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Molecular techniques in the characterization of *Leishmania* isolates from Central America

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The public-health problems caused by leishmaniasis in most countries in Central America are becoming more severe. This is partly because of the increasing size of the human populations that are at risk and their migratory patterns. Annual incidence of the disease in Costa Rica, Honduras, Guatemala, Panama and Nicaragua is estimated to be as high as 20 000 cases. Regional changes in the epidemiology of the various *Leishmania* spp. present have emphasized the need for innovative, sensitive and accurate diagnostic tools. PCR and isoenzyme, monoclonal antibody, schizodeme, DNA-probe and random-amplified, polymorphic DNA analyses have been tested. Preliminary indications that *Leishmania chagasi* was present in Costa Rica and Honduras and that interspecific hybrids occurred in Nicaragua have been confirmed using these methods. The distribution of the *mexicana* complex was also found to be broader and more heterogeneous than initially expected. Overall, there was 87% concordance between the results produced using the different techniques.

The leishmaniases form a group of widespread and important diseases which are an increasing public health problem in many areas (WHO, 1990). Worldwide prevalence exceeds 12 million cases, with an estimated annual incidence of 400 000 cases in 80 countries, 15 000–20 000 of these cases being in Central America. This group of diseases causes serious economic loss in Central America, both in terms of the disability of affected individuals

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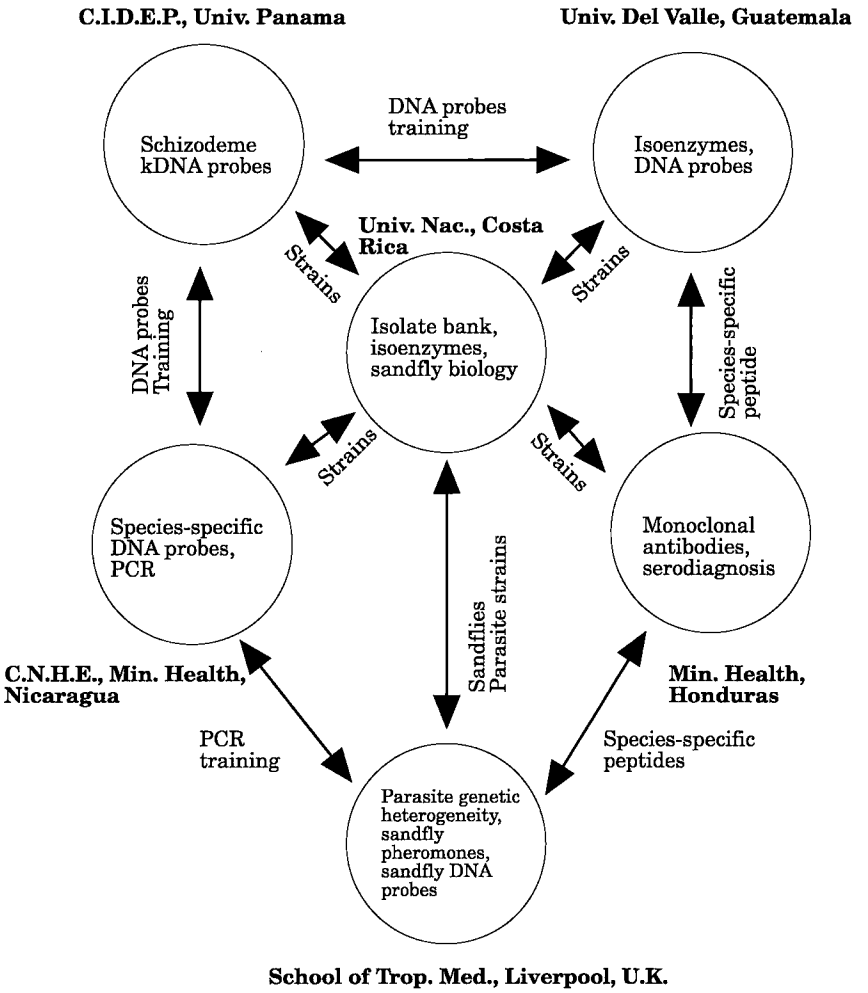

Fig. 1. The *Leishmania* Central America-European Network.

and in the cost of treatment, especially as most of those with leishmaniasis are on low incomes and live in rural areas.

