, unpublished).

Comparisons of relapse rates and acquired protective immunity are further complicated by the difficulty in distinguishing reinfections from reactivations. Some estimate of the proportion of secondary clinical infections due to relapses can be made by (a) comparing the molecular profile of parasites isolated from sequential infections (Saravia et al., 1990), (b) comparing the site on the body of primary and secondary lesions (Muñoz, 1998), and (c) investigating the relationship between time since the primary lesion and the risk of a secondary lesion (Davies et al., 1995b; Muñoz, 1998). In general, where incidence rates are low (e.g. in Nariño, where the MST conversion rate was 0.066/year: Weigle et al., 1993b) the proportion of incident cases in a population due to relapses rather than new infections is likely to be relatively high, in comparison with sites such as Santander where incidence rates are relatively high (MST conversion rate of 0.19/year: Muñoz, 1998)

Immunology

The immunological basis for any interspecific differences in pathogenicity or virulence has rarely been addressed. Most studies of the immunological response to *Leishmania* have followed the course of infection in experimental laboratory mouse models, which share a number of key features with human infections: (a) disease resolution is mediated by the cell-mediated immune response, not the humoral immune response; (b) primary activation of T-cell subsets, mainly CD4+, is of vital importance for the development of TH1 and TH2 responses and subsequent course of infection; and (c) there is a strong correlation between activation of different T-cell subsets and severity of disease. In particular, the chronic nature of LCL appears to be due to the dominance of TH2-like response at the site of infection in the skin (Tapia et al., 1994). The factors determining the CD4+ cell subset dominating the immune response upon infection remain to be conclusively identified, but include the initial cytokine response(s), the size of the infecting inoculum, sandfly saliva, and natural killer cells (Bogdan & Rollinghoff, 1998). For example, whereas the presence of interferon (IFN) γ and interleukin (IL) 12 early in infection promotes the development of TH1 responses, transforming growth factor (TGF) β and IL10 seem to suppress the development of TH1 cells but stimulates a TH2 response, leading to enhanced growth of *Leishmania* parasites. There is also some evidence that CD8+ cells play an important function in the host immune response, either by stimulating IFNγ secretion and activation of macrophages, or by a cytotoxic effect of CD8+ cells upon parasitized macrophages (Coutinho et al., 1998).

Epidemiological data from patient surveys are generally supportive of the TH1/TH2 dichotomy shown in experimental laboratory models. Briefly, LCL patients display a positive proliferative TH1-type response to leishmanial antigens: the delayed-type hypersensitivity response (DTH) is positive, with induration size correlated to lesion size and – sometimes – number (Mimori et al., 1987; Armijos et al.,

1997). DTH has also been shown to be lower among individuals experiencing recurrent leishmaniasis than among those patients with sub-clinical infections (i.e. with a positive DTH but no signs of the disease) (Bosque et al., 2000), and lower among patients with relapses than among those with reinfections (Saravia et al., 1990). This demonstrates the predominant role of a TH2-type response in chronic infections. There is some evidence that the cytokine profile varies with time during the course of infection (Ribeiro-de-Jesus et al., 1998): significant levels of IFNγ are produced upon leishmanial antigen presentation, but during the early phase of infection (the first 60 days) IFNγ production may be down-regulated, and IL10 production increase, possibly accounting for a transient period of high parasite multiplication. However, there is no evidence that patients with low IFNγ production are at risk of developing larger lesions or parasite dissemination (Ribeiro-de-Jesus et al., 1998), and patients with lower IFNγ production may have a better response to therapy with pentavalent antimonials. IL5 is also produced by LCL patients, though in lower amounts than diffuse cutaneous leishmaniasis (DCL) and MCL patients. DCL patients display primarily a TH2-type cytokine response, i.e. DCL patients have a complete anergy to leishmanial antigen, the DTH response being negative with the lymphocytes non-responsive to leishmanial antigen (as tested by either proliferation or by lymphokine production). DCL patients have low levels of IFNγ and IL2, but significant serum levels of IL4, IL5 and tumor necrosis factor (TNF) α. MCL patients display a mixture between a TH1 and TH2-type cytokine response (with significant levels of IL2, IL4, IL5, and TNFα), which could explain non-resolution of the disease, as the TH2-type response tends to dominate when both types of responses are activated (Pirmez et al., 1993). MCL patients tend to have a larger DTH than LCL patients, with comparatively high serum levels of IFNγ and IL2, as well as IL5 and TNFα. However, there are currently no immunological markers for LCL patients which might identify their future risk of developing MCL. Indeed studies showing differences in the immune response to different parasite strains or species are scarce. For example, the DTH response to *Leishmania* antigen is greater in *L. braziliensis*-infected patients than in *L. panamensis*-infected patients, even after adjusting for time of evolution and lesion type (i.e. LCL or MCL) (Saravia et al., 1989).

Heterogeneity in transmission ecology

Transmission ecology will determine who is most likely to be infected, where they are likely to be infected and when they are likely to be infected. Only with such information can a rational control program be designed. As the principal determinant of transmission ecology is probably the behavior and ecology of the sandfly vectors, this section focuses first on the entomological evidence. Secondly we discuss how the risk for humans of these zoonotic diseases also depends on factors associated with the reservoir hosts; and finally we look at the epidemiological evidence for the role of human components – such as behavioral activities or house design – which have been identified as risk factors for leishmaniasis.

Incrimination of vectors

Table 2 lists the sandfly species in the Andean region meeting the criteria outlined by Killick-Kendrick & Ward (1981) to incriminate vectors of human leishmaniasis: anthropophily, vector-human contact, and characterization of natural infections of the same *Leishmania* species within the vector as in human cases. In addition, several other Andean phlebotomines are strongly suspected as vectors. These include anthropophilic vectors in which natural infections of parasites which are morphologically identical to *Leishmania* have been observed but not characterized (e.g. *Lutzomyia youngi*), and species which are the only significant human-biters in areas with large numbers of leishmaniasis cases, such as *Lu. torbida* and *Lu. longiflocosa* in Colombia (M. C. Ferro & R. P. Pardo, personal communication). Although all the incriminated vectors may be competent to transmit infections to humans, their relative importance has rarely been quantified. The two approaches taken are (a) to compare entomological inoculation rates (Perez et al., 1994), and (b) to carry out regression analyses of the spatial or temporal relationship between the abundance of each sandfly species and the incidence of LCL (Davies et al., 1997). In addition to differences in vector competence (of which little is known for Andean sandfly vectors), vector importance is determined by a series of key ecological and behavioral characteristics.

