Studies on New World Leishmaniasis and its Transmission, with Particular Reference to Ecuador

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Studies on New World Leishmaniasis and its Transmission, with Particular Reference to Ecuador

edited by

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CONTENTS

Foreword.....xiii

Preface.....xv

Members of the research team.....xvii

Acknowledgements.....xix

Chapter 1. Introduction.....3

Chapter 2. A brief review of leishmaniasis in Ecuador.....5

Chapter 3. An ecological view of leishmaniasis endemic areas in Ecuador.....33

Chapter 4. Parasitology.....44

1. *Leishmania* isolates from humans.....44

2. *Leishmania* isolates from wild and domestic mammals.....52

3. Serodyme typing of *Leishmania* isolates using monoclonal antibodies.....58

Chapter 5. Vector entomology.....70

1. Natural infections of sand flies with *Leishmania* promastigotes.....70

2. A laboratory trial of sand fly rearing in Ecuador.....79

Chapter 6. Immunology.....95

1. Evaluation of skin test and ELISA in the screening of leishmaniasis.....95

2. Examination of reservoir hosts by counter immuno-electrophoresis.....108

Chapter 7. Epidemiology.....116

1. Andean leishmaniasis in Ecuador.....116

2. Bacterial flora isolated from two types of leishmanial ulcers in Ecuador.....132

3. Parasitologically-proven retrospective cases diagnosed in INHMT.....140

Chapter 8. Summary.....160

Chapter 9. Appendix (Abstract of related papers published)...165

1. An epidemiological study of leishmaniasis in a plantation "Cooperativa 23 de Febrero" newly established in Ecuador

(Jpn J Parasitol, 33, 393-401, 1984)

2. Natural infections with promastigotes in man-biting species of sand flies in leishmaniasis endemic areas of Ecuador

(Am J Trop Med & Hyg, 34, 440-446, 1985)

3. Biting activity of two anthropophilic species of sandflies, Lutzomyia, in an endemic area of leishmaniasis in Ecuador

(Ann Trop Med & Parasitol, 79, 533-538, 1985)

4. Leishmania isolated from wild mammals caught in endemic areas of leishmaniasis in Ecuador

(Trans Roy Soc Trop Med & Hyg, 79, 120-121, 1985)

5. A review of leishmaniasis in the New World with special reference to its transmission mode and epidemiology

(Jpn J Trop Med & Hyg, 13, 205-243, 1985)

6. Leishmaniasis in different altitudes on Andean slope of Ecuador

(Jpn J Trop Med & Hyg, 15, 7-15, 1987)

7. The relationship between severity of ulcerated lesions and immune responses in the early stage of cutaneous leishmaniasis in Ecuador

(Ann Trop Med & Parasitol, 81 (5), 1987)

Explanations for the Plates

Plate 1. Front pictures of the Instituto Nacional de Higiene y Medicina Tropical "Leopoldo Izquieta Perez", Guayaquil city, Ecuador. Our laboratory work was mainly done in this institute. Above: upper floar, Below: entrance and lower floar; note a bust of Doctor Leopoldo Izquieta Perez (left) and a profile of Doctor Hideyo Noguchi (right).

Plate 2. Pictures of areas endemic for leishmaniasis in Ecuador. A and B: highland areas in the Andes. A: central parts and outskirts of the Canton Paute, Department of Azuay, B: countryside around the housing area near the Canton, C: lowland leishmaniasis-endemic area, the Canton Pompeia, Department of Los Rios, in the Pacific coastal region; site of a preliminary survey by two of our research team (V.V.C. & M.K.).

Plate 3. Active leishmaniasis lesions. A: an ulcer found on the right wrist of a male (21 years of age), B: a typical crater shaped ulcer on the right forearm of a girl (9 years), C: an ulcer on the left forearm of a male (19 years) with other 4 ulcers (not shown) on his left elbow; note a pronounced lymphadenitis (arrows), D: active lesions on the ear of a male (19 years), E: mucocutaneous lesions on the nasopharynx of a female (64 years).

Plate 4. Active (A-C) and cured (D) leishmaniasis lesions. A: complete destruction of the external ear of a male (31 years), B: partial destruction of the nasal septum of a male (52 years), C: multiple cutaneous ulcers (arrows) of a male (24 years), D: cured lesions (scars) on the face of a boy (12 years) and a girl (11 years).

Plate 5. Pictures of field research activities. A: research members (E.G.L.A. & R.S.) riding horses to reach sand fly collection sites at the Andean leishmaniasis endemic area, B: sand fly collection using human bait (volunteer) by two collectors (Y.H. & R.S.) during the night at the Andean slope, C: sand fly dissections (E.G.L.A., M.L. & R.S.) in a relatively well-provisioned field laboratory supplied by Dr. Napoleon Urdillares at Ocaña, Department of Cañar.

Plate 6. Morphological features of Leishmania and the lesion development in hamsters. A: promastigotes stained with Wright's staining solution, B: Giemsa stained amastigotes (arrows), C: rosette formations of promastigotes in the posterior triangle of a naturally-infected sand fly. D: a nodular lesion (arrow) developed on the nose of a hamster injected with homogenized lesion materials from a patient. E: ulcer lesions (arrows) developed on the nose and left hind footpad of a hamster injected with culture materials of Leishmania; note the damaged footpad in comparison with the normal right one.

