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PARASITOLOGY

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Contents

17

New World leishmaniasis

RALPH LAINSON AND JEFFREY J. SHAW

H123.1 ## INTRODUCTION

P123.1 Neotropical cutaneous leishmaniasis (NCL) seems to be of great antiquity. Pottery from Peru and Ecuador, dated c. AD 400–900, commonly depicted human faces with mutilations that are remarkably similar to those caused by present day cutaneous and mucosal leishmaniasis (Figure 17.1) and, at the time of the conquistadores, Spanish historians wrote about ugly facial lesions that frequently afflicted the local Amerindians.

P123.2 The earliest traceable clinical description of the disease is probably that of a certain Dr L. Villar who, in 1859, wrote 'the disease (Peruvian ''uta'') is very like the Aleppo button' (i.e. oriental sore due to *Leishmania tropica*) (Matta 1918).

P123.3 The first suspicions that phlebotomine sandflies might be involved in the transmission of NCL seem to have been those of Cosme Bueno who, in 1764, implicated these insects in uta in endemic areas; uta is caused by *L. (Viannia) peruviana* in the Peruvian highlands (Herrer and Christensen 1975). Final proof that NCL was due to infection with *Leishmania* was to await publications by Lindenberg (1909) and Carini and Paranhos (1909), who independently demonstrated 'Leishman-Donovan bodies' in the skin lesions of patients from the State of São Paulo, Brazil. It was left to another Brazilian, Gaspar Vianna (1911) to give the name of *Leishmania brazilienses* to the parasite, later corrected to *L. braziliensis* by Matta (1916).

P123.4 It is quite extraordinary that until 1972, all cases of NCL in Brazil and a number of neighboring countries were attributed solely to this parasite, although specific species names were attributed in different regions: Velez (1913) had given the name *L. peruviana* to the causative agent of Peruvian uta; Biagi (1953) had named the parasite of Mexican chiclero's ulcer as *L. tropica mexicana,* emended to *L. mexicana* by Garnham (1962); and Floch (1954) considered pian-bois in French Guyana to be due to *L. tropica guyanensis,* later emended to *L. braziliensis guyanensis* by the Brazilian parasitologist Pessôa (1961). During the past 30 years, intensive ecological and epidemiological studies in the Americas have revolutionized previous ideas regarding the etiology of NCL and the classification of its causative parasites. No less than 21 species of *Leishmania* are now recognized within the neotropical region, of which 15 are known to cause either cutaneous or mucocutaneous leishmaniasis or both in humans. There is little doubt that others remain to be discovered and described, particularly in the rich sandfly and mammalian faunas of the great South American forests.

P123.5 Cutaneous leishmaniasis is widespread in Latin America, and the only two countries that seem to be free of the disease are Chile and Uruguay: cases have even been recorded in the extreme south of the USA in Texas, and rodents naturally infected with *L. (L.) mexicana* have been found in Arizona, USA. A reliable figure for the incidence of the human disease in the Americas is virtually impossible to obtain: it is not a notifiable disease in most of the countries where it occurs. Most of these countries also have poor facilities for unequivocal diagnosis so heavy reliance is placed on

FIG123.1 **Figure 17.1** *Peruvian pottery (huaco) showing facial mutilations thought to represent mucosal leishmaniasis. (From Pessôa and Barretto 1948)*

clinical aspects, which are often misleading. In Costa Rica, a small country, but with very good communications and medical assistance, the impressive figure has been given of more than 2 000 cases a year in a population of 2 000 000 (Walton 1987). In Brazil, the Ministry of Health reported an increase in the number of cases from 2 856 in 1977 to 4 821 in 1982, (Walton 1987). Lacerda (1994) suggested the considerably higher figure of 154 103 recorded cases between the years 1980 and 1990, with an incidence of c. 5 people in 1 000 infected between the years 1980 and 1984, rising to 25 per 1 000 during 1987–1990.

P123.6 In all known instances, NCL is a zoonosis: that is, the causative parasites are primarily those of wild animals. When the various sandfly vectors also feed on humans there may be transmission of a number of species of *Leishmania*; in this 'unnatural' host, they usually provoke an intense reaction and the eventual development of a skin lesion at the site of the bite. Some 7–10 days later, a tiny papule appears which, although usually painless, may itch considerably. In most cases, the papule eventually ulcerates, producing a steadily growing and crater-like lesion with a characteristically inflamed, elevated border (Figure 17.2). A lesion may remain single, but in some cases infected macrophages may transport the parasites to other parts of the body and establish secondary lesions. One parasite in particular, *L. (V.) braziliensis,* tends to produce

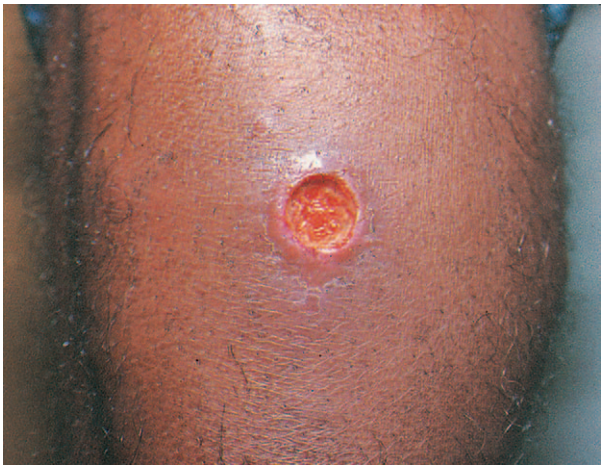

Figure 17.2 *Simple skin lesion of the arm, due to* Leishmania (Viannia) braziliensis*; Pará, Brazil* FIG123.2

such metastatic lesions in the nasal, pharyngeal and laryngeal mucosae. These may arise within a few months of the original skin lesion, or years later when the patient has supposedly been cured of the initial infection; they may be extremely mutilating (Figures 17.3 and 17.4).

Other neotropical *Leishmania* species may produce an even more serious and incurable condition in individuals who fail to mount a fully functional cell-mediated immune reaction against the parasite. Such patients, who develop large numbers of nodular lesions scattered over almost the whole skin surface (Figure 17.5), have been referred to as cases of diffuse cutaneous leishmaniasis (DCL). This is a misleading term as other perfectly curable cases of cutaneous leishmaniasis in immunologically competent patients may have numerous lesions P123.7

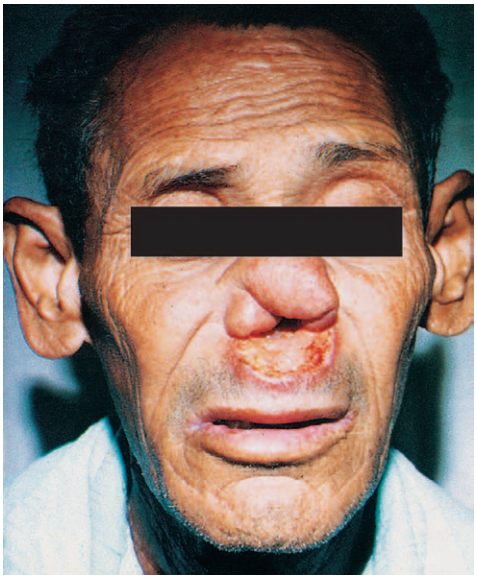

Figure 17.3 *Mucocutaneous leishmaniasis, due to* L. (V.) braziliensis*; Pará, Brazil* FIG123.3

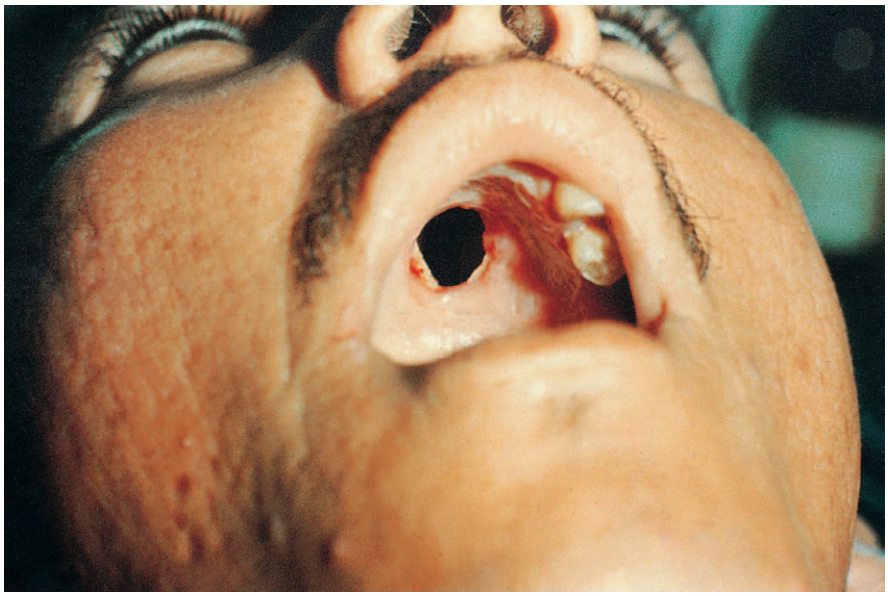

FIG123.4 **Figure 17.4** *Destruction of the palate, due to* L. (V.) braziliensis*; Pará, Brazil*

scattered over the body surface (Figure 17.6; see also *Leishmania (Viannia) guyanensis* Floch, 1954, below); the term anergic diffuse cutaneous leishmaniasis (ADCL) is more appropriate. In the Americas, this condition has been found associated only with infection by members of the *mexicana* complex, e.g. *L. (Leishmania) mexicana, L. (L.) pifanoi*, and *L. (L.) amazonensis.* In the Old World, another member of the subgenus *Leishmania*, *L. (L.) aethiopica,* causes a similar incurable disease in immunologically incompetent patients (see Chapter 16, Old World leishmaniasis).

P123.8 The fact that a number of *Leishmania* species cause disease in humans in the neotropics is reflected in variations in chemotherapeutic responses. Thus, drugs that work well in one region may not be so efficient in another because the species of the parasite infecting the patients are different. In Guatemala, for example, individuals infected with *L. (L.) mexicana* responded to treatment with ketoconazole better than those infected with *L. (V.) braziliensis* s.l., but the reverse applied when patients were treated with sodium stibogluconate (Navin et al. 1992).

P123.9 Unlike humans, the natural sylvatic hosts of the various neotropical *Leishmania* species rarely suffer disease from the infection, which is usually of a benign, inapparent nature. Under certain circumstances (see *Leishmania (Viannia) braziliensis* (Vianna, 1911) Matta, 1916), domestic animals such as dogs, mules, and horses may be found with extensive skin ulcers due to *Leishmania:* an indication that they, like humans, are unnatural and unaccustomed hosts.

P123.10 The known history of American visceral leishmaniasis (AVL) is comparatively short compared with that of the Old World (Chapter 16, Old World leishmaniasis). The first record was probably that of Migone (1913), who saw 'corpuscles', which he was convinced were amastigotes of *Leishmania,* in the blood of a sick man in Paraguay. The patient's symptoms were highly indicative of visceral leishmaniasis and, failing to respond to antimalarial treatment, he died. Prior to his illness, the man had been working on the construction of the São Paulo–Corumbá railway in Brazil, where he most probably became infected. The first undoubted cases to be registered in Latin America were documented by Mazza and Cornejo (1926) in two Argentinian children.

P123.11 Penna (1934) used the viscerotome to examine liver samples from patients suspected to have died from yellow fever in various parts of Brazil. He diagnosed 41 of them as being cases of visceral leishmaniasis, the largest number coming from the north-east of that country. Sporadic cases began to be recorded in Bolivia, Colombia, Guatemala, Paraguay, and El Salvador, but the full importance of the disease as a public health problem was not realized until as recently as 1953, when a dramatic outbreak was estimated to have been responsible for more than 100 deaths in the small country town of Sobral, in the State of Ceará, north-east Brazil (Deane 1956). Clearly, the history of AVL goes back much further than this and deaths from this highly lethal infection must have long been attributed to other causes, including malaria and yellow fever. To this day, AVL remains second in importance only to malaria among the Latin American tropical diseases, with the very conservative number of over 6 000 cases recorded in Brazil alone, up to 1980 (Deane and Grimaldi 1985).

P123.12 Although a wide variety of *Leishmania* species have been recorded in humans, the prevalence of each of these will clearly depend on just how anthropophilic their respective sandfly vectors are. Thus, by far the

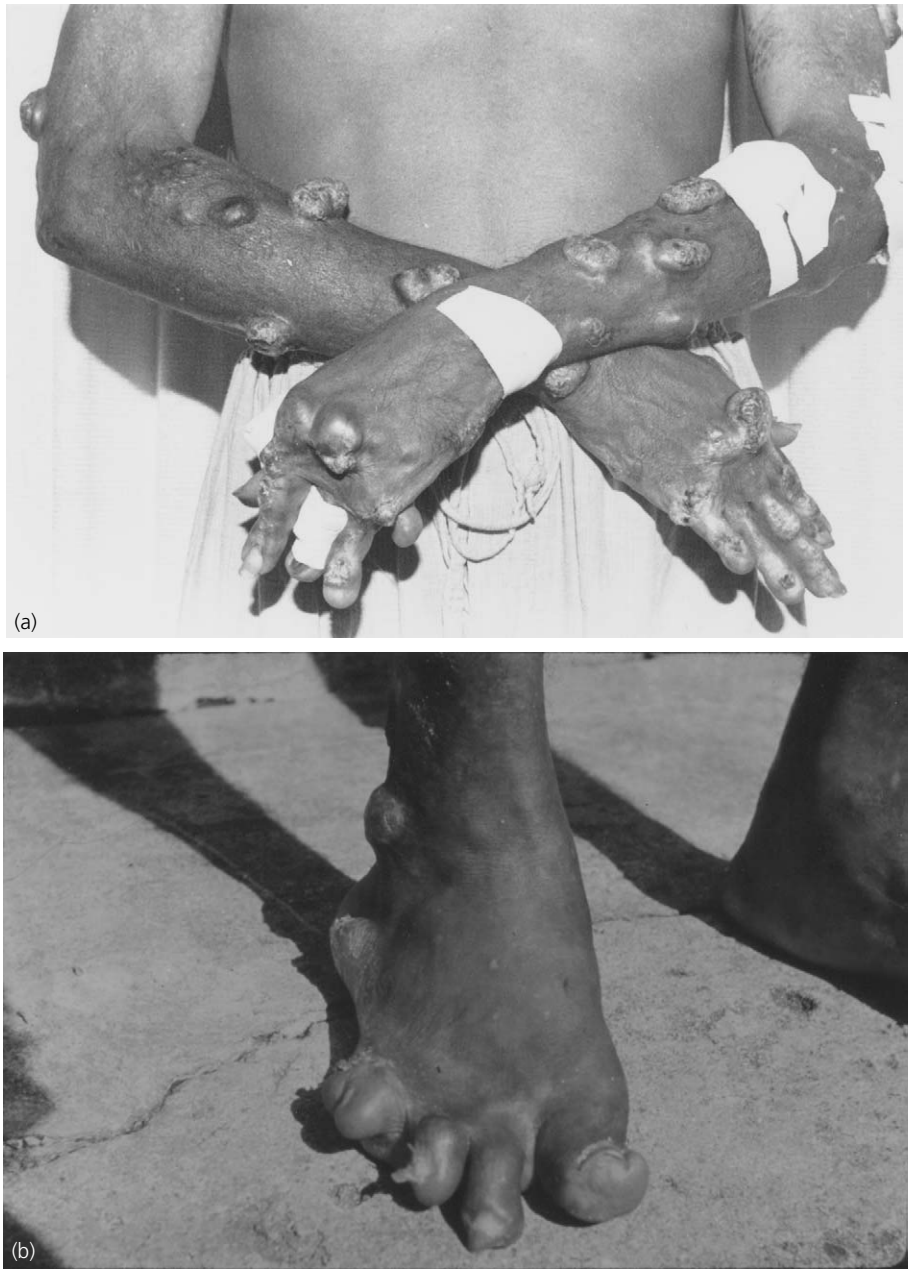

FIG123.5 **Figure 17.5** *ADCL due to* L. (L.) amazonensis. *Note the amputation of some fingers* **(a)** *and toes* **(b)**, *simulating lepromatous leprosy, with which disease ADCL is often confused; Pará, Brazil. (Figure 17.5a from Lainson 1982b)*

largest proportion of NCL cases in Brazil are due to *L. (V.) braziliensis* and *L. (V.) guyanensis*, both of which have sandfly vectors that feed avidly on humans. On the other hand, human infection with *L. (V.) lainsoni* and *L. (V.) naiffi* is relatively rare, as their vectors are disinclined to bite humans. Some neotropical *Leishmania* species, like *L. (L.) hertigi* and *L. (L.) deanei* of porcupines, are unknown in humans, possibly due to their inability to survive in human tissues, but more probably because their sandfly vectors never feed on humans.

