LEISHMANIASIS CONTROL STRATEGIES

A CRITICAL EVALUATION OF

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Esta serie incluye ponencias de reuniones, informes internos y documentos técnicos que pueden posteriormente conformar la base de una publicación formal. El informe recibe distribución limitada entre una audiencia altamente especializada.

Leishmaniasis control strategies

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Leishmaniasis control strategies: A critical evaluation of IDRC-supported research

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Risk Factors Associated with Cutaneous Leishmaniasis Infection and Disease in the State of Campeche, Peninsula of Yucatan, Mexico

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Introduction

In 1987 a review of knowledge regarding Cutaneous Leishmaniasis (Chiclero's Ulcer) in Mexico was presented (1). It can be summarized as follows: Seidelin in 1912 described the disease at the Peninsula of Yucatan and named it "Chiclero's ulcer"(2); Shattuck in 1933 (3), Beltrán & Bustamante in 1942 (4) and Martínez in 1951 (5) reported cases in the same geographic area; Biagi et al. working at the same endemic area from 1953 to 1965 carried out studies regarding clinical picture, histopathological pattern, geographical distribution, parasite and vector identification (6-10).

However, there is a complete absence of knowledge about reservoirs in Mexico. In summary the parasite responsible of Mexican Localized Cutaneous Leishmaniasis (Mex LCL), well known as Chiclero's Ulcer, has been identified as Leishmania mexicana mexicana (11,12); and the only proven vector identified is Lutzomyia olmeca olmeca (13,14). Lainson and Strangways-Dixon in 1962 working at Belize indentified Ototylomys phyllotis, Heteromys desmarestianus, and Nictomys sumichrasti as animal reservoirs of Leishmania mexicana mexicana (15). On the basis of this limited knowledge it was considered justified to initiate a comprehensive study of leishmaniasis in the Peninsula of Yucatan, Mexico.

The general objective of the Phase I project (1987-89) was to assess the relative importance of Mexican Localized Cutaneous Leishmaniasis (Mex LCL) in an endemic area of Mexico, the State of Campeche, Peninsula of Yucatan, through the development of diagnostic measures as a first step in the control of disease both in the individual and in the community. Specific objectives were: 1) to assess the incidence of leishmaniasis; 2) to determine sensitivity and specificity of the ELISA assay for IgG antibodies to L. mex. mex.; 3) to evaluate safety and efficacy of treatment of Mex LCL with meglumine antimoniate.

Upon completion of the Phase I project, a Phase II project (1990-92) was undertaken to study risk factors associated with acquisition of infection and/or disease. Its specific objectives are: 1) to identify risk factors associated with acquisition of

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infection and/or disease in a population living in the endemic area; 2) to identify potential sandfly vectors; 3) to identify parasites isolated from human cases; and 4) to determine sensitivity and specificity of the ELISA assay for IgM antibodies to L. mex. mex.

Materials & Methodology

Phase I, 1987-89 studies:

In order to obtain reliable knowledge about the incidence of Mex LCL in the State of Campeche, we interviewed and performed skin tests (Montenegro intradermal test) on a sample of 449 persons randomly selected from men aged 15-45 years, from 7 Rural Health Posts (RHP)[2] (16). Diagnosis of cases detected from January to December 1987 was established based on clinical examination, parasitological demonstration (smear, isolation-culture, biopsy) when possible, histophatology (to exclude other pathology) and immunological tests, skin-test and ELISA (17,18).

Two-hundred and twenty-three sera samples were assayed to assess the sensitivity and specificity of ELISA for IgG antibodies to L. mex. mex. (A = 75 sera samples from healthy negative skin-test; B = 56 from healthy positive skin-test; C = 74 from patients with Mex LCL; and D = 18 from patients suffering other diseases). ELISA for IgG was carried out according to El Amin et al. (19) employing a promastigote suspension of $1x10^7$/ml and a goat anti-IgG heavy chain conjugated to peroxidase (SIGMA A6029) diluted 1/1000 in PBS-T. Absorbances were read at 490mm. Results were determined as positive or negative according 2x2 Table and Chi-square test (20).

In order to evaluate the safety and efficacy of treatment a total of 53 males ranging in age from 8 to 75 years (average age 26 years) were treated for Mex LCL with meglumine antimoniate during 1987-1988. Diagnosis was established as described (17-19). Criterion for the application of treatment followed those established by CHEMLEISH/WHO (21).