Leishmaniasis occurs in different clinical forms and in a variety of ecological and epidemiological patterns in Central America (Zeledon, 1985). The cutaneous form is the most common in the region, with up to 10% of the infections metastasizing to the nasopharyngeal mucosa. Diffuse cutaneous leishmaniasis and visceral leishmaniasis also occur.

Response to treatment can sometimes give clues to the identity of the parasite species involved. In Guatemala, for example, lesions caused by *L. braziliensis*, the most common species in the country, respond well to treatment with antimonial drugs, whereas those caused by *L. mexicana* respond better to treatment with imidazoles (Navin *et al.*, 1992).

There appear to have been recent, significant changes in the distribution of *Leishmania infantum* (=*L. chagasi*) in Costa Rica and Honduras. Visceral leishmaniasis caused by *L. infantum* is becoming common although this species has been isolated from the skin lesions

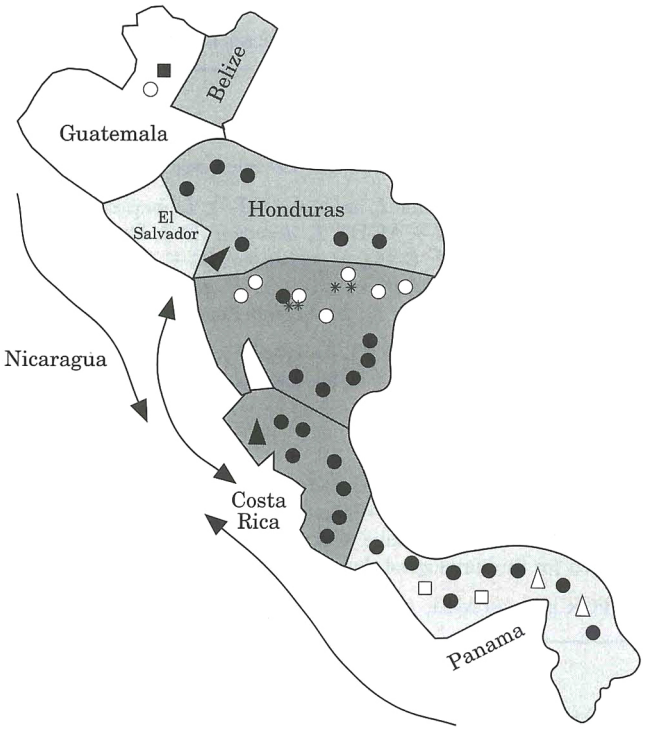

Fig. 2. Regional distribution of *Leishmania* spp. in Central America. ○, *L. braziliensis*; ●, *L. panamensis*; *, interspecific hybrids; □, *L. mexicana*-like; △, *L. amazonensis*; ▲, *L. chagasi*; ■, *L. mexicana*.

of 'atypical cases of cutaneous leishmaniasis' in the area (Zeledon *et al.*,1989; Ponce *et al.*, 1991).

Although hybrids of *L. braziliensis* and *L. panamensis* have been detected in Nicaragua, little is known about their clinical manifestations and response to treatment (Belli *et al.*, 1994).

Species of the *L. mexicana* complex have been reported in Panama (De Vasquez *et al.*, 1990). Many leishmaniasis infections in endemic areas of Panama recur, especially where *L. panamensis*, the major causative agent of cutaneous leishmaniasis, and *L. amazonensis* co-circulate (Carreira, 1992).

THE PRESENT PROJECT

The recent changes seen in the epidemiology of leishmaniases throughout Central America prompted the present attempt to characterize *Leishmania* isolates from human cases and infected sandflies. The aims were:

(1) to elucidate the specific problems related to the epidemiology of leishmaniasis in Central America (by creating a network between participating laboratories and using it to exchange and introduce advanced technology);
(2) to study the intra-specific heterogeneity of *Leishmania* isolates from selected foci;
(3) to improve the methods used to detect and identify *Leishmania* parasites in man or the sandfly vectors in the region.