P123.13 It is likely that all neotropical *Leishmania* species once shared a sylvatic ecology, as is still the case in the remaining areas of extensive primary and secondary forest in the Amazon Region. Following the Iberian colonization and ensuing destruction of the forests, some phlebotomine sandfly species adapted to a peridomestic habitat. The persistence of cutaneous leishmaniasis in such ecologically disturbed, although still essentially rural, areas has been taken to indicate the evolution of a secondary, peridomestic transmission of the causative parasite; the occurrence of leishmanial skin lesions in village dogs, mules, and horses suggests that such domestic animals might have now become secondary reservoir hosts. Their development of extensive skin

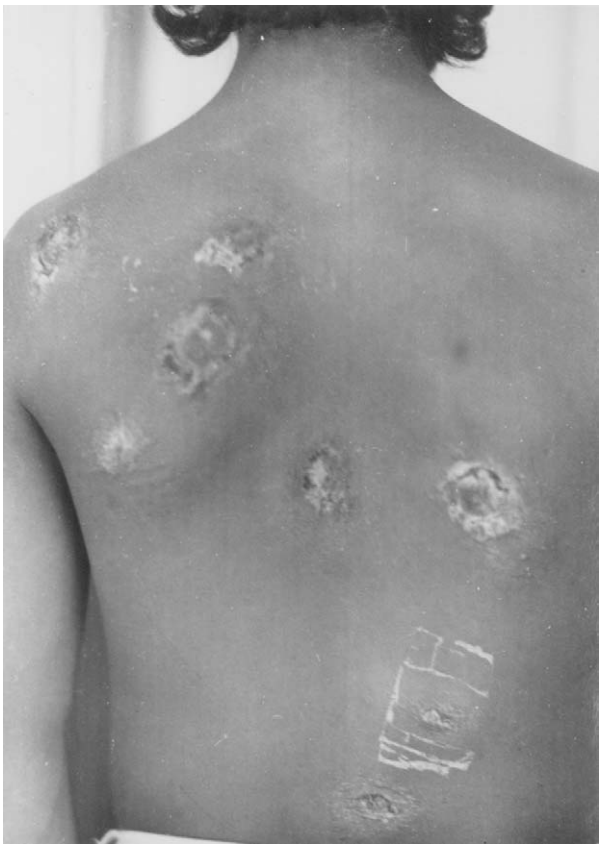

FIG123.6 **Figure 17.6** *Multiple skin lesions due to* L. (V.) guyanensis. *Similar lesions were present on this man's legs, arms and face; Pará, Brazil. (From Lainson 1982b)*

lesions tends to suggest, however, that, like humans, these animals still remain unnatural and unaccustomed 'victim' hosts. Firm evidence is required to show that infected humans, dogs, and equines are capable of infecting sandflies fed on them and thus maintaining the parasite in the absence of another source of infection in wild animals still existing in nearby surviving pockets of woodland. Reithinger and Davies (1999), in a review of the literature, concluded that evidence suggesting the domestic dog as a reservoir for human American cutaneous leishmaniasis was largely circumstantial. This conclusion was supported by Savani et al. (1999) who found no serological evidence of infection in 973 stray dogs examined in an endemic area of cutaneous leishmaniasis in São Paulo State, Brazil. Tolezano et al. (1998) carried out serological surveys of dogs in two endemic areas of the Ribeira River Valley, São Paulo State. It was suggested that dogs played only a minor role in maintaining the parasite population in one area, with only six of 77 (7.8 percent) animals positive to the skin test: in the other area, a positivity of 10 out of 56 (17.9 percent) led to their conclusion that dogs are involved in the maintenance of *L. (V.) braziliensis* to different degrees in different regions of Brazil. Positive skin tests are no indication, however, that the dogs are a source of parasites for the sandfly vector(s).

P123.14 The role of humans as a source of infection for sandfly vectors of cutaneous leishmaniasis in the Americas remains controversial. The Brazilian parasitologist Aragão (1922) succeeded in infecting *Lu. intermedia* on the skin lesions of patients, and Strangways-Dixon and Lainson (1966) infected six out of eight sandflies *(Lu. cruciata* and *Lu. ylephilator*) by feeding them on the indurated margin of lesions due to *L. (L.) mexicana* in Belize. The fact that sandflies fed on healthy skin of the same patients consistently failed to become infected and that the insects showed a marked reluctance to feed on old, scabbed lesions, led them to conclude that only on rare occasions were humans likely to serve as a reservoir of infection for the sandfly vector. In a similar experiment, Montoya-Lerma et al. (1998) obtained two infected *Lu. longipalpis* out of 103 that took a bloodmeal on the edges of lesions due to *L. (V.) braziliensis*, but none among those that had fed on normal skin of the same patients. Understandably, the systemic nature of visceral leishmaniasis provides a more ready source of amastigotes in the blood of persons with active infections. Costa et al. (2000) fed 3 747 laboratory-bred *Lu. longipalpis* on persons with active, asymptomatic and apparently cured infections, in an area endemic for AVL: 26 flies acquired infection from 11 of 44 persons with active infections, but none of those fed on 137 asymptomatic individuals became infected. This supports the observation of Deane (1956) that man is a poor source of infection compared with the acutely infected dog.

P123.15 Although *Lutzomyia longipalpis*, the major vector of AVL, is better known as a peridomestic or intra-domiciliary sandfly, its origin is sylvatic (Lainson 1989; Lainson et al. 1990a). In Amazonian Brazil, for example, it has been captured in primary rain forest far from human habitation and when crude roads are cut through such forest, newly constructed houses and animal sheds are soon invaded by this insect. It follows that whether the source of the disease is local foxes (*Cerdocyon thous*), in which the infection rate may be more than 50 percent, or infected dogs brought to the area from distant endemic foci of AVL, transmission of the parasite will ensue and a new focus of the canine and human disease will be established.

P123.16 Studies on the ecology of the New World leishmanial parasites have strongly suggested the existence of environmental barriers that limit the different species of *Leishmania* to specific sandfly species that transmit to certain mammalian hosts in distinct ecotopes. Extreme care and considerable field research is needed, therefore, before conclusions can be reached as to the principal sandfly vectors of the different parasites. The mere presence of a *Leishmania* in a specimen of a given sandfly species does not necessarily mean that this insect is the vector of that parasite. Before such a conclusion can be reached, the organism must be found with frequency in that particular sandfly and must show

abundant proliferation in the alimentary tract, with migration to the foregut and mouthparts. Ideally, a definite association with the wild mammalian host should be shown to exist and, if the parasite is a cause of human leishmaniasis, the fly must be shown to bite humans.

P123.17 Similarly, the efficiency of a given mammal in maintaining a *Leishmania* species in nature and in serving as a source of infection for the sandfly vector must be considered. Determining a significant infection rate in the mammalian host and providing experimental proof that the sandfly vector can be infected by feeding on it are prerequisites in labeling the animal as a reservoir host.

P123.18 A number of the neotropical *Leishmania* species were first described in their wild mammalian or sandfly hosts, considerable time elapsing before they were incriminated as a cause of human leishmaniasis. For this reason, this chapter discusses all the known New World species, regardless of the fact that some have yet to be found in humans. Chapter 16, Old World leishmaniasis, deals with the systematic position of the genus *Leishmania*, morphology and development of the organism in the mammalian and sandfly hosts, the application of biochemistry and molecular biology to identification of the different species of the parasite, genetics and immunology, clinical features and the treatment of human cutaneous and visceral leishmaniasis. Many features of these topics are common to both Old World and New World leishmanial parasites and this chapter discusses only those that seem to be peculiar to the American leishmaniases and their causative agents. Lengthy discussions on the evolution, ecology, epidemiology, and classification of the parasites have been given elsewhere (Lainson and Shaw 1979, 1987; Lainson 1983; Lainson et al. 1994; Shaw and Lainson 1987; Shaw 1994), and reviews on the history of the neotropical leishmaniases have also been dealt with in other publications (Lainson and Shaw 1992; Lainson 1996). For methods in the laboratory diagnosis of these diseases, see Lainson and Shaw (1981) and Chapter 16, Old World leishmaniasis.

H123.2 ## CLASSIFICATION OF THE RECOGNIZED NEOTROPICAL *LEISHMANIA* SPECIES

P123.19 The genus *Leishmania* has been subdivided into two subgenera, as follows.

The subgenus *Leishmania* Ross, 1903

P123.23 This subgenus possesses the characteristics of the genus (Chapter 16, Old World leishmaniasis). The life-cycle in the natural sandfly vector is limited to the midgut and foregut of the alimentary tract. Species occur in both the Old World and the New World (type species *Leishmania (Leishmania) donovani* (Laveran and Mesnil, 1903) Ross, 1903 of the Old World). The recorded neotropical species are:

1 *Leishmania (Leishmania) infantum chagasi** Cunha and Chagas, 1937
2 *L. (L.) enriettii* Muniz and Medina, 1948
3 *L. (L.) mexicana** (Biagi, 1953) Garnham, 1962
4 *L. (L.) pifanoi** (Medina and Romero, 1959) Medina and Romero, 1962
5 *L. (L.) hertigi* Herrer, 1971
6 *L. (L.) amazonensis** Lainson and Shaw, 1972
7 *L. (L.) deanei* Lainson and Shaw, 1977
8 *L. (L.) aristidesi* Lainson and Shaw, 1979
9 *L. (L.) garnhami** Scorza et al., 1979
10 *L. (L.) venezuelensis** Bonfante-Garrido, 1980
11 *L. (L.) forattinii* Yoshida et al., 1993.

*Recorded from humans. For distribution, see Table 17.1.

The subgenus *Viannia* Lainson and Shaw, 1987

P123.24 This subgenus possesses the characteristics of the genus (Chapter 16, Old World leishmaniasis). The developmental cycle in the natural sandfly vector includes a prolific and prolonged phase of division of rounded or ovoid paramastigotes and promastigotes attached to the wall of the hindgut (pylorus and ileum, see Figure 17.7, p. 321) by flagellar hemidesmosomes, followed by migration of free promastigotes to the midgut and foregut. Members of this subgenus are known only in the neotropical region and the recorded species are as follows:

1 *Leishmania (Viannia) braziliensis** (Vianna, 1911) Matta, 1916; type species
2 *L. (V.) peruviana** Velez, 1913
3 *L. (V.) guyanensis** Floch, 1954
4 *L. (V.) panamensis** Lainson and Shaw, 1972
5 *L. (V.) lainsoni** Silveira et al., 1987
6 *L. (V.) shawi** Lainson et al., 1989
7 *L. (V.) naiffi** Lainson and Shaw, 1989
8 *L. (V.) colombiensis** Kreutzer et al., 1991
9 *L. (V.) equatorensis* Grimaldi et al., 1992
10 *L. (V.) lindenbergi** Silveira et al., 2002
11 *L. (V.) utingensis* Braga et al., 2003

*Recorded from humans. For distribution, see Table 17.1.

Species within the subgenus *Leishmania* Ross, 1903

LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI CUNHA AND CHAGAS, 1937

Known geographical distribution

P123.25 Distribution has been noted throughout most of the Latin American continent: Argentina, Bolivia, Brazil,

TBL123.1 **Table 17.1** *A country-by-country list of the neotropical* Leishmania *species recorded in humans and the resultant pathologies*

Country	Species	Disease forms recorded[g]
Argentina	*L. (L.) infantum chagasi*	VL
	L. (V.) braziliensis s.l.	CL
Belize	*L. (L.) mexicana*	CL
	L. (V.) braziliensis s.l.	CL
Bolivia	*L. (L.) amazonensis*	CL, ADCL
	L. (L.) infantum chagasi	VL
	L. (L.) sp.	CL
	L. (V.) braziliensis s.l.	CL, MCL
	L.(V.) lainsoni[o]	CL
Brazil	*L. (L.) amazonensis*	CL, ADCL, MCL[h], VL[i]
	L. (L.) infantum chagasi	VL, (CL[j])
	L. (L.) sp.	CL[k]
	L. (V.) braziliensis	CL, MCL
	L. (V.) guyanensis	CL, MCL
	L. (V.) lainsoni	CL
	L. (V.) naiffi	CL
	L.(V) shawi	CL
	L.(V.) lindenbergi[p]	CL
Colombia	*L. (L.) amazonensis*	CL, ADCL
	L. (L.) infantum chagasi	VL
	L. (L.) mexicana	CL, ADCL
	L. (V.) braziliensis s.l.	CL, MCL
	L. (V.) colombiensis	CL
	L. (V.) guyanensis	CL
	L. (V.) panamensis	CL, MCL
Costa Rica	*L. (L.) infantum chagasi*	CL[l], VL[q]
	L. (L.) mexicana	CL
	L. (V.) braziliensis s.l.	CL, MCL
	L. (V.) panamensis	CL
Dominican Republic	*L. (L.) mexicana*-like	ADCL
Ecuador	*L. (L.)* sp.[a]	CL
	L. (L.) mexicana[a]	CL
	L. (V.) braziliensis s.l.	CL, MCL
	L. (V.) braziliensis / *L.(V.) panamensis*?	CL
	L. (V.) panamensis / *L.(V.) guyanensis*?	CL
El Salvador	*L. (L.) infantum chagasi*	VL
	L. (L.) mexicana	CL
French Guyana	*L. (L.) amazonensis*	CL, ADCL
	L. (V.) braziliensis s.l.[b]	CL, MCL
	L. (V.) guyanensis	CL
	L. (V.) naiffi[c]	CL
Guadeloupe	*L. (L.) infantum chagasi*	VL
	Leishmania sp.	CL
Guatemala	*L. (L.) chagasi*	VL
	L. (L.) mexicana	CL
	L. (V.) braziliensis s.l.	CL
Guyana	*L. (V.) guyanensis*	CL
	Leishmania sp.	MCL
Honduras	*L. (L.) infantum chagasi*	VL, CL[l]
	L. (L.) mexicana	CL, ADCL
	L. (V.) braziliensis s.l.	CL, MCL
	L. (V.) panamensis	CL, MCL
Martinique	*L. (L.)* sp.	CL

(*Continued over*)

Table 17.1 *A country-by-country list of the neotropical* Leishmania species recorded in humans and the resultant pathologies (*Continued*)

Country	Species	Disease forms recorded[g]
Mexico	*L. (L.) infantum* chagasi	VL
	L. (L.) mexicana	CL, ADCL
	L. (L.) sp.	CL, ADCL[m]
	L. (V.) braziliensis	CL
Nicaragua	*L. (L.) infantum chagasi*	VL, CL[r]
	L. (V.) braziliensis s.l.	CL, MCL
	L. (V.) panamensis	CL, MCL
	L. (V.) braziliensis / panamensis[d]	CL
Panama	*L. (V.) braziliensis* s.l.	CL
	L. (V.) panamensis	CL
	Leishmania sp.	MCL
Paraguay	*L. (L.) amazonensis*	CL, ADCL
	L. (L.) infantum chagasi	VL
	L. (V.) braziliensis s.l.	CL, MCL
Peru	*L. (V.) braziliensis* s.l.	CL, MCL
	L. (V.) peruviana	CL, MCL
	L. (V.) braziliensis / L. (V.) peruviana[e]	CL
	L. (V.) lainsoni[s]	CL
Surinam	*Leishmania* sp.	CL
USA	*L. (L.) mexicana*	CL, ADCL[m]
Venezuela	*L. (L.) infantum chagasi*	VL
	L. (L.) garnhami	CL
	L. (L.) pifanoi	CL, ADCL
	L. (L.) venezuelensis	CL
	L. (V.) braziliensis s.l.	CL, MCL
	L. (V.) colombiensis	VL[n]
	L. (V.) braziliensis / L. (V.) guyanensis[f]	CL

Data from the review articles of Lainson and Shaw 1979, 1987; Shaw and Lainson 1987; Grimaldi et al. 1989 except where otherwise indicated.
a) Katakura et al. 1993. b) Raccurt et al. 1995. c) Darie et al. 1995. d) Darce et al. 1991. e) Dujardin et al. 1995. f) Bonfante-Garrido et al.1992. g) ADCL, anergic diffuse cutaneous leishmaniasis; CL, cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; VL, visceral leishmaniasis. h) In cases of ADCL. i) Only recorded in a small region of Bahia State, BR. j) Only in Rio de Janeiro, BR. k) Localized in Minas Gerais, BR. l) Zelodón et al. 1989; Ponce et al. 1991. m) In Texas, southern USA. n) Bone marrow only. o) Martinez et al. (2001). p) Silveira et al. (2002). q) Carrillo et al. (1999). r) Belli et al. (1999). s) Lucas et al. (1994).

Colombia, Ecuador, El Salvador, Guadeloupe, Guatemala, Honduras, Martinique, Mexico, Nicaragua, Paraguay, Surinam, and Venezuela.

Known mammalian hosts

P123.26 Known mammalian hosts include humans and the domestic dog, *Canis familiaris*. Among wild animals the fox, *Cerdocyon thous*, appears to be an important natural reservoir in Brazil in the northeast (Ceará), north (Pará), and southeast (Mato Grosso do Sul) (Deane 1956; Lainson et al. 1987; Mello et al. 1988; respectively). Evidence has been presented suggesting that the infected foxes identified by Deane (1956) in Ceará as *Lycalopex vetulus* were most probably *C. thous* (Courtenay et al. 1996).

P123.27 There are reports of isolates of the parasite from domestic rats in Honduras (Walton, personal communication) and, on rare occasions, from opossums of the genus *Didelphis* in Bahia, northeast Brazil (Sherlock et al. 1984). In Colombia, *D. marsupialis* is considered to be an important reservoir host (Corredor et al. 1989; Travi et al. 1994).

P123.28 The latter authors (Travi et al. 1998) obtained positive results using the polymerase chain reaction (PCR)-hybridization test for three out of 34 specimens of the 'spiny-rat', *Proechimys canicollis* from forest in Colombia and suggested that these animals had active infections, although no parasites were isolated from them. Continuing their studies on the epidemiology of visceral leishmaniasis in Colombia, Travi et al. (2002) tested the susceptibility of another species of *Proechimys* (*P. semispinosus*) to *L. infantum chagasi* following inoculation of promastigotes by the intracardial and intradermal routes. No parasites could be detected in smears of the viscera from all of the 10 animals when they were sacrificed 7 months later, but positive cultures were obtained from splenic material of two of five animals inoculated by the intracardial route and three of five inoculated by the intradermal route. Attempts to infect sandflies (*Lu. longipalpis*) after two or three xenodiagnoses on each animal, during a period of 1–6 months, all failed: PCR-

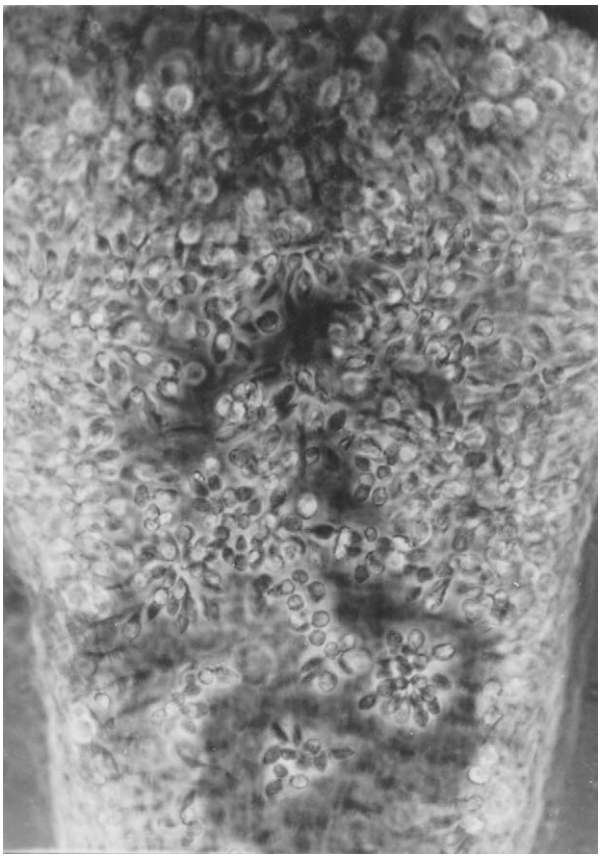

FIG123.7 **Figure 17.7** *The pylorus ('hindgut triangle') of a sandfly infected with* L. (V.) braziliensis. *Prolific division of rounded or ovoid promastigotes and paramastigotes attached by flagellar hemidesmosomes to the gut wall, a characteristic of* Leishmania *species within the subgenus* Viannia.

hybridization tests gave negative results. It was concluded that the infection in *P. canicollis* 'is contained and compartmentalized', but that there may be differing degrees of susceptibility among different species of *Proechimys*.