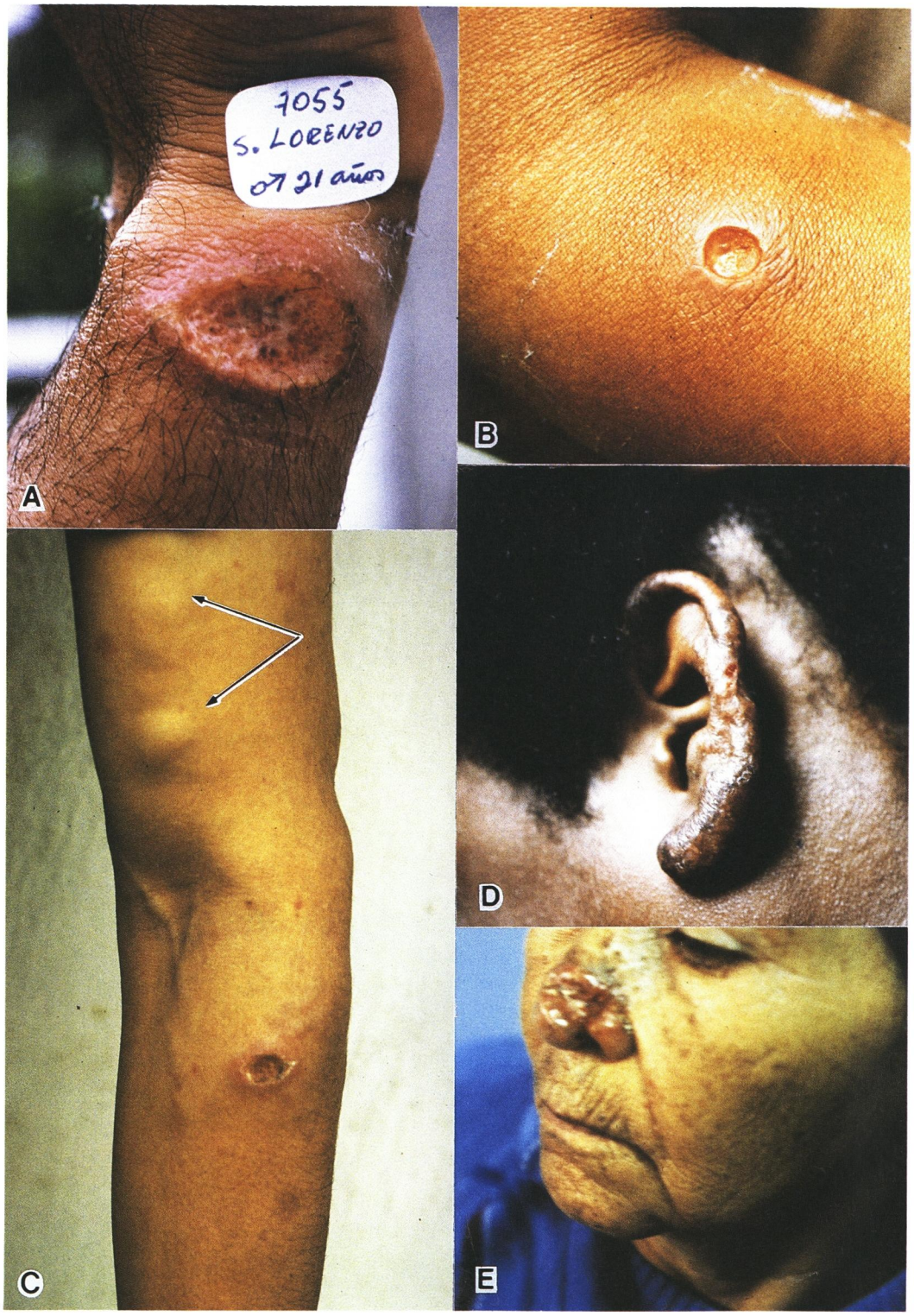
See illustrations pages, vi-xi

Plate 1


INSTITUTO
NACIONAL DE HIGIENE
LEOPOLDO IZQUIETA PEREZ
1941

Plate 2

Plate 3



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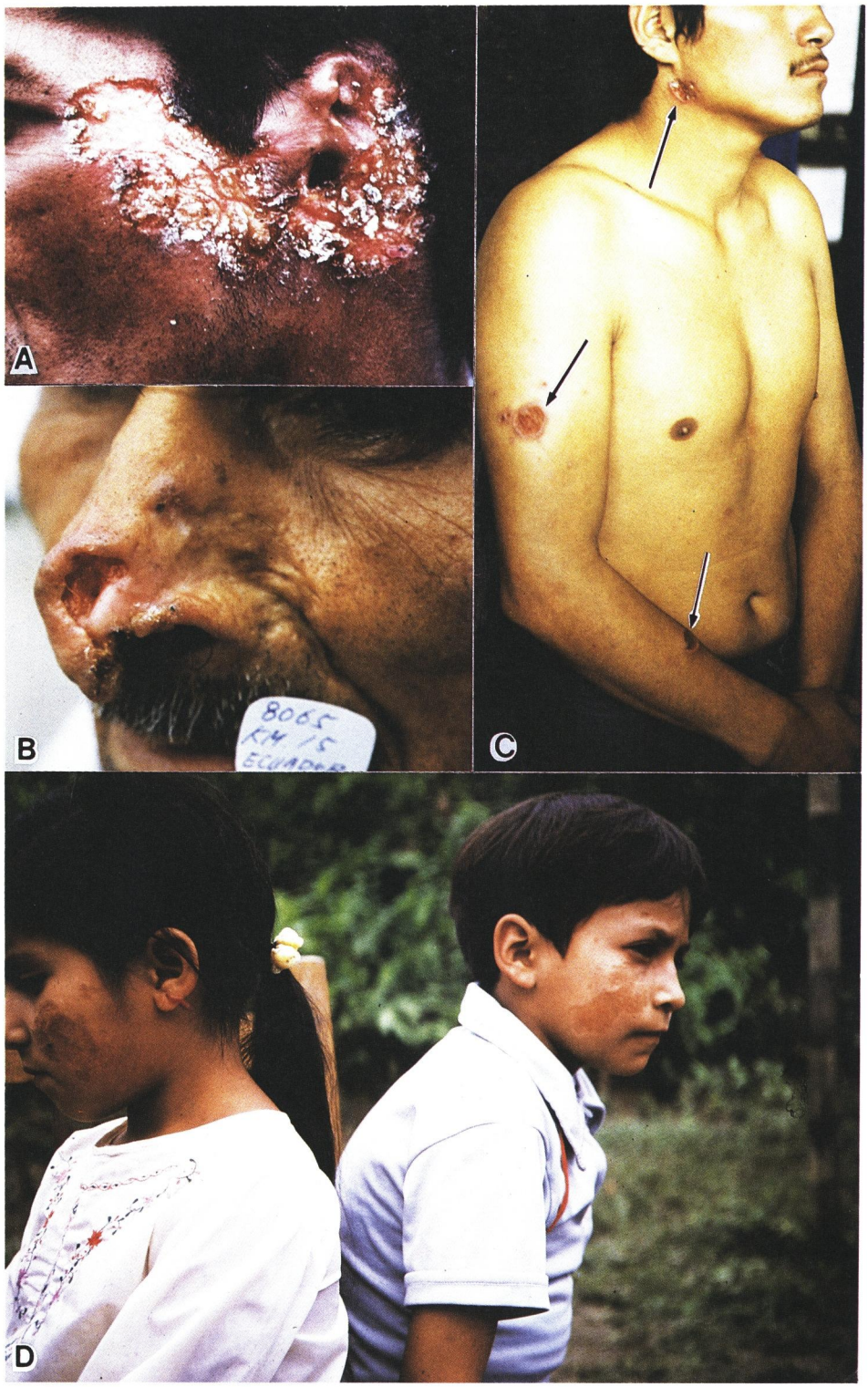
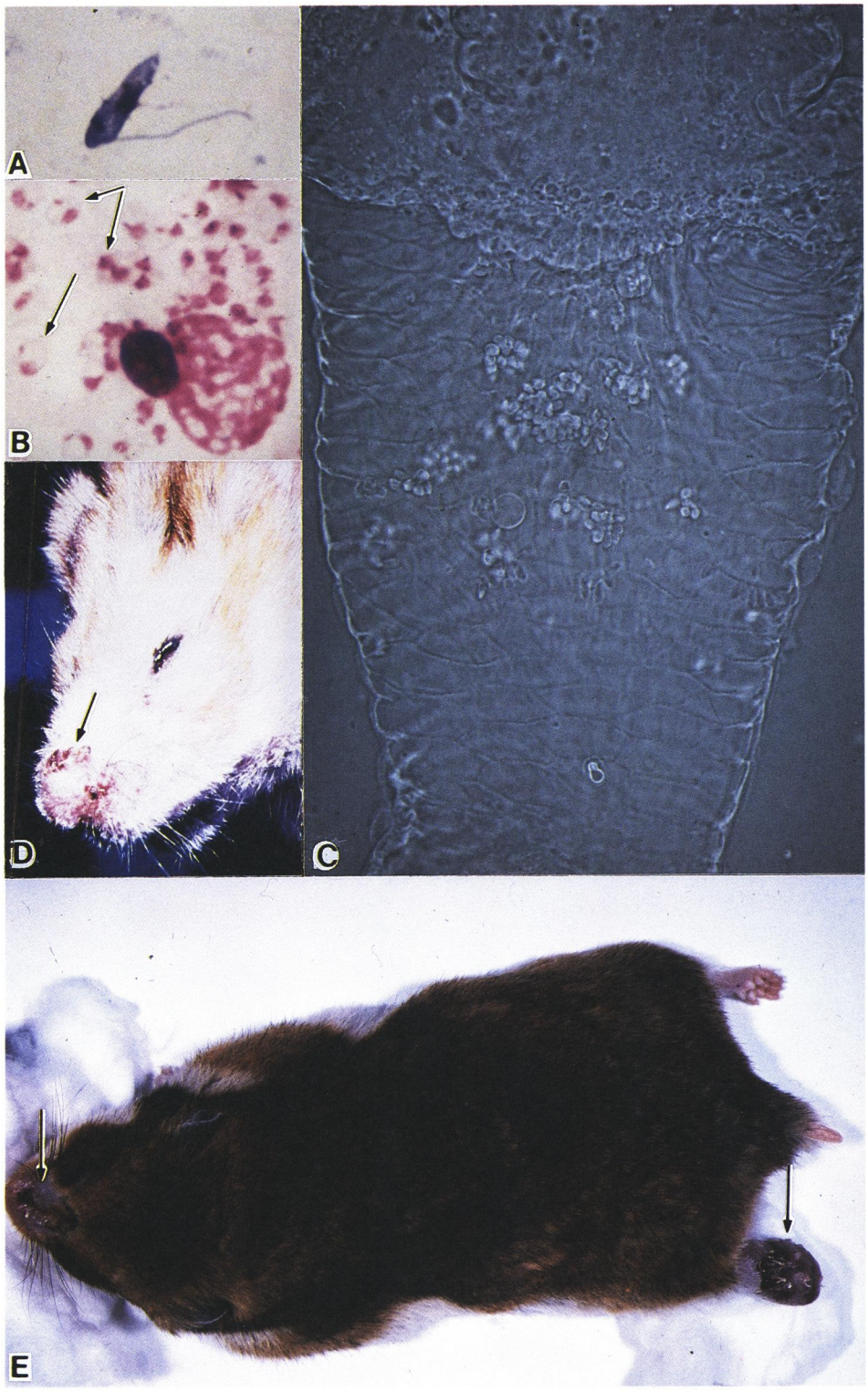


Plate 5

Plate 6



FOREWORD

I would like to take this opportunity to outline a brief history of our leishmaniasis research activity in Ecuador. In 1982, while I was performing my routine parasitological studies in the Departamento de Parasitologia of the Instituto Nacional de Higiene y Medicina Tropical, "Leopoldo Izquieta Perez", Guayaquil, Ecuador, I received a visit from Dr. Yoshihisa Hashiguchi, a Japanese researcher who had planned to study onchocerciasis in Ecuador. For a variety of reasons this was not possible and he therefore decided to initiate a program of research into leishmaniasis.

Little had previously been known on the transmission mode of leishmaniasis in Ecuador. Studying the epidemiology of this disease often involved field trips into the dense tropical forest, and I initially felt concerned for Dr. Hashiguchi's safety. This concern proved needless, as he proved to be a calm, unflappable field worker.

Thus began our investigation of leishmaniasis transmission in the densely forested endemic areas of Ecuador. Together with our capable assistant, Mr. Roberto Sud, we made several field trips into the rain forest, experiencing together the attendant hazards of heat, high humidity, difficult terrain and venomous snakes.

Our work revealed many features of leishmaniasis ecology in Ecuador, and much time was devoted to the study of the mammal reservoirs and sand fly vectors of the disease during 1982-1984.

In 1986, Dr. Hashiguchi and his research team came to Ecuador

once more, in order to continue leishmaniasis research. The results obtained will be summarized in the current report.

In 1987, he invited me to visit his country, and here I am, immersed in a pile of research papers with the results of our work; looking at them, I feel that perhaps, control of leishmaniasis in Ecuador is not too far from becoming a reality. When that day comes, I think that few people will realize the debt owed to the efffort of Dr. Hashiguchi. We in Ecuador salute his effort and look forward to continuing intensive leishmaniasis research with his collaboration in the future.

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At Nankoku City, Kochi, Japan:
10 September 1987

PREFACE

During the period 1982-1984, we made preliminary studies of the transmission of leishmaniasis in Ecuador, with the support of the Ministry of Public Health, Republic of Ecuador, and the Japan International Cooperation Agency (JICA). From initial research experience of leishmaniasis in Ecuador, we strongly felt that continued investigation was necessary, in order to accumulate epidemiological data for future control of the disease in that country.

In 1986 we were again able to perform a study of New World leishmaniasis and its transmission with particular reference to Ecuador, through the financial support of the Japanese Ministry of Education, Science and Culture.

In this report, original data collected on the survey are briefly summarized in each chapter. Some brief reviews and details of retrospective cases diagnosed in medical centers and institutions are also included for the convenience of domestic investigators and health officers in Ecuador. The generous collaboration of many persons in different institutions of the Ministry of Public Health, Republic of Ecuador, is gratefully acknowledged. Without their support and collaboration, accumulation of the information contained within this report would not have been possible.

It was not possible to include in this report all the data obtained from a field study made in Ecuador from July to September 1986. Much of the materials and data collected on the survey

have yet to be examined and analyzed. Results will be published in detail elsewhere at a later date, under the authorship of all research workers involved in the study.

A further cooperative study of leishmaniasis and its transmission in Ecuador will be performed in 1988, with the intention of further elucidating features of the epidemiology of this disease in the New World as a whole.

Yoshihisa Hashiguchi,

Representative of an Overseas Scientific Research Team funded by the Ministry of Education, Science & Culture, Japan

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We are extremely grateful to Dr. Philippe Desjeux, PDP, World Health Organization, Geneva, Switzerland (formerly Co-Director, IBBA, La Paz, Bolivia) for his kind supply of Leishmania reference strains, and to Dr. Alfredo Davila, Departamento de Bacteriologia, Instituto Nacional de Higiene y Medicina Tropical, Guayaquil, Ecuador for identification of bacterial organisms. Thanks are also due to Dr. C. E. Caceres M. (Director, Hospital Cantonal Paute, Azuay, Ecuador) for allowing us to review Andean leishmaniasis cases diagnosed in the hospital, and to Dr. Encalada, Hospital Centro de Salud, Cuenca, Ecuador and Dr. H. S. Mendes, Hospital Cantonal Paute for their invaluable information and support in this study. We are particularly indebted to our

staff for their devoted and efficient support: Srs. Roberto Sud and Miguel Leyton, Srta. Teresa Flor, and Sra. Morima Gonzalez.

Special thanks go to Dr. Manabu Sasa, President, Toyama Medical and Pharmaceutical University, Toyama, Japan, Dr. Rokuro Kano, President, Tokyo Medical and Dental University, Tokyo, Dr. Nakao Ishida, President, Tohoku University, Dr. Isao Tada, Professor, Kumamoto University Medical School, Kumamoto, Dr. Kinya Kawano, Professor, Nihon University School of Medicine, Tokyo, Dr. Yoshitsugu Ohsumi, Professor, Kochi Medical School, Nankoku, Dr. Kenjiro Kawashima, Professor, Kyushu University, Fukuoka, Dr. Takesumi Yoshimura, Professor, University of Occupational and Environmental Health, Japan, Kitakyushu, Dr. Shigeo Nonaka, Associate Professor, Nagasaki University School of Medicine, Nagasaki, Dr. Humio Osaki, Ex-Vice President, Kochi Medical School, Nankoku, Dr. Jyutaro Tawara, President, Kochi Medical School, Nankoku, Drs. Isamu Kitamura and Ken-ichi Ito, Vice Presidents, Kochi Medical School, Nankoku and Dr. Noriji Suzuki, Professor, Kochi Medical School, Nankoku, for the encouragement and support throughout the study.

Finally, we would like to show our sincere thanks to Dr. Napoleon Urdillares, Hospital de Troncal, Cañar, Ecuador, for his generous cooperation in the field phase of the present research, and to Dr. Takeshi Agatsuma, Kochi Medical School, Nankoku, Japan for the isoenzyme characterization. Editing assistance by Dr. J. Bruce Alexander, Florida University, Gainesville, U.S.A., is gratefully acknowledged.

Studies on New World Leishmaniasis and its Transmission, with Particular Reference to Ecuador

Chapter 1

INTRODUCTION

Leishmaniases are important and very widespread protozoan diseases, which continue to plague rural populations in the New World from the southern U.S. to northern Argentina. The disease is principally divided into three forms, i.e., cutaneous, mucocutaneous and visceral leishmaniasis, mainly based on the clinical manifestations in patients and on the species of the causative agents, Leishmania. Recent investigations in the field and in the laboratory have led us to realize that the disease is highly complex in all aspects, such as epidemiology, symptomatology, immunology, parasitology, vector entomology, and etc. Epidemiologically, new cases are reported from previously unidentified endemic areas involving unclassifiable Leishmania isolates and different modes of transmission. Intensive leishmaniasis research has been performed in several Central and South American countries, such as Belize, Panama, Venezuela and Brazil. Leishmaniasis has however been less well-studied in many other countries of the New World, particularly with regard to delimiting endemic areas or incriminating vectors and reservoirs of the disease.

In order to determine suitable future control measures against leishmaniasis in endemic areas of different New World countries, it is necessary to clarify the epidemiological characteristics of the diseases including their transmission modes, causative agents and clinical forms. Most leishmaniasis trans-

mission in the New World has been recorded from dense tropical rain forest and involves various species of Leishmania, sand flies and mammals. The difficulty of prophylaxis and control of New World leishmaniasis under these cconditions has frequently been pointed out by several investigators. At present, in the absence of a suitable vaccine and insufficient epidemiological data, it has been suggested that the only control measure for New World leishmaniasis is to remove all the inhabitants of communities from regions at risk of the disease, or to perform thorough deforestration around dwelling or working places. Such measures are impractical because of political, socioeconomic and logistic reasons (Marinkelle, 1980). In order to overcome these problems, more intensive and comprehensive studies of American cutaneous, muco-cutaneous and visceral leishmaniasis should be performed throughout the Neotropics. With this in mind, we initiated research on New World leishmaniasis and its transmission mode, especially in Ecuador. The present report briefly deals with the results obtained in leishmaniasis-epidemiological surveys carried out in different endemic areas.