Phase II, 1990-92 studies:

To identify risk factors associated with the acqusition of infection and/or disease the study was designed as a "case control" study including three "controls" per "case". A total of four "ejidos" (small village) were selected to carry out this study. A questionnaire including age, sex, occupation(s), time of living in the "ejido", workplace, home-working-play distance, going or not going to the field, household living conditions, house location, house building materials and protective measures against insects was conducted as was a skin-test. A complete clinical history, parasitological and immunological diagnosis test were done in all "cases" detected.

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[2] These were located in the isthmus with a tropical climate, humidity of over 80% and an annual average rainfall of over 1400mm.

La Libertad, a small village with a total of 628 inhabitants located at 24 km from Escarcega (Biagi's studies area) and with 12 "well defined" TDR/WHO/LEISH criterion "cases" during 1989-90 was chosen as the study area for the identification of potential sandfly vectors of Leishmania sp. The methodology for sandfly sampling included: 1) Searching resting sites approximately 1km surrounding the "ejido"; 2) Use of tent traps (Shannon) for all-night anthropophilic collections; and 3) Use of CDC light traps in sylvatic areas without air movements between 18:00 to 21:00 hours. This study was conducted in the surrounding area of La Libertad from October 1990 to September 1991. Taxonomic classification was completed according established methodology (22-23).

Identification of parasites isolated from human cases was achieved through isolation, culture, and maintenance on Senekjie's biphasic medium, hamster inoculation, cryopreservation and isoenzyme characterization by cellulose acetate electrophoresis employing six enzymes (NH, GGPDH, PGM, GPI, MPI, GPGDH) according to established methodology (25-26).

To determine the sensitivity and specificity of the ELISA assay for IgM antibodies to L. mex. mex. a total of 79 sera were collected. These consisted of: Group I - 40 samples from healthy negative skin-test patients; and Group II - a) 21 samples from patients with Mex LCL parasite-positive demonstration and IgG anti-L. mex. mex. ELISA positive and b) 9 samples from patients parasite-positive and IgG negative and 9 from patients parasite-negative demonstration and IgG ELISA positive, all of them with less than 12 months of clinical evolution. ELISA was carried out according El Amin et al. (19) employing a promastigote suspension at $1x10^7$/ml. and a goat anti-IgM heavy chain conjugated to peroxidase diluted 1/1000 in PBS-T. All sera were treated with 2-ME to expose IgM and eliminate IgG (28). Absorbances were read at 490 nm. Results were considered positive when an absorbance value was more than 0.374 using a 1/16 dilution, this was the mean plus 2 SD at Group I control sera (29).

Results

Phase I, 1987-89 studies:

A positivity rate of 24-90% (mean 43%) for Mex LCL was found in the seven Rural Health Posts studied (Table 1). Furthermore, 72 patients were examined from January to December 1987 and 56 of them acquired the disease in 1987. Therefore, we found amongst men aged 15-45 years in the focus area an annual incidence rate of 508 per 100,000 inhabitants (16).

The ELISA results for the four groups of sera are shown in Table 2. A total of 56 of the 74 sera from patients with Mex LCL, gave positive reactions (i.e. 76% sensitivity) of a titre of 1:40 and the positivity rate was lower in patients with long-standing infections. Table 3 shows differences in reactivity considering parasite positivity and time of evolution (17).

Table 1. Montenegro skin test positivity in seven rural communities in Campeche, Mexico

Rural community	No. of subjects	No. positive
Constitucion	80	46 (57%)
Don Samuel	31	28 (90%)
A. Obregon	81	20 (24%)
Yohaltun	30	15 (50%)
Centenario	131	33 (25%)
Carmen II	33	20 (60%)
R. Payro	63	34 (53%)
Total	449	196 (43%)

Table 2. Enzyme-linked immunosorbent assay results for the different groups of sera tested.

Group	No. of sera	No. positive	Percentage reactivity
A. Healthy human beings M-a	75	0	0
B. Healthy human beings M+b	56	4	7
C. Patients with leishmaniasis	74	56	76
D. Patients with other diseases	18	6	33

aM- = negative Montenegro test.
bM+ = positive Montenegro test.