P123.29 Lainson et al. (2002) attempted to infect 12 laboratory-bred *Proechimys guyanensis* with a strain of *L. i. chagasi* from Amazonian Brazil, by massive intraperitoneal inoculation of amastigotes or promastigotes Although control hamsters succumbed to acute infection 6 months p.i., no infection could be registered in any of the *P. guyanensis* when sacrificed at 6 or 12 months p.i., by stained smears of liver and spleen, culture of these tissues and their inoculation into hamsters, and the PCR-hybridization test. Furthermore, during the experiment laboratory-bred *Lu. longipalpis* showed a complete reluctance to feed on the experimental animals. Based on these results, previous unsuccessful attempts to capture *Lu. longipalpis* in Disney-traps baited with *P. guyanensis* and placed in the backyards of sandfly-infested houses, and a failure to isolate *L. i. chagasi* from large numbers of this rodent captured in foci of AVL in northern Brazil (Lainson et al. 1987), the authors concluded that *P. guyanensis* is an unsuitable host for this parasite and plays no important part in the eco-epidemiology of the disease in north Brazil.

Recorded sandfly hosts

P123.30 *Lutzomyia (Lutzomyia) longipalpis* is the major vector of *L. (L.) infantum chagasi* throughout its geographic range (Deane and Grimaldi 1985; Lainson 1989) and the parasite has been transmitted experimentally by the bite of this sandfly, using both laboratory-infected and naturally infected flies (Lainson et al. 1977, 1985). Strong evidence was provided, however, that *Lu. longipalpis* represents a species complex of at least two taxa (Ward et al. 1988), based on slight morphological differences (males with pale spots on terga 3 and 4, versus others with a similar spotted pheromonal gland on only the 4th tergum). Attempts to cross-breed the two forms failed, adding support to this suggestion (Ward et al. 1983). Following a study of population genetics and phylogenetic analyses of *Lu. longipalpis* from Central and South America, Soto et al. (2001) suggested that specific allopatric populations had differentiated to the extent of forming sibling species, with four distinct lineages corresponding to central and northern South America, Brazil and an isolated population in Colombia.

P123.31 Lanzaro et al. (1993) compared populations of *Lu. longipalpis* from Costa Rica, Colombia and Brazil, using isoenzyme electrophoresis and cross-breeding experiments. They concluded that the three populations represented three different species. This finding has helped to explain why different clinical manifestations of *L. (L.) infantum chagasi* infection in humans occur in some geographic regions (see Clinical features).

P123.32 In one particular region endemic for visceral leishmaniasis, in the Córdoba Department of Colombia, 87 percent of the sandflies captured were found to be *Lutzomyia evansi* and as one of these flies was infected with *L. (L.) chagasi* it was suggested that this sandfly might be acting as an alternative vector (Travi et al. 1990). In later studies (Travi et al. 1996) infections were found in nine more specimens of *Lu. evansi*, and, taking into account the apparent absence of *Lu. longipalpis* and the peridomestic and intradomiciliary habits of *Lu. evansi*, these authors concluded that this species was the principal vector in that part of Colombia. *Lu. evansi* had long been suspected as an alternative vector of *L. (L.) infantum chagasi* in Venezuela, following failure to find *Lu. longipalpis* in some foci of AVL where *Lu. evansi* was abundant (Potenza and Anduze 1942; Pifano and Romero 1964). The conclusions of these authors have gained strong support from the recent isolation of promastigotes from this sandfly in Venezuela and the finding that k-DNA restriction analysis showed high homologies between the cultures and *L. (L.) infantum chagasi* (Feliciangeli et al. 1999).

P123.33 In the State of Mato Grosso do Sul, Brazil, suspicions have been raised that *Lu. cruzi* might be another alter-

native vector (dos Santos et al. 1998). Unfortunately, the females of this sandfly are morphologically indistinguishable from those of *Lu. longipalpis* and conclusions were based on an infection in a single female sandfly (*Lu. cruzi* or *Lu. longipalpis*?) and the apparent absence of *Lu. longipalpis* males. The limited geographical distribution of these two 'alternative' vectors compared with that of *Lu. longipalpis*, however, leaves no doubt regarding the overwhelming importance of the latter as the principal sandfly host of *L. (L.) infantum chagasi*.

Disease caused by the parasite in humans

P123.34 The parasite predominantly causes visceral leishmaniasis, commonly with a fatal outcome unless treated (see Chapter 16, Old World leishmaniasis). On rare occasions, the visceral disease may be preceded by a cutaneous lesion, and in some geographical regions the parasite is responsible for an almost exclusively cutaneous disease (see Clinical features). Until recently, only cutaneous lesions were recorded in Costa Rica, but a single case of visceral leishmaniasis has now been recorded (Carrillo et al. 1999). Common names for the visceral disease include kala-azar or calazar but it is more appropriate to reserve these terms for Indian visceral leishmaniasis and to use the name American visceral leishmaniasis or AVL.

P123.223 Opinions have been divided as to whether the causative agent of AVL is indigenous to the Americas, or whether it is simply *L. (L.) infantum*, which was introduced into the New World by immigrants from the Iberian peninsula in post-Columbian times. Points in favor of the first hypothesis and retention of the specific name *L. (L.) chagasi*, originally given to the parasite, are as follows:

- There is a high incidence of infection in the native fox, *Cerdocyon thous*, in relatively remote areas of Amazonian Brazil and the benign and inapparent nature of these infections is indicative of an ancient host–parasite relationship.
- Wild canids are considered to be the source from which members of the *L. (L.) donovani* complex originated (Lysenko 197l) and canids were present in the Americas as long ago as the Pleistocene era, some 2–3 million years ago.
- *Lu. longipalpis* is a vector of *L. (L.) chagasi* throughout almost the entire geographic range of the parasite, but is not known to transmit any other neotropical species of *Leishmania*; the vectors of *L. (L.) infantum* belong to a different sandfly genus. The host specificity of *Leishmania* species in nature is most pronounced among their sandfly vectors, thus it seems unlikely that introduced *L. (L.) infantum* could have made the relatively sudden jump from one phlebotomine genus to another.
- There are differences in both the kinetoplast DNA fragment patterns and the radio-respirometry profiles of *L. (L.) infantum* and *L. (L.) chagasi* (Jackson et al. 1982, 1984; Decker-Jackson and Tang 1982) and the two parasites are antigenically different (Santoro et al. 1986). The hypothesis that American visceral leishmaniasis is the result of imported *L. (L.) infantum* is based on the similarity of those isoenzyme profiles of the two parasites that have been studied (Rioux et al. 1990), the dual role of the domestic dog as an amplification host in their respective epidemiologies (Killick-Kendrick 1985) and recent studies on the genomic diversity in the *Leishmania donovani* complex (Maurício et al. 1999). The authors of this chapter have long used the name *L. (L.) chagasi*. From the close biochemical and molecular similarities that have now been shown, however, we feel it best to separate the two parasites only at subspecific level, with the names *L. (L.) infantum infantum* and *L. (L.) infantum chagasi*.

LEISHMANIA (LEISHMANIA) ENRIETTII MUNIZ AND MEDINA, 1948

Known geographical distribution

P123.40 To date, this parasite has been found only in the States of Paraná and São Paulo, Brazil.

Known mammalian hosts

P123.41 Natural infections have, until now, only been found in domestic guinea-pigs (*Cavia porcellus*). When this strange parasite was first discovered, producing large tumor-like lesions on the ears of two laboratory guinea-pigs (Medina 1946), the origin of the infected animals was obscure. As phlebotomine sandflies are the only known vectors of *Leishmania* species, however, it is difficult to imagine that domestic guinea-pigs are the principal natural hosts of *L. (L.) enriettii*, and it can only be assumed that the animals had spent some time in or near a rural area where transmission was occurring among the true, wild mammalian hosts. There have been two further spontaneous reappearances of the parasite in domestic guinea-pigs, again in Curitiba, Paraná (Luz et al. 1967) and more recently in a rural district of São Paulo State (Machado et al. 1994). Although the exact locality of the animals was on these occasions well documented, attempts to discover the wild animal source of the parasite were not made.

Recorded sandfly hosts

P123.42 The natural vector of *L. (L.) enriettii* remains to be discovered. Luz et al. (1967) examined the sandfly population of neighboring forest around the site of isolation, where *Lu. monticola* and *Lu. correalimai* were the only species encountered. *Lu. monticola* was caught on tree trunks, in the nests of opossums (*Didelphis*) and from human bait, and six of 10 specimens fed on the lesions of guinea-pigs became heavily infected.

Absence of infection in humans

P123.43 Human infection has not yet been reported. Muniz and Medina (1948) attempted to infect human volunteers, rhesus monkeys, dogs, mice, and the wild guinea-pig (preá) by the intradermal inoculation of promastigotes from in vitro cultures, without success. Out of eight hamsters inoculated, only one developed an inconspicuous lesion containing scanty amastigotes. Failure to infect the closely related wild guinea-pig (*Cavia aperea*) at first seems surprising. Lainson and Shaw (1979) suggested, however, that if this animal is the natural host of *L. (L.) enriettii* the inoculated preá may well have developed an inapparent infection that went unnoticed.

LEISHMANIA (LEISHMANIA) MEXICANA (BIAGI, 1953) GARNHAM, 1962

Known geographical distribution

P123.44 The areas of known geographical distribution are Southern USA (Arizona and Texas), Mexico, Belize, Guatemala, Honduras, and Costa Rica. In view of extensive geographic separation from the type locality of *L. (L.) mexicana* and considerable differences in the mammalian and phlebotomine sandfly faunas, reports of this parasite in Panama and some South American countries must be viewed with caution.

Known mammalian hosts

P123.45 The known mammalian hosts are humans and the forest rodents *Ototylomys phyllotis* (primary host), *Nyctomys sumichrasti*, *Heteromys desmarestianus* and *Sigmodon hispidus* (secondary hosts). In southern USA, the rodent *Neotoma albigula* has been found infected in Pima County, Arizona.

Recorded sandfly hosts

P123.46 *Lutzomyia olmeca olmeca* is the only proven vector, but *Lu. (Lu.) diabolica* has been suspected as a vector in foci of cutaneous leishmaniasis due to *L. (L.) mexicana* in southern Texas and northern Mexico, and *Lu. anthophora* in Arizona.

Disease caused by the parasite in humans

P123.47 This parasite causes cutaneous leishmaniasis, with a pronounced tendency towards long-lasting and destructive lesions of the external ear (Figure 17.8). Relatively rare cases of ADCL have been recorded, principally from southern Texas and northern Mexico. Local names include chiclero's ulcer, chiclero's ear, and bay-sore.

P123.48 Parasites clearly related to *L. (L.) mexicana* have been reported from humans and wild animals in Panama, Colombia, Venezuela, Peru, and parts of Brazil. In the absence of the vector, *Lu. olmeca olmeca,* it is perhaps unlikely that any of these are true *L. (L.) mexicana*.

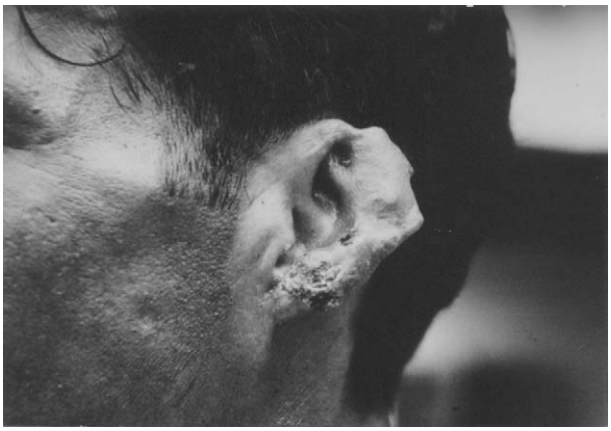

Figure 17.8 *Chiclero's ulcer, due to* L. (L.) mexicana*. Almost total destruction of the the external ear in a chiclero from Belize with an infection of many years' duration. (From Lainson and Strangways-Dixon 1963)* FIG123.8

Sandflies of the *Lu. flaviscutellata* complex, to which subspecies of *Lu. olmeca* belong, range through these South American countries, however, and are probably the vectors of a number of closely related parasites within the *mexicana* complex: at present this includes *L. (L.) pifanoi*, *L. (L.) amazonensis*, *L. (L.) aristidesi*, *L. (L.) garnhami*, *L. (L.) venezuelensis* and *L. (L.) forattinii*.

LEISHMANIA (LEISHMANIA) PIFANOI (MEDINA AND ROMERO, 1959) MEDINA AND ROMERO, 1962

Known geographical distribution

P123.49 Known distribution is limited to Venezuela, specifically in the States of Yaracuy, Lara and Miranda.

Known mammalian hosts

P123.50 Humans are the only known mammalian host: the wild mammalian hosts of the parasite have yet to be discovered. A parasite isolated from the forest rodent *Heteromys anomalus* was shown to behave in hamsters in the same way as *L. (L.) pifanoi* isolated from humans, but its conclusive identification remains in doubt.

Recorded sandfly hosts

P123.51 A parasite with similar characteristics was also found in a specimen of *Lu. flaviscutellata* but, once again, has not been conclusively identified. This sandfly is the proven vector of *L. (L.) amazonensis*, another member of the *mexicana* complex often found in rodents in South American forests and it remains likely that the Venezuelan workers were dealing with this parasite.

Disease caused by the parasite in humans

P123.52 All isolates found to date have been from cases of ADCL. It is most likely, however, that simple, curable lesions also occur in immunologically competent

individuals. There has been much controversy regarding the validity of this member of the *mexicana* complex, some authors regarding it merely as an enzymic variant of either *L. (L.) amazonensis* or *L. (L.) mexicana*. Much confusion has certainly resulted from the laboratory mix-up of parasites. Although there are relatively few isolates of *L. (L.) pifanoi*, interest in the clinical features of the human infection has resulted in a wide distribution of the parasite to laboratories throughout the world.

LEISHMANIA (LEISHMANIA) AMAZONENSIS LAINSON AND SHAW, 1972

Known geographical distribution

P123.53 The parasite has been recorded in Bolivia, Brazil, Colombia, French Guyana, and Paraguay. It is also very likely to occur in other South American countries where the sandfly vector is found.

Known mammalian hosts

P123.54 Known mammalian hosts are the forest rodents *Proechimys* (principal host), *Oryzomys*, *Neacomys*, *Nectomys*, and *Dasyprocta*; the marsupials *Marmosa*, *Metachirus*, *Didelphis*, and *Philander*; and the fox *Cerdocyon*.

Recorded sandfly hosts

P123.55 The principal vector of the parasite is *Lutzomyia (Nyssomyia) flaviscutellata*. Occasional infections have been found in the closely related flies *Lu. (N.) olmeca nociva* and *Lu. (N.) reducta* but, if these are capable of transmitting the parasite, they probably play a small role in its ecology and epidemiology. In Bolivia, a parasite identified as *L. (L.) amazonensis* has been isolated from 16 of 1 715 *Lu. nuneztovari* dissected (Martinez et al. 1999).

Disease caused by the parasite in humans

P123.56 The parasite causes cutaneous leishmaniasis, usually of the single sore type and ADCL in individuals with a defective cell-mediated immune system (Figures 17.5 and 17.9). If it does occur, classical mucocutaneous leishmaniasis following metastasis to the nasopharyngeal mucosae from a simple cutaneous lesion is extremely rare. In advanced cases of ADCL, however, the disseminated infection may also include those tissues.

P123.57 Typical visceral leishmaniasis in patients from one particular region of Bahia State Brazil, has been attributed to *L. (L.) amazonensis* (Barral et al. 1986). Conversely, no records exist to confirm this anywhere else in the geographical range of the parasite and cases of ADCL of very long duration show no signs or symptoms of visceral involvement, in spite of their defective immune system. Silveira examined the tissues from a patient who suffered from ADCL due to *L. (L.) amazonensis* from the age of 5 years until his death at the age of 57 years (Figure 17.5a) (F.T. Silveira, unpublished observations). At the time of his death, scarcely any of his body surface remained unaffected and the cutaneous lesions contained enormous numbers of parasites (Figure 17.10). No macroscopic or microscopic pathological changes were seen in the viscera and no amastigotes were found in stained impression smears prepared from the spleen, liver and lungs, and bone marrow. Finally, hamsters inoculated intradermally with triturates of these visceral tissues failed to become infected.

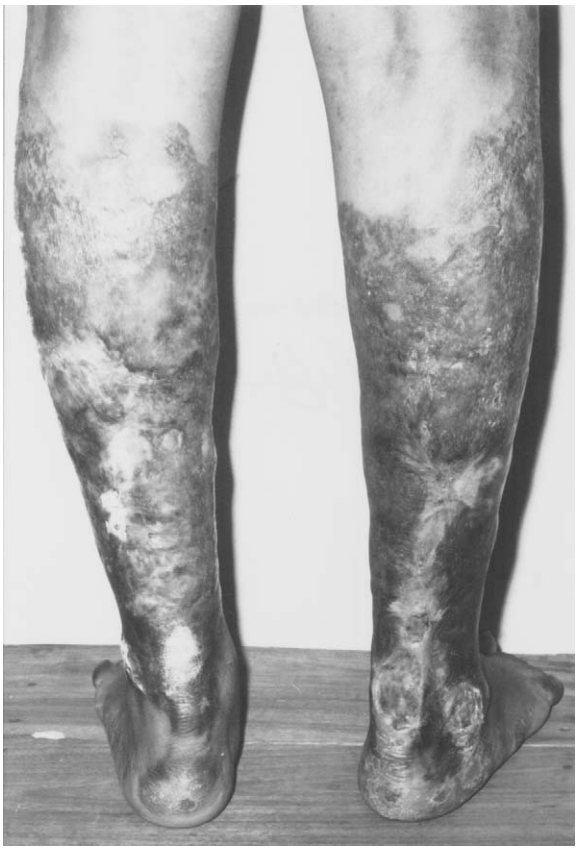

Figure 17.9 *ADCL due to* L. (L.) amazonensis *in a young woman from Pará, Brazil, showing active lesions and extensive scarring of the legs in an infection of some 15 years' duration.* FIG123.9

LEISHMANIA (LEISHMANIA) ARISTIDESI LAINSON AND SHAW, 1979

Known geographical distribution

The parasite is known to occur in the Sasardi forest, San Blas Territory, Eastern Panama. P123.58

Known mammalian hosts

Known mammalian hosts are the rodents *Oryzomys capito*, *Proechimys semispinosus*, and *Dasyprocta punctata*; and the marsupial *Marmosa robinsoni*. P123.59

Recorded sandfly hosts

The species most suspected is *Lutzomyia (Nyssomyia) olmeca bicolor*. Christensen et al. (1972) showed it to be P123.60

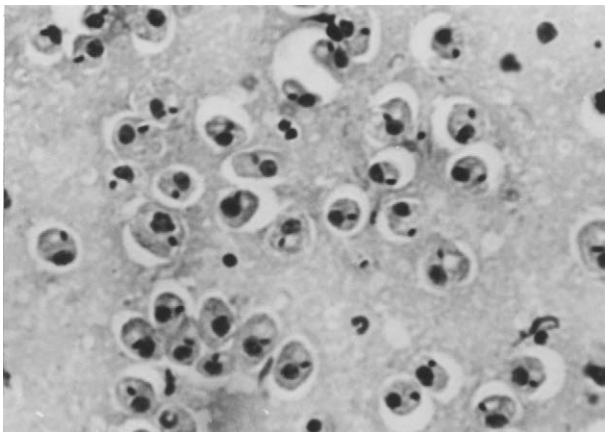

FIG123.10 **Figure 17.10** *Amastigotes of* L. (L.) amazonensis *in a Giemsa-stained smear from one of the nodular lesions of the patient shown in Figure 17.5a.*

the dominant fly on Disney-traps baited with rodents and opossums in the area where infected animals had been captured and the most common species collected among leaf litter on the forest floor.