Yoshihisa Hashiguchi

Reference

1. Marinkelle, C. J., 1980. The control of leishmaniasis. Bull. Wld. Hlth. Org., 58, 807-818.

Chapter 2

A BRIEF REVIEW OF LEISHMANIASIS IN ECUADOR

Abstract. The current state of knowledge on Ecuadorian leishmaniasis was briefly reviewed, largely from previous literature dealing with the disease in the country. The particular aspects reviewed were 1) historical aspects, 2) geographical distribution of cases, 3) disease occurrence in endemic areas, 4) clinical aspects, 5) transmission studies, 6) parasite isolations, 7) diagnosis of leishmaniasis in Ecuador and 8) its treatment in the country. Present knowledge of Ecuadorian leishmaniasis can be summarized as follows: 1) Major research activities and discoveries from 1920, when the first human case was reported in Ecuador, to the present, are cited in the text, following published papers. 2) Fourteen of the 20 Departments of the Republic of Ecuador, i.e., Esmeraldas, Pichincha, Bolivar, Manabi, Los Rios, Guayas, Cañar, Azuay, El Oro, Loja, Napo, Pastaza, Morona Santiago and Zamora Chinchipe, are endemic areas of the disease. 3) No registration system at the moment is available to provide statistical information. We therefore briefly compiled the clinically and/or parasitologically-diagnosed cases registered at some institutions of the Ministry of Health, over four years (1983-1986), in order to know the disease occurrence in each endemic area mentioned above. 4) In order to determine the clinical forms, cases appeared in the literatures were reviewed. Out of 260 cases reported, 239 (91.9%) were cutaneous (CL) forms, while 18 (6.9%) were muco-cutaneous (MCL) ones. Only one case each of visceral (VL) and diffuse cutaneous (DCL) forms was reported from 1920 to 1987. 5) Until 1982, studies on the transmission were limited to a few taxonomical studies of sand flies. A search for leishmanial infections in the vectors and reservoirs has since been initiated by the present workers. Current knowledge of the principal Ecuadorian man-biting sand fly species are reviewed in the text, including details on their distribution and incidence of leishmanial infections. Current information on natural infections of mammals (reservoirs) with *Leishmania* in Ecuador are also summarized. 6) Eleven *Leishmania* isolates, six from humans and five from wild mammals have been made to date from Ecuador. 7) Direct diagnosis using smear specimens has long been the principal method used in the country, although even this technique has not been employed in most Ecuadorian medical centers. A skin test using promastigote antigen has recently been utilized. 8) Antimonial drugs are regularly used in Ecuador for the treatment of leishmaniasis, as in other countries. Some Indian medicines have also been used as a traditional medication in the Amazonian regions.

Leishmaniasis was first reported in Ecuador, in 1920 by Valenzuela (Rodriguez, 1974); however it has remained one of the least studied of Ecuadorian tropical diseases until recently. For many years the main research activity on the disease has been involved with clinical diagnosis, and this produced some eventually confirmed case reports. No well-organized medical registration system for leishmaniasis is available in Ecuador at the moment. A variety of factors has contributed to this problem: first of all, leishmaniasis in Ecuador has always been a rural disease as in other South American countries. Therefore, patients are usually poorly-educated; some suffer benign infections which cure spontaneously, while others with longer more chronic infections go to rural doctors, who are unable to confirm the infection, mainly because of the lack of laboratory facilities, and can only make clinical diagnosis. Thus, many cases registered as leishmaniasis may be misdiagnoses of other diseases such as leprosy, anthrax, sporotrichosis, paracoccidiodomycosis, syphilis, bacterial abcess and dermal cancer. Some patients are sent to city laboratories, to have suspected leishmaniasis confirmed, and to be registered again; positive cases are then sent to hospitals which frequently lack antimonials, and registered for a third time. Thus the same individual can be registered two or three times in statistic compilations. Other patients never consult a doctor; instead they consult anyone with past experience of the disease about the medicine to be used, and buy it if available. Such cases will never appear in statistics. Therefore, as in many South and Central American countries, leishmaniasis

statistics in Ecuador do not closely reflect the actual incidence of the disease in the country, and only serve in identification of endemic foci, or show where accidental vector-human contacts have occurred.

Since 1920, many clinical cases have been diagnosed, and different clinical aspects have been discussed among the Ecuadorian medical community, but the transmission mode of leishmaniasis, and the identification of vectors and reservoirs of the disease remained unknown very recently.

Historical Aspects

It is at present unclear whether leishmaniasis evolved independently as a zoonosis in the Old and New World. In the past, the continents were linked and it is therefore difficult to hypothesize on the origin of the disease. It is however known that the original parasite has diverged, adapting to different vectors and reservoirs in each continent.

According to Ala-Vedra (1952) who worked with Ecuadorian ceramics in relation to leishmaniasis, the disease has existed in Ecuador for hundreds or perhaps thousands of years before the arrival of white men; some Precolumbian ceramics show typical leishmanial lesions, suggesting that the disease was very prevalent in that era. Ceramics from other South American countries, such as Colombia and Peru, also suggest that leishmaniasis was widespread in the north-western South America (Ala-Vedra, 1952; Werner and Barreto, 1981).

Leishmaniasis research in Ecuador did not go farther than

investigation of clinical and therapeutic aspects, until 1982 when serious transmission studies of leishmaniasis began (Hashiguchi et al., 1985a, b, c). Important events in history of leishmaniasis research in Ecuador are listed below.

1920 Valenzuela described the first recorded case, of a female patient with leishmanial ulcers on the forearm and thorax (Rodriguez, 1974).

1924 Heinert reported the first case of muco-cutaneous leishmaniasis in one of his patients at the general hospital in Guayaquil (Heinert, 1924).

1928 Valenzuela reported a case of muco-cutaneous leishmaniasis with osteoperiostitis, based on some x-ray films which he had taken on the patient (Valenzuela, 1928). This is the only record of this complication in a leishmaniasis patient.

1931 Trujillo reported a case of visceral leishmaniasis. The patient also had a single ulcer on his leg, from which no amastigote forms were isolated (Trujillo, 1931). This was the first report of visceral leishmaniasis in Ecuador, although it apparently represented an incorrect diagnosis. Valenzuela reported a new type of muco-cutaneous leishmaniasis causing laryngeal ulcers, although no parasites were observed in smear specimens (Valenzuela, 1931).

1945 Carrera reported the first case of leishmaniasis from the Amazon region of Ecuador (Carrera, 1945).

1949 Leon demonstrated the first case of a 3-year-old boy with visceral leishmaniasis from the Department of Esmeraldas. In the patient, hepatic and splenic biopsies were reported

positive. However, there have been no more cases of visceral leishmaniasis in the area and other areas of Ecuador to date (Rodriguez, 1974).

1950 Rodriguez started the first taxonomic studies on Ecuadorian sand flies. He described a new species, Phlebotomus camposi (Rodriguez, 1950).

1952 Ala-Vedra mentioned Precolumbian cases of the disease, based on ceramic evidence. He also presented and listed clinical aspects of the disease and hypothesized on the transmission mechanisms, vectors and reservoirs of the zoonosis. Several chemotherapeutic treatments against Ecuadorian leishmaniasis were first considered and compared in his text (Ala-Vedra, 1952). Rodriguez made a review of knowledge on sand fly taxonomy, especially on P. camposi (Rodriguez, 1952, 1953a).

1953 Rodriguez reported his observation of P. dysponetus in copula in Ecuador (Rodriguez, 1953b). Rodriguez and Aviles made a bibliographic review of all known leishmanial cases in Ecuador, adding 29 cases that they themselves had diagnosed. They evaluated Ecuadorian leishmaniasis research done on the parasite, clinical aspects, vector taxonomy and histopathological diagnosis. They did not believe that the ceramic pieces were enough to suggest Precolumbian existence of leishmaniasis, emphasizing that the involvement of indigenous American mammals as leishmanial reservoirs was a better argument (Rodriguez and Aviles, 1953). Rodriguez described a new sand fly species named P. leopoldoi; this species is still considered valid as Brumptomyia leopoldoi (Rodriguez, 1953c). Carrera reported seven cases of leish-

maniasis from Suscal, Guayeturo and Cochancay, Department of Cañar, 800 m to 1,000 m above sea level on the Andean slope. All the smear specimens from their ulcerous lesions were positive for Leishmania amastigotes. These cases were the first time reported from the Andean slope of Ecuador. Carrera also suspected but not incriminated probable vectors and reservoirs from where his patients came, after observing the ecological conditions. He also made some recommendations for epidemiological surveillance and future control of the disease in Ecuador (Carrera, 1953).

1954 Leon published analysis on the probable causative or predisposing factors of mucosal lesions of American leishmaniases, and on the probable mechanisms of dissemination from skin to mucous membrane. He also discussed the general clinical aspects of otic, rhinal, bucal, pharyngo-laryngeal and ophthalmic (palpebral) leishmaniasis found in the New World (Leon, 1954).

1956 Rodriguez described a new species of sand fly, P. guayasi, and included a modified check list of Ecuadorian sand flies (Rodriguez, 1956). This species, however, was determined later to be a synonym of Lutzomyia serrana.

1960 Arzube recorded P. sallesi and P. cayannensis cayannensis for the first time in Ecuador and added these two species to Ecuadorian sand fly list (Arzube, 1960).

1961 Zerega described the first case of diffuse cutaneous leishmaniasis (DCL) in Ecuador. The patient was thoroughly studied, including clinical, parasitological, histopathological and immunological aspects (Zerega, 1961). This type of leishman-

iasis has not been recorded in Ecuador since.

1962 Arzube published a tentative plan of investigation of leishmaniasis in the Department of Esmeraldas, Ecuador. He made vector and human case surveys in different areas of the Department, and concluded that control should involve poisoning of wild animal reservoirs living in their burrows (Arzube, 1962).

1967 Leon made a brief review on the tegumentary forms of the leishmaniasis of children, based on the cases reported from different areas of Ecuador (Leon, 1967).

1969 Rodriguez reported a new focus of leishmaniasis in Los Bancos, Department of Pichincha, 1,150 m above sea level. He also made a brief survey of the sand fly fauna, and made some recommendations for control of the disease (Rodriguez, 1969).

1975 Leon modified the classification of clinical forms of American tegumentary leishmaniasis, based on personal experience and the published literature (Leon, 1975).

1978 Tafur and De Tafur devised a therapeutic assay for tegumentary leishmaniasis, using metronidazole, in the Department of Los Rios (Tafur and De Tafur, 1978). Preliminary results were good, but unfortunately treatment of their patients was not followed up for enough time to establish whether permanent cures were made.

1979 Leon and Leon published an epidemiological evaluation of nasal muco-cutaneous leishmaniasis; they presented information on the diverse clinical aspects of this form of the disease, and made recommendations for its treatment (Leon and Leon, 1979).

1981 Calero and De Coronel carried out an epidemiological

study of leishmaniasis in a village on the Andean slope where the disease was epidemic (Calero and De Coronel, 1981).

1982 Amunarriz made a careful study of human leishmaniasis cases in the Amazon region of Ecuador. He studied the clinical forms and different treatments of the disease, making follow-up studies of two years or more (Amunarriz, 1982). The present workers initiated research on the transmission of leishmaniasis in Ecuador. This was the first attempt to determine the vectors and reservoirs in leishmaniasis-endemic areas of the country. The main purpose of our research was to establish some pilot endemic areas for studies on leishmaniasis transmission with special reference to the vectors and reservoirs.

1984 Amunarriz published an abstract of his investigations on leishmaniasis in the Amazon region of Ecuador, with special reference to treatment of the patients; he followed up cases for a long period after treatment, lending credibility to his conclusions (Amunarriz, 1984). Hashiguchi et al. (1984) published the results of an epidemiological survey on leishmaniasis performed in September 1982 in "Cooperativa 23 de Febrero", a newly established plantation in the Andean region of Ecuador. The results obtained indicated that leishmaniasis transmission had been occurring in a wide range of working and housing areas in the plantation. Young and Rogers (1984) gave a checklist of 49 species and subspecies of sand flies found in Ecuador, with additional comments on some species. In their text, three closely related and anthropophilic sand flies, Lu. amazonensis, Lu. davisi and Lu. claustrei, all of which occurred in many parts

of the Amazon Basin, were keyed and illustrated.