Habitat preferences

The habitat preferences of sandfly species will influence both the degree to which they come into contact with humans and the particular

Table 2

Confirmed natural *Leishmania* infections in proven sandfly vector species in the Andean countries.

Sub-genus, species	Distribution within region[1]	Parasite identified (total nº infections)*	Diagnostic method[1]	Reference
Helcocyrtomyia				
Lu. ayacuchensis	EP	*L. peruviana* (2) *L. mexicana* (1)	DNA IE, mAb	Dujardin et al., 1993 Gomez & Hashiguchi, 1991
Lu. hartmanni	CE	*L. colombiensis* [a] (6)	IE, mAb	Kreutzer et al., 1991
Lu. peruensis	P	*L. peruviana* [b] (3)	IE, PCR	Perez et al., 1991, 1994
Lu. tejadai	P	*L. braziliensis* [c] (3)	PCR	Davies & Roncal, unpublished
Lutzomyia				
Lu. gomezi	CEPV	*L. braziliensis* (7)	PCR, DNA	Feliciangeli et al., 1994; Rodriguez et al., 1999
Lu. longipalpis	BCV	*L. infantum* [d] (5)	IE	Moreno et al., 1984; Rodriguez, unpublished
Nyssomyia				
Lu. flaviscutellata	BCEPV	*L. amazonensis* (27) ‡	IE, mAb	Lainson & Shaw, 1968; Ward et al., 1973; Arias et al., 1985, 1987; Ryan et al., 1987
Lu. olmeca	CEPV	*L. amazonensis* (4) ‡ *L. mexicana* (1) ‡	IE, mAb IE	Arias et al., 1987; Grimaldi Jr. et al., 1991 Williams, 1970
Lu. trapidoi	CE	*L. panamensis* (7)	IE, mAb, PCR	Young et al., 1987; Travi et al., 1988; Le Pont et al., 1994a; Muñoz, 1998
Lu. umbratilis	BCPV	*L. guyanensis* (1)	IE	Young et al., 1987
Psychodopygus				
Lu. carrerai	BCEPV	*L. braziliensis* (4)	mAb	Le Pont et al., 1988
Lu. llanosmartinsi	BP	*L. braziliensis* (1)	IE	Le Pont & Desjeux, 1986
Lu. panamensis	CV	*L. panamensis* (1) ‡	IE	Christensen et al., 1983
Lu. yucumensis	BP	*L. braziliensis* (1)	IE	Le Pont & Desjeux, 1986
Verrucarum				
Lu. evansi	CV	*L. infantum* (7)	IE, PCR, DNA	Travi et al., 1990; Feliciangeli et al., 1999
Lu. nuneztovari	BCPV	*L. amazonensis* [e] (3)	IE	Martinez et al., 1999
Lu. ovallesi	CV	*L. braziliensis* (50) *L. mexicana* (1)	mAb, PCR, DNA mAb, DNA	Barrios et al., 1994; Feliciangeli et al., 1994 Barrios et al., 1994
Lu. spinicrassa	CV	*L. braziliensis* (27)	IE, PCR	Young et al.,1987; Perruolo, 2000
Lu. verrucarum	PV	*L. peruviana* (6)	PCR	Perez et al., 1994

* Only infections detected within the region are listed, except for those sandfly species which are suspected to be vectors in the region, but whose natural infections (marked by ‡) have only been detected outside the region to date.
[1] mAb = monoclonal antibody; IE = isoenzyme; PCR = polymerase chain reaction; DNA = DNA probe; B = Bolivia; C = Colombia; E = Ecuador; P = Peru; V = Venezuela.
a = also suspected vector of *L. panamensis* (Alexander et al., 1992b); b = taxonomic status of first isolate, LP52, is now in doubt (A.-L. Bañuls, personal communication); c = PCR identification as *L. braziliensis* complex; on basis of human isolates in region, probably *L. braziliensis*, possibly *L. peruviana*; d = it is now generally believed that *L. infantum* and *L. chagasi* are synonymous; e = also suspected vector of *L. braziliensis* (Torrez et al., 1998).

sub-populations that are most likely to become infected: sylvatic vectors usually cause more cases among adult males who work in the forest, while domestic vectors are more of a threat to women and children. The *Psychodopygus* vectors *Lutzomyia yucumensis*, *Lutzomyia llanosmartinsi*, and *Lutzomyia carrerai* are closely associated with primary rainforest (Le Pont & Desjeux, 1986; Le Pont et al., 1989b). *Lutzomyia umbratilis* is also a primary forest species, both within the Andean region (Feliciangeli et al., 1985) and elsewhere (Pajot et al., 1982). *Lutzomyia flaviscutellata*, while found in primary forest, also appears to be well adapted to secondary regrowth, and may be a particular threat following deforestation (Ready et al., 1983). Several species which are found in the forest are also abundant in agricultural crops (especially coffee and cacao), thereby causing a risk to people living and working within the plantations. These include *Lu. spinicrassa*, *Lu. ovallesi*, *Lu. gomezi*, *Lu. nuneztovari*, *Lu. trapi-*

doi, Lu. hartmanni, and the strongly suspected vector *Lu. youngi* (Travi et al., 1988; Le Pont et al., 1989b, 1994b; Alexander et al., 1992a, 1995a; Maingon et al., 1994; Mouchet et al., 1994). Many of the most important vectors have even closer associations with humans and are abundant within small settlements. These include the VL vectors *Lutzomyia longipalpis* and *Lutzomyia evansi* and the LCL vectors *Lu. gomezi*, *Lutzomyia verrucarum*, *Lu. ovallesi*, *Lutzomyia tejadai*, *Lu. nuneztovari*, *Lutzomyia ayacuchensis*, and *Lutzomyia peruensis*, and to a lesser extent *Lu. trapidoi* (Feliciangeli et al., 1987b, 1999; Hashiguchi et al., 1991; Caceres, 1993; Villaseca et al., 1993; Davies et al., 1995a; Morrison et al., 1995a; Lopez et al., 1996; Travi et al., 1996; Feliciangeli & Rabinovich, 1998; Martinez et al., 1999). Some species vary geographically in their habitat associations. For example, whereas *Lu. nuneztovari* and *Lu. trapidoi* are mainly sylvatic in pristine rainforest regions, both species are abundant in coffee plantations and around houses in areas with a longer history of deforestation. This suggests that these species may be adapting to land-use changes associated with human colonization (Le Pont et al., 1989b; Mouchet et al., 1994; Dujardin et al., 1996).

