LEISHMANIASIS CONTROL STRATEGIES

IDRC
CRDI
CIID

CANADA

A CRITICAL EVALUATION OF IDRC-SUPPORTED RESEARCH

The International Development Research Centre is a public corporation created by the Parliament of Canada in 1970 to support research designed to adapt science and technology to the needs of developing countries. The Centre's activity is concentrated in six sectors: agriculture, food and nutrition sciences; health sciences; information sciences; social sciences; earth and engineering sciences; and communications. IDRC is financed solely by the Parliament of Canada; its policies, however, are set by an international Board of Governors. The Centre's headquarters are in Ottawa, Canada. Regional offices are located in Africa, Asia, Latin America, and the Middle East.

Le Centre de recherches pour le développement international, société publique créée en 1970 par une loi du Parlement canadien, a pour mission d'appuyer des recherches visant à adapter la science et la technologie aux besoins des pays en développement; il concentre son activité dans six secteurs : agriculture, alimentation et nutrition; information; santé; sciences sociales; sciences de la terre et du génie et communications. Le CRDI est financé entièrement par le Parlement canadien, mais c'est un Conseil des gouverneurs international qui en détermine l'orientation et les politiques. Établi à Ottawa (Canada), il a des bureaux régionaux en Afrique, en Asie, en Amérique latine et au Moyen-Orient.

El Centro Internacional de Investigaciones para el Desarrollo es una corporación pública creada en 1970 por el Parlamento de Canadá con el objeto de apoyar la investigación destinada a adaptar la ciencia y la tecnología a las necesidades de los países en desarrollo. Su actividad se concentra en seis sectores: ciencias agrícolas, alimentos y nutrición; ciencias de la salud; ciencias de la información; ciencias sociales; ciencias de la tierra e ingeniería; y comunicaciones. El Centro es financiado exclusivamente por el Parlamento de Canadá; sin embargo, sus políticas son trazadas por un Consejo de Gobernadores de carácter internacional. La sede del Centro está en Ottawa, Canadá, y sus oficinas regionales en América Latina, Africa, Asia y el Medio Oriente.

This series includes meeting documents, internal reports, and preliminary technical documents that may later form the basis of a formal publication. A Manuscript Report is given a small distribution to a highly specialized audience.

La présente série est réservée aux documents issus de colloques, aux rapports internes et aux documents techniques susceptibles d'être publiés plus tard dans une série de publications plus soignées. D'un tirage restreint, le rapport manuscrit est destiné à un public très spécialisé.

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

IDRC-MR322e
June 1992

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

Proceedings of a workshop held in Mérida, Mexico, November 25–29, 1991, sponsored by the International Development Research Centre, in collaboration with the Universidad Autónoma de Yucatán (UADY) and the Universidad Peruana Cayetano Heredia (UPCH)

Edited by
Pandu Wijeyaratne, Tracey Goodman
and Carlos Espinal

INTERNATIONAL DEVELOPMENT RESEARCH CENTRE
Ottawa • Cairo • Dakar • Montevideo • Nairobi • New Delhi • Singapore

ISBN 088936-653-5

TABLE OF CONTENTS

Epidemiological Studies on Andean Cutaneous Leishmaniasis and their Significance for Designing a Control Strategy

A. Llanos-Cuentas[1] and C. Davies[2]

Introduction

The Workshop "Research on Control Strategies for the Leishmaniasis" held in Ottawa, Canada in 1987, identified the priorities for research to aid control efforts. It recognized that each clinical-epidemiological form has unique characteristics, so that general recommendations are not feasible for control approaches. Four disease groups were considered separately. For one of them, New World cutaneous and mucocutaneous leishmaniasis, the major recommendations were: (1) the identification of high risk groups; (2) identification of the factors associated with transmission; (3) development of vaccines; and (4) pilot control studies.

Summary of the Status of Leishmaniasis in Perú

In Perú four Leishmania species have been described: L.(V.) braziliensis, L.(V.) peruviana, L.(V.) guyanensis, L. mexicana (Llanos-Cuentas 1990). Only the first two have epidemiological importance in Perú. L.(V.) peruviana causes Andean Cutaneous Leishmaniasis (Uta). It occurs in the Andean valleys along the Pacific slope and in some Inter-Andean valleys between 800 and 3,000 meters. Incidence also varies with latitude, and below 13° south there are no reported cases of Uta. In contrast with other subspecies of the L.(V.) braziliensis complex, L.(V.) peruviana is not transmitted in forested regions. In the endemic valleys of the western Andes, the vegetation in the uncultivated areas is usually low scrub. Uta patients always suffer cutaneous lesions, but mucous membranes can occasionally be involved via two processes: by extension of a contiguous lesion (Lumbreras 1985; Miranda 1988), or (very rarely) by metastasis (Llanos-Cuentas unpublished data).

L.(V.) braziliensis is the agent of cutaneous and mucocutaneous leishmaniasis (MCL) and it causes the majority of mucosal cases. It is present on the eastern slopes of the Andes, from 3,000 meters (in the Department of Huanuco) down to sea level and the Amazonian jungle.

---

[1] Working Group of Leishmaniasis, Instituto de Medicina Tropical "Alexander von Humboldt" (IMTAvH), Universidad Peruana Cayetano Heredia (UPCH), Lima, Perú. Centro de Investigación en Salud "Dr. Hugo Lumbreras Cruz", INS, MINSA.

[2] London School of Hygiene and Tropical Medicine (LSHTM), U.K.

Leishmaniasis is a disease that is increasing in Peru; its incidence (as recorded by passive detection) has recently doubled from 9.1 cases/10^5 in 1978 to 20.8/10^5 in 1988 (Fig. 1). Both Uta and MCL have increased in this time (Fig. 2 and Fig. 3).

In some departments, such as Madre de Dios, Huanuco, Cusco, Ucayali and Ancash, incidence rates were above 70 cases/100,000 inhabitants in 1988; and in some areas, such as Ambo (Huanuco department), leishmaniasis has the same importance as respiratory disease, diarrheal disease or tuberculosis (Table 1).

Table 1. Principal causes of morbidity in Ambo from January to October, 1988

Disease	No. of patients
Leishmaniasis	364
Diarrheal disease	277
Respiratory disease	269
Tuberculosis	15

The Ministry of Health (MINSA) has estimated approximately 15,000 new cases of tegumentary leishmaniasis for 1992 (Chumbe 1991, manuscript document). There is no specific program for control in leishmaniasis in Peru, and the main measure taken by MINSA is the provision of drug supplies. Some operative units of MINSA have programmed spraying activities, but no quantitative analysis of their efficacy has been carried out.