1985 Hashiguchi et al. published the results of studies done from 1982 to 1984, with special reference to the vectors and reservoirs. Among six anthropophilic species of sand flies examined, two species, Lu. trapidoi and Lu. hartmanni, were for the first time incriminated as probable vectors of leishmaniasis in Ecuador. Furthermore, three probable reservoirs of the disease were identified, after the examination of a considerable number of wild mammals. These naturally-infected animals were the sloth Choloepus hoffmani dydactilus, the squirrel Sciurus granatensis and the kinkajou Potos flavus. Some ecological studies of the vector sand flies, such as biting behavior and cycle of activity, were also performed in leishmaniasis-endemic areas and related to climatic conditions. Vertical distibutions of sand flies were also investigated at different altitudes from 350 m to 2,000 m above sea level, along the road from Cochancay to Cuenca in Ocaña, Department of Cañar, Ecuador (Hashiguchi et al., 1985a, b, c).

1986 Calero et al. reported two cases of muco-cutaneous leishmaniasis from the Amazon region of Ecuador (Calero et al., 1986). Ferreti et al. (1986) demonstrated a case of ganglionar leishmaniasis; this was thoroughly investigated and confirmed.

1987 Hashiguchi et al. reported the results of an epidemiological survey of leishmaniasis in different altitudes of the endemic areas on the Andean slope of Ecuador. These results suggested that the intensity of transmission was markedly influenced by the altitudes of human dwelling sites, as measured

by natural infection rates of sand flies with Leishmnania promastigotes at each site studied. Mimori et al. (1987) examined the relationship between severity of ulcerated lesions and immune responses in the early stage of cutaneous leishmaniasis in Ecuador, and suggested that this related to the activation of both the humoral and cell-mediated immune systems.

Thus from 1920 to 1981 Ecuadorian leishmaniasis research only involved clinical case studies, mainly in city hospitals. Since 1982 studies have been made in leishmaniasis-endemic areas, involving the collection of data and materials for analysis; these materials are at present being subjected to isoenzyme analysis, monoclonal antibody binding and k-DNA probe examinations, experimental infection and other laboratory studies.

Geographical Distribution of Cases

Leishmaniasis probably exists as a zoonosis in most parts of the tropical and subtropical humid forest of Ecuador. Based on analysis of the data registered in the National Institute of Health and Tropical Medicine, Guayaquil (see Chapter 7-3 in this text), and also on our epidemiological surveys in the country over several years, it appears that there is a principal endemic area which traverses Ecuador from north to south, forming a wide belt along the west Andean slopes. The disease is also endemic on the Pacific coast and in the Amazonian regions of Ecuador (Fig. 1). A new type of leishmaniasis was recently recorded from the Andean highlands (see Chapter 7-1). Fourteen of the 20 Departments of Ecuador lie within leishmaniasis-endemic areas, viz.,

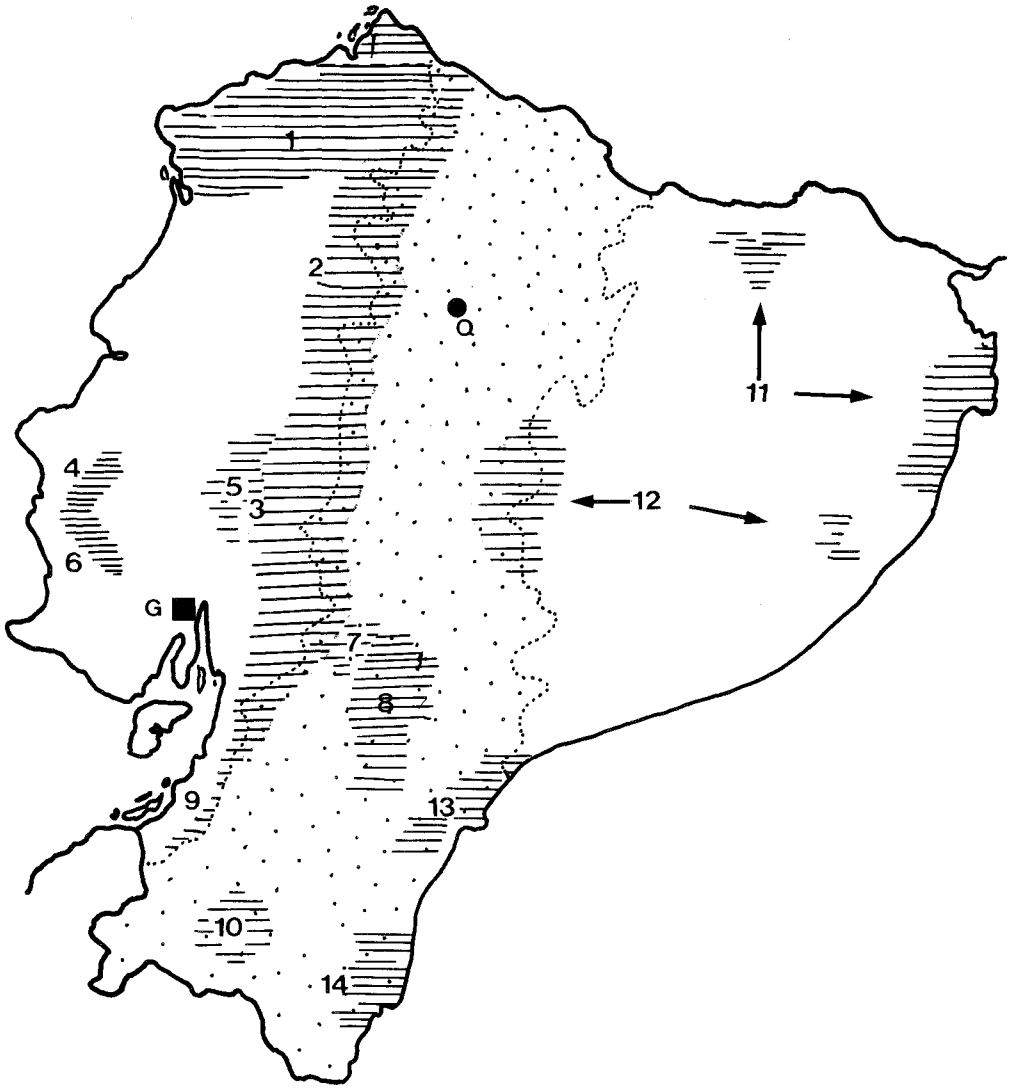

Figure 1. Map of the Republic of Ecuador, showing the geographical distribution of leishmaniasis (shaded areas) and the Andean highlands (dotted areas). The number indicates each Department of the endemic areas: 1, Esmeraldas; 2, Pichincha; 3, Bolivar; 4, Manabi; 5, Los Rios; 6, Guayas; 7, Cañar; 8, Azuay; 9, El Oro; 10, Loja; 11, Napo; 12, Pastaza; 13, Morona Santiago; 14, Zamora Chinchipe. Q shows Quito, the capital of Ecuador, and G, Guayaquil where Instituto Nacional de Higiene y Medicina Tropical (INHMT) and Subsecretary (II) of the Ministry of Health are located.

Esmeraldas, Pichincha, Bolivar, Manabi, Los Rios, Guayas, Cañar, Azuay, El Oro, Loja, Napo, Pastaza, Morona Santiago and Zamora Chinchipe.

Disease Occurrence in Endemic Areas

Because of the lack of an adequate system for registering an epidemiological information and total absence of a surveillance and control program, there is no statistical information on the prevalence and incidence of human leishmaniasis in Ecuador.

From 1920 to 1952, there were only a few reports of human cases, the disease being little-known in the country at that time. During the period from 1953 to 1987, most of studies were done on human cases coming for medical care to health centers and general hospitals, with a few epidemiological surveys made in endemic areas of the disease (Rodriguez and Aviles, 1953; Rodriguez, 1969; Calero and De Coronel, 1981; Amunarriz, 1982, 1984; Hashiguchi et al., 1984, 1987).

In Ecuador, certain national institutions, such as the Instituto Nacional de Higiene y Medicina Tropical (INHMT), have their own statistics of diagnosed human leishmaniasis cases, but most of the patients are sent there from hospitals or rural health centers to confirm the diagnosis. Thus, a patient is registered once in a hospital or health center and then registered in INHMT again. Moreover, if the patient is diagnosed at INHMT as positive for leishmaniasis, he is sent to a dermatological hospital for having chemotherapy, where he is registered again. Later, the Ministry of Health collects statistical information from

different sources under its charge. Many patients will therefore be probably registered two or more times.

For the above reasons, we have compiled a partial statistical record, in order to estimate the occurrence of leishmaniasis cases in each endemic area of the country. The cases included were clinically and/or parasitologically diagnosed, and then registered in the Departamento de Estadisticas, INHMT and the Epidemiology Division, Subsecretary (II) of the Ministry of Health, Guayaquil (Table 1). The majority of cases had occurred in the Departments, situated in the Pacific lowlands and western slope of the Andes, such as Esmeraldas, Pichincha, Manabi, Los Rios and Guayas.

Clinical Aspects

Clinical forms of the leishmaniasis in Ecuador are mainly limited to cutaneous (CL) and muco-cutaneous (MCL) lesions (Table 2). Only one case each of diffuse cutaneous (DCL) and visceral (VL) forms has been reported to date, both of these based on clinical diagnosis without confirming the parasite in smear specimens or cultures. There is therefore insufficient evidence for the existence of these two forms in Ecuador and more detailed investigations are required.

Cutaneous leishmaniasis (simple and multiple ulcers) is the most frequent form found in endemic areas of Ecuador. There have been relatively few confirmed cases of the MCL form, in spite of the fact that it is usually registered. Because of the severity of this disease form, almost all of MCL go to doctors for medical

Table 1. Human leishmaniasis cases registered in the Instituto Nacional de Higiene y Medicina Tropical and Epidemiology Division, Subsecretary (II) of the Ministry of Health, during the period from 1983 to 1986

Departments (Provinces)	Years				Total
	1983	1984	1985	1986	
Esmeraldas	220	270	295	307	1,092
Pichincha	150	110	210	215	685
Bolivar	-	-	-	24*	24
Manabi	210	272	253	-	735
Los Rios	12	8	391	73	484
Guayas	142	156	240	140	678**
Cañar	95	-	-	-	95**
Azuay	12	29	70	52	163
El Oro	1	4	-	-	5
Loja	5	22	-	1	28
Napo	7	5	12	17	41
Pastaza	7	11	9	6	33
Morona Santiago	11	-	-	1	12
Zamora Chinchipe	2	-	4	17	23
Total	874	887	1,484	853	4,098

* Our unpublished data.

** Including our own data in part (Hashiguchi et al., 1984, 1987).

Table 2. Leishmaniasis cases reported in principal Ecuadorian medical journals during the period from 1920 to 1987

Year	Total cases reported	Clinical form and sex of patients*										Reference no.
		CL			MCL			DCL		VL		
		M	?	F	M	?	F	M	F	M	F	
1920	2	1		1								36, 37
1922	1				1							19, 37
1925	1	1										37
1928	2	2										41, 37
1931	2	1			1							40, 37
1945	1	1										36, 37
1949	3	2								1		36, 37
1951	1			1								37
1952	14	9		4	1							1
1953	39	16	10	10	3							37, 10
1961	1							1				48
1969	28	16		10	2							35
1978	13		13									38
1981	10	5		5								7
1982	32		22			10						2, 3
1984	15	7		8								14
1987	95	32	31	32								18
Total	260	93	76	70	8	10		1		1		

* CL, cutaneous; MCL, mucocutaneous; DCL, diffuse cutaneous; VL, visceral; M, male; F, female; ?, sex was not mentioned.

treatment at same point in its development. The majority of MCL cases might therefore be registered once or more times in several medical care systems, such as those of rural or city hospitals and health centers.

The case reported as VL 38 years ago by Leon (Rodriguez, 1974), came from a CL-endemic area of Esmeraldas, Ecuador. However, no other such case has been reported to date from the area. The diagnosis was not confirmed by visceral biopsy and parasite isolation, and this infection might have been a common CL form manifesting a visceral phase in an immunologically-deficient patient.

The case reported as diffuse cutaneous leishmaniasis was thoroughly studied 26 years ago by Zerega (1961), who reported that the clinical, immunological and histopathological aspects indicated this form of the disease. Unfortunately, however, there was no information on the drug resistance usually found in DCL; furthermore, no parasite isolation was performed for the definitive diagnosis, though a biopsy specimen was positive for amastigotes. There have been no more probable DCL cases in Ecuador to date.