Possibility of infection in humans

P123.61 Human infection has not yet been reported. *Lu. olmeca bicolor* does bite humans on rare occasions and it is likely that the parasite may eventually be found infecting a human. In this respect, it should be remembered that following the discovery of *L. (V.) naiffi* in armadillos in 1979, 11 years were to pass before cases of human cutaneous leishmaniasis due to this parasite were diagnosed (Lainson et al. 1979, 1990b; Naiff et al. 1989).

LEISHMANIA (LEISHMANIA) GARNHAMI SCORZA ET AL., 1979

P123.62 Difference of opinion exists regarding the validity of *L. (L.) garnhami*, which is indistinguishable from *L. (L.) amazonensis* on isoenzyme profiles (Rioux et al. 1990). Guevara et al. (1992), however, noted clear differences between the nontranscribed rDNA intergenic spacer sequences of these two parasites and, in our own laboratory, they have been separated by monoclonal antibodies (Shaw, Ishikawa, and Lainson, unpublished observations).

Known geographical distribution

P123.63 The parasite has been found only in the Venezuelan Andes.

Known mammalian hosts

P123.64 Humans are known hosts and a single infection has been recorded in the marsupial *Didelphis marsupialis*.

Recorded sandfly hosts

P123.65 Experimental infections in the sandfly *Lu. youngi* (*verrucarum* group) in Venezuela have led some authors to suggest this species to be the vector (Grimaldi et al. 1989; Young and Duncan 1994; Killick-Kendrick 1990). In addition, Scorza (in Màrquez and Scorza 1982) reported that he had found natural infections with flagellates in *Lu. youngi* (at the time identified as *Lu. townsendi*) that, on inoculation into the skin of hamsters, produced 'amastigotes de *Leishmania garnhami*'. Unfortunately, the parasite was not specifically identified and the role of *Lu. youngi* as the vector still remains in doubt.

Disease caused by the parasite in humans

P123.66 The parasite causes cutaneous leishmaniasis, but there are no recorded cases of mucocutaneous leishmaniasis or ADCL.

LEISHMANIA (LEISHMANIA) VENEZUELENSIS BONFANTE-GARRIDO, 1980

Known geographical distribution

P123.67 The parasite has been found in the Lara and Yaracuy States, Venezuela.

Known mammalian hosts

P123.68 The known mammalian hosts are humans, equines, and the domestic cat. These are best regarded as 'victim' hosts and the wild animal source of infection has yet to be ascertained.

Recorded sandfly hosts

P123.69 *Lu. olmeca bicolor* and *Lu. rangeliana* are suspected as possible vectors.

Disease caused by the parasite in humans

P123.70 The parasite causes single or multiple skin lesions, sometimes of a disseminated, nodular type simulating ADCL but curable by the current method of antimonial treatment.

LEISHMANIA (LEISHMANIA) FORATTINII YOSHIDA ET AL., 1993

Known geographical distribution

P123.71 The parasite has been found in the States of São Paulo, Bahia and Espirito Santo, Brazil.

Known mammalian hosts

P123.72 The opossum *Didelphis marsupialis aurita* (São Paulo) and the rodent *Proechimys iheringi denigratus* (Bahia

and Espirito Santo, Brazil) are the known mammalian hosts.

Recorded sandfly hosts

P123.73 The sandfly vector is unknown. Barretto et al. (1985) showed experimentally that the parasite was capable of development in the sandflies *Psychodopygus ayrozai* and *Lutzomyia yuilli* from the Três Braços area, Bahia, where the two insects are very common.

Possibility of infection in humans

P123.74 The parasite has not yet been reported to cause human disease. Both *Ps. ayrozai* and *Lu. yuilli* occasionally feed on humans and if one or other of these sandflies is indeed the vector among the wild animal hosts, human infection with *L. (L.) forattini* may well occur.

Species within the subgenus *Viannia* Lainson and Shaw, 1987

P123.75 These parasites have been separated by their isoenzymatic profiles, the use of monoclonal antibodies, and their biological characteristics. As is clear from Supplement 1 to volume 96 of *Trans R Soc Trop Med Hyg*, molecular biology techniques are now important tools in the field of parasitology and a set of microsatellite markers has recently been developed that enables separation of all known species of *Leishmania* within the subgenus *Viannia* and also indicates possible species hybrids (Russell et al. 1999). It will be particularly useful in the detection and identification of the more closely related pair *L. (V.) braziliensis* and *L. (V.) peruviana*, and the trio *L. (V.) panamensis*, *L. (V.) guyanensis* and *L. (V.) shawi*. A rapid method for the identification of *Leishmania* species using formalin-fixed biopsy samples and PCR is another promising new development (Mimori et al. 1998), as is the claim that these parasites can be reliably detected and identified, at least to the *L. (V.) braziliensis* complex level, by PCR using boiled dermal scrapings instead of skin biopsies (Belli et al. 1998).

LEISHMANIA (VIANNIA) BRAZILIENSIS (VIANNA, 1911) MATTA, 1916

Known geographical distribution

P123.76 The distribution of this important parasite is badly defined, due to inadequate methods of identification used in the past. Parasites variously described as '*L. braziliensis*', '*L. braziliensis braziliensis*' or '*L. braziliensis* sensu lato' have been reported from most Latin American countries, including Argentina, Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guyana, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Surinam, and Venezuela.

P123.77 Environmental factors seem to govern the combinations of sandfly vector and wild mammalian host in the natural history of the different species of *Leishmania*. It seems unlikely, therefore, that *L. (V.) braziliensis* sensu stricto can have such an enormous geographic range and the parasites recorded in many of these regions may represent related, but different, parasites of the *braziliensis* complex. The situation is aggravated by the fact that the type material of *L. (V.) braziliensis* Vianna, 1911 from Além Paraiba, Minas Gerais, Brazil, is no longer available for comparison. It is further complicated by distinctly different ecological and epidemiological features of cutaneous and mucocutaneous leishmaniasis due to *L. (V.) braziliensis* s.l. in different regions, sometimes within the same country, due to human destruction of the sylvatic habitat. Finally, a high degree of enzymic polymorphism has been shown to exist in isolates of *L. (V.) braziliensis* s.l. from Central and South America, with as many as 44 zymodemes obtained in a total of 137 isolates examined by 10 enzyme systems (Chouicha et al. 1997). In a similar manner, a variety of different serodemes have been shown to exist in the parasite from Brazil (Shaw et al. 1986).

Known mammalian hosts

P123.224 In primary forest in the Serra dos Carajás, Pará State, Brazil, the low-flying habit and high attraction of the principal sandfly vector to both humans and rodent-baited traps led to the conclusion that terrestrial mammals, such as rodents, were probably hosts of *L. (V.) braziliensis* (Ward et al. 1973; Lainson et al. 1973). Subsequent records of parasites considered as this species in the Brazilian rodents *Oryzomys concolor, O. capito*, *O. nigripes*, *Akodon arviculoides*, *Proechimys* spp., *Rattus rattus* and *Rhipidomys leucodactylus*, and the opossum *Didelphis marsupialis* did much to support this suggestion (Forattini et al. 1972, 1973; Lainson and Shaw 1970, 1979; Lainson et al. 1981a; Rocha et al. 1988). In Venezuela, records have been given for the rodents *R. rattus* and *Sigmodon hispidus* (De Lima et al. 2002). In most cases, identification of the parasite was based on only biological features and the isolates are no longer available for confirmation by modern biochemical methods, monoclonal antibodies or molecular techniques. Recently, however, a definitive identification of isolates from the Brazilian rodents *Bolomys lasiurus* and *R. rattus* as *L. (V.) braziliensis* by multilocus enzyme electrophoresis (Brandão-Filho et al. 2003) does suggest that the earlier records of rodent hosts of *L. (V.) braziliensis* were probably correct. It now remains to show that these animals serve as an efficient source of infection for the sandfly vectors.

P123.79 Domestic animals such as dogs, mules, horses, and (very rarely) cats have been found with skin lesions produced by parasites regarded as *L. (V.) braziliensis* s.l. or simply recorded as leishmanias of the *braziliensis*

complex. These reports, in areas of suspected peridomestic transmission of the parasite, come principally from localities of extensive deforestation in Argentina, southern Brazil, Bolivia, Colombia, and Venezuela.

Recorded sandfly hosts

P123.80 Uncertainties regarding the exact distribution of *L. (V.) braziliensis* make it difficult to indicate its vector (or vectors). An isolate of the parasite responsible for human cutaneous leishmaniasis in primary Amazonian rain forest in the Carajás highlands of Pará State, Brazil, has been used as a reference strain of *L. (V.) braziliensis* (MHOM/BR/1975/M2903) and in this area the vector is undoubtedly *Psychodopygus wellcomei*. This sandfly, referred to by some as *Lutzomyia (Psychodopygus) wellcomei*, has been found heavily infected on numerous occasions (Lainson et al. 1973) and *L. (V.) braziliensis* has been experimentally transmitted to a hamster by the bite of a naturally infected specimen (Ryan et al. 1987).

P123.81 *Ps. wellcomei* is essentially sylvatic and avidly feeds on humans, not only at night but also in the daylight hours during overcast weather. It is extremely abundant in the rainy season (November–April), when it may represent c. 65 percent of the total catch of some 25 different species of sandflies taken off human bait. Captures of sandflies from humans stationed at different heights on tree-ladders have shown that *Ps. wellcomei* has a vertical flight range of only 1–2 meters above ground level: this, and the insect's abundance (25.5 percent) in catches of different sandflies taken from rodent-baited traps, suggest that the principal wild mammalian hosts of *L. (V.) braziliensis*, in the area in question, are likely to be terrestrial animals, probably rodents.

P123.82 In the lowland regions of Pará State, a parasite identified as *L. (V.) braziliensis* was isolated from a single specimen of a closely related and highly anthropophilic sandfly, *Ps. complexus* (de Souza et al. 1996). The females of this species are morphologically indistinguishable from those of *Ps. wellcomei*, but the males of each species are quite distinct.

P123.83 In the State of Amazonas another highly anthrophilic species, *Ps. carrerai*, has been found infected with *L. (V.) braziliensis* s.l. (Grimaldi et al. 1989). The strain was biochemically similar to *L. (V.) braziliensis* of humans from the same region but antigenically different from those from other areas of Brazil, including the lower Amazon region.

P123.84 In other parts of Brazil, occasional isolates of *L. (V.) braziliensis* s.l. have been made from the sandfly *Lutzomyia (Nyssomyia) whitmani* sensu stricto, caught in and around houses in rural parts of Bahia and Ceará States, north-east Brazil (Hoch et al. 1986; Ryan et al. 1990; de Queiroz et al. 1994) and in the States of São Paulo and Minas Gerais (south-east Brazil) it is again suspected as a vector due to its highly anthropophilic feeding habits and its high density in and around human dwelling places and animal sheds in the endemic areas of cutaneous leishmaniasis. In the northern part of Paraná *Lu. whitmani* was shown to form 62 percent of the sandfly population captured, and to be highly anthropophilic. Of 1 628 specimens dissected, three were infected (0.2 percent) and in each case the isolated parasite was identified as *L. (V.) braziliensis* on enzyme profiles using the standard World Health Organization (WHO) reference strains for comparison (Luz et al. 2000).

P123.225 In the State of Rondônia, Brazil, a parasite identified as *L. (V.) braziliensis* has been isolated from three specimens of *Lutzomyia davisi* (Grimaldi et al. 1991). This sandfly is also highly anthropophilic.

P123.85 *Lutzomyia (Nyssomyia) intermedia* is another highly anthropophilic sandfly that, although originally sylvatic, has now adapted well to a peridomestic habitat in deforested, rural areas. It is highly suspected as a vector of *L. (V.) braziliensis* s.l. in the State of Rio de Janeiro and some parts of the State of São Paulo, Brazil and certain regions of Argentina. Flagellates thought to have been promastigotes of *Leishmania* were on one occasion seen in histological sections of the intestines of the sandflies *Lutzomyia migonei* and *Lu. (Pintomyia) pessoai* caught in endemic areas of cutaneous leishmaniasis in São Paulo State (Pessôa and Barretto 1948). Their true nature, however, remains obscure.

P123.86 In Bolivia *L. (V.) braziliensis* s.l. has been isolated from *Ps. carrerai carrerai* (Le Pont et al. 1988), *Ps. llanosmartini*, and *Ps. yucumensis* (Le Pont and Desjeux 1986), whereas in Colombia (Young et al. 1987) and Venezuela (Young and Duncan 1994) similar parasites have been found in *Lu. spinicrassa*. In north central Venezuela, parasites isolated from *Lu. ovallesi* and *Lu. gomezi* have been typed as *L. (V.) braziliensis* and these sandflies are considered as primary and secondary vectors, respectively, on epidemiological grounds (Feliciangeli et al. 1994). In the Andean region, there is strong evidence that *Lu. youngi* is the vector of local cutaneous leishmaniasis, but the parasites encountered in this sandfly require precise identification. *Lu. spinicrassa* may be a vector throughout areas bordering the endemic Colombian foci.

Disease caused by the parasite in humans

P123.87 The parasite causes cutaneous leishmaniasis, usually with one or few lesions and also mucocutaneous and mucosal leishmaniasis (Figures 17.2–17.4). Common names include: úlcera de Bauru, ferida brava, ferida sêca, bouba, buba, nariz de anta (tapir nose), and espundia.

P123.88 The various zymodemes of *L. (V.) braziliensis* s.l. are placed in the *braziliensis* complex, together with the closely related parasite *L. (V.) peruviana*.

LEISHMANIA (VIANNIA) PERUVIANA VELEZ, 1913

Known geographical distribution

P123.89 The parasite has been noted in Peru, on the western slopes of the Andes and in the inter-Andean valleys. Its range may possibly extend into the Argentinian highlands and it is probably more widely distributed in the Andean countries than previously suspected. Transmission seems to take place in relatively barren, mountainous areas with scant vegetation and a relatively restricted wild mammalian fauna.

Known mammalian hosts

P123.90 Until recently humans and dogs (*Canis familiaris*) were the only known mammalian hosts (Herrer 1951) The role of the dog in the epidemiology of the human disease has remained obscure, however, and it has long been postulated that both humans and dogs are merely 'victim hosts' of a parasite maintained in wild animals of the Peruvian Andes. Recent studies (Llanos-Cuentas et al. 1999) report that isolations of a parasite from two specimens of the rodent *Phyllotis andinum* and an opossum, *Didelphis marsupialis* have been characterized as *L. (V.) braziliensis* by their isoenzyme profiles. Five other isolates from the rodent *Akodon* sp., were identified only to the subgenus *Viannia*. That the prevalence in the wild animals is much the same as in dogs, supports the existence of enzootic disease in wild animals.

Recorded sandfly hosts

P123.91 *Lutzomyia (Helcocyrtomyia) peruensis* and *Lutzomyia verrucarum* have long been suspected as probable vectors, in view of their anthropophilic feeding habits. Isolation of a parasite with the biological characteristics of *L. (V.) peruviana* from the former fly, captured in an endemic area, implies its involvement in transmission (Herrer 1982), but much research in the field is needed before significant evidence can be obtained.

Disease caused by the parasite in humans

P123.92 The parasite causes simple cutaneous leishmaniasis, not associated with the mucocutaneous disease. It is particularly frequent in school children, commonly resulting in extensive facial scars. Ulcers are usually self-healing and a firm immunity to reinfection with the same parasite is usually imparted. Common names include uta, tiaccaraña and llaga.

LEISHMANIA (VIANNIA) GUYANENSIS FLOCH, 1954

Known geographical distribution

P123.93 This is an essentially sylvatic species that is an extremely common cause of human cutaneous leishmaniasis, particularly in Brazil, north of the Amazon river and the Guyanas. Its range is reported to also extend into Colombia, Ecuador, Venezuela, and the lowland forests of Peru.

Known mammalian hosts

P123.94 Known mammalian hosts include humans and, in primary forest, the major reservoir hosts are the sloth *Choloepus didactylus* and the lesser anteater *Tamandua tetradactyla* (Xenarthra) (Lainson et al. 1981b; Gentile et al. 1981) with occasional infections found in rodents and opossums (Lainson et al. 1981a; Gentile et al. 1981) The infection is always inapparent, with parasites located in apparently normal skin and in viscera such as the spleen and liver.

Recorded sandfly hosts

P123.95 The principal vector among wild animals and to humans is the sandfly *Lutzomyia (Nyssomyia) umbratilis*, with infections relatively infrequently found in a closely related fly, *Lu. (N.) anduzei* and *Lu. (N.) whitmani* s.l. Some early records of the parasite in the latter sandfly in Amazonian Brazil, possibly refer to *Leishmania (V.) shawi.*

P123.96 *Lu. (N.) umbratilis* is a sandfly that dwells in the forest canopy and on the larger tree trunks. During the early hours of daylight, it may be found in large numbers resting on the larger tree trunks from which the flies will readily fly off and attack humans when disturbed. Although infection of this sandfly clearly takes place when it feeds on the reservoir hosts at night in the canopy, transmission to humans is principally during the day (early morning), when gangs of forest laborers are engaged in their work, particularly deforestation. Others at risk include the collectors of Brazil nuts and other fruits, topographers, visiting botanists and zoologists, and even the occasional tourist.