Table 3. Enzyme-linked immunosorbent assay results for sera from patients with chiclero's ulcer according to parasitological diagnosis and stage of development

Patients	No. of sera reactivity	No. positive	Percentage
Acute p+a	34	31	91
Acute p-b	16	12	75
Chronic	24	13	54

aP+= Parasites demonstrated
bP-= Parasites not demonstrated

A total of 42 patients with Mex LCL completed their course of treatment of with meglumina antimoniate. From these, three cases were classified as "acute" (i.e. less than a year of clinical infection), and 10 as "chronic" (i.e. 2 to 38 years from onset). The response to treatment in both groups is shown in Tables 4 and 5. Importantly, Table 5 shows that when systemic treatment was given to both "acute" and "chronic" cases, no significant difference in the results, regardless average doses, was observed (27).

Table 4. Response to Treatment depending on via administration with Meglumine Antimoniate in patients with Mex L.C. Leishmaniasis

Time of Evolution	Systemic		Via Intralesional		Total	
	Cured	Failure	Cured	Failure	Cured	Failure
Acute (n=32)	25 (90%)	3 (10%)	3 (75%)	1 (25%)	28 (88%)	4 (12%)
Chronic (n=10)	7 (88%)	1 (12%)	2 (100%)	0 (0%)	9 (90%)	1 (10%)
Total	32 (89%)	4 (11%)	5 (84%)	1 (16%)	37 (88%)	5 (12%)

Table 5. Mex LCL Response to Treatment with Meglumine Antimoniate. Average doses in cured patients

Time of Evolution	Dose (No. of ampules)	
	Systemic Range (average)	Intralesional Range (average)
Acute	8-33 (23)	5-9 (5)
Chronic	10-30 (22)	13-18 (15)

Phase II, 1990-92 studies:

It was possible to detect 58 well defined "cases" of Mex LCL according TDR/WHO/LEISH (i.e. parasitological demonstration positive) in the four "ejidos" selected for the study and 213 "controls" from the 2,907 total population of inhabitants who were studied (questionaire and skin-test). In Table 6 the main risk factors associated with acquisition of infection and/or diseases identified after all statistical data analysis are shown. From all data analysed we can conclude that the most important risk factor is "exposure" and as a consequence age, sex, occupation, working place and going to the field are closely related.

Table 6. Risk factors associated with acquisition of infection and/or diseases in an endemic area of Mex CLC.

Risk Factor	No. of Cases (%)	No. of Controls (%)	Average	p^2	Odds Ratio
Age:					
> 15 years	47 (81)	106 (49.8)	16.88	0.00	4.31
< 15 years	11 (19)	107 (50.2)			
Sex:					
Male	52 (89.7)	78 (36.6)	49.27	0.00	15
Female	6 (10.3)	135 (63.4)			
Going to Field*:					
Yes	55 (94.8)	97 (45.5)	42.98	0.00	21.92
No	3 (5.2)	116 (54.5)			
Skin test (IDR):					
Positive	34 (87.2)	20 (9.4)	113.9	0.00	65.62
Negative	5 (12.8)	193 (90.6)			

*Going to Field: Yes, going often and for sometime; No, not going to the field.

A total of 362 (100%) Lutzomyia sp. were collected employing the methodology described previously. Table 7 presents the frequency of species collected by sex. In Table 8 it is possible compare the different sandfly sampling methods used throughout one year (the CDC light traps were the best). Of the 169 (46.6%) Lutzomyias collected 137 (81%) were females. It is important to point out that only one of them was Lutzomyia olmeca, whereas 96 (56.8%) were L. deleoni and 26 (15.4%) were L. carpenteri. Searches of resting sites yielded 133 (36.7%) sandflys however, only 26 (19.5%) were females and only L. deleoni, L. cayannensis, L. shanoni and L. cruciata were found. Shannon traps capatured 60 (16.6%) sandflys and 54 (90%) were females including L. cruciata 39 (65%), L. olmeca 5 (8.3%), L. deleoni, 5 *8.3%) and L. panamensis 3 (5.0%), but were not relevant as results.

Regarding anthropophilic activity by all-night collections employing Shannon traps, the highest activity was observed from 18:00 to 22:00 hours with a peak at 19:00 hours, and L. cruciata was the most anthropophilic species followed by L. olmeca and L. deleoni.