As shown in Table 2, out of 260 cases reported, 239 (91.9%) were CL forms, while 18 (6.9%) were MCL. As mentioned above, only one case each of VL and DCL was reported during the 67 years from 1920 to 1987.

Transmission Studies

Since the first diagnosed case of human leishmaniasis in

Ecuador, the study of this disease has concentrated on clinical and therapeutic aspects; reports of many cases presenting different clinical features of leishmaniasis were published. Unfortunately, however, these were not followed by field research in endemic areas. Therefore, much of the present knowledge of Ecuadorian leishmaniasis to date has remained largely speculative.

The first studies relating to transmission of leishmaniasis in Ecuador were done by Rodriguez during the period from 1950 to 1956 (Rodriguez, 1950, 1952, 1953a, b, c, 1956), and by Arzube (1960) and Young and Rogers (1984), all of whom made taxonomical studies on Ecuadorian sand flies. A total of 49 species and subspecies were recorded, including seven new species (Young and Rogers, 1984). In 1982, we began research to investigate the transmission mechanism of leishmaniasis in endemic areas of Ecuador, with special reference to the vectors and reservoirs; part of our data have already been published (see Appendix in this text; Hashiguchi et al., 1984, 1985a, b, c).

Vectors

In 1982-1984, a survey for Ecuadorian vectors of leishmaniasis was performed in different endemic areas; six man biting species of Lutzomyia were collected using human bait. From dissections of the sand flies collected, two species, Lu. trapidoi and Lu. hartmanni were found to be naturally-infected with Leishmania promastigotes. Four other species which have not yet been incriminated are Lu. panamensis, Lu. gomezi, Lu. shannoni and Lu. serrana; the first two of these have been incriminated as vectors of leishmaniasis in neighboring countries. With regard to the

two Ecuadorian species from which infections were recorded, Lu. trapidoi has been incriminated as a vector in other South American countries, but the findings from Lu. hartmanni represent the first time incrimination of that species as a vector of the New World leishmaniasis. Moreover, in our recent survey Lu. gomezi collected at Palenque, Department of Los Rios, was found positive for Leishmania promastigotes (see Chapter 5-1). Some of the sand fly species reported hitherto in Ecuador have been also incriminated as vectors of the disease in other countries, i.e., Lu. flaviscutellata, Lu. olmeca bicolor, Lu. ylephiletor and Lu. paraensis (Table 3).

All efforts were made to isolate the parasite from suspected vector sand flies. This has unfortunately not been possible as yet, largely because of the problem of contamination. Laboratory rearing of Lu. trapidoi was recently accomplished for the first time in Ecuador (see Chapter 5-2). Laboratory colonies of sand flies will permit us to carry out various experiments and observations, in relation to our studies of leishmaniasis transmission in the country.

Reservoirs

A survey for Ecuadorian leishmaniasis reservoirs was initiated in 1982, when the vector research was also done. Forty-eight wild mammals belonging to 12 species and 12 genera were caught in leishmaniasis-endemic areas , and examined for cutaneous lesions and then necropsied. Samples from the liver and spleen were homogenized and inoculated into special culture medium, and then examined for Leishmania promastigotes. Three

Table 3. Principal man-biting sand fly species reported in Ecuador, in relation to leishmaniasis transmission

Lutzomyia species	Locality recorded	Country where suspected or incriminated as vectors	*Leishmania* sp. transmitted in other countries	Ref. no.
Lu. gomezi	Esmeraldas Manabi Guayas Los Rios Azuay Pichincha Napo El Oro	Panama	*L.b. braziliensis*	11,15, 45
Lu. serrana	Guayas Napo Pichincha El Oro			15,45
Lu. shannoni	Guayas Manabi Napo Pichincha Los Rios			15,45
Lu. flavi-scutellata	Napo	Brazil Venezuela Trinidad	*L. m. amazonensis*	20,27, 39,45
Lu. o.bicolor	Los Rios Napo	Panama	*L. b. aristedesi*	12,45
Lu. trapido	Pichincha Los Rios El Oro Cañar* Esmeraldas Guayas	Panama Colombia Costa Rica	*L. b. panamensis*	11,15, 26,45, 46
Lu. yleph-iletor	Guayas	Panama Costa Rica	*L. b. panamensis*	11,45, 47
Lu. panamensis	Pichincha	Panama	*L. b. panamensis*	11,15, 45
Lu. paraensis	Paztaza	Brazil	*Leishmania* sp.	4,45
Lu. hartmanni	Cañar* Pichincha Esmeraldas Guayas El Oro Los Rios			15,45

* In the area, naturally infected sand flies with *Leishmania* promastigotes were found (Hashiguchi *et al.*, 1985a).

mammalian species, Choloepus hoffmani didactylus, Sciurus granatensis and Potos flavus tested were positive for the parasites, while others, Didelphis marsupialis, Tamandua tetradactyla, Sylvilagus braziliensis, Dasypus novemcinctus, Proechimys semispinosus (Rattus espinosus), Rattus rattus, Coendou bicolor, Agouti paca and Dasyprocta punctata, were negative. Isolates obtained were kept for identification. The research was performed in two localities, Naranjal, Department of Guayas and Ocaña, Department of Cañar. The results obtained are summarized in Table 4 (Hashiguchi et al., 1985b). Recently, two mammalian species, Sciurus vulgaris and T. tetradactyla, were added to the reservoir list in Ecuador (see Chapter 4-2). It is interesting to note that only cultures from the liver showed positive in our survey, though the parasites could be isolated from the liver, spleen, blood and skin of wild caught mammals.

Parasite Isolations

Since 1920, many attempts have been made to isolate Leishmania from human lesions in Ecuador, with no positive results. In 1986, a strain of Leishmania was isolated for the first time by us from a human being in Paute, Department of Azuay, Ecuador, located on the Andean highlands of the southern Ecuador (see Chapter 7-1). In 1987, we isolated a total of five more Leishmania organisms from human beings who came from the endemic areas of the coastal regions of Ecuador (see Chapter 4-1). Additionally, a total of five wild animals have been found naturally infected with leishmanial parasites so far (Hashiguchi et al.,

Table 4 Natural infections of wild caught mammals with *Leishmania* in leishmaniasis-endemic areas of Ecuador between 1982 and 1984*

Mammalian** species examined	Ecuadorian common name (English name)	No. of animals examined in each locality		No. of positive animals
		Naranjal	Ocaña	
Di. marsupialis	Zorro (opossum)	9	5	0
T. tetradactyla	Oso hormiguero (tamandua)	1		0
C. h.didactylus	Perico ligero (two-toed sloth)	1		1
Sy. brasiliensis	Conejo (rabbit)	1		0
Da. novemcinctus	Armadillo (armadillo)	1	1	0
Sc. granatensis	Ardilla (squirrel)	4	1	1
Pr. semispinosus	Cuy de monte (guinea pig)	6		0
R. rattus	Rata (rat)	1		0
Co. bicolor	Puercoespin (coendou, tree porcupine)	2		0
A. paca	Paca (Guanta) (paca)	2		0
D. punctata	Aguti (guatusa) (agouti)	2		0
Po. flavus	Cuzumbo (kinkajou)	11		1
Total		41	7	3

* Hashiguchi *et al.*, 1985b.

** Generic names of mammals in full: *Di.*, *Didelphis*; *T.*, *Tamandua*; *C.*, *Choloepus*; *Sy.*, *Sylvilagus*; *Da.*, *Dasypus*; *Sc.*, *Sciurus*; *Pr.*, *Proechimys*; *R.*, *Rattus*; *Co.*, *Coendou*; *A.*, *Agouti*; *D.*, *Dasyprocta*; *Po.*, *Potos*.

1985b). No previous attempts had been made to isolate Leishmania from Ecuadorian mammals in the past, although some mammals were suspected to be leishmaniasis reservoirs, based on the observation that sand flies were found in their nest or burrows. All the isolates from human beings and animals were kept for identification (see Chapter 4-3).

Leishmaniasis Diagnosis in Ecuador

Direct diagnosis using smears from the ulcerous lesions or nodules is the principal method used in Ecuador. However, this has only been performed in a few Ecuadorian medical centers. Rural doctors rarely employ this technique and many cases of dermal lesions have therefore been treated for leishmaniasis without differential diagnosis when drugs are available. Immunological diagnosis had not been routinely performed in the country, but skin test (Montenegro reaction) using promastigote antigen prepared by the method of Reed et al. (1986) was recently employed as a diagnosis tool in INHMT (Instituto Nacional de Higiene y Medicina Tropical), Guayaquil, Ecuador, with good results.

Leishmaniasis Treatment in Ecuador

Antimonials are regularly used in Ecuador for the treatment of leishmaniasis patients; Gulcantime (Meglumine antimonate) seems to be the most effective drug, though Fuadin (Stibophen B. P.) is probably equally patent. Repodral (Stibophen) is also sometimes used for leishmaniasis treatment in Ecuador. Other drugs, such as Amphotericin B (Fungizone), Pyrimethamine and

Lampit are used occasionally, and there have been same satisfactory results. A few doctors in Ecuador have used Metronidazol for leishmaniasis treatment, and reported some good results (Tafur and De Tafur, 1978); the drug, however, may only act as an anti-inflamatory agent, since there is no biochemical explanation to qualify it as a curative agent (Walton et al., 1974).

In general, therapeutic assays with a correspondent follow-up of patients for a long period, have not as yet been done in Ecuador. Ammunarriz (1984) published the data on a carefully designed therapeutic research procedure using antimonials and five kinds of traditional Amazonian Indian medicines. His information on the effectiveness of the Indian treatments against leishmaniasis is noteworthy. However, it will be necessary to study more cases using Indian medicines, before any definitive conclusions can be reached.

Conclusions

Review of past leishmaniasis research in Ecuador, reveals one undoubted fact: main attention was paid to clinical and therapeutic aspects of the disease for a long period, and other than some sporadic attempts, transmission research was unfortunately ignored. A detailed study of leishmaniasis transmission is necessary in order to obtain enough information to develop a future control plan for leishmaniasis in Ecuador. Although much time has been lost, emphasis should now be placed on transmission studies, in order to establish a good surveillance and control program as soon as possible.

Several stocks of Leishmania from animals and human beings have already been isolated from Ecuador. This survey should be continued in order to collect and identify the largest possible number of isolates. Special efforts should be made to isolate the parasite from vector sand flies for the Ecuadorian leishmaniasis stocks collection; all endemic areas should be surveyed. If possible, all the stocks should be cloned and maintained in laboratory animals or under modern maintenance systems; cryopreservation of promastigotes should be performed as soon as possible after primary isolation. Cryopreservation would be specially necessary for species such as L. braziliensis braziliensis, which is very difficult to maintain in culture.

We are sure that more than three species of sand fly are involved in leishmaniasis transmission in Ecuador; therefore, studies in this area will be continued and extended. The biology of these three suspected or incriminated vectors (Lu. trapidoi, Lu. hartmanni and Lu. gomezi), should be extensively studied. Fortunately, our advances in laboratory rearing of sand flies will permit us to investigate this in detail. To date, five wild animals, i.e., Choloepus h. didactylus, Sciurus granatensis, S. vulgaris, Potos flavus and Tamandua tetradactyla, have been found naturally infected with Leishmania in Ecuador. Their true and potential roles as reservoirs should be determined in each leishmaniasis-endemic area of the country in future studies.

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Chapter 3

AN ECOLOGICAL VIEW OF LEISHMANIASIS-ENDEMIC AREAS IN ECUADOR

Abstract. The Andes divide Ecuador into three distinct biogeographical regions: two lowland areas, one lying along the Pacific coast (littoral) and the other in the upper Amazonian basin (oriente), and one highland area (sierra) including the Andean slope. In the text, ecological features of each area which relate to the mammalian and sand fly fauna were taken into special consideration in relation to the disease transmission ecology.

Ecuador is, in all aspects, a highly varied country, and geographic, climatic, ecological, ethnical, socioeconomical and pathological differences are found between each natural region of the country (Barrera et al., 1978). A good description of the country would therefore require much more than a short article. However, in order to present a clearer picture of the relationship between the wide distribution of leishmaniasis and the ecological aspects of each area, we shall briefly discuss the most important points of interest for each region of continental Ecuador, excluding Galapagos Islands since these are so far free of the disease.