Endophagy/endophily

Sandfly species which are encountered in and around human settlements vary in their tendency to enter houses to feed (endophagy) and rest (endophily). These behavioral characteristics are important determinants both of their vectorial threat and their susceptibility to control measures such as residual spraying and insecticide-treated curtains and nets. Most peridomestic species have been shown to feed inside houses, including *Lu. longipalpis*, *Lu. evansi*, *Lu. peruensis*, *Lu. verrucarum*, *Lu. ayacuchensis*, *Lu. nuneztovari*, *Lu. gomezi*, *Lu. ovallesi*, and *Lutzomyia olmeca* (Feliciangeli, 1987a, 1997; Le Pont et al., 1989b; Hashiguchi et al., 1991; Villaseca et al., 1993; Davies et al., 1995a; Travi et al., 1996; Martinez et al., 1999). The relative rates of biting indoors and outdoors, however, are rarely compared. Furthermore, comparisons between species in different sites are usually hampered by variation in local conditions and study techniques. However, *Lu. ovallesi* was shown to be relatively more likely than *Lu. gomezi* to enter houses in the same site in Venezuela (Feliciangeli, 1987a), and the latter was in turn shown to be more likely to enter houses than *Lu. trapidoi* in Ecuador (Mouchet et al., 1994). The observation that *Lu. trapidoi* and *Lu. gomezi* are found outside rather than inside houses in Nariño, Colombia (Travi et al., 1988) suggests that these species may be amongst the less endophagic vectors.

Diurnal rhythms

Another important determinant of the threat posed by vector species is the timing of their biting activity. Outdoor feeders are more likely to encounter humans in the early evening. In contrast, indoor feeders which feed later at night are more likely to find sleeping, non-defensive, human hosts. *Lu. longipalpis* in Colombia are most active between 2030-2330 hours, followed by a steady decline in activity (Morrison et al., 1995b), whereas *Lu. evansi* in Venezuela show peak activity at 2400 and 0300 hours (Gonzalez et al., 1999). *Lu trapidoi* was most active at dusk and again towards dawn in studies in both Nariño, Colombia (Travi et al., 1988) and Ecuador (Hashiguchi et al., 1985). The same studies report biting throughout the night for *Lu. hartmanni* and *Lu. gomezi*. In contrast, the activity of *Lu. gomezi* in Antioquia, Colombia (Porter & Defoliart, 1981), and in Miranda, Venezuela, is largely restricted to between 1900 and 2000 hours (Feliciangeli, 1997). *Lu. ovallesi* activity in the same Venezuelan site is greatest between 2000 to 2400 hours, followed by a gradual decrease. In Bolivia, *Lu. nuneztovari* enters houses only after 2200 hours and leaves before 0600. Unusually, the same species has also been observed to bite during the day in coffee plantations (Le Pont et al., 1989b). In the Peruvian Andes, *Lu. peruensis* and *Lu. verrucarum* tend to feed outdoors only in the early evening, whereas activity inside the relatively warmer houses persists later into the night. The restriction of outdoor activity becomes more pronounced at higher altitudes (Villaseca et al., 1993). Less is known of the biting rhythm of the forest vectors. It is feasible that the relatively stable temperatures in the forest (compared to peridomestic environments) may contribute to more constant biting activity for forest species. *Lu. umbratilis*, for example, is known to bite throughout the night, as well as during the day if disturbed (Ready et al., 1986); and both *Lu. trapido*i and *Lu. hartmanni* remain highly active throughout the night in forest sites in Antioquia, Colombia, whereas activity steadily declines after a dusk peak in clearings (Porter & Defoliart, 1981).

Seasonal variations

Rainfall and humidity are important determinants of seasonal patterns. For VL vectors, *Lu.*

evansi is more abundant in baited traps in the wet season (Montoya-Lerma & Lane, 1996) in Colombia, and *Lu. longipalpis* abundance also correlates positively with humidity and rainfall in the preceding three weeks (Morrison et al., 1995a). A direct comparison of these species in Venezuela demonstrates that *Lu. evansi* is more abundant at the end of the wet season, but is replaced by *Lu. longipalpis* during the dry season (Feliciangeli et al., 1999). Among the LCL vectors, *Lu. spinicrassa* abundance increases markedly in the wet season in Colombia (Alexander et al., 1992a), as does *Lu. ayacuchensis* in the Ecuadorian Andes (Gomez & Hashiguchi, 1991). *Lu. gomezi* was strongly associated with the wet season in Ecuador (Le Pont et al., 1994b), less so in Colombia (Alexander et al., 1992a), and not at all in Venezuela, where temperature effects seem more important (Feliciangeli, 1987b). Species which are more abundant in the dry season include *Lu. trapidoi* in Ecuador (Le Pont et al., 1994b), and *Lu. ovallesi* in Venezuela (Feliciangeli, 1987b). Forest sandflies may be more protected from seasonal as well as diurnal variations. *Lu. umbratilis* and other forest species are found throughout the year in Brazil, although there is some decrease in abundance at the end of the dry season (Ready et al., 1986). The problems of comparing between sites are illustrated by *Lu. flaviscutellata*, which is a dry season species in the Brazilian Amazon, but a wet season species in Panama, which is relatively drier (Shaw & Lainson, 1972). In addition to abundance, other epidemiologically important factors may vary seasonally. In the Peruvian Andes, *Lu. peruensis* and *Lu. verrucarum* are more abundant outside during the dry season, but more endophagic during the wet season (Villaseca et al., 1993). In the Ecuadorian Andes the infection rate of *Lu. ayacuchensis* is highest at the end of the wet season and beginning of the dry season (Gomez & Hashiguchi, 1991). The observation that parous rates increase for many species during the wet season (Anez et al., 1994) suggests that effects on adult longevity may be an important driving factor for both vector abundance and infection rate. Greater understanding of the patterns and mechanisms of seasonal variations may help inform the timing of control interventions, such as insecticide spraying.