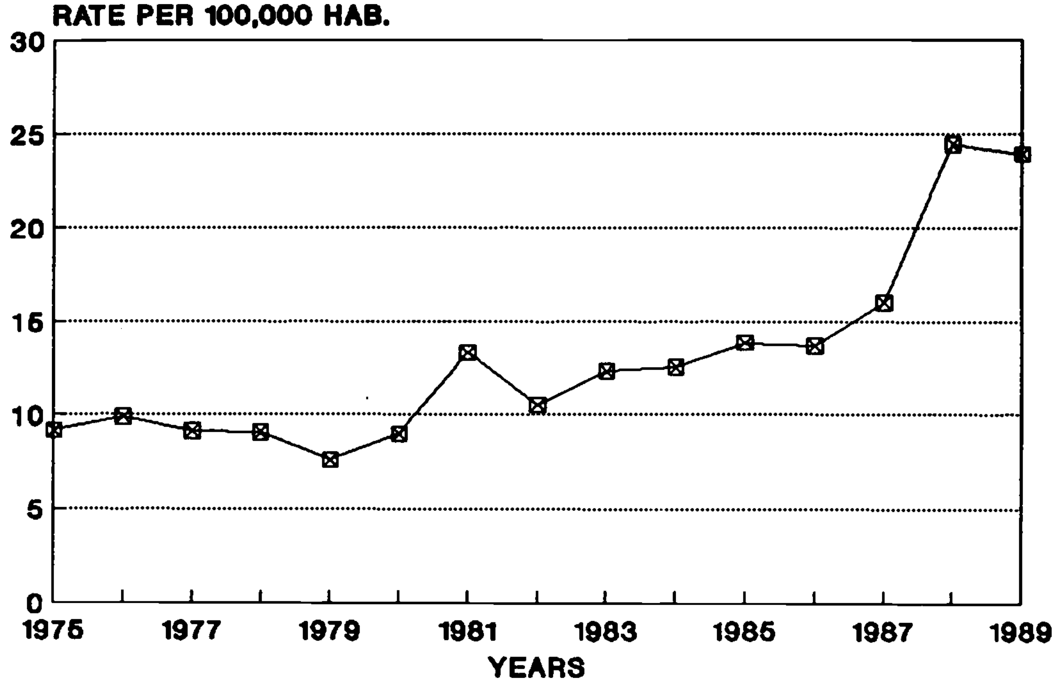
Current Status of our Knowledge of the Epidemiology of Uta, and its Relevance for Control Strategies

In this meeting we would like to address the following questions: (a) Where and when does transmission of Uta occur? and (b) How does the epidemiology relate to the entomological parameters? These questions are potentially the most relevant for choosing an appropriate control strategy. We shall be referring to the results of recent and on-going studies that are being carried out in several endemic areas by the Working Group of Leishmaniasis at the Instituto de Medicina Tropical "Alexander von Humboldt"- UPCH and Centro de investigación en salud "Dr. Hugo Lumbreras"-INS-MINSA.

The identification of the vector species is one of the pre-requisites for any control program. Prior to our current studies, the evidence for distinguishing the vector status of the various potential vectors of Uta was largely circumstantial. At least nine Lutzomyia (Lu.) species (Diptera: Psychodidae: Phlebotominae) have been identified in sandfly collections made in endemic areas of Uta. The most important anthropophilic species are Lu. peruensis, Lu. verrucarum, and Lu. ayacuchensis.

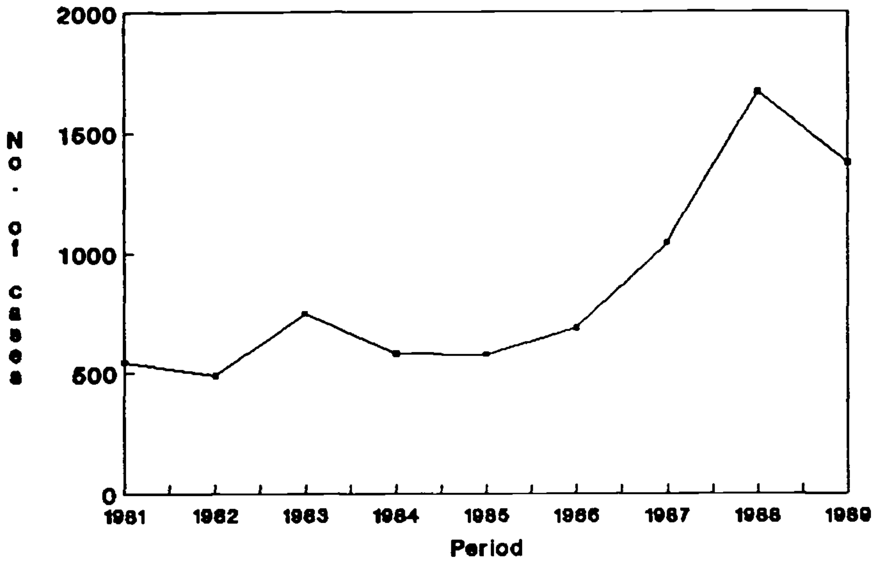
FIGURE 1:

INCIDENCE OF LEISHMANIASIS IN PERU 1975 - 1989



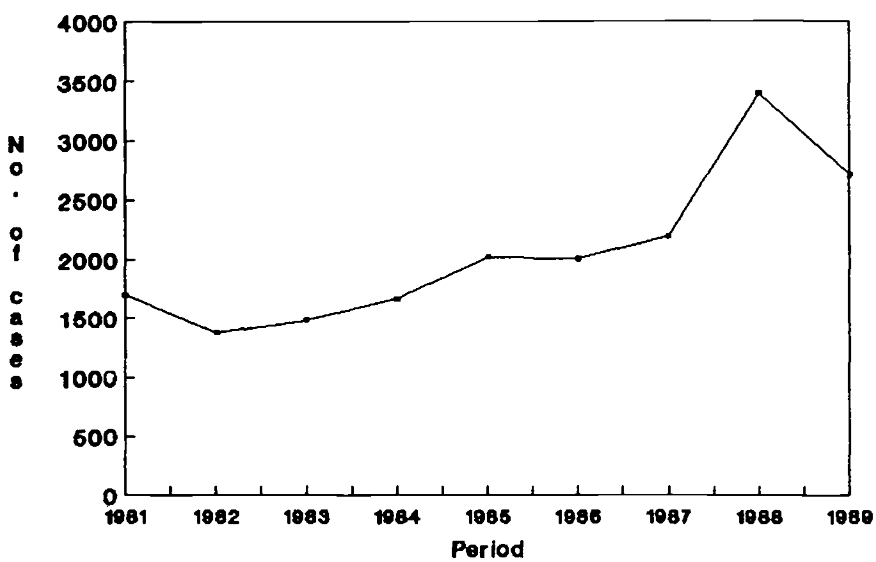
Source: DTID/OGE

FIG. 2. NEW CASES OF UTA IN PERU DURING THE PERIOD 1981 TO 1989



Source:DTIG/OGE/MINSA

FIG 3. NEW CASES OF MUCOCUTANEOUS LEISHMANIASIS IN PERU, PERIOD 1981-89



Source: DTIG/OGE/MINSA

Following the early studies of Townsend (1913), Lu. verrucarum was incriminated as the vector of Bartonella baciliformis, and transmission was shown to be closely associated with human dwellings. As Uta and bartonellosis are closely associated geographically, and frequently coexist in some areas, the vector and the place of transmission were assumed to be the same (Weiss 1943; Herrer 1951a; Herrer & Battistini 1957). No specific attempts were made to identify the vector of Uta until 1982 when Herrer (1982) induced Leishmania infections in hamsters (Mesocricetus auratus) inoculated with triturates of pooled field-caught Lu. peruensis. He concluded that this sandfly may be the natural vector of L.(V.) peruviana; he also suggested that Lu. peruensis has less domesticated habits than Lu. verrucarum (Herrer 1982). Later, Cruzado in La Libertad (1987) and Pérez in Ancash (Purisima valley) detected natural infections of Leishmania promastigotes in individually dissected Lu. peruensis (Pérez et al. 1991). The strain isolated by Pérez was identified by isoenzymes as L.(V.) peruviana. Recently, the group of Dr. Arevalo of IMTAvH, using the polymerase chain reaction technique with a sequence of kinetoplast DNA specific for the L.(V.) braziliensis complex (López et al. 1991), have identified natural infections in two other species of sandflies: Lu. verrucarum and Lu. ayacuchensis (Arévalo, Cáceres & Pérez Personal communication). Lu. ayacuchensis is the likely vector in the endemic valleys of Ayacucho (and possibly in Piura), whilst in Western valleys along the Pacific slopes Lu. peruensis and Lu. verrucarum are the potential vectors. The relative importance of Lu. peruensis and Lu. verrucarum as vectors is hard to distinguish, as they are frequently sympatric and both show a close geographic correlation with the disease.

One approach is to relate altitudinal variation in the proportion of Lu. peruensis with variation in the incidence of Uta. In Figure 4, we can see clearly that the ratio of Lu. peruensis to Lu. verrucarum (collected in Shannon traps) increases with altitude, indicating that although the two species overlap, the mean altitude of the Lu. peruensis population is greater and correlates with an increase in the incidence of Uta with altitude (Fig. 5). Sandfly collections and prospective epidemiological surveys (by active search) were undertaken in Purísima valley from 1987 to 1989 (Phase I). The line of best fit was calculated by logistic regression. Thus, these results suggest that in Purísima valley transmission is mainly by Lu. peruensis, although a role for Lu. verrucarum cannot yet be discounted.

The second approach we are taking is to investigate temporal correlations between sandfly densities and monthly incidence rates. In Purísima valley, we correlated the monthly incidences in the whole valley with concurrent monthly variation in sandfly numbers in one highly endemic village (Huanchoc) estimated by three methods (CDC in houses, Shannon traps outside, and daytime captures from outdoor resting places by aspiration). The only relationship identified was between the number of Lu. peruensis caught inside houses and incidence one month later (Fig. 6). The correlation coefficient was 0.42 (just above the critical value (0.50) for significance ($p < 0.05$). No correlations were observed for any other time shifts (from 0-6 months were tested) or for outdoor Lu. peruensis captures (by Shannon or aspiration); neither were any correlations apparent

FIGURE 4:
PERCENTAGE L. PERUENSIS
(Shannon traps in Purisima Valley)

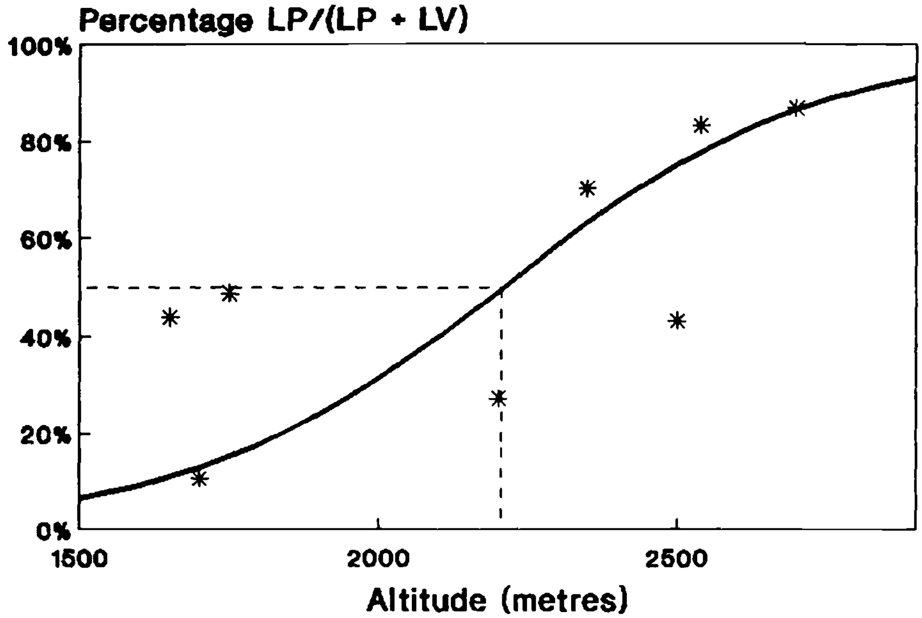
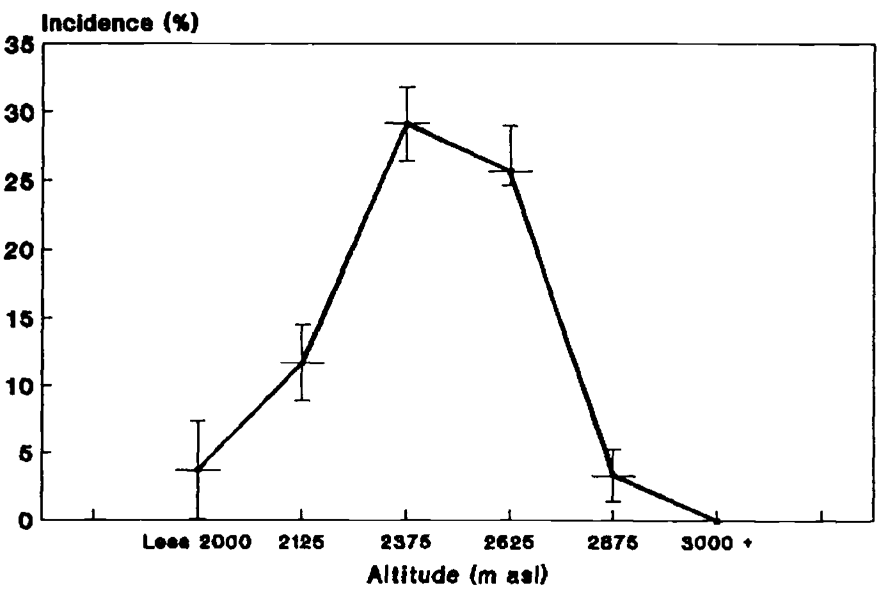
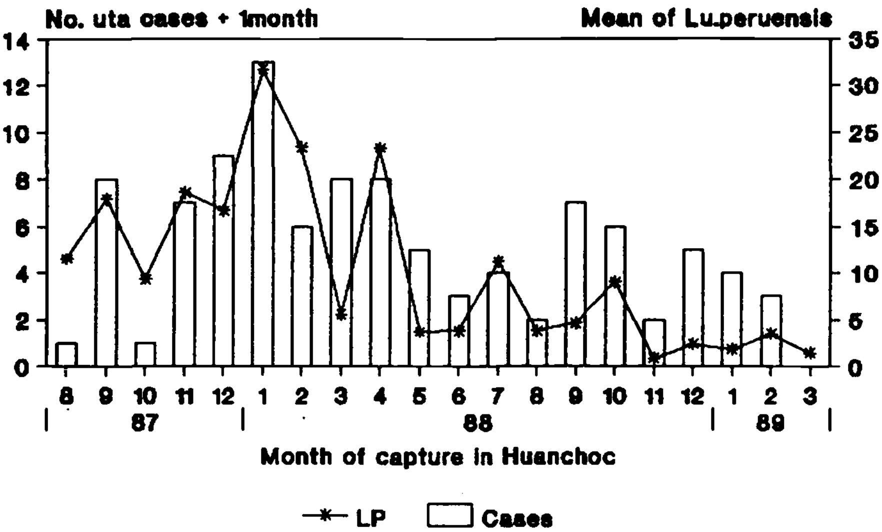