Natural Regions of Continental Ecuador

The Andes, a range of mountains which traverses Ecuador from north to south, divide the country into three natural regions (Fig. 1): the Littoral (costa) or Pacific coast region, the Sierra or Andean region, and the Oriente or Amazonian region. According to Teran (1984), the population of Ecuador is approxi-

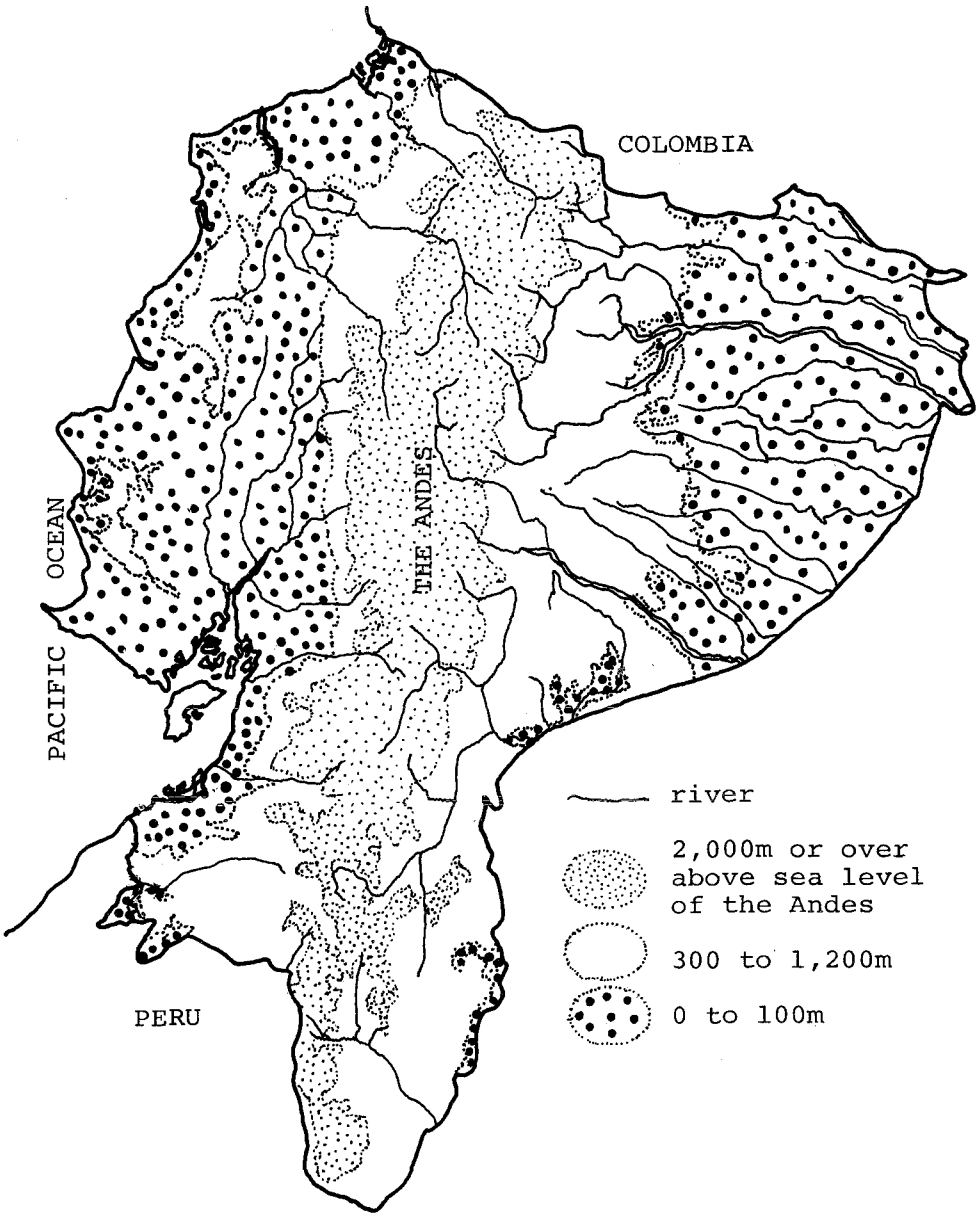

Figure 1. Outline map of the Republic of Ecuador, showing three natural regions, the Littoral or Pacific coast, the Sierra or Andes and the Oriente or Amazon, with rivers at different altitudes above sea level. 2,000 m or over, Andean highlands; 300 to 1,200 m, Andean slopes at bilateral regions of the Andes; 0 to 100 m, lowlands at both the Amazonian and the Pacific coast regions.

mately 8,000,000. The population of Department in the three geographic areas is as follows: Littoral region, 4,057,000 in total, viz., Esmeraldas, 266,000; Manabi, 995,000; Los Rios, 496,000; Guayas, 1,957,000; and El Oro, 343,000, Sierra region, 3,611,000 in total, viz., Carchi, 142,000; Imbabura, 254,000; Pichincha, 1,276,000; Cotopaxi, 272,000; Tungurahua, 323,000; Chimborazo, 347,000; Bolivar, 165,000; Cañar, 174,000; Azuay, 429,000; and Loja, 402,000, and Oriente region, 231,000 in total, viz., Napo, 86,000; Pastaza, 27,000; Morona Santiago, 68,000; and Zamora Chinchipe, 51,000. The country is one of the smallest of the South American countries, with an area of about 284,000 km^2.

Littoral (costa) or Pacific coast region

The Pacific coast region is a wide band of land situated between the west Andean slopes and the Pacific Ocean, ranging from approximately 500 m above sea level at the Andean slopes to sea level. This region consists of about 70,000 km^2 of lowlands, with sporadic groups of low mountains at some points (Teran, 1984).

Climatically, this region is divided into two areas: the northern area, which is hot (26 C - 32 C) and rainy because of the influence of the El Niño sea current; and the southern area, which is less hot (23 C - 28 C) and humid (Benalcazar, 1981). Endemic areas of leishmaniasis, which form a long ribbon from north to south of the littoral near the west Andean slopes, are geographically included in these two climatic areas. Esmeraldas province, which is one of the principal leishmaniasis-endemic

area of the littoral region, lies totally within the northern climatic area; the remaining endemic areas of the littoral are included in the southern climatic area.

These two climatic regions of the littoral, both containing leishmaniasis-endemic areas each have a characteristic vegetation. Many crops are cultivated in the littoral region, including banana, rice, coffee, cocoa, sugar cane, and many varieties of tropical fruit. In the west part of the littoral rice, cotton, coffee and citrus fruits are grown (Benalcazar, 1981). The climate of the littoral is not as "equatorial" as might be expected, because of the influence of the Humbolt cold current in the west, and the Andes in the east; it is neither as hot nor as humid as the Amazonian region (Teran, 1984). The littoral region however has extensive areas of rain forest especially in the north and near the Andean slopes, with a great variety of mammals and blood-sucking insects providing ideal for the existence of a tropical zoonosis like leishmaniasis. Because of the excellent agricultural conditions and water supply, most Ecuadorian rural inhabitants live in these areas, in scattered dwellings or small villages, close to the crops among the vegetation (Barrera et al., 1978).

Leishmaniasis can exist as a zoonosis for many years among wild mammals and vector sand flies, without producing human cases. However, when men enter the forest and establish their settlements, they are exposed to the bite of infected sand flies and become infected (Hashiguchi et al., 1985). The future status of leishmaniasis in a newly-established village depends on the

behavior of inhabitants and the growth of the village, as pointed out by Herrer and Christensen (1976a, b). For instance, if inhabitants regularly enter the jungle very often to hunt or work, and if vegetation around the village is abundant and close to the houses, leishmaniasis may become endemic; in this case newly-born children are usually affected. If the human population does not have contact with forest, and houses are kept away from and free of vegetation, leishmaniasis is more likely to be manifested as occasional epidemics. When unusual situations force men to enter the forest, or when climatic conditions such as heavy rain assists growth of vegetation close to houses, persons of all age-groups may become infected. Leishmaniasis in the Ecuadorian littoral region is therefore sporadic, and occurs when accidental or sporadic contact occurs between persons and infected sand flies.

The flora and fauna of the littoral region are very diverse, including several mammals and phlebotomine sand flies which may be involved in the maintenance of human leishmaniasis as a zoonosis, as reservoirs and vectors respectively. In Ecuador, several arboreal mammals are suspected reservoirs of the disease; these include the sloth Choloepus h. didactylus, the squirrels Sciurus granatensis and S. vulgaris, the kinkajou Potos flavus and the anteater Tamandua tetradctyla, all of which have been found naturally-infected with Leishmania in the littoral region (Hashiguchi et al., 1985a; see Chapter 4-2). Three species of sand flies have been found naturally infected with promastigotes of Leishmania, and incriminated as probable vectors of the di-

sease. These three anthropophilic species are Lutzomyia trapidoi, Lu. hartmanni and Lu. gomezi (Hashiguchi et al., 1985b; see Chapter 5-1). The wild mammals and sand flies mentioned above are nocturnal (Hashiguchi et al., 1985c), and spend the day resting. It appears that the Ecuadorian littoral has an ideal ecological conditions for leishmaniasis transmission, particularly in the rainy season when high humidity would probably support increases in sand fly population size. Apart from the large leishmaniasis-endemic areas of the littoral region lying near the west Andean slope, there are some other limited foci, near the coast in small mountain systems, especially in the southern part of the Department of Manabi (see Chapter 2).

Sierra or Andean region

The "Sierra" is the long, narrow territory situated between the two principal branches of the Andes. These Andean branches are the "Occidental" (west) and "Oriental" (east), they are partially or totally linked at different sites along the mountain range. Whithin the principal and crossed branches (knots) of the Ecuadorian Andes are many valleys, where several cities are situated (Benalcazar, 1981). The climate is generally temperate (10 C - 15 C), but at lower elevation it is Andean subtropical (15 C - 20 C). At higher places (3,200 m - 6,300 m) temperature ranges from 0 C to 9 C (Teran, 1984; Benalcazar, 1981).

Because of the different altitudes, the natural conditions of the Andean region in Ecuador, such as atmospheric pressure, luminosity, humidity and vegetation, are quite different from

those in the littoral region. Agricultural products in the Andean region are potatoes, onions, beans, corn, lentils, field beans, lettuce, and fruit such as apples, peaches, pears, grapes, strawberries and cherries (Teran, 1984; Benalcazar, 1981). There is no humid forest in this region. However in this area, we have discovered an autochthonous leishmaniasis with a quite different ecology from the littoral one (see Chapter 7-1). The causative agent has been hypothesized, at least on the basis of current knowledge, as Leishmania braziliensis peruviana, which causes uta in the Peruvian Andes. Altitude of the Ecuadorian endemic area is 2,300 m to 2,700 m above sea level, where vegetaion is very scarce in a mostly rocky terrain with relatively low humidity and temperature. In this area, only one species of anthropophilic sand fly has been found, suspected to be Lu. peruensis; the ecology of this species seems to be quite different from that of flies found in the Pacific coast and Amazonian regions.

In the Ecuadorian Andes, habits and activities of people are exclusively diurnal because of low temperatures. The local population therefore does not usually enter the vector's habitat for hunting or other purposes during the night, and transmission probably involves the vector sand flies entering human habitations. At the moment, the suspected reservoir is the domestic dog, though no positive isolates of parasites have yet been made from these animals. Relatively few wild mammals occur in the Andean region of Ecuador. Those present in this newly-recognized leishmaniasis-endemic area include rodents, opossums, two species of mustelids and a wild canine, Dusicyon culpaeus, the "paramo

wild dog" (Patzelt, 1978). These Andean wild mammals should be thoroughly investigated in order to clarify their potential roles as reservoir hosts of leishmaniasis in Ecuador.

Oriente or Amazonian region

The "Oriente" or Amazonian region occupies the eastern part of Ecuador and is relatively sparsely populated. The area is almost completely covered by tropical rain forest, and can be divided into two principal parts: a higher part, which is situated along the eastern Andean slope with an average altitude of 600 m, and a lower part, the Amazonian lowlands (Teran, 1984). This Amazonian plain is traversed by many tributaries of the Amazon river, all of them rising in the Andes. Vegetation of the Amazonian rain forest is very diverse.