Epidemiological evidence for risk factors

Risk factors for infection require active search data, as passively reported case data are notoriously biased according to age, gender, economic status, and other sociological factors. Cross-sectional survey data can provide important evidence for demographic risk factors, in particular gender. For example, comparisons of cumulative prevalence (the proportion of a population with a positive MST response or evidence of either past or current disease) distinguish clearly between those sites where there is no gender bias (e.g. Hashiguchi et al., 1984; Torres-Espejo et al., 1989a; Nonaka et al., 1990; Barrerra et al., 1994; Davies et al., 1995b), to sites where the cumulative prevalence ratio for males:females is about 2:1 (Barrera et al., 1994; Alcais et al., 1997b), 3:1 (Weigle et al., 1993b; Armijos et al., 1997), or even 8:1 (De Muynck et al., 1978; Bermudez et al., 1993). Lack of gender bias is usually an indicator of domestic transmission, whereas male bias suggests occupational exposure, especially when entering forests. Female bias has also been occasionally detected (Scorza et al., 1983; Aguilar et al., 1984, Scorza et al., 1985; Velez et al., 1987; Aguilar et al., 1989), but has rarely been fully explained.

Cross-sectional surveys can also provide evidence for age bias, by examination of the relationship between age and cumulative prevalence (sigmoid if risk increases with age, monotonic increase to an asymptote of 100% if risk is age-independent). However, these patterns are confounded by temporal changes in incidence, e.g. due to a long-term house-spraying campaign (Davies et al., 1994). Interpretation of active prevalence data needs to take into account the increase in acquired immunity with age. In particular, as incidence rates increase the mean age of cases will naturally decrease, as demonstrated by both spatial (Davies et al., 1995b) and temporal comparisons (Rodriguez, 1992). Prospective surveys of incident cases amongst the healthy (i.e. susceptible) population should provide a more reliable measure of age bias (Davies et al., 1995b). However, a reduction in incidence with age does not necessarily mean that exposure decreases with age, but could be explained by age-independent heterogeneity in exposure (i.e. those most at risk are infected when they are young, so that the population remaining susceptible into adulthood are those least at risk).

The principle techniques for identifying non-demographic risk factors for *Leishmania* infection are comparative analyses of incidence in cohorts with defined attributes (e.g. living in different villages: Davies et al., 1997) or case-control studies (Llanos-Cuentas, 1993). Such analyses can be used to estimate the relative importance (i.e. the "population-attributable risk") of risk factors associated with indoor house characteristics, outdoor environmental

characteristics, and human behavioral patterns. For example, a case-control study in endemic valleys for *L. peruviana* in Lima and Ancash, Peru, found that the population-attributable risk for factors associated with indoor transmission (e.g. use of a kerosene lamp, having a chimney, and living in a stone house) was 79%, indicating that transmission is largely indoors (Llanos-Cuentas, 1993). In contrast, a case-control study of *L. panamensis* transmission in Nariño, Colombia (Weigle et al., 1993c), showed that the principle risk factors were occupational (farming, hunting, lumbering, or fishing). Risk was most strongly related to entering the forest after sunset, correlating with the hours spent there.

Evidence for reservoir hosts

Natural *Leishmania* infections in the Andean region have been detected in a range of non-human hosts (principally marsupials, rodents, edentates, and carnivores: Table 3); but so far, only reservoir hosts for *L. infantum* (*Canis familiaris, Cerdocyon thous, Didelphis marsupialis*), *L. amazonensis* (*Proechimys* spp.*), L. mexicana* (*Ototylomys phyllotis), L. guyanensis* (*Choloepus didactylus*), and *L. panamensis* (*Ch. hoffmani*) have been fully incriminated (Ashford, 1997). The identification of leishmaniasis reservoir hosts is complicated as it becomes increasingly clear that the disease transmission cycle can adapt to environmental (e. g. deforestation) and sociological (e. g. human migration) changes. For example, because domestic dogs are incriminated reservoir hosts of *L. infantum*, and because there have been numerous reports of American cutaneous leishmaniasis (ACL) in dogs, it is widely believed that they may be reservoirs of ACL as well (Reithinger & Davies, 1999). In particular, dogs have often been referred to as reservoir hosts of *L. peruviana*, although parasites have only be isolated and characterized fairly recently (Llanos-Cuentas et al., 1999). Circumstantial evidence that dogs might act as either primary or secondary reservoir hosts comes from two observations: (a) *Leishmania* strains isolated sympatrically from dogs and humans are indistinguishable; and (b) the risk of ACL infection in dogs is correlated with the risk of ACL in humans (Reithinger & Davies, 1999). The identification of parasites from dogs, however, does not distinguish whether dogs are accidental or reservoir hosts of the disease. The reported coincidence between households with ACL patients and the presence of infected dogs only indicates that humans and dogs are exposed in the same way to the sandfly vector, but is not evidence for dogs being reservoirs of disease. There is currently no evidence, for example, that people who own dogs are at any greater risk of ACL (Llanos-Cuentas, 1993) or that villages with higher dog densities have a greater population risk (Davies et al., 1997). In practice, three main parameters determine the potential epidemiological role of a putative reservoir host population: (a) the prevalence of infection in the host; (b) the biting rate of sandfly vectors on the host; and (c) the infectiousness of an infected host to sandfly vectors. In general, the reported measurements of prevalence are difficult to interpret (due to differences in study protocol), and most studies reporting infection rates in putative reservoir hosts fail to report comparable data on the *Leishmania* infection rates of other animals found in the same endemic and epidemiological setting. For example, *L. braziliensis* has been isolated from cats, equines, rodents, and opossums; both *L. panamensis* and *L. peruviana* have been isolated from opossums and rodents; and *L. amazonensis* has been isolated from or detected in rodents, marsupials, and carnivores (Table 3). Furthermore, measurements of sandfly biting rate on the host and host infectiousness are scarce (Reithinger & Davies, 1999). *Lu. whitmani* (a vector of ACL in Brazil) became infected when permitted to feed on the lesions of 3 of 9 *L. braziliensis*-infected dogs, with a mean infection rate on the three infective dogs of 2.7% (5/186). No generalizations can be extrapolated from those results due to the low number of replicates, and because flies were artificially fed on lesions. Indeed, the reported infection rate is less than that reported on humans: 10.4% (10/96) for *Lu. youngi* fed directly on lesions of eight human patients, all of whom were infectious. *Lu. youngi* have also been infected with *L. braziliensis* when fed on 3 of 6 opossums experimentally infected with *L. braziliensis,* with a mean infection rate on the 3 infectious hosts of 7.3% (4/ 55); and *Lu. sanguinaria* were infected with *L. panamensis* when fed on 3 of 7 naturally infected two-toed sloths in Panama, with a mean infection rate on the 3 infectious hosts of 15.6% (7/45) (Christensen et al., 1983; Scorza et al., 1984; Vexenat et al., 1986; Rojas & Scorza, 1989).