P123.97 The enzootic of *L. (V.) guyanensis*, as studied in primary rain forest, is unlikely to survive in secondary forests or man-made plantations of non-indigenous trees. The small girth of young trees provides a microhabitat that is unsuitable for resting sandflies due to the low surface humidity of the smooth trunks. In addition, such immature trees are an equally unsuitable environment for relatively large and heavy animals, such as sloths and anteaters. Finally, in monoculture plantations (e.g. pine and gmelina) sloths are deprived of their normal diet of indigenous fruits and foliage.

P123.98 Cutaneous leishmaniasis due to *L. (V.) guyanensis* may reach high prevalence in human communities situated in or very near primary forest, leading to an erroneous impression that the sandfly vector, *Lu. (N.) umbratilis*, has adapted to a peridomestic habitat. There is, as yet, no evidence that this occurs and peridomestic acquisition of the disease is doubtless due to infected flies that have been attracted to the lights of houses at night, from nearby forest. Esterre et al. (1986) showed

experimentally that clearing forest to c. 500 meters from a village situated in primary forest in French Guyana completely interrupted the transmission of *L. (V.) guyanensis* among its inhabitants.

P123.99 The marsupial *Didelphis marsupialis* has rarely been found infected with *L. (V.) guyanensis* in primary forest where there is intensive transmission of the parasite among sloths and anteaters and to humans. Strangely, however, a high rate of infection has been recorded in the abnormally large populations of this opossum that are attracted to human refuse in villages on the borders of virgin forest (Arias and Naiff 1981). Reasons for this are not clear, nor is it certain whether opossums serve as a source of *L. (V.) guyanensis* for the sandfly vector, or if they merely represent dead-ends in the life-cycle of the parasite.

Disease caused by the parasite in humans

P123.100 The parasite causes cutaneous leishmaniasis, very frequently with multiple skin lesions (Figure 17.6). Cases of mucocutaneous disease appear to be rare. Common names for the disease include pian-bois, bosch-yaws, and forest-yaws.

P123.101 Multiplicity of the skin lesions arises in two very different ways. First, sloths are rather sedentary animals and an infected animal may remain in a given spot for a considerable period. The infection rate of *Lu. (N.) umbratilis* resting on neighboring tree trunks will thus tend to rise to a high level, with levels as high as 25 percent found among many hundreds of specimens taken from a single tree. It follows that people attacked by sandflies in the area may receive numerous infective bites at the same time on all exposed parts of the body. Forest workers tend to be shirtless and frequently wear shorts: for this reason many patients present with lesions scattered over the face, trunk, arms, and legs (Figure 17.6). The developing multiple lesions of such individuals tend to be of a similar size and evolution. Secondly, there is abundant clinical evidence indicating the formation of metastatic lesions in persons originally presenting with a single skin lesion. These lesions tend to follow a distinct migratory course along the lymphatics and because of this evolution they are frequently nodular and, when ulcerated, of very unequal size.

P123.102 *L. (V.) guyanensis* gives its name to the *guyanensis* complex of closely related parasites including *L. (V.) panamensis* and *L. (V.) shawi*.

LEISHMANIA (VIANNIA) PANAMENSIS LAINSON AND SHAW, 1972

Known geographical distribution

P123.103 As the name suggests, most information on this parasite comes from Panama and the Canal Zone, where the very frequent acquisition of cutaneous leishmaniasis by American military personnel prompted intensive eco-epidemiological studies. It is also recorded in west and central Colombia, Ecuador, Venezuela, Costa Rica, Honduras, and Nicaragua.

Known mammalian hosts

P123.104 Humans are a host to the parasite. The eco-epidemiology of *L. (V.) panamensis* follows a very similar pattern to that of *L. (V.) guyanensis*, which is not surprising in view of the close biological and biochemical relationship of the two parasites. The major host is the two-toed sloth *Choloepus hoffmanni* with occasional infections reported in the three-toed sloth *Bradypus infuscatus* and *B. griseus* (Herrer et al. 1973). More rarely, infections have been registered in other sylvatic animals such as *Bassaricyon gabbi*, *Nasua nasua* and *Potos flavus* (Carnivora: Procyonidae), *Aotus trivirgatus*, and *Saguinus geoffroyi* (Primates: Cebidae and Callitrichidae) and *Heteromys* (Rodentia). Hunting dogs occasionally develop skin lesions due to *L.(V.) panamensis*: like humans they are 'victim hosts' that rarely, if ever, serve as a source of parasites for the sandfly vector(s), or as a means of maintaining the enzootic.

Recorded sandfly hosts

P123.105 The major sandfly vector is considered to be *Lu. (N.) trapidoi*, whereas *Lu.(N.) ylephiletor*, *Lu. (Lu.) gomezi*, and *Psychodopygus panamensis* may act as secondary vectors (Johnson et al. 1963; Christensen et al. 1969)

Disease caused by the parasite in humans

P123.106 The parasite usually causes single or a limited number of skin lesions. Rare cases of mucocutaneous leishmaniasis have been attributed to *L. (V.) panamensis*.

P123.107 Studies on populations of *Leishmania* in Ecuador have shown a cluster in which the isoenzyme profile of 6-phosphate gluconate dehydrogenase (6PGD) showed considerable variation (Bañuls et al. 1999). As it is this enzyme that has been extensively used to separate *L. (V.) panamensis* and *L. (V.) guyanensis*, these authors suggested that these parasites are not separate species. Within the cluster of these species in eastern Amazonia, however, *L. (V.) guyanensis* strains all grouped together, while isolates from Ecuador grouped with *L. (V.) panamensis* markers. For this reason, and taking eco-epidemiological and clinical data into consideration, we prefer to maintain specific separation of the two parasites.

LEISHMANIA (VIANNIA) LAINSONI SILVEIRA ET AL., 1987

Known geographical distribution

P123.108 Until recently, this parasite was recorded only in the State of Pará, north Brazil. Its presence has now been noted, however, in forested areas of Peru and Bolivia (Lucas et al. 1994; Martinez et al. 2001). It probably exists in other regions where the known mammalian and sandfly hosts coexist.

Known mammalian hosts

P123.109 Humans are a known mammalian host. The only known host among wild animals is the rodent *Agouti paca* (Rodentia: Dasyproctidae) (Silveira et al. 1991a).

Recorded sandfly hosts

P123.110 *Lu. (Trichophoromyia) ubiquitalis* is a known host and the first representative of the subgenus *Trichophoromyia* to be incriminated as a vector of a *Leishmania* species.

P123.111 *L. (V.) lainsoni* was isolated only from this sandfly, among many other species dissected in forested areas where patients had become infected with this parasite (Silveira et al. 1991b). The puzzling fact remained that *Lu. (T.) ubiquitalis* had not been caught biting humans in the forest. It was found, however, that this sandfly would feed avidly on humans if maintained for some hours in the laboratory after capture and this prompted the conclusion that under certain conditions it must also feed on humans in its natural habitat. Continuing field studies confirmed this (Lainson et al. 1992), although the factors influencing the sandfly's biting habits remain obscure. *Lu. (T.) ubiquitalis* is clearly not particularly fond of human blood, which accounts for the relatively low rate of infection with *L. (V.) lainsoni* in humans, compared with other species of *Leishmania*, such as *L. (V.) braziliensis* and *L. (V.) guyanensis*; these have highly anthropophilic sandfly vectors. In Bolivia, promastigote infections have been recorded in the sandfly *Lu. velascoi*, but the parasite was not isolated for characterization. As this fly is the only species of the subgenus *Trichophoromyia* in a region where *L.(V.) lainsoni* was encountered infecting man, it remains highly likely that it is the local vector of this parasite (Martinez et al. 2001).

Disease caused by the parasite in humans

P123.112 The parasite causes cutaneous leishmaniasis, usually with a single ulcerating skin lesion. Cases of mucocutaneous leishmaniasis due to this parasite have not yet been encountered.

LEISHMANIA (VIANNIA) SHAWI LAINSON ET AL., 1989

Geographical distribution

P123.113 To date, the parasite has been found in various localities in the Amazon Region of north Brazil, south of the Amazon river.

Known mammalian hosts

P123.114 Humans are commonly infected. Hosts among the forest animals include the monkeys, *Cebus apella* and *Chiropotes satanas* (Cebidae); the sloths *Choloepus didactylus* and *Bradypus tridactylus* (Xenarthra); and the coatimundi *Nasua nasua* (Procyonidae) (Lainson et al. 1988, 1989). It remains likely that other arboreal animals harbor the parasite.

Recorded sandfly hosts

P123.115 Infections have so far been recorded in only one species of sandfly, which was provisionally identified as *Lutzomyia (Nyssomyia) whitmani* (Lainson et al. 1989). Morphometric differences have been noted, however, between this insect and the type material of *Lu. (N.) whitmani* sensu stricto from Bahia, northeast Brazil. These, and separation by DNA probes, suggested the vector to be a 'cryptic' species of a *Lu. (N.) whitmani* complex (Rangel et al. 1996). A recent phylogenetic analysis of the described mitochondrial (cytochrome b) haplotypes of *Lu. whitmani*, however, disputes this hypothesis, and suggests the existence of clades of haplotypes and a continuum of interbreeding populations of *Lu. whitmani* in the rain-forest regions of Brazil (Ishikawa et al. 1999). The differences in behavior of the fly in the type locality in Bahia, northeast Brazil, and that in Pará State, north Brazil are nevertheless striking. The former is highly anthropophilic, abundant in human dwelling places, and transmits *L. (V.) braziliensis*; the latter rarely bites man, until now has not been recorded in houses even when these are situated very close to the fly's normal habitat in primary forest, and is a vector of *L. (V.) shawi*.

Disease caused by the parasite in humans

P123.116 The parasite causes cutaneous leishmaniasis, usually with a single ulcerating skin lesion, but cases of multiple lesions of varying pathologies have been observed (Figure 17.11). Cases of mucocutaneous leishmaniasis due to this parasite have not yet been encountered.

LEISHMANIA (VIANNIA) NAIFFI LAINSON AND SHAW, 1989

Known geographical distribution

P123.117 Isolates of the parasite are registered in the Brazilian States of Pará and Amazonas and in French Guyana. The range of this parasite will almost certainly extend into other parts of Latin America where the wild animal host and the sandfly vector coexist.

Known mammalian hosts

P123.118 Humans are a known mammalian host (Lainson et al. 1990b). To date, the only known wild animal host is the nine-banded armadillo, *Dasypus novemcinctus* (Xenarthra: Dasypodidae), in which there is a high infection rate in apparently normal skin and viscera.

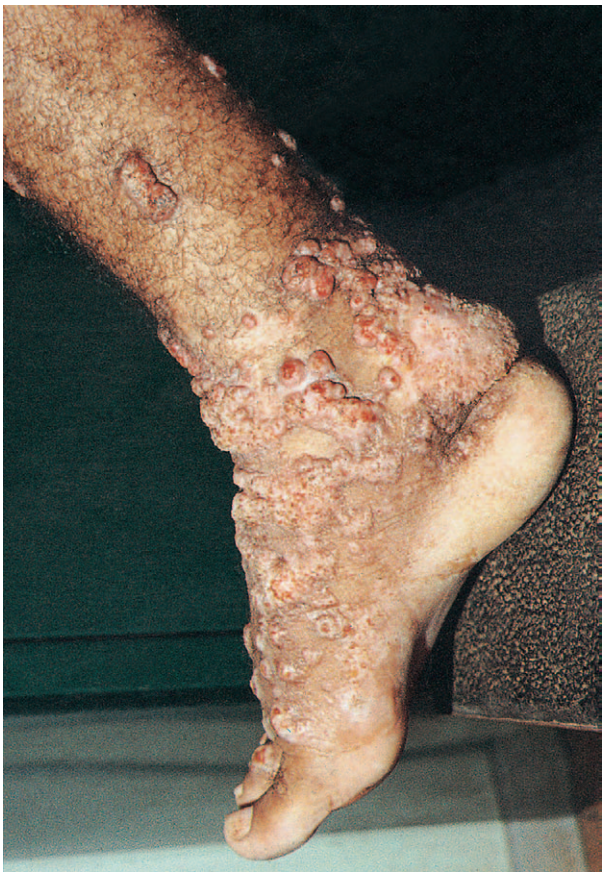

FIG123.11 **Figure 17.11** *Strange multiple nodules on the foot due to* L. (V.) shawi*, clinically resembling mycotic disease; Pará, Brazil*

Recorded sandfly hosts

P123.119 Most recorded sandfly infections (Arias et al. 1985; Naiff et al. 1989) involve the sandfly *Psychodopygus ayrozai* which is, therefore, most suspected as the vector among armadillos. This fly is not highly anthropophilic, which possibly accounts for the paucity of human infection with *L. (V.) naiffi*. On the other hand, the parasite has, on rarer occasions, also been found in *Ps. paraensis* and *Ps. s. squamiventris*, both of which are highly anthropophilic and possibly involved in transmission to humans. Further studies are clearly necessary to establish the respective roles of these three sandflies in the eco-epidemiology of *L. (V.) naiffi*.

Disease caused by the parasite in humans

P123.120 The parasite causes cutaneous leishmaniasis, usually with single, small, ulcerating lesions. No case of mucocutaneous leishmaniasis has yet been attributed to this parasite.

P123.121 Unlike most species of *Leishmania*, *L. (V.) naiffi* rarely produces a visible lesion when inoculated into the skin of hamsters, although the parasite may be re-isolated following culture of skin from the inoculation site in blood agar media after at least 1 year. For this reason, human infection with this parasite may have remained undiagnosed in the past, when inoculation of hamsters has been the sole method of isolation attempted from human skin lesions. In addition, it may be that *L. (V.) naiffi* can also produce an occult, benign infection in the skin of humans, as it does in the hamster and that transmission to humans is more frequent than has been suspected.

LEISHMANIA (VIANNIA) COLOMBIENSIS KREUTZER ET AL., 1991

Known geographical distribution

P123.122 The parasite exists in Colombia, Panama, and Venezuela, probably extending into the neighboring forests of Brazil, the Peruvian lowlands, and other Latin American countries where the wild mammalian and sandfly hosts coexist.

Known mammalian hosts

P123.123 The parasite has been recorded in humans and the sloth, *Choloepus hoffmanni* (Panama).

Recorded sandfly hosts

P123.124 *Lutzomyia (Helcocyrtomyia) hartmanni* has been found infected in Colombia and *Lu. (Lutzomyia) gomezi* and *Psychodopygus panamensis* in Panama (Kreutzer et al. 1991).

Disease caused by the parasite in humans

P123.125 The parasite causes single to multiple ulcerating skin lesions. Cases of mucocutaneous leishmaniasis caused by the parasite have not yet been seen. The strain identified from Venezuela by Delgado et al. (1993) was isolated from a bone marrow aspirate of a patient with visceral leishmaniasis. It is, however, uncertain whether or not this was the parasite responsible for the clinical symptoms observed.

LEISHMANIA (VIANNIA) EQUATORENSIS GRIMALDI ET AL., 1992

Known geographical distribution

P123.126 The parasite exists along the Pacific coast of Ecuador.

Known mammalian hosts

P123.127 The sloth, *Choloepus hoffmanni*, and the squirrel, *Sciurus granatensis* have been identified as hosts. In both animals, the parasite was isolated from the liver and spleen but was not found in the skin.

Recorded sandfly hosts

P123.128 *Lutzomyia hartmanni* has been found infected in Ecuador (Furuya et al. 1998).

Disease caused by the parasite in humans

P123.129 As yet, the parasite has not been found in humans.

LEISHMANIA (VIANNIA) LINDENBERGI SILVEIRA ET AL., 2002

Known geographical distribution

P123.130 At present, this recently described parasite has been recorded only from degraded forest on the outskirts of Belém, Pará State, north Brazil, with isolates made from a number of soldiers who acquired their infections while undertaking maneuvers in this forest at night.

Known mammalian hosts

P123.131 Humans are the only recorded host so far. From the behavior of the suspected vector and the circumstances in which the soldiers became infected, the wild animal host(s) is (are) most likely terrestrial.

Recorded sandfly hosts

P123.132 *Lutzomyia (Nyssomyia) antunesi* is by far the most common sandfly avidly biting man in the type locality. It is of particular interest that the infected soldiers spent much of their time in slit-trenches, so that their heads and resting arms were at ground level: most of their lesions were on these parts of the body. Previous workers recorded heavy promastigote infections in three specimens of *Lu. (N.) antunesi* from Marajó island, near Belém (Ryan et al. 1984), but they failed to identify the parasite. Its development in the sandfly gut was described as being 'suprapylarian in nature' (i.e. characteristic of the subgenus *Leishmania*), whereas Silveira et al. (2002) clearly showed the development of *L. (V.) lindenbergi* to be peripylarian in experimentally infected laboratory-bred *Lu. longipalpis*.

Disease caused by the parasite in humans

P123.133 Localized cutaneous lesions: as yet, no cases of mucosal lesions have been recorded. *L. (V.) lindenbergi* is distinguished from other members of the subgenus *Viannia* by its enzyme profiles and the use of monoclonal antibodies. It is clearly closely related to *L. (V.) naiffi* and also produces an inapparent infection in the skin of hamsters.

LEISHMANIA (VIANNIA) UTINGENSIS BRAGA ET AL., 2003

Known geographical distribution

P123.228 As yet, this parasite is recorded only from the Utinga forest, on the outskirts of Belém, Pará State, north Brazil.

Known mammalian hosts

P123.229 At present, mammalian hosts are unknown, but given the habits of the vector are likely to be arboreal.

Recorded sandfly hosts

P123.230 The parasite was isolated from a single specimen of the sandfly *Lutzomyia tuberculata*. This sandfly is commonly found on the larger tree-trunks of primary and secondary forests.

Disease caused by the parasite in humans

P123.231 Not yet found infecting humans. As the vector shows no tendency to bite man, human infections with *L. (V.) utingensis* are unlikely to occur by way of this insect. If any anthropophilic sandflies are also hosts of the parasite, however, human infection may well be found in the future.

'Hybrid' *Leishmania* of the subgenus *Viannia*

P123.135 Strains with phenotypic and genotypic characters of two species have been recorded in different geographical areas of Latin America. There are two possible interpretations for such strains: either they represent strains that originated directly from a common ancestor or they are the result of genetic exchange.