FIG 5. INCIDENCE BY ALTITUDE
(Purisima valley, Oct 86 - Mar 89)



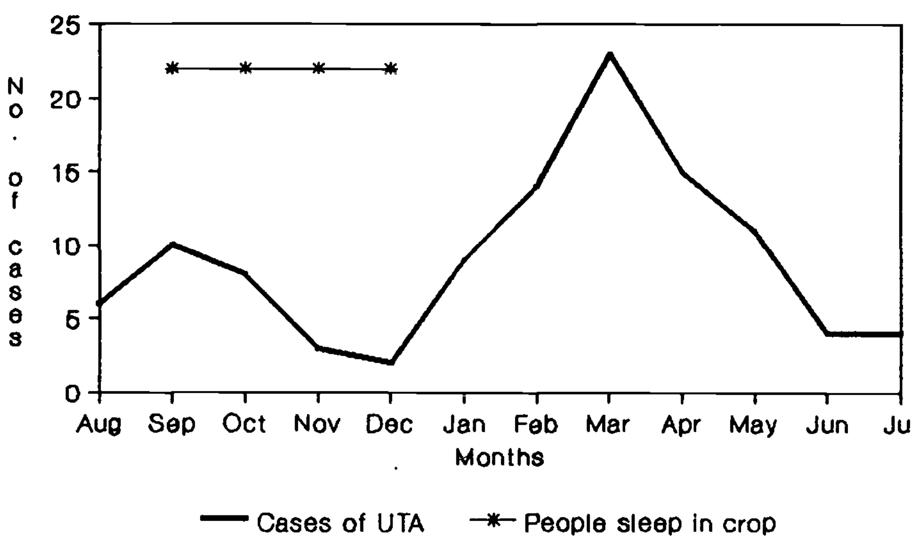
Incidence and confidence interval 95%

FIG 6. LU. PERUENSIS DENSITY AND MONTHLY DISTRIBUTION OF UTA CASES (CDC inside houses; uta cases + 1 month)



Period August 87 to March 89

FIG.7. RELATIONSHIP BETWEEN DISTRIBUTION OF UTA CASES AND CROPS ACTIVITIES (Purisima valley, August 87 to July 88)



with variation in the Lu. verrucarum population. These results suggest: (1) an incubation period of one month between sandfly bite and onset of lesion development; and (2) a key role for intra-domiciliary transmission by Lu. peruensis in the epidemiology of Uta in the Purísima valley.

Further on-going comparative studies of Lu. peruensis and Lu. verrucarum aim to: (1) relate incidence with sandfly densities in different endemic areas; (2) compare infection rates in the field; and (3) compare vectorial competence in laboratory transmission studies with colonised flies.

Additional observations in the Purísima valley indicate the possibility of extra-domiciliary rural transmission foci. The working habits of the population involve the displacement of all members of the family to their crop fields, where they remain for different periods (days), using provisional shelters. As these shelters are open, their occupants are very much exposed to sandfly bites. Sandfly captures were carried out at provisional shelters (both occupied and empty) in different locations of the valley, during the peak sandfly activity period (18:00 to 21:00 hours). The mean number of sandflies caught per night was from 20-31 in the occupied shelters, and less than 2 in the empty ones. Casual observation of (and conversation with) the population suggests that families remain in the crop fields (mainly corn) during the harvesting periods just some months before the peak of incidence in the valley (Fig. 7). A quantitative analysis of the time spent in these temporary shelters in relation to seasonal variation in incidence is currently being carried out.

It is vital to verify and define the different sites of transmission in order to account for the failure of past fumigation programs to eradicate the disease, and to design appropriate control strategies for the future.

Two further approaches are being taken to definitively identify the sites of transmission. Firstly, we are investigating the risk factors associated with recent transmission of Uta using a case control study. The exposure factors are the characteristics of the house, the peridomiciliary environmental characteristics, and the behavior patterns of the people in the endemic areas. Secondly, we are comparing monthly incidences and sandfly densities captured inside houses, in the peridomestic arena and within crops belonging to some 16 villages. The aim is to collect one year's base-line data, and then to manipulate the domiciliary sandfly populations (but not those in the crops) by house fumigation, and -- in a longitudinal active search study -- identify whether any reduction in incidence ensues.

The relative risk of intra-domiciliary infection versus the risk of infection associated with agricultural activities may well be age-related. A comparison of risk for different age groups could therefore provide clues to the relative importance of these two potential sites of transmission. Figures 8, 9, and 10 show the cumulative percentage of people infected in different age groups in four villages of Purísima valley (Ancash