Control strategies

In most endemic areas for ACL, the disease is well known, and the local people have a range of "folk methods" for preventing and treating it

Table 3

Confirmed natural *Leishmania* infections in non-human mammals in the Andean countries.

Parasite species[1]	**Host (order)**	**Country**[2]	**Reference**[3]
L. braziliensis	*Canis familiaris* (Carnivora)	CPV	Reithinger & Davies, 1999
	Cerdocyon thous (Carnivora)	V	Guevara et al., 1994
	Conepatus chinga rex (Carnivora)	B	Telleria et al., 1999
	Equus asinus (Perissodactyla)	CV	Grimaldi Jr. et al., 1989; Bonfante-Garrido et al., 1992
	Oryzomys concolur (Rodentia)	V	Grimaldi Jr. et al., 1989
	Zygodontomys microtynus (Rodentia)	V	Grimaldi Jr. et al., 1989
	Rattus rattus (Rodentia)	E	Mimori et al., 1989
L. panamensis	*C. familiaris*	EC	Reithinger & Davies, 1999
	Bradypus griseus (Edentata)	C	Corredor et al., 1990
	Choloepus hoffmani (Edentata)	C	Grimaldi Jr. et al., 1989
	Heteromys dermarestianus (Rodentia)	C	Grimaldi Jr. et al., 1989
L. braziliensis OR	*Melanomys caliginosus* (Rodentia)	C	Alexander et al., 1998
*L. panamensis**	*Microyzomys minutus* (Rodentia)	C	Alexander et al., 1998
	R. rattus	C	Alexander et al., 1998
	Sylvilagus brasiliensis (Lagomorpha)	C	Alexander et al., 1998
	Didelphis marsupialis (Marsupiala)	C	Alexander et al., 1998
	Micoureus demerarae (Marsupiala)	C	Alexander et al., 1998
L. peruviana	*C. familiaris*	P	Llanos-Cuentas et al., 1999
	Phyllotis andinum (Rodentia)	P	Llanos-Cuentas et al., 1999
	D. albiventris (Marsupiala)	P	Llanos-Cuentas et al., 1999
L. peruviana OR	*Akodon* sp. (Rodentia)	P	Llanos-Cuentas et al., 1999
*L. guyanensis**			
L. colombiensis	*C. familiaris*	V	Reithinger & Davies, 1999
	Ch. hoffmani	C	Kreutzer et al., 1991
L. garnhami	*D. marsupialis*	V	Grimaldi Jr. et al., 1989
L. amazonensis	*Potos flavus* (Carnivora)	E	Grimaldi Jr. et al., 1989
	Co. chinga rex	B	Telleria et al., 1999
	Tamandua tetradactyla (Edentata)	E	Grimaldi Jr. et al., 1989
	Akodon sp.	B	Telleria et al., 1999
	Oligoryzomys sp. (Rodentia)	B	Telleria et al., 1999
	Sciuris vulgaris (Rodentia)	B	Telleria et al., 1999
L. mexicana	*C. familiaris*	E	Reithinger & Davies, 1999
L. venezuelensis	*Felis domesticus* (Carnivora)	V	Bonfante-Garrido et al., 1992
L. infantum	*C. familiaris*	BCV	Grimaldi Jr. et al., 1989
	Coendou prehensilis (Rodentia)	B	Le Pont et al., 1989a
	Proechimys canicollis (Rodentia)	C	Travi et al., 1998
	D. marsupialis	C	Grimaldi Jr. et al., 1989; Travi et al., 1994, 1998

[1] *L. lainsoni* is endemic in Andean countries, but confirmed infections in non-human hosts have only been detected outside the region (in the rodent *Agouti paca*: Silveira et al., 1991).
[2] Country of isolation: B = Bolivia; C = Colombia; E = Ecuador; P = Peru; V = Venezuela.
[3] Primary references only given when not referred to in Grimaldi Jr. et al. (1989) or Reithinger & Davies (1999).
* Identification unsure as *L. braziliensis* complex-specific PCR used, where 2 species sympatric.

(Vasquez et al., 1991; Llanos-Cuentas et al., 1992; Weigel et al., 1994; Isaza et al., 1999). However, the efficacy of the various herbal remedies used has yet to be tested. Except for infrequent and sporadic spraying of houses with insecticides, government-run leishmaniasis control programs in the region are limited to the detection and treatment of active cases. Although the biochemical basis for their effectiveness remains unknown (Lucumi et al., 1998), the mainstay of anti-leishmanial therapy (except in Venezuela) are pentavalent antimonials (i.e. meglumine antimoniate – Glucantime® – or, less commonly, sodium stibogluconate – Pentostam®), at a recommended 20-28 doses of 20mg/kg/day (Berman, 1997). The drugs are typically administered intramuscularly, although in areas where MCL is rare, intralesional inoculation is becoming more regular (A. Llanos-Cuentas, personal communication). In each country, the official policy is to provide free treatment to all patients, although this is often not feasible in practice as drugs may be in limited supply in the highly dispersed rural hamlets where ACL is prevalent. The demand for anti-leishmanial drugs during the 1980s amongst jungle migrants from Cusco, Peru, who were at particularly high risk of MCL, led remarkably to the formation of self-help patient associations. These successfully lobbied for improvements in drug availability and provided a health education service which encouraged patients to seek early treatment (Guerra et al., 1993, Wong-Un, 1998). In endemic areas, such as in the departments of La Paz and Beni, Bolivia (Dedet et al., 1995), early diagnosis and treatment of patients has been greatly facilitated by nongovernmental organizations, usually funded by international aid. With respect to the Andean governments themselves, in most countries there has been a trend in the last 10 years towards a policy of providing diagnosis and treatment at local health posts rather than at hospitals, in order (a) to increase the proportion of patients seeking and receiving treatment, (b) to reduce the duration of disease prior to treatment, and (c) to increase the percentage of patients who complete their course of drugs (Llanos-Cuentas et al., 1992; Rojas, 1992). However, we are unaware of any studies which are monitoring changes in any of these parameters. Should patients not respond to antimonial treatment, 20-40 doses of 1mg/kg/day of amphotericin B (Fungizone®) is the second-line drug of choice, which has to be administered in hospitals. In Venezuela, control policy differs radically from the other countries in the region, as the majority of all LCL patients treated by the Ministry of Health each year since 1989 have been provided with immunotherapy (i.e. a combination of heat-killed *Leishmania* promastigotes and viable BCG), rather than antimonials; and currently (i.e. in 1999) immunotherapy is applied to about 83% of all LCL patients in Venezuela. Although trials with LCL patients in Miranda, Venezuela (Convit et al., 1989), showed that immunotherapy was as effective as standard chemotherapy (as well as being cheaper and with fewer and less severe side effects), we are unaware of any similar trial being carried out elsewhere; and it remains uncertain whether immunotherapy would be suitable in populations at greater risk of MCL.