The climate of the Amazonian region is generally hot and wet, but there are some differences between the upper and lower parts. The higher part, the "Alto Oriente" has an average temperature of 23 C to 24 C, due to its average altitude of 600 m. However it is the wettest area of Ecuador, with humidity of over 90% during several months of the year (Teran, 1984). The lower part, "Bajo Oriente" has a higher temperature (over 25 C - 26 C) and lower humidity than the higher part, although rains occur in almost all months of the year. The temperature in this part should be higher, but continuous rain water evaporation of the river, and the protection of dense trees, have a mediating effect (Teran, 1984).

The forest in the Amazonian region has special character-

istics, particularly in the lower part. Massive, tall trees growing in close proximity to one another, form a luxuriant canopy of foliage, blocking most of the sunlight and causing a permanent day-darkness, which maintains a high humidity and allows the build up of an incredibly thick humus layer (Barrera et al., 1978; Teran, 1984; Benalcazar, 1981). Such conditions provide an ideal habitat for the life cycle of certain sand fly vectors of leishmaniasis. This abundance of resting and breeding sites is complemented by the large populations of wild animals which provide abundant blood meal sources. Several large rodents occur in the lower part, including capybara (Hydrochoerus hydrochaeris), paca (Cuniculus paca), and pacarana (Dasyorocta punctata). Other common rodents include squirrels, mice and rats (Patzelt, 1978). There are about than ten species of monkeys, and superpredators such as jaguar (Panthera onca), puma (Felis concolor), ocelot (Felis pardalis) and some other small wild cats are also present. Several species of edentates (two kinds of sloths, two kinds of anteaters, and three kinds of armadillos), small carnivores (kinkajou Potos flavus; coati Nasua narica, and tayra Tayra barbara), marsupials and many species of bird are also present in the habitat, in addition to a great variety of insects and other invertebrates. Thus ecological conditions of the Amazonian region appears well-suited to the maintenance of leishmaniasis as a permanent zoonosis. However, relatively few leishmaniasis cases have been reported from the region compared with the Pacific coast. This may be mainly due to the lack of an adequate system of medical care and registration. Traditional

treatments involving the use of plant medicines may be effective in alleviating severe infections which might otherwise be referred to medical doctors (Amunarriz, 1982).

Conclusion

Leishmaniasis is the most widespread tropical zoonosis in Ecuador. In this study Ecuadorian leishmaniasis-endemic areas were detected on the basis of the occurrence of human cases. The biotic potential of the parasite, Leishmania, depends on the biological richness of its habitat, and is estimated by measurement of the incidence of natural infections in the vectors and reservoir hosts. Tropical rain forest is the prefered habit for many New World, and human Leishmania. Human cases will always be the result of accidental transmission. Thus we conclude that the Amazonian region is an important endemic area of leishmaniasis as a zoonosis although with relatively few human cases, while the Pacific coast (littoral) region is an ecologically less-diverse endemic area of the disease, with many more human cases.

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Chapter 4

PARASITOLOGY

1. *Leishmania* Isolates from Humans

Abstract. Five *Leishmania* isolates were obtained from ulcerous lesions of patients living in the Pacific coast region. The locality of each isolate was as follows: MHOM/EC/87/G05, Esmeraldas Department; MHOM/EC/87/G06, Esmeraldas; MHOM/EC/87/G07, Pichincha; MHOM/EC/87/G08, Pichincha; MHOM/EC/87/G09, Esmeraldas. Each isolate was inoculated into hamsters. Only one (MHOM/EC/87 /G09) of these 5 isolates however, developed a small lesion, after 4.5 months of the infection, though all the inoculated animals were positive for amastigotes at the site of inoculation. In the text, the isolation procedures, especially in the field condition, are also mentioned. It is emphasized that trial of this type, involving *Leishmania* isolations from patients, reservoirs and vector sand flies, should be done in different endemic areas, in order to clarify the epidemiological features in Ecuador as a whole.

The first recorded human case of leishmaniasis in Ecuador was diagnosed in 1920 by Valenzuela (Rodriguez, 1974) without parasite isolation. In 1986, the organism was isolated for the first time from a little girl during an epidemiological survey at Paute, a village situated at about 2,500 m above sea level in the Andean highlands of the country (see Chapter 7-1). The present paper deals with the isolation procedures and features of five *Leishmania* isolates obtained from patients living in the most economically important region of Ecuador, the Pacific coastal area. These represent the first stocks isolated from human beings in the Ecuadorian coast region.

Materials and Methods

Materials for culture

Culture medium: The medium was slightly modified from that reported by Walton et al. (1972). This was prepared from 40g Difico Blood Agar Base (Code B45, Difico Laboratories, Detroit, Michigan, U.S.A.) per 1,000 ml distilled water with 20% defibrinated rabbit blood. Two ml of melted media were poured into each vacuum tube, and the tubes sealed with rubber caps. The blood agar slants were left at room temperature for several hours to allow formation of condensation fluid, and then stored at 4 C until used. When cool, an overlay of sterile physiological saline (0.9% w/v) was added to each tube. Two drops of 20% gentamycin were also added on occasion to combat fungal contamination, without any adverse effect on the parasites.

The following materials were also used for isolations, especially in the field. One ml tuberculin syringe with needle (#22 or 23G); alcohol burners; antiseptic washing solutions, as used by surgeons; a small thermic box for cooling; a large thermic box for incubating and storing culture tubes with Leishmania isolates; environmental thermometers; plastic cups for ice; slides and staining solution.

Procedures

Before beginning the isolations, ulcerous lesions were washed very carefully with antiseptic solution in order to eliminate bacteria and fungi; use of mercurial components was avoided since these might have killed the Leishmania parasites.

One ml of sterile saline solution was taken up into a tuberculin syringe and the syringe needle introduced intradermally along the border of a lesion and material taken up, ensuring that no saline was injected into the patient's skin. This suction caused the saline solution to more back to the distal part of syringe, and caused the formation of a large air bubble appeared in the proximal end. The syringe was then rotated while maintaining suction and the needle moved alternatively to the left and to the right. This movement drew tissue cells with parasites through the needle and into the air bubble. When a bloody liquid appeared in the base and air bubble, the extraction process was completed and the needle was retracted. The syringe contents were immediately inoculated into culture tubes with media through the rubber cap to avoid contamination, close to an alcohol burner. The saline solution in the syringe aided in pushing aspirated materials into the culture tubes.

As soon as aspirated material was inoculated, tubes were placed in the refrigerator (4 C) for 1 or 2 hours; this facilitated adaptation of amastigotes to the culture medium. Thereafter, the inoculated tubes were kept in a thermic box containing small cups of ice to maintain temperature to within 21 C - 25 C; this temperature facilitates transformation of amastigotes into promastigote forms. Ice was renewed regularly to maintain a stable temperature inside the thermic box during field work. When available, an electric adjustable 21 C incubator should be used in place of the thermic box.

Culture tubes were checked every two or three days. Some

isolates of Leishmania, especially those belonging to braziliensis complex take 10 or more days to grow in the medium. Others such as mexicana complex strains take only three or four days to grow. Isolated stocks were also inoculated immediately into hamsters, a more traditional method which served as a back-up.

Results and Comments

Five isolates of Leishmania were obtained from ulcerous lesions of human beings in the Pacific coast region of Ecuador, using the procedures mentioned above. The results are shown in Table 1. The growing time of isolates in the original culture tube to which the lesion materials from patients were directly inoculated ranged from 3 to 12 days after inoculation. Variation was also evident in the subculture time, parasite numbers and motility among the different isolates obtained.

The results of general examination of each patient, from whom the present isolates have been obtained, are summarized as follows.

MHOM/EC/87/G05, (female, 20 years old): 1) residence, Quininde, Department of Esmeraldas; 2) lesion type, ulcer; 3) duration of lesion, 4 months; 4) lymphadenitis, generalized; 5) number of lesions, 2 (10x8 mm; 15x12 mm); 6) location of the lesion from which isolate was made, wrist; 7) smear specimen, -; 8) culture, +; and 9) skin tsest using leishmanin antigen, +.

MHOM/EC/87/G06, (male, 21 years old): 1) Zapallo Grande, Esmeraldas; 2) ulcer; 3) 2 months; 4) not found; 5) 1 (9x25 mm); 6) left arm; 7) +; 8) +; and 9) +.

Table 1. Five Leishmania isolates from the patients living in the Pacific coast region of Ecuador

Stock code	Growing time (day)*	No. of parasites**	Motility***	Subculture time (days)****
MHOM/EC/87/G05	3	++++	++++	8-10
MHOM/EC/87/G06	10	+	++	15-20
MHOM/EC/87/G07	12	++	++	10-15
MHOM/EC/87/G08	8	++	++	10-15
MHOM/EC/87/G09	6	+++	+++	8-10

* The day when promastigotes were first observed in culture after amastigotes isolation from patient's ulcers.

** Number of promastigotes at the first detection in the original culture tube: +, 1-5; ++, 6-10; +++, 11-15; ++++, more than 16, at a field under x100 magnification

*** Activity of promastigotes in culture: +, very slow; ++, slow; +++, active; ++++, highly active.

**** Adequate subculture times (days).

MHOM/EC/87/G07, (female, 38 years old): 1) Santo Domingo de Los Colorados, Pichincha; 2) ulcer; 3) 3 months; 4) not found; 5) 1 (15x10 mm); 6) left arm; 7) +; 8) +; and 9) +.

MHOM/EC/87/G08, (male, 25 years old): 1) Guayaquil, but he visited Santo Domingo de Los Colorados, Pichincha; 2) ulcer; 3) 3 months; 4) not found; 5) 1 (15x10 mm); 6) right wrist; 7) +; 8) +; and 9) +.

MHOM/EC/87/G09, (female, 18 years old): 1) Quininde, Esmeraldas; 1) ulcer; 3) 4 months; 4) not found; 5) 1 (10x10 mm); 6) lower right leg; 7) +; 8) +; and 9) +.

The above present five isolates were from patients living in or visiting two endemic areas of leishmaniasis, Esmeraldas and Pichincha, which are located in the north west Pacific coast region of Ecuador. Three (MHOM/EC/87/G05, MHOM/EC/87/G06 and MHOM/EC/87/G07) of the five isolates have been identified as L. braziliensis panamensis, while the rmainings are still in characterization (see Chapter 4-3). In order to clarify the characteristics of causative agents of the Ecuadorian leishmaniasis, more extensive and country-wide collection of material should be performed. Such attempts will be continued in future studies of this program.

The results of inoculation into hamsters are shown for each Leishmania isolate in Table 2. Each of the isolates was inoculated into the noses of two Chinese hamsters, after 10 to 15 days of cultivation of material aspirated from patients. All the animals inoculated with each Leishmania isolate were positive for amastigotes at the site of inoculation (Table 2). However, only

Table 2. Results of hamster inoculation with Leishmania isolates from human beings

Stock code*	Date of isolates inoculation	Date of examination at inoculation sites**		Date of the last examination on the lesions	
		date	+ or -	date	+ or -
G05	13 Feb 87	4 Aug 87	+	8 Oct 87	-
G06	4 May 87	4 Aug 87	+	8 Oct 87	-
G07	13 May 87	4 Aug 87	+	8 Oct 87	-
G08	17 May 87	24 Spt 87	+***	8 Oct 87	-
G09	23 May 87	4 Aug 87	+	8 Oct 87	+****

* See Table 1.

** Examination was made on the culture materials taken from the inoculation site of hamsters with isolates by the method mentioned above (see Chapter 4-1).

*** Materials taken on the day 4 August 1987 were examined, but revealed negative in culture specimens.

**** A very small lesion was observed, demonstrating many amastigotes on smear specimens.

one of the 10 hamsters demonstrated a small lesion, 4.5 months after the inoculation. Among the remainder, furthermore, one isolate (MHOM/EC/87/G05), inoculated into a hamster's nose on 13 February 1987 had still not caused development of a lesion by 8 October of that year; although amastigotes were present at the inoculation site. Thus, lesion evolution in hamsters infected with our Leishmania isolates from Ecuadorian patients was an extremely lengthy process.

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2. *Leishmania* Isolates from Wild and Domestic Mammals

Abstract. In order to identify reservoir hosts of Ecuadorian leishmaniasis in lowland and highland areas, a total of 167 animals belonging to 17 mammalian species were thoroughly examined by performing culture *in vitro* and hamster inoculation of organ homogenates. One individual each of three species, i.e., *Potos flavus* and *Sciurus vulgaris* from Palenque, Los Rios, and *Tamandua tetradactyla* from Echeandia, Bolivar, revealed positive for *Leishmania* parasites. The last two mammal species were newly recorded as reservoir hosts of *Leishmania* in Ecuador. No animals positive for the Andean highland leishmaniasis were found, though 71 dogs and six wild mammals were examined.