Table 7. Number of Lutzomyia species collected by sex (% in brackets)

Species	Male	Female	Total
L. deleoni	104 (28.7)	115 (31.8)	219 (60.5)
L. cruciata	2 (0.5)	47 (12.9)	49 (13.5)
L. carpenteri	14 (3.9)	27 (7.4)	41 (11.3)
L. panamensis	9 (2.5)	6 (1.6)	15 (4.1)
L. shannoni	7 (1.9)	4 (1.1)	11 (3.0)
L. cayennensis	6 (1.6)	5 (1.4)	11 (3.0)
L. olmeca	0	6 (1.6)	6 (1.7)
L. gomezi	1 (0.2)	2 (0.6)	3 (0.8)
Ph. permirus	0	3 (0.8)	3 (0.8)
L. ovallesi	2 (0.6)	0	2 (0.6)
No identification	2 (0.6)	0	2 (0.6)
Total	147 (40.6)	215 (59.4)	362 (100%)

Table 8. Number of Lutzomyia sp. caught (% in brackets) employing three different collection methods.

Month	Shannon	C.D.C	Resting Sites	T o t a l
Oct. 90	3 (0.8)	3 (0.8)	12 (3.3)	18 (5.0)
Nov. 90	2 (0.5)	9 (2.5)	12 (3.3)	23 (6.4)
Dec. 90	4 (1.1)	9 (2.5)	14 (3.9)	27 (7.5)
Jan. 91	6 (1.7)	36 (9.9)	36 (9.9)	78 (21.5)
Feb. 91	15 (4.1)	60 (16.6)	22 (6.1)	97 (26.8)
Mar. 91	18 (5.0)	26 (7.2)	0	44 (12.1)
Apr. 91	2 (0.5)	1 (0.3)	6 (1.7)	9 (2.5)
May. 91	0	0	2 (0.5)	2 (0.5)
Jun. 91	0	3 (0.8)	8 (2.2)	11 (3.0)
Jul. 91	5	10 (2.8)	3 (0.8)	18 (5.0)
Aug. 91	0	1 (0.3)	3 (0.8)	4 (1.1)
Sep. 91	5 (1.4)	11 (3.0)	15 (4.10)	31 (8.6)
	60 (16.6)	169 (46.6)	133.(36.7)	362 (100%)

Phase II, 1990-92 studies:

Studies for the identification of parasites isolated from human cases only began in June 1991 due to equipment and chemical reagents obtained during first semester 1991. However, it has been possible since January 1990 to date, isolate and maintain a total of 85 stocks from patients with a Mex LCL. Cryopreservation and isoenzyme characterization by cellulose acetate electrophoresis employing 6 enzymes mentioned previously, has been standarized. We have recently identified 16 strains and all of them are L. mex. mex.

The ELISA results for groups of sera studied are shown in Table 9. It is possible to observe that 22/39 (56%) cases were positive at titres > 1:16. Therefore, employing the methodology described previously, we have obtained a sensitivity of 56% and specificity of 92%. However, as shown in Table 10, when comparing parasite-positive cases and parasite-negative cases there is a difference in the percentage of reactivity. The biological significance of this finding, even though the sample was small, remains unknown. Positivity relationship with age was significant in high risk groups (ages 10-40 years old) and also regarding time of evolution (83% of positive cases were < 6 months).

Table 9. ELISA results for the different groups studied

Group	No. of sera	No. of positive	Percentage reactivity
I. Healthy-skin test negative	40	12	30 %
II. A) Mex.LCL, P(+), ELISA (+)	21	9	7.5 %
B) Mex.LCL, P(+), ELISA (-)	9	5	55.55%
C) Mex.LCL, P(-), ELISA (+)	9	9	88.88%

Mex.LCL= cases; P(+)= parasite-positive; ELISA (+/-)= ELISA IgG.

Table 10. Sensitivity of IgM-ELISA to detect antibodies anti-L. mex. mex. in patients with Mex. LCL related to parasitological demonstration

Group	No. of patients	No. of positives	Percentage of reactivity
Parasite-positive	30	14	46.66%
Parasite-negative	9	8	88.88%
Total	39	22	56%

Results and Application to Prevention and Control

Health research is the generation of new knowledge using the scientific method to identify and deal with health problems. Knowledge, worldwide or local, is essential for effective action. Local knowledge is essential to: 1) identify and set priorities; 2) guide and accelerate application of knowledge to solve health problems; 3) develop new tools and strategies; and 4) advance basic understanding (30). The present review of Phase I and II projects results supported by IDRC from 1987 to 1991 regarding leishmaniasis in the State of Campeche, Yucatan Peninsula, an endemic area recognized worldwide for Chiclero's ulcer, has generated the following benefits.