RESULTS

Research strengthening and technology transfer was established through a network of

TABLE 1
The methods of characterization tested

Method	*Details*	*Reference*
Isoenzyme analysis	GPI, MPI, 6GPDH, PGM and LP examined	Kreutzer *et al.* (1987)
Monoclonal antibodies, by IFAT and ELISA	Antibodies specific for *L. mexicana* (M-7), *L. mexicana/ L. amazonensis* (M-9, M-11), *L. donovani* (D-2, D-13), *L. braziliensis* (B-2, B-16), *L. panamensis* (B-4), *L. panamensis/L. braziliensis* (B-5), or *L. guyanensis* (B-19), all provided by D. McMahon-Pratt	McMahon and David (1981)
PCR	Primers MP1L and MP3H, provided by M. Lopez. Amplification product is 70-bp fragment specific for *L. braziliensis*	Lopez *et al.* (1993)
Schizodeme analysis	Using *Msp*I and *Rsa*I	Goncalves *et al.* (1984)
DNA probes	Probes specific for *Leishmania* (*Viannia*) spp. (B4 insert), *L. mexicana* complex (V4 insert) or *L. chagasi* (503), all provided by W. DeGrave. Commercial digoxigenin kit used for immunological detection.	
RAPD	PCR primers M13, A1, A7 and IL0525	Adamson *et al.* (1993)

collaborating Central American and European scientists (Fig. 1). The validity, accuracy and efficiency of different molecular techniques was evaluated after following standard methodologies (Table 1). Exchange of a selected panel of *Leishmania* isolates between participating laboratories provided a common pool of parasites for the study.

The results of using the different molecular techniques on isolates from Panama, Honduras and Nicaragua were compared (Table 2). Overall, there was a generally good (87%) concordance. However, there were clear discrepancies between results for some isolates, particularly in the analysis of isolate HN29 (Table 2). Schizodeme analysis indicated that this isolate was *L. mexicana* whereas monoclonal antibodies indicated it to be *L. chagasi*. None of the isolates had been cloned, however, so the possibility of selecting, by amplification, a particular population of the parasite, cannot be excluded.

Some methods were more suited to some applications than others. Whereas PCR can provide information rapidly, use of monoclonal antibodies permitted a wider range of phenotypic variations to be observed. Random-amplified, polymorphic DNA (RAPD) and schizodeme analysis permitted a better evaluation of the genotypic heterogeneity within complexes and within individual isolates than the other methods.

PROSPECTS

This collaborative project has already produced a substantial amount of information on the heterogeneity of *Leishmania* spp. in Central America. The heterogeneity and complexity of these parasites increases towards the centre of the region, where hybrids and several complexes may co-exist. Curiously, both foci of *L. infantum* detected, one in Costa Rica and one in Honduras, are on the Pacific coast of the isthmus; one is probably the result of migration of infected individuals from the other.

It is not known what factors have contributed to the complexity seen in the region. However, the increasingly detailed information becoming available should permit parasite

TABLE 2
Comparison of the results obtained using the various molecular techniques to characterize the isolates

Isolate	*Result using:**				
	Isoenzymes	*Monoclonals*	*PCR*	*Schizodemes*	*DNA probes*
PANAMANIAN					
(all from cases of atypical cutaneous leishmaniasis)					
CIDEP001	Lp	Lp	+	Lp	B4+
CIDEP002	Lp	Lp	+	Lp	B4+
CIDEP003	Lp	Lp	+	Lp	B4+
CIDEP004	Lp	Lp	+	Lp	B4+
CIDEP005	Lp	Lp	+	Lp	B4+
CIDEP006	Lm	La	−	Lm†	V4+
CIDEP007	Lm	La	−	Lm†	V4+
CIDEP008	ND	La	−	Lm	V4+
CIDEP009	Lp	Lp	+	Lp	B4+
HONDURAN					
HN29 (from visceral leishmaniasis patient)	ND	Lc‡	−	Lm	−
HN116 (from atypical cutaneous leishmaniasis)	ND	Lc	−	Lc	503+
HN125 (from vector)	ND	Lc‡	−	Lc	503+
NICARAGUAN					
(all from cases of atypical cutaneous leishmaniasis)					
XD8839	ND	Lp	+	Lp	ND
XD9002	ND	Lb	+	ND	ND
ZF9101	ND	Lb	+	Lm	ND

*Lp, *L. panamensis*; +, amplification product present; Lm, *L. mexicana*; La, *L. amazonensis*; -, amplification product absent; ND, not determined; Lc; *L. chagasi*; Lb, *L. braziliensis*.
†Recognized by V4 but not indicated as Lm or La by schizodeme analysis.
‡Reacted with D-2 and M-7.

genotype and other contributing factors to be associated with specific clinical manifestations and response to treatment. As an example, the behaviour of *L. infantum* isolates, which may be visceral or dermotropic, might be explained.

It is clear from this study that the development of joint projects, by the creation of networks between participating scientists, can improve regional scientific capabilities and help all the participating laboratories to undertake question-driven research.

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