FIGURE 8: Percentage of infected people in four villages of Purisima Valley

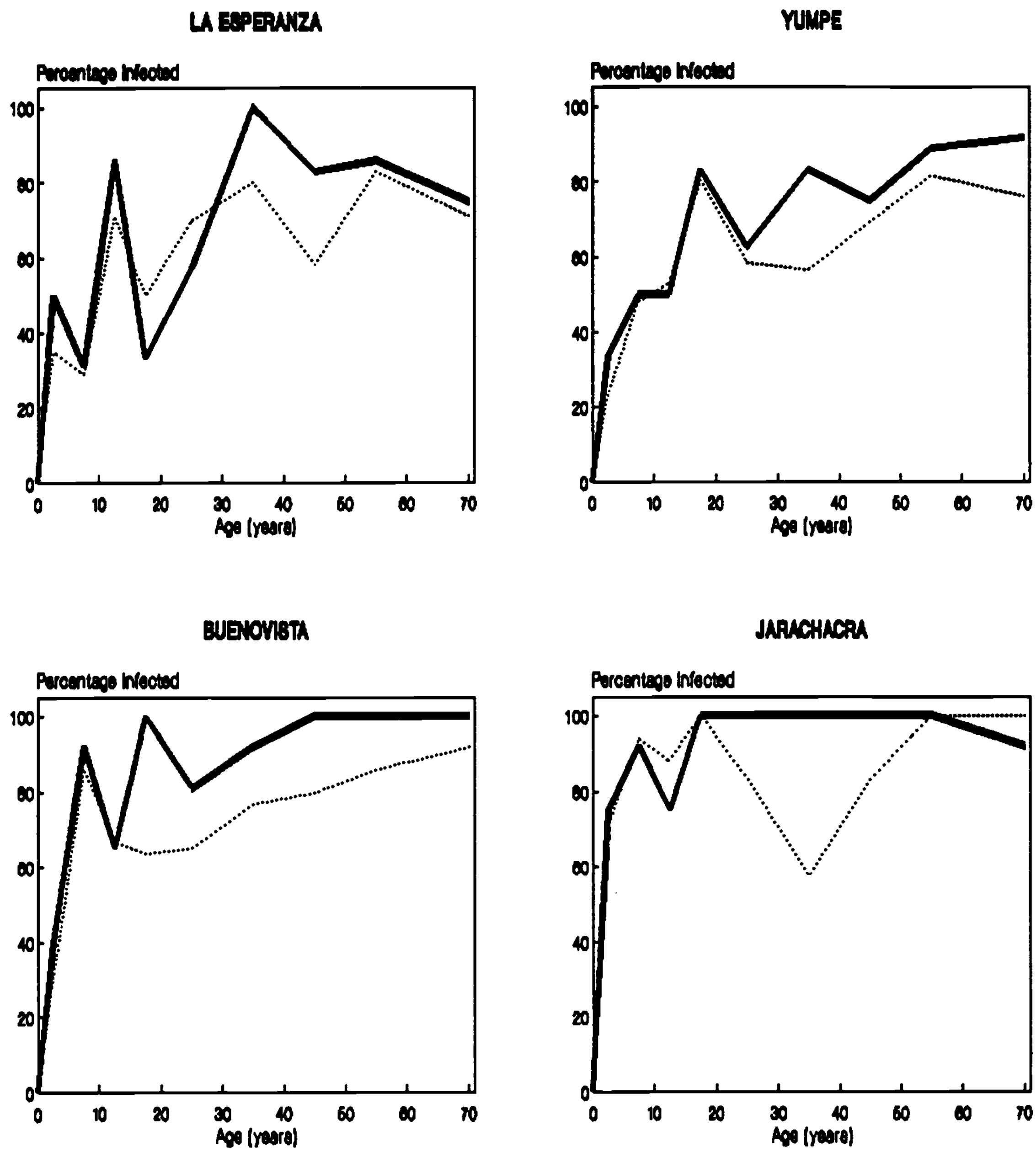


FIGURE 9: Percentage of infected people in four villages of Lurin Valley

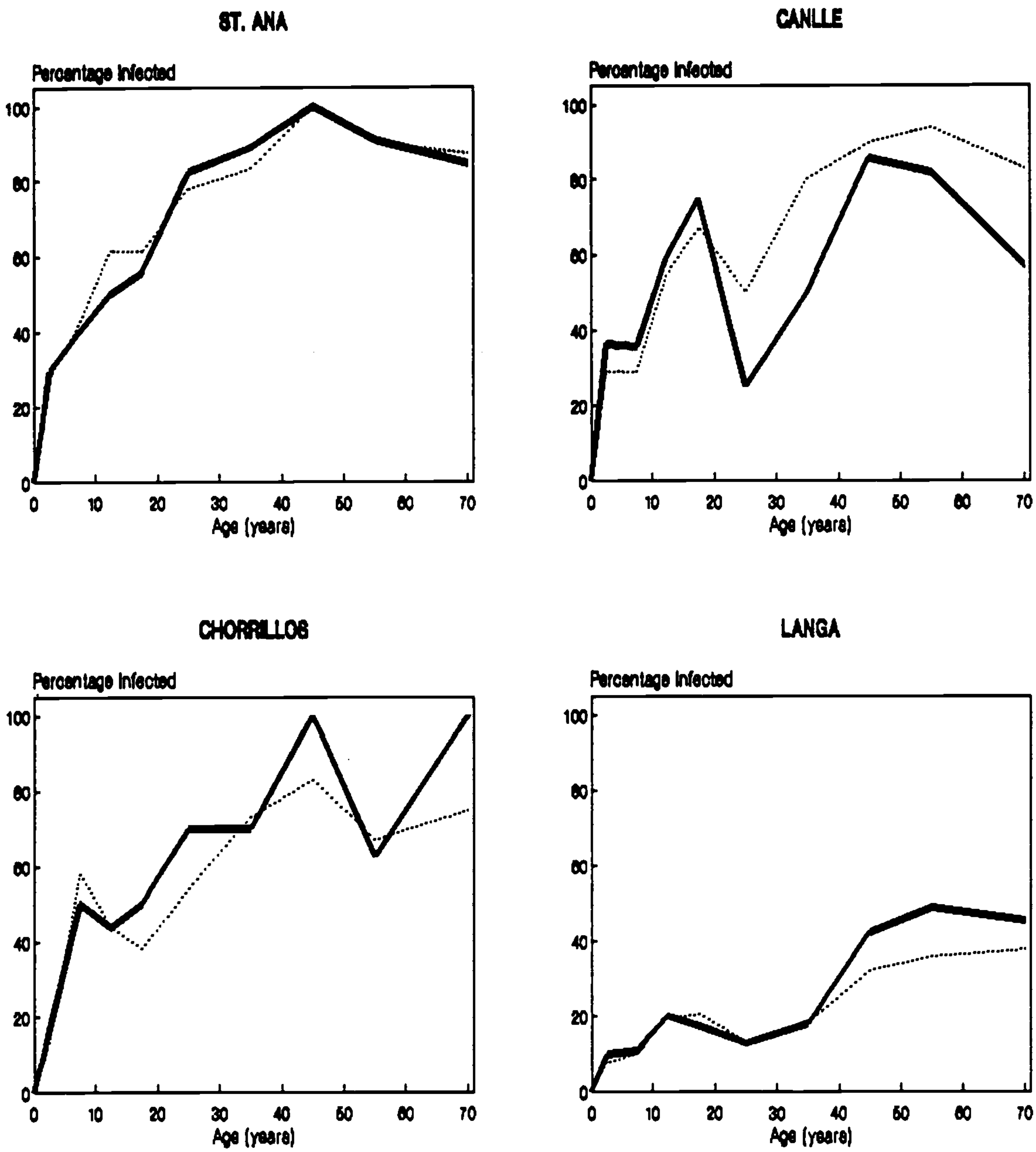
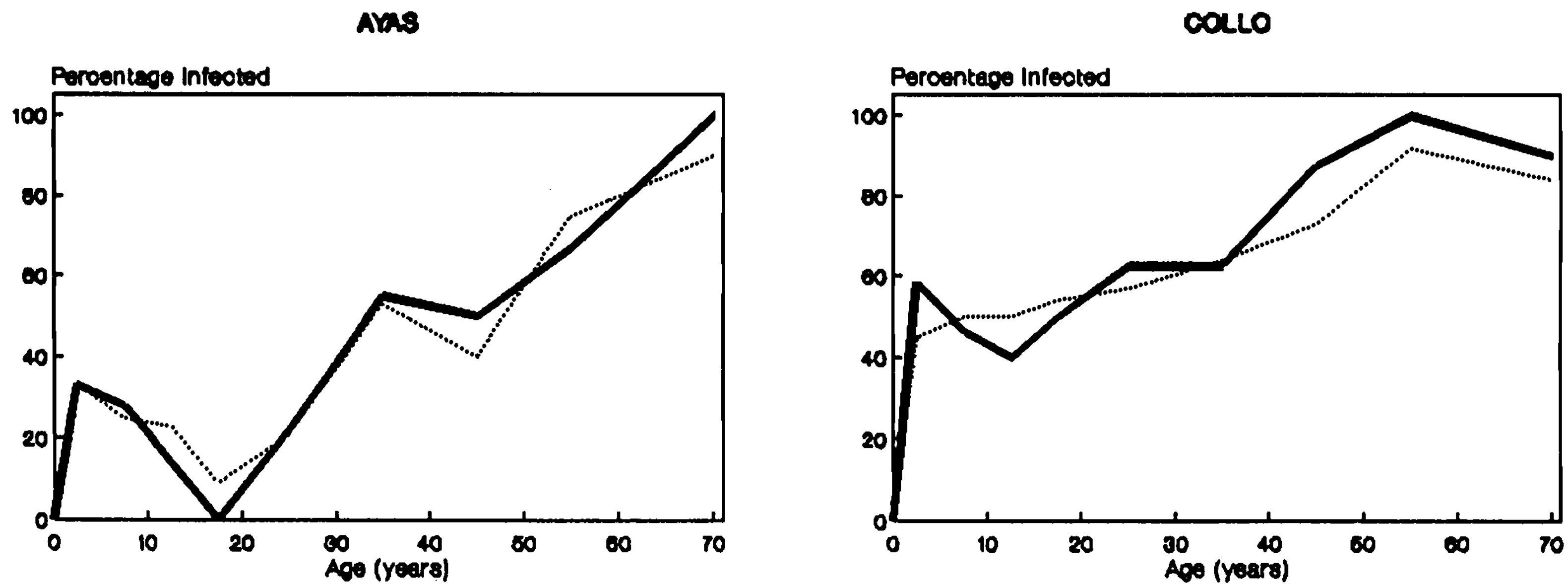