Advances in diagnosis

Because the leishmaniases have a broad clinical spectrum, diagnosis of both present and past cases can be difficult. Differential diagnosis is important because diseases of other etiologies (e.g. leprosy, skin cancers, tuberculosis) with a similar clinical spectrum to leishmaniasis are often present in leishmaniasis-endemic areas (Weigle et al., 1993a). Although many researchers have suggested that taxonomic discrimination of *Leishmania* infecting LCL patients (at least distinguishing *L. (Viannia)* and *L. (Leishmania)* subgenera) is important for therapeutic decisions (Rodriguez et al., 1994; Mimori et al., 1998), this is not practiced in any country, not least because there is currently insufficient evidence to tailor treatment protocols according to parasite type. Nevertheless, taxonomic discrimination is crucial in any research study or in epidemiological surveillance. In most endemic areas the diagnosis of leishmaniasis by health practitioners is based largely on the clinical presentation, frequently with confirmation by microscopic examination of Giemsa-stained biopsy smears or aspirates, and occasionally by MST (Escobar et al., 1992). Although very simple to use and of high sensitivity and specificity, the main disadvantage of MST is that it does not distinguish between past and present infections. Serological diagnosis is rarely used to diagnose LCL as the number of circulating antibodies against LCL-causing parasites appears to be very low, especially if previous chemotherapy has been administered (Kar et al., 1995).

Parasitological diagnosis is the "gold standard" for leishmaniasis patients, as it is highly specific (i.e. there are few false positives). It is generally based on the microscopic examination of Giemsa-stained lesion biopsy or aspirate impression smears, histopathological ex-

amination of fixed lesion biopsies, or *in vitro* culture of biopsy triturates or aspirates. The latter is the most informative method, as it allows species identification and characterization, but the process is time-consuming and expensive. The sensitivity of this technique can be highly variable, depending on the number and dispersion of parasites in biopsy samples. Hence, parasite isolation success from MCL patients is especially low, due to the scarce numbers of parasites in mucosal lesions (Dimier-David et al., 1991a; Lopez et al., 1993). Successful culture of the *Leishmania* isolate is also dependent on the culture media (e.g. NNN, LIT, Schneider's) and the risk of microbial contamination (Escobar et al., 1992). Biopsy triturates and aspirates can also been used to inoculate hamsters, which reduces the risk of contamination, but is otherwise less sensitive than *in vitro* culture, probably because a minimal number of parasites in the inoculate is required to establish infection in the animal (Weigle et al., 1987, De Bruijn et al., 1993). The sensitivity of histopathology is also very low, due to the scarcity of parasites in the fixed biopsy or shrinkage and/or distortion of amastigotes during fixing of the histopathological slides (Weigle et al., 1987; Dimier-David et al., 1991a). Thus, although the detection and identification of parasites provides a conclusive test for infection, there are many false negatives.

Recently, several Polymerase Chain Reaction (PCR)-based protocols have been developed to diagnose ACL, and most studies (Table 4) have found them to be consistently more sensitive (88% on average for ACL) than other parasitological diagnostic methods, including biopsy impression smears (51%), culture (47%), and histopathology (35%). While many of the protocols employ relatively conserved primers for amplifying kDNA minicircles (e.g. Pirmez et al., 1999), the products can be hybridized with more specific DNA probes, which may allow identification to subgenus or species as well as enhancing sensitivity (Rodriguez et al., 1994, Reithinger et al., 2000). The other advantages of PCR-based methods are that (a) diagnosis can be made fairly rapidly and (b) contamination of samples is less of an issue than for culturing of parasites, as successful PCR diagnosis can be carried out on biopsy samples which are either frozen (DeBruijn et al., 1993; Lopez et al.,1993; Uezato et al., 1998; Aviles et al., 1999, Pirmez et al., 1999), cooled (Reithinger et al., 2000), stored at room temperature (provided they have been stored in an adequate PCR buffer) (Rodriguez et al., 1994; Uezato et al., 1998), or on formalin (or ethanol) fixed and paraffin-embedded histological sections (Schubach et al., 1998, Uezato et al., 1998). Nevertheless, successful PCR diagnosis is comparatively expensive, and its sensitivity is de-

Table 4

Sensitivity of Polymerase Chain Reaction for detecting Leishmania in the lesions of american cutaneous leishmaniasis patients in comparison with classical parasitological diagnostic methods. Summary of published comparative studies.

PCR		**Culture**		**Biopsy smear**		**Histopathology**		**Reference**
+	Total	+	Total	+	Total	+	Total	
15	24	7	25	0	24	ND		Lopez et al., 1993
14	18	13	18	12	18	ND		Lopez et al., 1993
14	19	12	35	12	18	2	8	De Bruijn et al., 1993
226	233	100	233	147	233	ND		Rodriguez et al., 1994
18	25	14	25	10	24	6	25	Calvopiña et al., 1997
14	24	ND		13	16	ND		Harada et al., 1997
14	21	8	15	8	15	ND		Uezato et al., 1998
19	20	9	14	9	17	7	19	Schubach et al., 1998
20	24	1	24	16	24	ND		Belli et al., 1998
178	184	86	144	76	176	65	174	Pirmez et al., 1999
7	7	0	1	4	7	5	7	Pirmez et al., 1999
9	24	10	24	4	23	5	23	Aviles et al., 1999
18	23	11	13	7	10	ND		Matsumoto et al., 1999
566	646	271	571	311	606	90	256	**Total**
(88%)		(47%)		(51%)		(35%)		Percent positive