On the basis of diagnosis tool development as the first step in the control (both at the individual and community levels), it was possible to obtain reliable knowledge

regarding the magnitude of this health problem in an endemic area of Mexico. As a consequence, the national health authorities included leishmaniasis as a notifiable disease in Mexico and designated our Tropical Disease Research Unit, Research Center "Dr. Hideyo Noguchi" as a Reference Center responsible of the "Programme for the Study, Surveillance and Control of leishmaniasis at the Peninsula of Yucatan".

This program includes four main objectives: 1) To carry out multi- and interdisciplinary research including social, economical, cultural, epidemiological, clinical, parasitological, entomological, zoological, immunological and cellular-molecular biological studies; 2) To organize an epidemiological surveillance system; 3) To organize an efficient system of medical care; and 4) to promote community participation.

In order to achieve these objectives it has been necessary to improve and increase resources and staff development (training activities, post-graduate courses, etc.). It has also been necessary to establish accurate coordination with Rural Health Post personnel from both IMSS-Solidaridad Programme and Secretaria de Salud. This has included training for detection (suspicious "cases"), standardizing notification procedurs and records (specific format, age, sex, place of infection, date, lesion, and skin-test), and monthly field work to confirm diagnosis (clinical history, parasitological demonstration by smear, isolation-culture, biopsy and immunological studies). It has also been necessary to carry out clinical trials to evaluate the safety and efficacy of anti-leishmania drugs; to organize and supervise Rural Health Committees and Health Promoters in each "ejido" (small village); to inform to local authorities and communities of the activities (research proposals) prior to initiation, during development, and at the conclusion of studies (conveying findings, results and benefits).

Main Conclusions

Diagnostic tools: It is well known that diagnosis tests, such as the ELISA of this project, applied to "community diagnosis" are a pre-requisite to the planning, implementation and evaluation cycle to determine the effects of the disease and the control strategies selected according cost/benefit analysis.

Epidemiological studies: Since Phase I and subsequently through the Phase II Risk Factor Study, it has been possible to determine: incidence; prevalence; distribution by time, location, age, sex, occupation etc,. It has been remarked that the most important knowledge obtained has been the identification of the "high risk population", i.e. target to which to apply any selected control strategies.

Clinical studies: To date we have studied more than 400 leishmaniasis patients. It has been possible to study clinical presentation (patterns); histopathological patterns; and evaluate the safety and efficacy of anti-leishmanial drugs.

Parasitological study: We are just at the begining of this very important study to

characterize the focus, i.e. identify specie or species responsible of the clinical forms studied. We must point out, that we have developed and acquired staff, resources (equipment, supplies and chemical reagents) to carry out biochemical characterization of "stocks" isolated from human cases to date. Furthermore, we have also obtained the resource necessary to identify parasites isolated from vectors and reservoirs (to be initiated in 1992).

Entomologial study: We have completed the one-year first study in the endemic area. Results have been briefly commented upon. We must remark that this base (control) study was done mainly to evaluate and exclude peri-domiciliary and/or intradomicilary transmission. Data obtained have been analysed in correlation to epidemiological and clinical studies (case detection, place of infection) and reinforce the possible role of sylvatic transmission (study to be initiated in 1992).

Community participation: The research team has received stimulating acceptance from the community with respect to each proposal presented to them and developed. They have even said "we will accept any suggestion to prevent and control this disease". We most mention that we have also started social-economic studies in collaboration with the University of Calgary, Canada, which are being supported by TDR/WHO.

Immunological studies: It has been possible to develop enzyme-linked immunoassays to detect IgG and IgM antibodies to L. mex. mex. promastigotes.

Possible application to prevention and control: With all these data analysed as a total and representative sample of "reality" from the endemic area studied, it is possible now to establish "well defined" case diagnosis based on epidemiological, clinical, therapeutic response, histopathological, parasitological and immunological criterion (which are included in the Leishmaniasis Programme). Through active community participation it is now possible to detect, notify, confirm diagnosis and treat cases. It has also been possible to carry out biological (immunological, parasitological, molecular biology studies to advance in our host-parasite relationship knowledge), and follow-up on a yearly basis on the the patients who have been studied. This feature will be very important and useful for the vaccine research which is to be initiated in 1992. Due mainly to the positive and supportive attitude of the community, our primary objective is now to design realistic control measures based on community participation. Research in this area is to be initiated in 1992.

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