FIGURE 10: Percentage of infected people in two villages
(Rimac Valley and Canta Valley)



department), four in Lurín (Lima department) and two in Rimac Valley (Lima department). Present or past infections were identified in a cross-sectional house-to-house survey of the population by Montenegro test (solid line), as well as clinical signs -- characteristic scars or lesions (dotted line). Infections with L. (V.) peruviana lead to long-term protection against further infections, as illustrated by the relative rarity of second episodes of Uta in patients whose lesions have healed (i.e with the characteristic scar). Thus, the villages with the highest infection rates (as measured by the cumulative prevalence for the whole population -- the percentage Montenegro positive), should have the lowest average age for cases. This can be visualised by comparing the graphs for Jarachacra, with the highest infection rate and a very high proportion of cases in children less than five, and Langa, with the lowest infection rate and the majority of cases in adults.

This is the pattern one would predict if the risk for susceptible (Montenegro negative) children and susceptible adults was the same. But the epidemiological patterns derived from the various villages in the study indicates significant deviations from any simple relationship between transmission rate and the proportion of cases in children. This can best be illustrated in Figure 11, which plots mean annual incidence in susceptible (Montenegro negative) adults against the mean annual incidence (from 1989-1991) in susceptible children (under 15 years) for 16 endemic villages. Points near the x-axis represent villages where the infection rate in susceptible children is very much greater than that in susceptible adults; whilst points close to the y-axis represent the reverse. Points close to the dotted line represent sites with no change in incidence with age. The wide dispersion of points suggest the existence of different patterns of transmission, even for villages in the same valley. In some cases, such as Licahuasi, Canye and La Esperanza, the risk in susceptible children is eight times that in susceptible adults; whereas in Huatiacaya (Lurin) the risk to adults is four times greater than in children. Between these two extremes there are several intermediate patterns. Thus, these data suggest: (i) different patterns of age-related risk of exposure for people in different villages and/or different valleys; and/or (ii) differences in the immune status of the populations living in different endemic areas.

A similar pattern was generated from the results of phase I (epidemiological, clinical and parasitological characteristics of Uta) in the Purísima valley. There, the incidence risk in persons without signs of disease (scar and/or active lesions) fell rapidly with age (Fig. 12). The inference that asymptomatic infections (or at least those with no detectable scar) are frequent in this valley, and that immunity is developed by adults (probably as a result of multiple repeated infections), was not supported by our recent studies. Some 70% of the populations of the villages mentioned above (Fig. 8, 9 and 10) have undergone Montenegro skin tests. Ninety percent of all Montenegro positive inhabitants have detectable scars or lesions, and there is no significant difference in the percentage for children less than 15 years old (92%) and adults (89%). Thus, the drop in incidence rate with age for the unscarred population is apparently not generated by an increase in their immune status (at least as determined by the Montenegro test).

FIGURE 11:

Age-related incidence in susceptibles
Mean annual incidence in children(<15)
and adults in 16 villages: 1989-1991

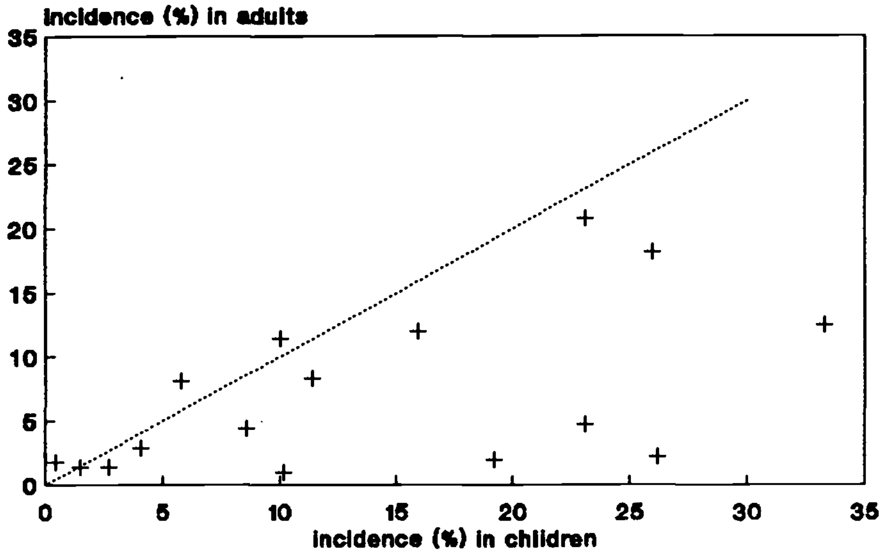
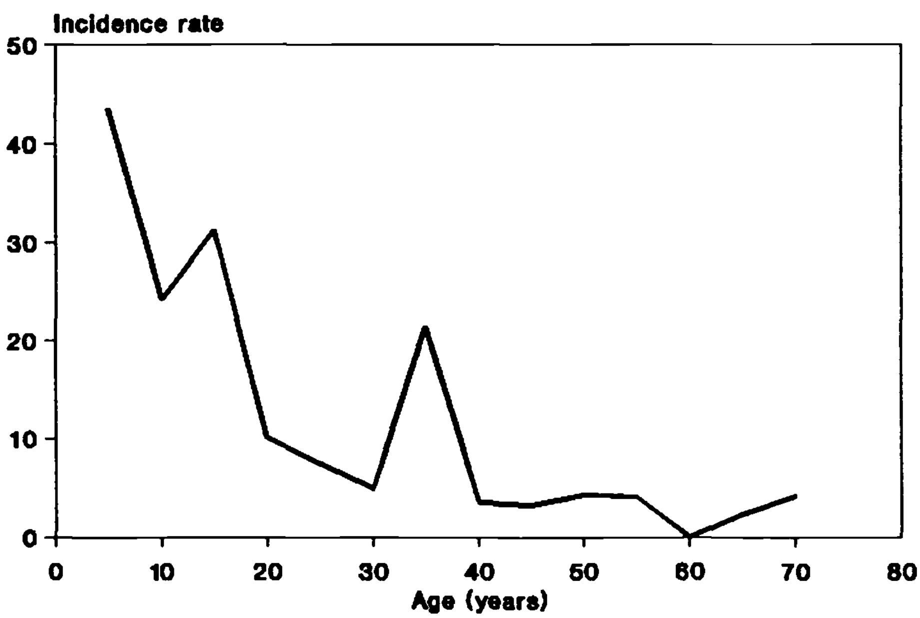