ND = not done.

pendent on the origin of sample biopsy (e.g. skin biopsy, dermal scraping, or lesion aspirate), DNA extraction methods and PCR primers used (Reithinger et al., 2000). Though there has been considerable effort in applying molecular diagnostics such as PCR to the field (De Bruijn et al., 1993, Belli et al., 1998, Harris et al., 1998), and more user-friendly PCR-ELISA techniques have now been developed (Pinero et al., 1999), a reference diagnostic center remains necessary to process the samples. The use of PCR-based methods should, however, have an important epidemiological application in studies that monitor the clinical and chemotherapeutic follow-up of leishmaniasis patients. For example, the detection of disseminating *Leishmania* parasites in the blood of LCL patients could indicate that they are at risk of developing mucocutaneous disease. Also, PCR combined with specific DNA probing and sequencing should help identify and characterize those *Leishmania* species or strains that are drug-resistant and that cause the different pathologies associated with LCL.

Advances in treatment

The main problems in treating VL and CL are that both pentavalent antimonials and amphotericin B can have severe side-effects (e.g. myalgia, pancreatitis, musculoskeletal pains, renal failure, peripheral neuropathy, hepatotoxicity, cardiotoxicity) (Berman, 1997) and are of variable efficacy against mucocutaneous disease (Franke et al., 1990; Llanos-Cuentas et al., 1997). Also, the drugs and medical attention due to side effects make courses of treatment expensive, and there are an increasing number of reports on patients non-responsive to the drugs either due to the emergence of drug-resistant parasite strains (Grogl et al., 1992; Lucumi et al., 1998, Robledo et al., 1999) or to immunosuppression (as is the case in HIV-infected patients) (Mattos et al., 1998). Finally, the invasiveness of the standard procedure – a lengthy course of daily intramuscular inoculations – means that a significant proportion of patients fail to complete their full course of treatment. Hence, most research has focused on the development of alternative dosage schedules or treatments (e.g. pentamidine, allopurinol, dapsone, mefloquine) including immunotherapy, thermotherapy, and phytotherapy.

In several trials, pentavalent antimonials (Sb^v) at 20mg/kg/day have shown to be more efficient than a dosage of 10mg/kg/day, 13mg/kg/d, or 15mg/kg/d in treating both VL and ACL (Berman, 1997). Reducing the duration of treatment with 20mg/kg/d Sb^v from 20d to 10d was successful in a trial in Guatemala (10d instead of 20d, cure rate: 64% [9/14]) (Berman, 1997), but less so in two trials in Colombia where only 36% [12/33] and 40% [19/57] of patients cured when treated with 20mg/kg/d Sb^v for 15d (Martinez & Marr, 1992; Martinez et al., 1997). Extending the treatment from 28 to 40 days did not increase rates of clinical cure of patients with MCL, with clinical cure rates being the same for both 28d and 40d patient groups (63% [10/16] and [12/19], respectively) (Franke et al., 1994). Recently, a Brazilian study reported a cure rate of 84% [120/143] for patients treated with 5mg/kg/d Sb^v for 30d (Oliveira-Neto et al., 1997). Several less toxic formulations of amphotericin B, such as AmBisome®, Amphocil®, and Abelcet® have been developed and tested *in vitro* and *in vivo* (Yardley & Croft, 2000), and in clinical trials in the Old World, but they await scrutiny in clinical trials against ACL (Berman, 1997).

To circumvent clinical resistance to antimonials or amphotericin B, and the side-effects associated with their use, a variety of new drugs have been tested. So far the results have been either inconclusive or negative (Berman, 1997). Pentamidine, which has been tested against ACL in Guyana (Berman, 1997) and Colombia (Soto et al., 1993; 1994a), offers significant advantages in terms of duration of treatment, and costs of medical attention (hospitalization). Seven injections of pentamidine (2mg/kg) given every other day was as efficient in curing LCL patients (cure rate: 95% [19/20]) as the standard course of Sb^v (20d 20mg/kg/d, cure rate: 91% [21/23]) (Soto et al., 1993). Increasing the dose from 2mg/kg to 3mg/kg increased the cure rate from 84% (32/38) to 96% (49/51) (Soto et al., 1994a). However, pentamidine is not cheap and has serious side effects, which include hypoglycemia, hypotension, myalgias, and nausea. Another new treatment, paromomycin (aminosidine) monotherapy, although effective against VL in Kenya (Berman, 1997), has been shown to be rather ineffective against ACL, with clinical cure in only 10-50% of tested patients, whether applied by inoculation (Soto et al., 1994b) or as a cream (Nonaka et al., 1992; Krause & Kroeger, 1994). Applying paromomycin cream in combination with reduced dosages of inoculated Sb^v (i.e. polytherapy) was also less effective than the standard recommended 20d course of 20mg/kg/d Sb^v (with cure rates of 20% or 58% for the former as compared with 84% for the latter) (Soto et al. 1998). Although a topical ointment may be the most desirable formulation, an oral treatment

for leishmaniasis would also significantly increase compliance rates of patients undergoing treatment, and a number of candidate oral treatments have now been tested against ACL patients, though with no real success. Despite a pilot study reporting cure rates of 100% using mefloquine (Gomez et al., 1997a, 1997b), a subsequent study showed that cure rates were no higher than for the placebo controls and less than half that observed in the Sb^v positive control group of patients (Hendrickx et al., 1998). In contrast to studies on *L. donovani* in India (Berman, 1997), dapsone is apparently ineffective for ACL: 4/6 (67%) LCL patients who completed the course of treatment were clinical failures, and patients experienced several side effects, mainly anemia and methemoglobinemia (Osorio et al., 1998b). In Colombia, 19/20 (95%) patients treated with itraconazole, a triazole, were treatment failures, the cure rate being lower than that in untreated controls (Soto et al., 1993). Also, in a study of MCL patients in Bolivia no difference was observed between amphotericin B monotherapy and amphotericin B/itraconazole polytherapy respective to time to clinical cure and treatment dosage (Rodriguez et al., 1995). Two studies showed that Sb^v/allopurinol polytherapy might be more efficient than Sb^v monotherapy in treating LCL cases (Martinez & Marr, 1992; Martinez et al., 1997), but no significant positive effect was detected for allopurinol as monotherapy against LCL (Guderian et al., 1991; Velez et al., 1997) or for Sb^V/allopurinol polytherapy against MCL patients (Llanos-Cuentas et al., 1997). Data from trials in Brazil and Panama appear to confirm that allopurinol is ineffective against ACL (Berman, 1997). Clinical trials with ketoconazole, an imidazole, are unusual as they provide rare evidence that treatment success among ACL patients may be dependent on the *Leishmania* species responsible. Ketakonazole was shown to be effective against rapidly self-curing ACL caused by *L. mexicana* (cure rate: 89% [8/9] patients), but less so against slowly self-curing ACL due to *L. braziliensis* (cure rate: 30% [7/23]) (Berman, 1997). Ketoconazole has also been shown to be as effective as Sb^v in treating *L. panamensis*-infected patients, the cure rates being 76% (16/21) and 68% (13/19), respectively (Berman, 1997), i.e. the cure rate amongst *L. panamensis* patients is apparently intermediate between that for *L. mexicana* and *L. braziliensis* patients. Finally, a few studies have looked at phytotherapy as an alternative to chemotherapy to treat leishmaniasis, but to date compounds with leishmanicidal activity have only been isolated or tested *in vitro* or *in vivo* experimental models (Fournet et al., 1996).