L. (V.) BRAZILIENSIS/*L. (V.) PANAMENSIS* HYBRID

Known geographical distribution

P123.136 The parasite has been found in northern Nicaragua (Darce et al. 1991) close to the border with Honduras. To the south, the strains are *L. (V.) panamensis* and to the north, *L. (V.) braziliensis*. Bañuls et al. (1997) recorded what appears to be the result of hybridization between *L.(V.) braziliensis*/*L.(V.) panamensis* and *L. (V.) panamensis*/*L. (V.) guyanensis* in isolates from human infections in Ecuador.

Known mammalian hosts

P123.137 So far, the parasite is only known in humans.

Recorded sandfly hosts

P123.138 No sandfly host has been recorded to date.

Disease caused by the parasite in humans

P123.139 The parasite causes ulcerating skin lesions. Cases of mucocutaneous infections have not yet been seen.

L. (V.) BRAZILIENSIS/*L. (V.) GUYANENSIS* HYBRIDS

Known geographical distribution

P123.140 In Venezuela, the parasite has been found in Lara State; Tachira; DF, El Junquito; and Miranda, Guarenas. The isolates from the last three localities were similar, and their hybrid alleles differed from those of the isolate previously made in Lara (Bonfante-Garrido et al. 1992; Delgado et al. 1997).

Known mammalian hosts

P123.141 So far, the parasite has only been recorded from humans.

Recorded sandfly hosts

P123.142 No sandfly hosts have yet been found.

Disease caused by the parasite in humans

P123.143 The parasite causes ulcerating skin lesions. Cases of mucocutaneous leishmaniasis caused by this parasite have not yet been seen.

L. (V.) BRAZILIENSIS/*L. (V.) PERUVIANA* HYBRID

Known geographical distribution

P123.144 To date, four strains have been isolated from patients in the Limapampa region of the Huanuco Valley, Peru (Dujardin et al. 1995).

Known mammalian hosts

P123.145 Humans are the only known mammalian host to date.

Recorded sandfly hosts

P123.146 No sandfly vectors have been recorded.

Disease caused by the parasite in humans

P123.147 Ulcerating skin lesions were noted in all patients and one also had a mucosal lesion.

Leishmania-like parasites of uncertain taxonomic position

P123.148 Molecular studies suggest that the following parasites are more closely related to *Endotrypanum* (an intra-erythrocytic flagellate of sloths) than they are to *Leishmania*. Until more information is available, however, we feel it best to retain their present names.

LEISHMANIA (LEISHMANIA) HERTIGI HERRER, 1971

Known geographical distribution

P123.149 The known distribution is limited to Panama and Costa Rica.

Known mammalian hosts

P123.150 The tree-porcupine *Coendou rothschildi* is the only known mammalian host.

Recorded sandfly hosts

P123.151 The sandfly vector of the parasite has still to be discovered. The remarkably high infection rate of 88 percent found in the porcupines studied suggests a close association of the vector with the mammalian host, possibly in the hollow trees where these animals live.

Absence of infection in humans

P123.152 Human infection has not been recorded. *L. (L.) hertigi* seems to be specific to *Coendou rothschildi*, as the parasite has not been found in a wide variety of other forest mammals studied in the Panamanian forests. The apparent absence of human infection may be due to the failure of the parasite to survive in human tissues, or because the sandfly vector does not bite humans.

P123.153 *L. (L.) hertigi* is included in the *hertigi* complex, together with another closely related parasite of porcupines, *L. (L.) deanei*.

LEISHMANIA (LEISHMANIA) DEANEI LAINSON AND SHAW, 1977

Known geographical distribution

P123.154 The parasite has been noted only in Amazonian Brazil.

Known mammalian hosts

P123.155 The known mammalian hosts are the tree-porcupine *Coendou p. prehensilis* and another, as yet unnamed, species of *Coendou*.

Recorded sandfly hosts

P123.156 The vector of *L. (L.) deanei* remains to be discovered. As with *L. (L.) hertigi*, the infection rate in Brazilian porcupines is very high, again suggesting a close association of the vector with the mammalian host, probably in the animal's home in hollow trees. A tree-inhabiting sandfly, *Lu. (Viannamyia) furcata*, taken from a tree-hole in which an infected porcupine was living, was shown to have promastigotes of *L. (L.) deanei* in its midgut (Miles et al. 1980). There was, however, no evidence of migration of the parasites to the anterior station of the gut and, in subsequent experimental infections of this sandfly, it was shown that the promastigotes

disappeared following complete digestion of the bloodmeal (Lainson and Shaw 1987).

Absence of infection in humans

P123.157 Human infection has not been recorded. As with *L. (L.) hertigi*, this could be because the sandfly vector is not attracted to humans or because the parasite cannot survive in human tissues.

P123.158 *L. (L.) deanei*, like *L. (L.) hertigi*, seems to be restricted to species of the porcupine *Coendou* and an exhaustive examination of other animals in areas of forest where porcupines are commonly infected failed to indicate any other mammalian hosts (Lainson and Shaw, unpublished observations). Although both parasites seem to be peculiar to porcupines, they are readily distinguishable by their isoenzyme profiles and the morphology of their amastigote stages; those of *L. (L.) hertigi* are strangely elongated and measure from 3.5 × 1.2 to 4.8 × 2.5 μm. The amastigotes of *L. (L.) deanei* are the largest of all known species of *Leishmania*, measuring from 5.1 × 3.1 to 6.8 × 3.7 μm.

P123.159 Working with 12 isolates of *L. (L.) deanei* from Pará, north Brazil, Miles et al. (1980) found that they were separable into groups by the enzyme profiles of:

- malate dehydrogenase (oxaloacetate-decarboxylating) (NADP) E.C.1.1.1.40 (ME)
- phosphoglucomutase E.C.2.7.5.1. (PGM)
- malate dehydrogenase E.C.1.1.1.37 (MDH).

Whether or not this is indicative of a third species of *Leishmania* in the *hertigi* complex is debatable and further study is indicated on the biology, biochemistry and molecular biology of the two zymodemes.

Questionable leishmanial parasites

LEISHMANIA HERRERI ZELODÓN ET AL. 1979

P123.160 This parasite was isolated in Costa Rica from the sloth *Bradypus griseus* and regarded as a new species of *Leishmania*. Mention was made that the DNA buoyant densities and enzyme profiles of the organism were 'totally different from other known hemoflagellates', but no details of the biochemistry were given and, until further isolates of the parasite are available, there must remain considerable doubt as to its true nature.

Infection of human skin by nonleishmanial amastigotes

P123.161 Amastigotes found in skin lesions of individuals infected with human immunodeficiency virus (HIV) and non-infected individuals on the Island of Martinique have been considered to be those of monoxenous insect flagellates (Boisseau-Garsaud et al. 2000). It is likely that some such lesions have, in the past, been wrongly attributed to *Leishmania*.

GENETICS H123.3

P123.162 The New World *Leishmania* have 20–25 chromosomes that exhibit high degrees of intra- and interspecies size polymorphism. This is thought to reflect a high level of genomic plasticity. Leishmanial genomic DNA is composed of repetitive, moderately repetitive and unique sequences that, respectively, form ca. 25, 13, and 60 percent of the total. Repeated sequences are useful for identification regardless of their distribution, function or copy numbers. Fernandes et al. (1994) found that the mini-exon repeat units of four *Viannia* species were different. Considerable genomic differences have been noted between the two subgenera, but they appear greater for species of *Viannia*. Mendoza-León et al. (1995) found that the β-tubulin gene regions showed a much greater degree of heterogeneity within and between parasites of the *braziliensis* complex than those of the *mexicana* complex. This suggests that there are more random mutations in the former than in the latter. The same gene may be located in more than one chromosome. This may be the result of gene duplication and transposition to another chromosome or chromosomal duplication followed by size divergence (Spithill and Samaras 1985). A useful summary of the recent developments from the *Leishmania* genome project have been given by Myler and Stuart (2000).

P123.163 Genetic exchange between neotropical *Leishmania* has not been demonstrated experimentally, but the finding of apparent hybrids among *Viannia* species suggests that it may occur. Hybrids only occur in some localities and it is possible that ecological conditions may be important in determining the contact between the species involved. No hybrids have been noted among neotropical species of the subgenus *Leishmania*. Extreme care is needed in the characterization of flagellates from sandflies, as they may sometimes harbor more than one parasite. Monoxenous flagellates, species of *Trypanosoma*, and *Endotrypanum* will contribute to confusion, in addition to the possible coexistence of two species of *Leishmania* in the same insect, as recorded by Barrios et al. (1994). Ideally, all isolates should be carefully cloned, especially if showing unusual characteristics by current methods of identification.

P123.164 The possible role in humans of genes and chromosome regions suggested by previous studies in mice to be linked to disease resistance or susceptibility to visceral leishmaniasis has been investigated in 638 individuals in 89 families with multiple cases of AVL in northeast Brazil, using complex segregation analyses (Blackwell 1998). Preliminary results indicated that the major histocompatibility complex showed only weak association with AVL; that there is no evidence for linkage between *nramp1* (the positionally cloned candidate for a murine

macrophage resistance gene) and susceptibility to AVL; and that the T helper 2 cytokine gene cluster is not linked to human susceptibility for this disease. It is suggested that the mouse model, together with knowledge of human response to infection, may lead to identification of important candidate gene regions in humans. Feitosa et al. (1999), used complex segregation analyses in a study of the intradermic reaction to antigens derived from *L. i. chagasi* in 502 individuals from 94 families in Bahia State, northeast Brazil. They concluded that the results gave evidence of a major genetic mechanism, with a frequency of a recessive susceptibility gene (q) of approximately 0.45, and that a small multifactorial component (H = 0.29), acting in combination with a major recessive gene (q = 0.37) could be a concomitant factor.

Viral infections in *Leishmania*

P123.165 Virus-like particles have been described in the cytoplasm of cultured promastigotes of five isolates of *L. (L.) hertigi* and three of *L. (L.) deanei* (Molyneux and Killick-Kendrick 1987). Although unidentified, they were shown to be associated with cytopathological changes in the mitochondrion of the promastigote. The number of virus-like particles was drastically reduced when *L. (L.) hertigi* were grown as amastigotes in mouse peritoneal macrophages and dog sarcoma cell lines incubated at 32°C. The particles were not transmissible to other *Leishmania* species, nor to cell lines susceptible to viruses.

P123.166 RNA virus particles belonging to the family Totaviridae were described by Tarr et al. (1988) in cultured promastigotes of a strain of *L. (V.) guyanensis* from French Guyana. Subsequent studies revealed the presence of similar viruses in promastigotes of 11 *Leishmania* strains isolated from humans, including both *L. (V.) guyanensis* and *L. (V.) braziliensis* from the Amazonian region of Brazil and Peru (Guilbride et al. 1992). All the viral isolates are considered to be different and are classified within a single genus, *Leishmaniavirus*. None, however, has been given specific status within this genus. The virus-bearing promastigotes were those of *Leishmania* isolated from uncomplicated cases of cutaneous leishmaniasis, but so far the viral particles have not been demonstrated in amastigotes from the skin lesions. The effect that such viruses may have on the *Leishmania* is not known but in experimental infections of *Leishmania major* in mice, it was noted that an infected line was less pathogenic than an uninfected one.

P123.167 When human biopsy material from some cases of cutaneous leishmaniasis from Peru were examined for the presence of *Leishmaniavirus* RNA by the reverse transcription polymerase chain reaction (RT-PCR), two samples showed RT-PCR bands of the expected size. Sequence analysis indicated them to share approximately 90 percent sequence with the neotropical strain LRV 1-4 of that virus (Saiz et al. 1998).

CLINICAL FEATURES H123.9

American visceral leishmaniasis

P123.168 Throughout most of its geographical range, the clinical features of AVL closely resemble those of infantile visceral leishmaniasis caused by *L. (L.) infantum* of the Old World (see Chapter 16, Old World leishmaniasis) and this similarity extends to the fact that the disease is seen mainly in children (Figure 17.12). However, It has been shown that the same parasite may produce almost exclusively nonulcerative cutaneous lesions in Costa Rica (Zelodón et al. 1989), and may cause both visceral and cutaneous disease in the same focus of AVL in Honduras and Nicaragua (Ponce et al. 1991; Belli et al. 1999).

P123.169 The saliva of *Lu. longipalpis* has been shown to contain a potent vasodilatory peptide, maxadilan (Lerner et al. 1991). It was found that when sandflies from Brazilian, Colombian, and Costa Rican populations were fed on the arms of volunteers, the degree of erythema was highest for the Brazilian flies, slightly less for the Colombian, and very low for the Costa Rican examples (Warburg et al. 1994). These authors considered this difference to be due to a variation in the *potency* of the peptide but similar recent experiments have suggested it to be due to differences in the *amount* of maxadilan in the saliva of the three populations (Yin et al. 2000). This is in agreement with the distribution of atypical cutaneous leishmaniasis due to *L. i. chagasi*, and lends credence to the involvement of maxadilan in visceralization of the parasite.

P123.170 There is evidence that *L. (L.) infantum chagasi* may produce a benign, inapparent infection in some individuals and that severity of the disease depends to some extent on the nutritional state of the infected person (Badaró et al. 1986). Differential diagnosis has to be made principally from malaria, schistosomiasis, cirrhosis of the liver, visceral syphilis and other causes of hepatosplenomegaly.

P123.171 Although serological methods such as the indirect fluorescent antibody, dot-enzyme-linked immunosorbent assay (ELISA) and direct agglutination tests are useful indicators, the novel use of a dipstick (InBios International, Inc) based on recombinant RK 39 antigen for the differential diagnosis of AVL from other sympatric endemic diseases, has been shown to be rapid and highly specific (Delgado et al. 2001). Also, new latex agglutination test (KATEX) is claimed to detect leishmanial antigen in the urine of patients with a 100 percent specificity (Attar et al. 2001). Unequivocal diagnosis of the disease, however, depends on the demonstration of amastigotes in stained smears of aspirates from the spleen, bone marrow or lymph glands. Usually, such smears contain abundant parasites; if not, the material can be cultured in a suitable blood agar medium (varieties of Novy, MacNeal, Nicolle (NNN) medium).

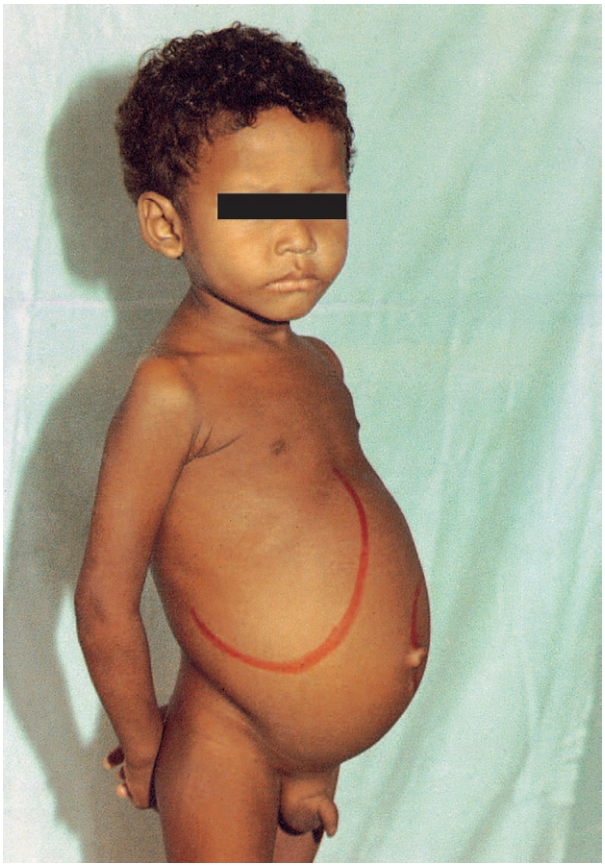

FIG123.12 **Figure 17.12** *AVL, due to* L. (L.) infantum chagasi*, in a boy from Marajó island, Pará, Brazil. Note the greatly distended abdomen resulting from hepatosplenomegaly.*

However, some difficulty may be experienced in culturing the parasite, especially when the aspirates contain scanty amastigotes. The intraperitoneal inoculation of aspirates into hamsters is by far the most reliable method of isolating *L. (L.) infantum chagasi* for further study but the long delay before parasites are detectable in these animals makes the method impractical for quick diagnosis. The PCR, together with specific hybridization techniques, will probably be the method of choice in the future (Noyes et al. 1996; Rodriguez et al. 1997; Breniere et al. 1999), at least in the larger and better-equipped hospitals, clinics, and research laboratories. Traditional methods of demonstrating the presence of the parasite will remain vitally important for many more years to come, however, particularly in the more remote rural areas where the majority of cases are concentrated.

Cutaneous leishmaniasis

P123.172 Simple cutaneous lesions are produced by 12 of the 14 neotropical species of *Leishmania* known to infect humans. It is not possible, however, to diagnose the causative species by the appearance of these lesions, which are in many cases indistinguishable from the classical leishmanial lesion of the Old World, forming a rounded, crater-like ulcer with a raised border (see Chapter 16, Old World leishmaniasis, and Figure 17.2). In addition, the simple lesion may vary greatly in appearance (Figures 17.11 and 17.13–17.15), evoking such descriptions as framboesiform, lichenoid, lupoid, nodular, vegetative, verrucose, ulcerative, etc. from clinicians and dermatologists. All of these forms have in common that they are painless; unfortunately, this means that the infected person often fails to seek medical advice until the lesion has reached large proportions. Differential diagnosis needs to consider tropical ulcer (painful and suppurative), sporotrichosis, cutaneous tuberculosis, yaws, blastomycosis, lupus, and tertiary syphilis. Bacterial infections of insect bites or skin abrasions are frequently misdiagnosed as early lesions due to *Leishmania* and are particularly common in children living in rural areas with an abundance of biting flies, such as *Simulium* and *Culicoides*.