Fig. 11

FIG 12. INCIDENCE BY AGE
(Purisima valley, Oct 86 - Mar 89)



These results indicate that any decrease in incidence (in susceptibles) with age is probably due to a difference in risk (exposure) associated with age-related differences in human activity. In endemic villages or valleys where this is the case, there is a significant implication for disease control. Prevention of Uta for several years will undoubtedly increase the percentage of susceptible adults in the population, but if for some reason the control program was terminated, these susceptible adults would have a relatively low risk of infection (compared with what they would have experienced as children). Thus, the success of control programs in reducing the cumulative prevalence in the population should not be immediately reversed if they are suddenly terminated.

In Perú, during the last 40 years the control activities for leishmaniasis have focussed on DDT spraying campaigns. The field experiments carried out by Hertig (1942) and Herrer (1957) in the 1940s and 1950s demonstrated that DDT house spraying reduces the number of sandflies both inside the house and in resting places up to 30 meters from the sprayed house for up to one year. However, none of these studies carried out any controlled analysis of the effect of the spraying on disease incidence. Similarly, the experiences of MINSA were never evaluated. However, it is possible to examine the effect of DDT house spraying during the nationwide campaign to control malaria during the 1960s and first years of the 1970s. Leishmaniasis and malaria used to coexist in many of the Western Andean valleys. Figure 13 shows a dramatic drop in the incidence of Uta in Ancash department during the spraying campaign, followed by a rather slower resurgence once it stopped.

Similar patterns are being recognised in most of the villages that we are currently working in. Figure 14 illustrates the estimated mean annual incidence (in susceptibles) per decade calculated retrospectively from a cross-sectional survey of the populations of four villages in Lurin Valley, in which age, date of immigration (where relevant), disease status and age when infected for each inhabitant was recorded.

These results give a strong indication of the efficacy of DDT for reducing transmission related with human dwellings (inside and/or peridomestic). They also suggest a significant lag period after the spraying campaign was terminated before the infection rates returned to previous levels. However, unlike malaria, the spraying campaign clearly failed to eradicate leishmaniasis, possibly because the putative sylvatic transmission cycle of Uta was not affected by the reduction of sandfly populations associated with human habitations.

Nothing is known with any certainty concerning a sylvatic transmission cycle involving wild reservoir hosts. Although reservoirs have been studied in Perú since 1927 (Weiss unpublished data, referred in Herrer 1955), effort has concentrated on domestic animals (mainly dogs), possibly because dogs were the first animals found infected with Leishmania (amastigotes in smear) in the New World (Pedroso 1913), and because (jointly with bartonellosis) transmission was accepted as peridomestic (Herrer 1951a, 1957a; Lumbreras 1985).

FIGURE 13:

INCIDENCE RATE IN ANCASH (1959-1989)

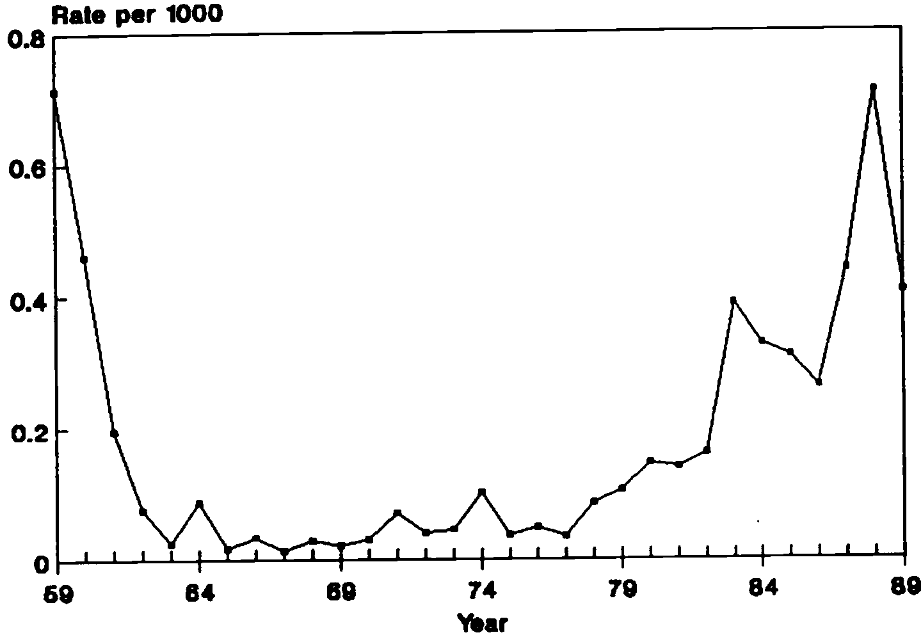
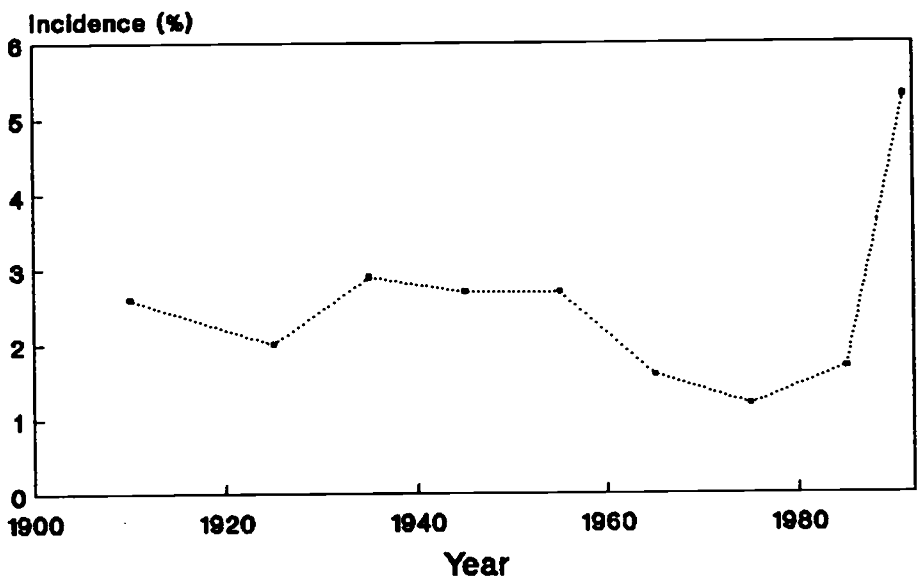


FIG.14. Change in incidence per decade Mean annual incidence in unscarred population in Lurin valley (LIMA)



Herrer & Battistini (1951), through experimental infection of dogs and foxes with promastigotes isolated from humans, demonstrated that although the majority of these animals did not develop ulcers, amastigotes could be detected in inconspicuous lesions. Based on this experience, Herrer carried out a field study (Herrer 1951b), in which he demonstrated amastigotes in stained skin smears (usually nasal) in 46 out of 165 dogs. No infections were found in 78 cats, 22 donkeys, 7 pigs or one horse. In a later study Herrer (1955) calculated a canine incidence of 29% in Huarochiri province, but his protocol for dog selection was not recorded. At that time a number of wild rodents and opossums were trapped and examined by the same method, but no parasites were demonstrated. Unfortunately, the descriptions of the methodology in Herrer's studies have frequently been incomplete making interpretation of his data awkward.