Despite the number of clinical trials that have tested different dosages, schedules, and drugs against leishmaniasis, comparisons between studies are difficult. Firstly, ACL is characterized by a tendency of lesions to self-cure, which can be as high as 75% (Guderian et al., 1991). Failure to include either negative (placebo) or positive (recommended standard treatment, i.e. Sb^v) controls in the studies (Falcoff et al., 1994; Krause & Kroeger, 1994; Soto et al., 1994a, 1994b; Gomez et al., 1997a, 1997b; Osorio et al., 1998b) makes the interpretation of an effect of either differences in drug, dosage, or schedule impossible. This is of particular importance in studies that have used small numbers of patients to evaluate treatment response. Secondly, infecting parasite species and strains are likely to vary in their sensitivities to drugs, and cure rates of ACL patients with moderate (LCL) or severe disease (MCL) are very different (e.g. Llanos-Cuentas et al., 1997). Healing rates also depend on host factors, such as localization and chronicity of lesions, underlying illness or concomitant infection, and acquired resistance to *Leishmania* infection. Thirdly, comparisons between studies are also difficult because the studies vary in experimental protocol (e.g. study design, duration of follow-up) and in particular in their definition of "clinical cure". Thus, whereas in one study clinical cure is defined as "when lesions had > 80% re-epithelialized by the first follow-up at 1.5 months" (Guderian et al., 1991), other studies define it as "complete re-epithelialization of all lesions at the end of treatment and no reactivation or mucosal involvement during the follow-up" (Osorio et al., 1998b). Even after apparent clinical cure, treated ACL patients can remain parasitologically positive, leading to persistent infections and possible relapse (Guevara et al., 1994). The ability of *Leishmania* parasites to persist in the host despite containment has implications for immunocompromised persons: MCL is likely to become an increasing risk for *Leishmania*-HIV co-infected patients in endemic regions (Mattos et al., 1998).

Advances in prevention

The principal leishmaniasis prevention strategy available is house spraying with residual insecticides, which is practiced infrequently and in a somewhat arbitrary fashion according to available funds and local political pressures. This policy has long been suspected to be effective against the domestic transmission of

ACL, at least where sandfly vectors are significantly endophagic and endophilic. Circumstantial evidence for this belief comes from the transient reduction in LCL incidence in Peru which appears to have been a beneficial side product of the national house-spraying campaign during the 1960s against malaria and bartonellosis (Davies et al., 1994). A recent randomized control trial in the Peruvian Andes has finally provided the first direct evidence in the Andean region that house spraying can reduce LCL incidence (by up to 81%: Davies et al., in press). It remains to be seen whether house spraying is equally effective against the whole range of domestic sandfly vectors found in the region.

As for malaria control, the long-term sustainability of a house-spraying leishmaniasis control program, whatever its efficacy, must be in doubt. Hence, in the absence of a vaccine in the foreseeable future (Armijos et al., 1998), researchers are actively seeking an alternative control strategy. Three areas of current research stand out. Firstly, a number of mostly small-scale trials have been carried out to test the effectiveness of insecticide-impregnated bed nets, curtains (Alexander et al., 1995b; Campbell-Lendrum et al., in press), or clothes (Soto et al. 1995). Secondly, the possibility of biological control agents against sandflies is being actively explored (Reithinger et al., 1997). Finally, the possibility that domestic dogs may be significant reservoir hosts for ACL indicates that they could be targeted in a control program (Reithinger & Davies, 1999). Instead of eliminating infected dogs, as in the highly controversial control programs for zoonotic VL, there is a very exciting possibility that the transmission cycle could be cut simply by preventing sandflies biting dogs – i.e. by the community-wide use of insecticide-impregnated dog collars (Halbig et al., 2000).

Conclusion

In conclusion, we suggest that the following research questions are the most relevant for public health. Firstly, we need more reliable information on the geographic distribution of disease and sandfly vectors. Armed with these risk maps, after carrying out a series of intervention trials, we can ask when and where it is cost-effective to introduce an intervention strategy, such as house spraying or impregnated bed nets. Alternative control strategies should also be tested, such as biological control for sandflies or impregnated dog collars. With respect to patient management, we need to know how to predict which LCL patients are likely to develop MCL; and we need to determine what is the most cost effective (site-specific) protocol for diagnosis and treatment of patients, including the method of delivery of drugs. The potential for expanding the use of immunotherapy should be explored in further trials, and alternative treatments such as thermotherapy should be tested. Finally the search for an effective vaccine should continue.

Acknowledgments

Unpublished data were kindly provided by Fernando Abad-French, Manuel Calvopiña, Rodrigo Armijos (Ecuador), Humberto Guerra, Alejandro Llanos-Cuentas (Peru), Raul Pardo, Gerardo Muñoz, Ricardo Palacios (Colombia), and Hernan Bermudez (Bolivia). Funding for many of CRD's epidemiological studies on leishmaniasis in Peru, Bolivia, and Colombia during the last 10 years was provided by the European Union, the Wellcome Trust, World Health Organization, COLCIENCIAS, the Sir Halley Stewart Trust, and the British Council. RR is supported by the Sir Halley Stewart Trust.

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