P123.173 Although patients under examination may show a positive Montenegro (leishmanin) skin-test, this may be due to a previous, subsequently eliminated infection with *Leishmania* and may be unrelated to present skin lesions. The demonstration of amastigotes in stained smears prepared from the border of the lesions and

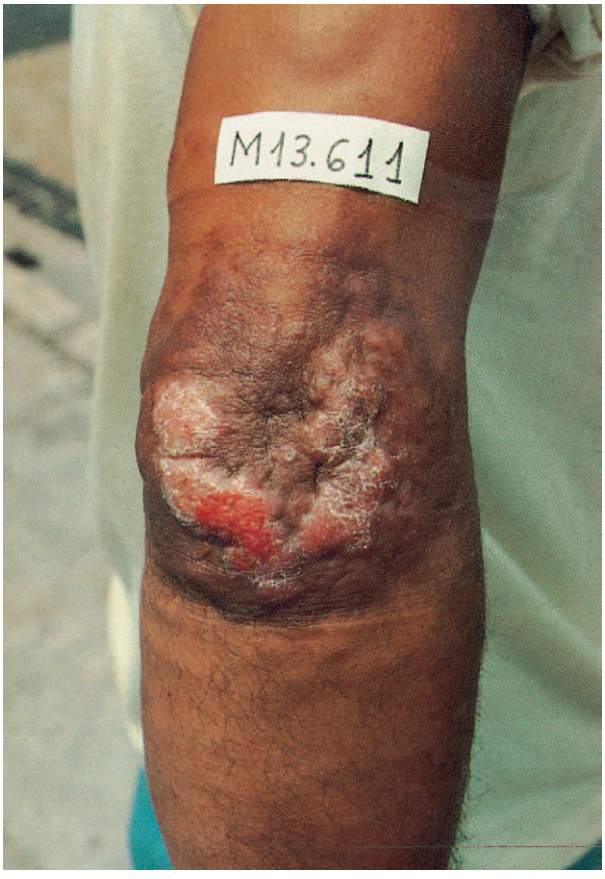

FIG123.13 **Figure 17.13** *Infection with* L. (L.) amazonensis*, on the elbow; Pará, Brazil*

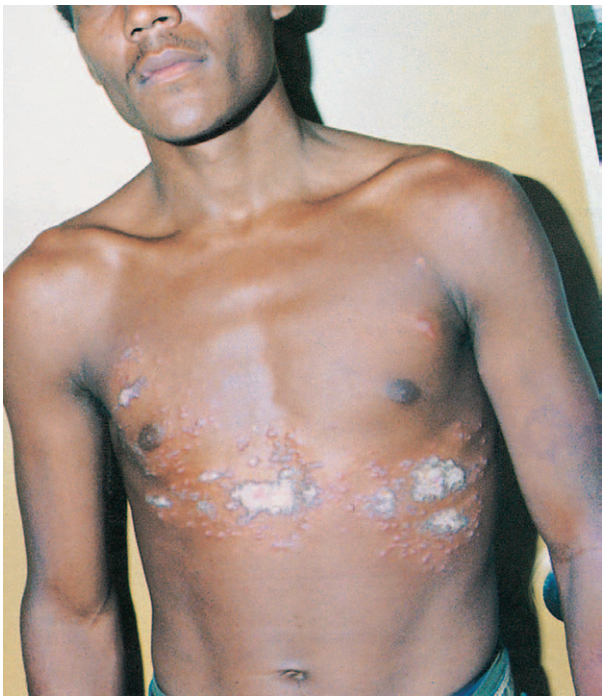

FIG123.14 **Figure 17.14** *A large number of small, papular satellite lesions surrounding the larger, primary lesions in a case of pian-bois due to* L. (V.) guyanensis*; Pará, Brazil*

isolation of the parasite in blood agar culture medium are prerequisite for diagnosis.

P123.174 Vacuum aspiration of amastigotes in material from human lymph nodes, first used in the examination of patients with visceral leishmaniasis, has been used in cases of cutaneous leishmaniasis resulting from *L. (V.) braziliensis* infection in Brazil (Marzochi et al. 1993; Romero et al. 1999). Aspiration directly into the culture medium, through the rubber cap, greatly reduces the risk of bacterial or fungal contamination.

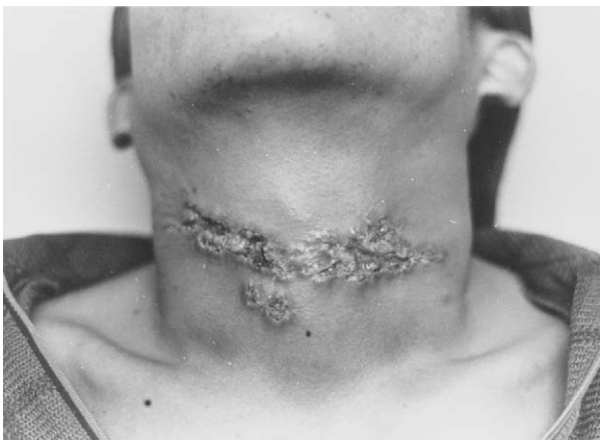

FIG123.15 **Figure 17.15** *Another atypical lesion, due to the same parasite; Pará, Brazil*

Mucocutaneous and mucosal leishmaniasis

For excellent general reading on this unique form of leishmaniasis, reference should be made to Pessôa and Barretto (1948), Marsden (1986), and Walton (1987). P123.175

Although the disease has, on rare occasions, been attributed to *L. (V.) guyanensis* and *L. (V.) panamensis*, the great villain is undoubtedly *L. (V.) braziliensis* s.l. The exact percentage of individuals infected with this parasite who develop mucosal lesions is difficult to calculate. In what was almost certainly a hospital series in São Paulo State, southern Brazil, Pessôa (1941) produced figures indicating that among 171 patients with cutaneous lesions of less than 1 year's duration, 105 lacked mucosal lesions and 66 (38.5 percent) had mucosal involvement. Of 110 individuals with cutaneous lesions of more than 1 year's duration, 21 had no mucosal lesions, and 89 (80.9 percent) had developed them. Marsden (1986), however, described Pessôa's figure of 80.9 percent as 'widely quoted out of text … giving the impression that the great majority (of cases) will proceed to mucosal metastasis'. P123.176

In field studies in an endemic area in Três Braços, Bahia State, north-east Brazil, Marsden and his colleagues found only 2.7 percent of 371 patients examined to have both cutaneous and mucosal lesions. Mucosal infection seems to be much more common in some Latin American countries (e.g. Bolivia and Ecuador) than in others, suggesting that certain parasites referred to as *L. (V.) braziliensis* have a greater propensity for producing mucosal lesions than others (these may, in fact, represent different subspecies, or even species, of the *braziliensis* complex). P123.178

In spite of its antiquity, the most detailed study of the mucosal lesions remains that of Klotz and Lindenberg (1923), but a wealth of clinical and pathological data is also available in the works mentioned above, and that of Ridley (1987). It is generally agreed that involvement of the mouth, nose, and throat is always the result of metastases from a simple skin lesion elsewhere on the body (Figure 17.2). Apparently, migration of the parasites to the mucosae, by way of the lymphatics or the bloodstream, may take place quite early in the infection, for amastigotes have been demonstrated in scrapings of the apparently normal nasal mucosae of 12 patients with skin lesions of only 1–11 months' evolution (Villela et al. 1939). The nose is the major site of the metastases (Figure 17.3) and it remains a mystery as to why this occurs in some individuals and not in others. From the observations of Villela et al. and the fact that years may elapse between the disappearance of the primary skin lesion and the onset of mucosal disease, it seems that the parasite remains dormant in the mucosae for a variable period. Exactly what triggers this occult infection into destructive activity is another mystery, although minor P123.179

injury to the mucosae may be one cause. The following account of the developing pathology of the mucosal disease is summarized from Ridley (1987).

P123.180 When a nasal lesion develops, it is initiated in the deep mucosa of the nose, an accumulation of plasma cells and lymphocytes forming around the small blood vessels. A few amastigotes may appear in the endothelial cells. The major part of the lesion stays in the deep mucosa, accompanied by congestion and edema, a pronounced infiltration of plasma cells and a characteristic proliferation of the vascular endothelial cells, which contain variable numbers of amastigotes. Inflammatory foci proceed towards the mucosal surface, resulting in a patchy desquamation, followed by hyalinization and necrosis of the exposed tissue, accompanied by polymorph infiltration. It should be stressed that the ulcer is due to the desquamation resulting from the inflammatory process and not to the necrosis. In the area of deep inflammation, the endothelial nodule, with its perivascular inflammatory cells, undergoes central necrosis or, in the case of large nodules, hyalinization. Scanty amastigotes may still be found, but they are absent in the necrotic area. Considerable endarteritis may be associated with thrombosis which, together with subsequent fibrosis, deforms and erodes the nasal septum. Liquefaction of cartilage continues, even some distance from the leishmanial nodule and the vascular supply is so reduced that only coarse fibrous tissue can survive.

P123.181 Blockage of the nasal passages due to the developing lesion usually results in respiratory distress, mouth breathing, and a high frequency of pulmonary infection that may lead to death. Palatal (Figure 17.4), laryngeal and tracheal lesions are less common; for first-hand accounts of these, reference should be made to Marsden (1986) and Walton (1987).

P123.182 Differential diagnosis of mucosal leishmaniasis must be made from nasal syphilis (no destruction of the septum), gangosa (ulcerating yaws), blastomycosis, rhinosporidiosis, midline granuloma, carcinoma, and cancrum oris.

P123.183 Sudanese and Ethiopian oro-nasal (mucosal) leishmaniasis bears a superficial resemblance to American espundia (see El-Hassan et al. 1995, for review). Due largely to *L. (L.) donovani* s.l. and, more rarely, *L. (L.) major*, it is not preceded by a cutaneous lesion. In addition, unlike patients with the South American disease, advanced cases of Sudanese mucosal leishmaniasis respond readily to treatment with pentavalent antimonials and ketoconazole.

Anergic, diffuse cutaneous leishmaniasis

P123.184 In Venezuela, Convit and Lapenta (1948) described a bizarre form of cutaneous leishmaniasis characterized by nodular lesions scattered all over the body and containing vast numbers of rather large amastigotes; a negative Montenegro skin-test reaction; and almost total resistance to chemotherapy. Further cases were recorded in that country and subsequently in Bolivia, Brazil, Colombia, the Dominican Republic, Honduras, northern Mexico, Texas, USA, and Peru. The causative parasites in Venezuela, Mexico, Texas, and Brazil are *L. (L.) pifanoi*, *L. (L.) mexicana* and *L. (L.) amazonensis* respectively and it is likely that this form of leishmaniasis elsewhere in the Americas is also due to members of the *mexicana* complex within the subgenus *Leishmania*. The disease, as its name suggests, is the outcome of infection by this group of parasites in individuals with little or no cell-mediated immunity. Curiously, parasites of the subgenus *Viannia* do not seem to possess the potential to produce this strange disease.

P123.185 At one time it appeared that ADCL was an irreversible condition and that the best treatment merely kept the disease in check, without eliminating the parasite. There is some evidence to suggest, however, that the prognosis is not always so grim. One of the several ADCL patients studied in the authors' laboratory acquired her infection with *L. (L.) amazonensis* when she was less than 5 years old and, in spite of constant chemotherapy, her condition had not improved greatly by the age of 28 years. However, the lesions faded away and the patient made a complete recovery following immunochemotherapy. We had noted that, unlike the lesions of other cases of ADCL, those of this young woman had occasionally ulcerated and healed, leaving her with very unsightly scars on her face and legs (Figure 17.9). This and the final recovery, suggests that there is a gradation in the degree of anergic condition, from total to partial. In many patients, the nodular lesions remain unulcerated, whereas in others, there may be ulceration and even amputation of fingers and toes, resembling that seen in leprosy (Figure 17.5).

P123.186 Usually, ADCL forms a minute proportion of the total number of cases of cutaneous leishmaniasis caused by parasites of the *mexicana* complex but an exception is found in the Dominican Republic, where ADCL occurs in the apparent absence of simple, curable cutaneous leishmaniasis. The causative parasite is clearly a member of the *mexicana* complex, but is, as yet, unnamed and its animal reservoir and sandfly vector are unknown. The occurrence of three cases in a single family in the Dominican Republic suggests involvement of an hereditary component (Walton 1987); Petersen et al. (1982) demonstrated a population of specific suppressor cells in four other patients. The deficient immune response in ADCL in general is considered to be related to a thymus-dependent system in both the New World and the Old World diseases (Convit et al. 1971; Bryceson 1970). The only other disease with which ADCL has frequently been confused is lepromatous leprosy; this mistake is unpardonable if Giemsa-stained smears of the skin lesions have been examined (Figure 17.10).

P123.232 In a recent paper, Silveira et al. (2004) discussed the clinical and immunopathological spectrum of American

cutaneous leishmaniasis, with particular reference to the disease in Amazonian Brazil. They emphasized the necessity of accurately identifying the species of *Leishmania* stimulating the patient's immune response, together with the quality and magnitude of this response. At the diagramatic center of the authors' clinical spectrum are the most commonly seen localized forms of cutaneous leishmaniasis (LCL), which may be caused by members of both subgenera, *Leishmania* and *Viannia*, and which usually respond well to conventional chemotherapy. The two pathogenicity poles of the spectrum generally recognized are the hyposensitivity pole, occupied by cases of ADCL, and the hypersensitivity pole represented by mucocutaneous leishmaniasis. They proposed the term borderline disseminated cutaneous leishmaniasis (BDCL) for the cases showing disseminated lesions due to some parasites of both the subgenera where there is a partial failure of the cellular immune response (unlike the complete failure seen in ADCL), enabling eventual cure of the patient provided that treatment is promptly given. If this is not undertaken, however, these infections may develop into either ADCL (due, for example, to *L. (L.) amazonensis*) or mucocutaneous leishmaniasis (due mainly to *L. (V.) braziliensis*).

Leishmania infections and the immunosuppressed patient

P123.187 Cutaneous and visceral leishmaniasis are now firmly established in the list of infections that the clinician must consider when dealing with immunosuppressed patients, in particular those with acquired immune deficiency syndrome (AIDS). Visceral leishmaniasis is the clinical form most commonly associated with HIV/AIDS and has become a public health problem of considerable proportions in south-western Europe. Strangely, although visceral leishmaniasis is far more common in many parts of Latin America (e.g. Brazil) than it is in Europe, cases of AVL/HIV infection are, until now, less common (WHO 1998). Possibly this is because, in the Americas, HIV is predominantly found in the larger cities; the situation will doubtless change as HIV spreads to the more rural areas where AVL is highly endemic or with the migration of leishmaniasis carriers into the large cities and their acquisition of HIV. One of the most striking examples of such migration is that of individuals from the rural areas of northeast Brazil, where AVL is very common, into São Paulo city in search of work. Co-infection of HIV and *Leishmania* species responsible for cutaneous leishmaniasis seems to be less frequent in the Americas, but may result in serious complications, ranging from disseminated skin lesions and mucosal lesions (Coura et al. 1987; Da-Cruz et al. 1999) to rectal involvement (Hernández et al. 1995). *L. (V.) guyanensis* has been isolated from the lesions of an HIV patient presenting with mucocutaneous leishmaniasis in the State of Amazonas, Brazil (de Souza e Souza et al. 1998), and visceral leishmaniasis due to *L. (L.) mexicana* has been recorded in a patient with HIV in Mexico (Ramos-Santos et al. 2000).

PREVENTION AND CONTROL H123.5

Visceral leishmaniasis

P123.188 Campaigns are of fundamental importance, with the distribution of illustrated pamphlets to alert the populations as to the early symptoms of the disease, the signs of infection in the dog and the appearance and habits of the sandfly vector. The staff of small, rural clinics must be trained to recognize visceral leishmaniasis and should have means of reporting suspected cases to centers where more conclusive diagnosis can be made. In view of the impecunious situation of many inhabitants of the rural districts of developing countries, the staff of such centers may need to travel to the patient's village, where the possibility of other cases must be investigated. Periodic surveillance of populations at risk may detect cases of early infection either clinically or, more effectively, by serological methods that can easily be carried out under field conditions, such as the direct agglutination test (DAT) or the dot-ELISA.

P123.189 In areas of high endemicity, such as the states of Ceará and Bahia in northeast Brazil, past control measures (i.e. destroying infected dogs, regular insecticide spraying of houses and animal sheds, and the early treatment of patients) resulted in a dramatic drop in the number of cases of AVL. A problem arises, however, in maintaining such a control program, which is costly and inevitably meets with considerable opposition on the part of dog owners, who fail to understand why their apparently healthy (but serologically positive) animals must be killed. Many deliberately conceal their dogs, and many strays are never examined. A critical evaluation of the cost-effectiveness of this dog-slaughtering policy (Akhavan 1996) points out the vast amount of work and expense expended in surveying dog populations, and the apparent failure of this method in control programs in some parts of Brazil has led to the question as to what extent the dog population needs to be reduced in order to eliminate AVL or to bring it under control. It has been suggested that it may be preferable to find improved methods of eliminating or controlling the sandfly vector.

P123.190 Insecticide (deltamethrin)-impregnated plastic dog collars have been found to provide an efficient and prolonged protection of dogs against the bites of sandfly vectors of *L. i. infantum*, thus breaking transmission of the parasite in its major reservoir host (Killick-Kendrick et al. 1997). Such collars have been shown to offer similar protection against the bites of *Lu. longipalpis* in Brazil (David et al. 2001), and preliminary results

following field-trials in three regions of Brazil have been discussed in the 2002 *Proceedings of the Second International Cannine Leishmaniasis Forum, Seville, Spain*. Only after the completion of more lengthy field-trials, however, will it be possible to assess both the cost-efficiency of deltamethrin-impregnated dog collars, or other methods of insecticide application, and the effects they may have on the incidence of human AVL. In the meantime, the development and use of an effective and long-lasting vaccine, preferably in a well-supervised, governmental program similar to that of antirabies campaigns, remains of great importance. Encouraging results have been obtained after the inoculation of dogs with a mixture of promastigotes of *L. (V.) braziliensis*, disrupted by ultrasound, and bacille Camette Guerin (BCG). Of 10 vaccinated dogs, only one succumbed to subsequent challenge with 2.3×10^6 infective promastigotes of *L. i. chagasi*, whereas all of nine nonvaccinated dogs became infected (Mayrink et al. 1996). In addition, field trials using a fucose–mannose ligand (FML)-vaccine prepared from *L. donovani* have afforded a 92 percent protection among dogs in an endemic area of AVL in Rio Grande do Norte, Brazil, 2 years after their vaccination. The 8 percent of dogs that did acquire infection showed only mild signs of disease with no deaths and, significantly, the number of human cases of AVL in the area decreased from 15 in 1996 to six in 1997 and none in 1998 (da Silva et al. 2001).

Cutaneous and mucosal leishmaniasis

P123.191 Most inhabitants of the endemic areas are very familiar with the dermal leishmaniases, under their wide variety of local names, but ignorance and negligence are all too frequently to blame for allowing these diseases to reach debilitating or mutilating proportions. The painless nature of the lesions makes them of no great inconvenience in their early stages and this encourages the tendency to wait and see if they will cure spontaneously. Again, as these diseases are predominantly zoonotic, most infections are acquired by those living in rural areas, often long distances from the simplest of medical attention. As a result, a high proportion of cases only seek help when the infection is well advanced and, in regions where the causal agent is frequently *L. (V.) braziliensis*, this may prove disastrous. As for visceral leishmaniasis, health education campaigns can help to indicate the importance of early treatment.