Recently, Llanos-Cuentas et al. (manuscript in preparation) studied the possible wild and domestic reservoirs in Purísima valley. The fauna was restricted: only three genera and eight species were found in monthly captures during 24 months. From 471 wild animals tested for Leishmania infection, flagellates were observed in 56. Of these strains, only three were identified as Leishmania (all L.(V.) peruviana) -- one from Didelphis albiventris and two from Phyllotis andinum. Of 867 domestic animals (643 dogs, 198 donkeys, 21 mules and 5 horses) examined for lesions compatible with Leishmania, all were negative except one dog; and sequential parasitological examinations of the dog were all negative. Serological evidence of Leishmania infection was demonstrated by DOT-ELISA in eleven of 51 (21%) sera collected from dogs in the same valley (Guevara et al. Personal communication). Recently, Leishmania parasites have been isolated from three dogs in Purísima valley, and one dog from Canta valley (Lima department). We are now evaluating the potential role of dogs as reservoirs by transmission experiments. Despite our parasite isolations from D. albiventris and P. andinum, the role of sylvatic reservoirs remains largely unexplored.

The aim of Phase II is to develop an appropriate control strategy for Uta using the information generated through our studies.

The studies proposed below for Phase II, will develop and apply an appropriate control strategy for Andean Cutaneous Leishmaniasis in several endemic areas (in the departments of Lima and Ancash). In each of two valleys, disease incidence and a variety of entomological parameters are being recorded in four villages. In one village, all houses and peri-domestic walled structures (e.g. animal houses) will be fumigated with insecticide at strategic periods before the peak of vector activity (October and April). In a second village, as well as domiciliary fumigation, ravines and other sites that have been incriminated as resting places (probable foci of transmission) will also undergo focal spraying and will simultaneously be reforested. Reforestation with eucalyptus trees will be employed in order to change ecological features that may be necessary for the maintenance of Leishmania transmission. The third and fourth villages in each valley will act as controls -- one to experience reforestation (but not fumigation) and the other to be left completely unaltered during the course of the experiment. In all villages, we are providing health education on leishmaniasis (plus other important health concerns), early diagnosis of lesions and free treatment. Forestation and fumigation activities will be conducted (under supervision) by the community, in conjunction with village health promoters who are being trained by our team.

Acknowledgements: The authors thank Drs. C.M. Dye (LSHTM) and M. Campos (UPCH) for their advice; and the researchers, field-workers and laboratory technicians in the Working Group of Leishmaniasis (IMTAvH).

Grant support: This work received financial support from the following institutions: the International Development Research Centre (IDRC, Canada); the Wellcome Trust; and the World Health Organisation.

REFERENCES

Chumbe, W.A., Ñavincopa, M., Dextre, J., Sipan, F., Chapiquillequen, F. and Arce, C. 1990. Dirección de Control de Malaria y otras enfermedades metaxénicas. Manuscript document.

Cruzado, L. 1987. Lutzomyia peruensis vector de Leishmania sp. en Pahash, Otuzco, Perú. Resúmenes de Trabajos científicos, 10° Congreso Latinoameriaco de Microbiología, Trujillo, 9:187.

Herrer, A. 1951a. Estudios sobre Leishmaniasis Tegumenatria en el Perú. VI. Relación entre Phlebotomus y leishmaniasis tegumentaria. Rev. Med. Exp. (Lima), 10:139-145.

Herrer, A. 1951b. Estudios sobre Leishmaniasis Tegumenatria en el Perú. V. Leishmaniasis natural en perros procedentes de localidades utógenas. Rev. Med. Exp. (Lima), 8:87-117.

Herrer, A. 1955. Consideraciones sobre el reservorio. Rev Viernes Med., 6(1):22-35.

Herrer, A. 1957. Phlebotomus y DDT en el Peru . Experiencias sobre control de la verruga y la uta. Rev. Med. Exp. (Lima), 10(2):99-138.

Herrer, A. 1982. Lutzomyia peruensis Shannon, 1929, posible vector natural de la uta (Leishmaniasis Tegumentaria). Rev Inst Med Trop Sâo Paulo, 24(3):168-172.

Herrer, A and Battistini, G.M. 1951. Estudios sobre Leishmaniasis Tegumenatria en el Perú. I. Infeción experimenatal de perro con cepas de leishmanias procedentes de casos de uta. Rev. Med. Exp. (Lima), 8:12-27.

Hertig, M. 1942. Phlebotomus and Carrion's Disease. Am. J. Trop. Med., 22 (Supply July):1-80.

López, M., Orrego, C., Cangalaya, M., Inga, R. and Arévalo, J. 1990. In vitro amplification of a kinetoplast DNA sequence to detect Leishmania parasites in biopsies. Biotechnolgy, 8:in press.

Lumbreras, H., Guerra, H. 1985. Leishmaniasis in Peru. In: Leishmaniasis, Chang, K.P. & Bray, R.S. (editors). Amsterdam: Elsevier Science Publishers BV, pp. 297-311.

Llanos-Cuentas, A. 1990. Epidemiology of Leishmaniasis in Perú. Rev. Brasil Med. Trop., 24(Supp 2):194-195.

Miranda, H. 1988. Andean Cutaneous Leishmaniasis in the Highlands of the Department of La Libertad in Peru. In: Research on Control Strategies for the Leishmaniasis, Walton BC, Wijeyaratne PM & Moddaber F (editors). Canada: IDRC. pp. 148-149.

Pessôa, S.B., Barretto, M.P. 1948. Leishmaniose Tegumentar Americana. Rio de Janeiro, Brasil: Ministerio da Educaçâo e Saude.

Pérez, E., Villaseca, P., Cáceres, A., López, M., Zolessi, A., Campos, M., Guerra, H. and Llanos-Cuentas, A. 1991. Leishmania (Viannia) peruviana isolated from the sandfly Lutzomyia peruensis (Diptera:Psychodidae) and a sentinel hamster in the Huayllacallan Valley, Ancash, Perú. Trans. R. Soc. Trop. Med., 85:60.

Townsend, C.H.T. 1913. The possible and probable etiology and transmission of verruga fever. J. Econ. Ent., 6:211-225.

Weiss, P. 1943. Epidemiología y clínica de las leishmaniasis tegumentarias en el Perú. Rev. Med. Exp. (Lima), 2(3):209-247.