P123.192 Personal avoidance of cutaneous leishmaniasis is, at present, limited to the use of insect repellents, protective clothing and the avoidance of danger areas, particularly at night when the sandfly vectors are most active. These are precautions that may be feasible for the visiting tourist, but they are not very practical for the shirtless forest-worker who can ill afford to be constantly purchasing insect repellents, who is most comfortable wearing shorts (far less expensive than trousers) and short-sleeved shirts and who has to eke out his living by hunting in the forest at night.

P123.193 The prevention or control of sylvatic leishmaniasis among gangs of laborers, topographers, and other forestry workers can be effective on a small scale by the following measures: placing the encampments of such men in adequate clearings; spraying the bases of the larger, nearby tree trunks with insecticides (e.g. in areas where the vectors are known to be arboreal – see *Leishmania* (*Viannia*) *guyanensis* Floch, 1954 above); and prohibiting night-time hunting. Destruction of the wild animal reservoirs of sylvatic leishmaniasis is clearly neither practical nor desirable. Finally, knowledge of the ecology of a vector can sometimes help in preventing acquisition of the disease under certain circumstances. Thus, *Psychodopygus wellcomei*, an important vector of *L. (V.) braziliensis* s.l. in the highland forests of Pará, north Brazil, is highly anthropophilic and attacks humans not only at night but also frequently during the day. Field studies have shown, however, that this sandfly is only active for about 6 months of the year, during the rainy season (November–April) and that it enters into diapause in the dry season during the rest of the year, when adult flies are rarely seen. The area in question is one of intense human activity due to the mining of iron ore and other minerals and the incidence of cutaneous leishmaniasis has been very high among those clearing the primary forest. Planning such work for the dry season clearly avoids contact with the sandfly vector.

P123.194 Although drastic ecological changes such as deforestation and the planting of non-indigenous pine, gmelina, and eucalyptus trees might lead to unfavorable conditions for the enzootics of some species of *Leishmania*, it can actually encourage others. Thus, the creation of vast monoculture plantations of non-indigenous trees for paper pulp production in north Brazil may eliminate cutaneous leishmaniasis due to *L. (V.) guyanensis* in the immediate areas, as neither the major reservoir host (the two-toed sloth) nor the sandfly vector (*Lu. umbratilis*) find this new environment suitable. On the other hand, the wild rodent and marsupial hosts of *L. (L.) amazonensis* and the sandfly vector, *Lu. flaviscutellata*, find it ideal.

P123.195 In those parts of Latin America where vector species have adapted to a peridomestic or domiciliary habitat, the use of insecticides is clearly indicated. In the absence of firm evidence that domestic animals with leishmanial skin lesions offer a source of infection to sandflies, it would seem unwise to recommend their destruction, even in the unlikely event of their owners' consent. Equines respond well to antimonial treatment.

Direct transmission risks

P123.196 Cases of congenital transmission of visceral leishmaniasis have been reported (Bialek and Knobloch 1999; Knobloch et al. 2001) but would appear to be rare.

P123.197 It has been estimated that in some endemic areas infection with leishmanial parasites may be as high as 70 percent of the population, and data on patients with *Leishmania*/HIV co-infection have indicated that viable parasites may persist in individuals long after initial infection, irrespective of the presence or absence of primary disease (WHO 1998). Leishmanial antibodies have been detected in 9 percent of a group of blood donors in the city of Natal, Rio Grande do Norte, Brazil, and in 37 percent of patients undergoing hemodialysis in the same locality (Luz et al. 1997); the latter figure was significantly higher than in any other group, including inhabitants in AVL foci on the outskirts of the city! The importance of transfusion transmission of visceral leishmaniasis in Europe has been clearly indicated in non-endemic regions where the sandfly vector is absent (Mauny et al. 1993). With increases in HIV infection and organ transplant surgery involving prolonged use of immunosuppressants, occult infections and contaminated blood or organs become life-threatening risks necessitating special precautions (Schulman 1994).

H123.6 ## TREATMENT

P123.198 Of the various drugs discussed for the treatment of leishmaniasis (see Chapter 16, Old World leishmaniasis; Lainson 1982a; Amato et al. 1996; de Carvalho et al. 2000), the pentavalent antimonials are those of first choice in dealing with the neotropical leishmaniases. It is of historical interest that the first use of antimony to treat leishmaniasis was in Brazil. A young clinician, Gaspar Vianna, impressed by the effectiveness of tartar emetic (antimony potassium tartrate) in the treatment of African trypanosomiasis (see Chapter 18, African trypanosomiasis), realized its potential against the related organism *Leishmania* and, in 1912, published his spectacular results obtained with this drug in advanced cases of mucocutaneous leishmaniasis (Vianna 1912). There followed development of the somewhat less toxic trivalent antimonials, which still produced unpleasant side-effects, and then the much better-tolerated pentavalent antimonials which, some five decades later, are still the clinician's principal armaments. Even so, these latter, more refined antimonials may sometimes show some cardiotoxicity (Ribeiro et al. 1999).

American visceral leishmaniasis

P123.199 Treatment usually follows that given in Chapter 16, Old world leishmaniasis. Unresponsiveness to the recommended antimonial dosage schedule, however, has been noted in some AVL patients from Bahia, Brazil. In such cases, there is little alternative other than elevating the dosage or using second-line drugs such as pentamidine. In this manner, Bryceson et al. (1985) cured four of ten unresponsive patients with Kenyan kala azar, but all ten suffered serious side-effects from both stibogluconate and pentamidine at the dosage levels used. Liposomal amphotericin B (AmBisome®) is recommended for the treatment of visceral leishmaniasis by the US Food and Drug Administration (Meyerhoff 1999).

Cutaneous leishmaniasis

P123.200 Once again, the treatment is much the same as that for Old World cutaneous leishmaniasis, using the pentavalent antimonials. Many clinicians prefer, however, to use the intravenous route for both pentostam and Glucantime, rather than intramuscular inoculation.

P123.201 In general, lesions due to the neotropical leishmanias tend to much greater chronicity than those of the Old World and there is not such a ready response to treatment. There is no justification in delaying treatment in anticipation of an early spontaneous cure, as recommended with *L. (L.) major* or *L. (L.) tropica* infections, or limiting treatment to intralesional injection of drugs or the topical application of these in ointments. The following hazards must be considered: subsequent mucosal disease due to *L. (V.) braziliensis*; ADCL due to parasites of the *mexicana* complex; and multiple lesions following lymphatic or hematogenous spread on the part of *L. (V.) guyanensis*. Treatment should, therefore, be systemic and immediate and identification of the causative parasite is most important. If it proves to be *L. (V.) braziliensis*, treatment should be particularly intensive.

P123.202 Effectiveness of the pentavalents Pentostam® and Glucantime may vary considerably, not only when dealing with different parasites, but in treating different patients infected with the same organism. Some patients with simple lesions due to *L. (V.) guyanensis*, for example, may be cured by a single course of treatment, whereas others may require three or four. In some cases of poor response, recourse must be made to other drugs, or combinations of drugs, in spite of their greater toxicity. The most commonly used second-line drug is pentamidine, which has been used routinely by French workers in treating cases due to *L. (V.) guyanensis* in French Guyana.

P123.203 Treatment of patients with the antimalarial drug mefloquine has produced conflicting results (Gómez et al. 1996; Hendrickx et al. 1998; Laguna-Torres et al. 1999).

Mucocutaneous and mucosal leishmaniasis

P123.204 Advanced cases are difficult to treat, with slow response to the pentavalent antimonials. The high dosage recommended (20 mg Sb^{5+} per kg body weight, given in a single daily injection for a mean of 30 days, or until no evidence of activity of the lesion has been noted for a week) may sometimes produce pronounced side-effects, so that treatment has to be suspended (Marsden 1986).

The considerably more toxic amphotericin B and pentamidine are used only when there is failure to respond to the pentavalent antimonials. In some patients, the early treatment provokes a severe inflammation around the lesion, presumed to be caused by antigen released from killed parasites. Although this reaction is regarded as a favorable prognostic sign, it may prove highly dangerous in patients with laryngeal or tracheal lesions. It is recommended that corticosteroids be used as a prophylactic measure when treating such cases and that there should be a gradual elevation of the drug dose (Marsden 1986). Successful treatment of mucosal leishmaniasis in patients unresponsive to Glucantime has been achieved with liposomal amphotericin B (Sampaio and Marsden 1997).

P123.205 Another difficulty confronting the clinician is in evaluating the effectiveness of treatment. What are the criteria of cure? Serological monitoring has used complement fixation and indirect fluorescent antibody tests, and it has been suggested that cure is indicated by decline and disappearance of antibody titers (Walton 1987). This method is likely to prove the most useful, provided that measurements of leishmanial antibody can be standardized. Both parasitological and histological examination entail biopsy trauma to the treated lesion and may reactivate a lesion that seems to be healed but still contains parasites. Serological follow-up should accompany testing for antigen: a negative antigen test together with reduced titer in serological tests indicates a lower likelihood of relapse (Amato et al. 1998), but an inflammatory process may persist, even in these patients, and could indicate continued presence of amastigotes.

P123.206 Mutilation following the mucosal lesions may be so extreme that even after cure the patient is ostracized and unable to lead a normal life. Plastic surgery is helpful, but only when there is no doubt of cure. Walton (1987) cites the case of one patient, with apparently complete healing, who underwent cosmetic surgery to reconstruct his nose: 'The results were disastrous, with widespread reactivation along the surgical wounds and, in the patient's words – the new nose fell off!'.

P123.208 Immunotherapy, utilizing vaccines with or without BCG (Convit et al. 1987, 1989; Mayrink et al. 1991) has proved effective, but has been shown to give much the same cure rate as conventional chemotherapy (Convit et al. 1987). As pointed out by the latter authors, however, it is considerably cheaper and safer.

Anergic diffuse cutaneous leishmaniasis

P123.209 A smooth, fleshy, unulcerated lesion containing very abundant, large amastigotes in a patient with a negative Montenegro skin-test reaction is a danger signal that should prompt immediate, high-dosage antimonial treatment. In the authors' laboratory in Amazonian Brazil, this is virtually diagnostic of an early lesion due to *L. (L.) amazonensis* which, in an individual with a defective cell-mediated immune response (suggested by his negative skin-test), will proceed to ADCL unless adequately treated. Fortunately, although simple, curable skin lesions due to this parasite are quite common, ADCL is relatively rare.

P123.210 Of all the forms of leishmaniasis, this disease undoubtedly represents the clinician's greatest challenge, because the patient is unable to offer the all-important immunological collaboration necessary for successful drug treatment. Advanced cases of ADCL, with multiple nodular lesions, may respond dramatically to the first antimonial treatment, with complete disappearance of the lesions. Unfortunately, this may be taken to indicate cure, but with the cessation of treatment the nodules reappear some time later, usually more abundantly. Repeated treatment with the same drug may again give good results, but the patient will relapse again and the response steadily diminishes until the treatment is virtually ineffective. Similar results may be obtained with a variety of other drugs, until the list is exhausted and the patient's situation becomes desperate.

P123.211 In patients with ADCL due to *L. (L.) amazonensis*, very hot baths taken daily, together with periodic chemotherapy, have been found to reduce the size of the nodules substantially, giving the patient a much improved appearance over periods of many years (Lainson 1982a). More recently, immunochemotherapy has been used with considerable success in the treatment of persons suffering from ADCL in Venezuela (Convit et al. 1989). 'Marked clinical improvement' was observed in nine out of ten patients given intradermal injections of a mixture of heat-killed promastigotes of '*L. mexicana amazonensis*' (*L. (L.) pifanoi*?) isolated from a Venezuelan case of ADCL, plus 'variable amounts' of BCG, together with the standard Glucantime treatment. A similar treatment of Brazilian ADCL patients infected with *L. (L.) amazonensis*, however, has met with limited success (F.T. Silveira, unpublished observations). Treatment with an association of Glucantime and paramomycin (Gabbrox, via oral) has been found useful (Costa et al. 1999).

VACCINATION

H123.7

P123.212 Immunology of infectious diseases in general is discussed in Chapter 4, Immunology and immunopathology of human parasitic infections. With regards to leishmaniasis, this is just as well, because a detailed review of the extensive literature on this subject over the past few years would fill many more pages here than space allows.

P123.213 The pioneer work of Mayrink and collaborators in Brazil, on the production of a vaccine against American cutaneous and mucocutaneous leishmaniasis (reviewed by Genaro et al. 1996), and that of Convit and coworkers in Venezuela on the use of vaccines in immu-

notherapy (reviewed by Convit 1996) have triggered a veritable explosion of studies directed towards the production of vaccines

P123.214 In early trials, the Mayrink vaccine was a mixture of killed, sonicated promastigotes of *L. (L.) amazonensis*, *L. (V.) braziliensis*, and a *Leishmania* of doubtful identity, without the use of an adjuvant. More recent versions have been a cocktail of promastigotes of *L. (L.) mexicana*, *L. (L.) amazonensis*, *L. (V.) guyanensis*, and *L. (V.) braziliensis*, with or without *Corynebacterium parvum* as an adjuvant. While apparently still some way from offering complete protection, the vaccine at least has the distinction of being the only one commercially available (Leishvacin®), and it is to be hoped that continuing studies will improve its efficacy. The protective efficacy of a similar vaccine against cutaneous leishmaniasis in Ecuador is claimed to be 72.9 percent (Armijos et al. 1998).

P123.215 The immune response of volunteers vaccinated with BCG plus killed promastigotes of *L. (L.) mexicana* in Venezuela indicated the vaccine to be potentially protective for the majority of the vaccinees (Sharples et al. 1994), and follow-up studies showed an immune response in more than 85 percent of the vaccines as indicated either by skin-test conversion, lymphocyte proliferation or interferon-γ production.

P123.216 Kenney et al. (1999) used rhesus monkeys to assess the safety, immunogenicity, and efficacy of a vaccine prepared from heat-killed *L. (L.) amazonensis* promastigotes combined with recombinant human interleukin-12 (rhIL-12) and aluminum hydroxide gel as adjuvants. Challenge with 10^7 metacyclic *L. (L.) amazonensis* promastigotes 4 weeks after vaccination demonstrated complete protection in 12 monkeys that received 2 μg rhIL-12 with alum/antigen. The safety and efficacy of this vaccine in monkeys suggest a basis for human trials.

P123.217 Handman (2001) gives a useful update of work on killed and live, attenuated vaccines; recombinant vaccines; synthetic peptides; nonprotein antigens; and 'naked' DNA vaccine. Transmission-blocking vaccines are discussed by Tonui (1999).

H123.8 CONCLUDING REMARKS

P123.218 We have discussed the possible origin of the parasitic Kinetoplastida, the family Trypanosomatidae, and the genus *Leishmania* elsewhere (Lainson and Shaw 1987). It is the general opinion that the trypanosomatids have their origin in monogenetic intestinal flagellates of invertebrates and that they subsequently adapted to spend a part of their life-cycle in vertebrates. Thus, it is more correct to consider the phlebotomine sandfly as the primary host of *Leishmania* species, rather than the vertebrate hosts that merely function as reservoirs of infection for the sandfly (Lainson 1997). The finding that promastigotes may undergo a form of conjugation (Lanotte and Rioux 1990), with possible exchange of nuclear material, supports this hypothesis on the reasonable assumption that such a process is more likely to take place in the definitive or primary host of a heteroxenous parasite.

P123.219 It is difficult to assess the specificity of the *Leishmania* species in their sandfly hosts and unwise to base one's conclusions on the results of laboratory experiments, when unnaturally large numbers of amastigotes or promastigotes, fed to laboratory-bred flies, may well overwhelm the natural resistance of a nonvector species. In nature, however, there is considerable evidence suggesting the limitation of the life-cycle of most leishmanial parasites to specific sandfly vectors. Thus, *Lu. longipalpis* is the major vector of *L. (L.) chagasi* throughout the whole geographical range of this parasite; the closely related *Lu. olmeca olmeca* and *Lu. flaviscutellata* are the only confirmed vectors of *L. (L.) mexicana* and *L. (L.) amazonensis* respectively, in Central and South America, in spite of the presence of many other species of sandflies known to feed on rodents in the endemic areas of these two parasites. As far as is known, *Ps. wellcomei* is the sole vector of *L. (V.) braziliensis* s.l. in the Carajás highlands of Pará and *Lu. umbratilis* is the major vector of *L. (V.) guyanensis* throughout its geographical distribution, again in spite of the presence of a large number of other species of sandflies. On the other hand, because some sandfly vectors feed on a variety of mammalian hosts in nature, the *Leishmania* of a given species of sandfly may sometimes be isolated from a variety of mammalian hosts sharing the same habitat. *Lu. flaviscutellata,* for example, is a low-flying sandfly and transmits *L. (L.) amazonensis* to a number of predominantly terrestrial rodents and marsupials; canopy and tree trunk-dwelling sandflies such as *Lu. umbratilis* and *Lu. whitmani* s.l. transmit *L. (V.) guyanensis* and *L. (V.) shawi*, respectively, to arboreal animals such as sloths, anteaters, monkeys, and procyonids.

P123.220 The number of *Leishmania* species in a given locality will largely be governed by the number of sandfly species, although some sandflies seem to be resistant to infection with this parasite. Thus, we (Lainson and Shaw, unpublished observations) have failed to infect the sandfly *Lu. carmelinoi* with any neotropical *Leishmania* species. The multiplicity of species within the genus is a relatively recent realization and much research is still needed to further our knowledge regarding the diversity, ecology, and taxonomy of the neotropical leishmanias. For the clinician, the continued isolation and characterization of parasites from cases of human leishmaniasis is sufficient to indicate the spectrum of *Leishmania* species commonly infecting humans in a given area. The parasitologist's interests, however, are much wider and include the wild mammalian reservoir and sandfly vectors of those parasites infecting humans, as well as the possible existence of other *Leishmania* species that rarely, if ever, infect humans.

P123.221 The number of *Leishmania* species in the neotropical region is anybody's guess and some idea of it will only be gained when sufficient numbers of mammalian and sandfly species have been examined. Clearly, it is easier and more economical to concentrate simply on the sandfly population, a truly gigantic task in itself considering that nearly 400 different species of these insects have been identified in the Americas (Young and Duncan 1994). The Amazon Region has already provided us with almost half of the recognized species of neotropical leishmanias and doubtless this great forest will continue to provide us with many more!

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