Leishmaniasis in Ecuador occurs in various areas from the lowlands of the Pacific coast and Amazonian region to the Andean slopes (Rodriguez, 1974). A variety of mammalian species have been incriminated as the reservoir hosts of New World leishmaniasis in neibouring South American countries (Lainson and Shaw, 1978), and the present workers have isolated leishmanial parasites from three mammalian species in endemic areas of Ecuador (Hashiguchi *et al*., 1985).

In this study we tried to isolate *Leishmania* parasites from wild and domestic mammals examined in the lowland areas of the Pacific coast, and also in the highland areas of the Andes where leishmaniasis was found to be endemic for the first time (see Chapter 7-1).

Materials and Methods

Study areas

The present study was carried out in and around the following areas: 1) Selva Alegre, Department of Esmeraldas, located at 0°

55' north latitude (NL) and 78°50' west longitude (WL), in a forested zone, 2) Echeandia, Department of Bolivar, 1°25' south latitude (SL) and 79°15' WL, 3) Palenque, Department of Los Rios, 1°25' SL and 79°45' WL, 4) Paute, Department of Azuay, 2°46' SL and 78°45' WL in the mid-Andean highlands (2,500 - 2,700 m above sea level).

Mammals examined and leishmanial isolation procedure

Ninety-six wild mammals, belonging to 17 species and 13 genera, viz., Didelphis marsupialis, D. paraguayensis, Philander opossum, Caluromys derbianus, C. lanatus, Chironectes minimus, Marmosa robinsoni mimetra, Potos flavus, Sciurus granatensis, S. vulgaris, Bradypus infuscatus ephippiger, Choloepus hoffmani didactylus, Tamandua tetradactyla, Sylvilagus brasiliensis, Rattus norvegicus and Mus musculus, and 71 domestic dogs (Canis familialis), were examined for Leishmania parasites. Sample materials from the liver and spleen of wild mammals and from small biopsies of ear skin of dogs were individually homogenized with normal saline. Thereafter, part of each of the homogenates was inoculated into two culture tubes, each containing medium, and the remainder was injected into the nose and foot pad of golden hamsters. In hamster inoculation, the animals were autopsied after one month, and the homogenates of samples from the nose, foot pads, liver and spleen were inoculated into culture medium. In another part of the examination, samples materials were taken from the livers of wild mammals without autopsy, using the same procedure employed in isolation from human beings (see Chapter 4-1). The culture medium was composed of 8 g Bacto-

agar, 5 g NaCl and 3 g beef extract in 1,000 ml of distilled water. After inoculation of each culture material, an overlay of 3 ml sterile physiological saline with 10% fetal calf serum containing 500 I.U. of penicilin and 500 ug of streptomycin per ml was added. Pan's medium (Pan, 1971) was also prepared for the isolation of parasites. The inoculated culture tubes were incubated at 25 C. If no organism was found in the culture by 40 days after incubation, the sample was regarded as negative.

Results and Discussion

The results of examination of wild and domestic mammals for Leishmania parasites are summarized in Table 1. In total, 167 mammals were examined in four Departments of Ecuador, Bolivar (Echeandia), Esmeraldas (Selva Alegre), Los Rios (Palenque) and Azuay (Paute). Out of 96 wild-caught mammals, 1 Potos flavus and 1 Sciurus vulgaris from Palenque, Los Rios and 1 Tamandua tetradactyla from Echeandia, Bolivar revealed positive for leishmanial parasites in both culture media and hamster inoculations. All of the three isolates (MSCI/EC/87/G02, MPOT/EC/87/G03 and MTAM /EC/G04) were identified as L. mexicana amazonensis (see Chapter 4-3).

Apart from these Leishmania positive animals, 1 Didelphis marsupialis and 1 Caluromys lanatus caught in Echeandia were positive for flagellates; the organisms isolated from hamster inoculations were morphologically identified as Trypanosoma sp., probably T. rangeli.

Hashiguchi et al. (1985) isolated Leishmania sp. from three

Table 1. Results of examination of wild caught mammals and domestic dogs for *Leishmania* parasites, from the endemic areas of Ecuador between 1986 and 1987

Mammalian species	No. caught in each Department and village				Total
	Bolivar (Echeandia)	Esmeraldas (Selva Alegre)	Los Rios (Palenque)	Azuay (Paute)	
D. marsupialis	24				24
D. paraguayensis				4	4
Ph. opossum	3				3
Ca. derbianus	16	1			17
Ca. lanatus	4				4
Ch. minimus		1			1
M. r. mimetra		2			2
*Po. flavus***	11		1 (+)*		12 (1+)
Sc. granatensis	1				1
*Sc. vulgaris****			1 (+)		1 (+)
B. i. ephippiger		2			2
Cho. h. didactylus	5				5
*T. tetradactyla*****	1 (+)				1 (+)
Sy. brasiliensis	1				1
R. norvegicus	6	1			7
M. musculus	7	2		2	11
C. familialis (dog)				71	71
Total	79 (1+)	9	2 (2+)	77	167 (3+)

* Positive for *Leishmania* parasites; **, stock code: MPOT/EC/87/G03; ***, MSCI/EC/87/G02; ****, MTAM/EC/87/G04.

species of mammals, i.e., Choloepus hoffmani didactylus (this species was misidentified as Bradypus variegatus ephippiger in their original text), Sciurus granatensis and Potos flavus, caught in Naranjal, Department of Guayas, Ecuador; species characterization of these isolates is in progress. To date, we have therefore incriminated five mammalian species, i.e., Choloepus h. didactylus, Sciurus granatensis, S. vulgaris, Potos flavus and Tamandua tetradactyla, as possible reservoir hosts of leishmaniasis in Ecuador from our surveys during 1982-1987. These incriminated wild mammals might play an important role as reservoir hosts in the transmission of the disease. Further intensive studies, however, would be necessary to identify the principal reservoir hosts of the disease in each endemic area of Ecuador.

A total of 71 domestic dogs were examined in Paute, an Andean highland village, where another type of autochthonous leishmaniasis (uta) was recognized for the first time (see Chapter 7-1). No positive case could be demonstrated among these dogs from culture or hamster inoculation. The negative results might be due to the small amount of dog's skin used for isolation of Leishmania parasites (the skin was taken from ear of dogs by Holth type scrorocorneal punch). The results of immunological examination of the dogs will be reported elsewhere.

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3. Serodeme Typing of *Leishmania* Isolates using Monoclonal Antibodies

Abstract. Six strains of *Leishmania* isolated from humans and wild mammals were identified at subspecies levels based on their reactivity to a cross-panel of specific monoclonal antibodies using a radioimmune binding assay. Three strains isolated from cutaneous ulcerous lesions of human patients were identified as *L. braziliensis panamensis*. Each organism isolated from wild mammals, i.e., *Tamandua tetradactyla*, *Potos flavus* and *Sciurus vulgaris* was identified as *L. mexicana amazonensis*. From the results obtained, it is suggested that these two species of *Leishmania* are found in a wide range of leishmaniasis-endemic areas in Ecuador, especially in the Pacific coast regions. The current study is the first trial of species and/or subspecies characterization of the genus *Leishmania* in Ecuador.

New World leishmaniasis is widely distributed in Central and South Americas, where it is a considerable health hazard (Molyneux and Ashford, 1983). These cutaneous, mucocutaneous and visceral leishmaniases have been characterized and identified at species and/or subspecies levels by isoenzyme electrophoresis, monoclonal antibodies and kinetoplast DNA (Lainson and Shaw, 1987; Grimaldi *et al.*, 1987; Barker, 1987). In Ecuador, since the first case was described in 1920 (Rodriguez, 1974), many human leishmaniasis cases were reported. *Leishmania* parasites were also isolated from three wild mammals in leishmaniasis-endemic areas of Ecuador (Hashiguchi *et al.*, 1985). In this country, however, the identification and taxonomy of *Leishmania* parasites have been done based on clinical manifestations in human patients, together with the epidemiological features, the lesion developments in hamster infections and the growth patterns of parasites *in vitro* cultures. It has however been often dif-

ficult to identify leishmanial parasites based on these criteria. In order to overcome such problems, a variety of techniques to characterize Leishmania parasites have been developed. The identification method using monoclonal antibodies has been recently established by performing the indirect radioimmune binding assay (McMahon-Pratt and David, 1981; McMahon-Pratt et al., 1982; Grimaldi et al., 1987).

The present paper describes on identifications of Ecuadorian Leishmania isolates based on results of serodeme typing using monoclonal antibodies; two Leishmania species, L. braziliensis panamensis from humans and L. mexicana amazonensis from wild mammals, were identified in this study.

Materials and Methods

Leishmania stocks

Three Leishmania strains were isolated from ulcerous lesions of humans living in leishmaniasis-endemic areas in the Pacific coast regions. The locality of each isolate was as follows: MHOM/EC/87/G05, Quininde (Rosa Zarate), Department of Esmeraldas; MHOM/EC/87/G06, Zapallo Grande, Department of Esmeraldas; MHOM /EC/87/G07, Santo Domingo de Los Colorados, Department of Pichincha. Leishmania parasites were also isolated from liver and spleen homogenates of three species of wild mammals: Sciurus vulgaris isolate (MSCI/EC/87/G02) and Potos flavus isolate (MPOT /EC/87/G03) from Palenque, Department of Los Rios, and Tamandua tetradactyla isolate (MTAM/EC/87/G04) from Echeandea, Department of Bolivar. The WHO recommended reference strains given in Tables

were examined together with Ecuadorian isolates in this study.

Preparation of samples

After culturing promastigotes in Schneider's medium, they were centrifuged (1,500 x g for 10 min. at 4 C) and washed in phosphate buffer saline (PBS), pH 7.3. The parasites were re-suspended in lysis buffer containing 0.04M NaCl, 0.01M sodium ethylenediamine tetraacetate, 0.01M phenylemethylsulfonylfluoride, 0.001M iodoacetamide, 0.01M Tris pH 8.0, for analysis with monoclonal antibodies. These materials were stored at -70 C until used. The samples were sonicated using a bath sonicator to assure a homogeneous distribution of antigen before analysis.

Monoclonal antibodies and indirect radioimmune assay

The monoclonal antibodies used in this study, specific for members of the L. braziliensis, L. mexicana and L. donovani complexes, have been described (McMahon-Pratt et al., 1982; Jaffe et al., 1984; McMahon-Pratt et al., 1985).

The characterization of Leishmania was performed with indirect radioimmune binding assay using whole parasite lysates as antigen. Cook U-bottom 96-well PVC plates were coated overnight at 5 C with sonicated homogenates of whole promastigotes diluted in PBS containing 0.02% sodium azide (NaN_3). The plates were washed five times in PBS containing 0.02% fetal bovine serum. Culture medium supernatants containing secreted antibodies were incubated with the antigen plates overnight at 5 C. After washing, affinity-purified ^{125}I-labeled rabbit $F(ab')_2$ anti-mouse immunoglobulin (5-10 uCi/ug protein 10^5 counts per min [cpm]) was added to the wells and incubated for 1 hour at 0 C. Excess

antibody was removed by washing. The plates were air dried and the radioactivity bound to each well was measured using a Packard Auto-Gamma Counter.

Results

The reactivity of Ecuadorian and WHO recommended reference strains to 22 monoclonal antibodies was examined. The homogenate antigens of parasites isolated from humans in Ecuador were not reactive to the specific monoclonal antibodies for L. mexicana and L. donovani complexes (data not shown). The reactivities of human isolates and reference strains for eight monoclonal antibodies, B3, B4, B7, B11, B12, B16, B18 and B19, are shown in Table 1. These monoclonal antibodies were not reactive against WHO recommended reference strains of L. d. chagasi (MHOM/BR/74/PP75), L. m. mexicana (MHOM/BZ/82/BEL21) and L. m. amazonensis (MHOM/BR/73/M2269).

Some monoclonal antibodies showed high and consistent qualitative specificity at species and/or subspecies level. The followings were the most specific: B4 and B11 for L. braziliensis panamensis (MHOM/BR/75/M2903); B19 for L. b. guyanensis (MHOM/BR/75/M4147); B7 for L. b. guyanensis and L. b. panamensis; B3 and B12 for L. b. braziliensis and L. b. panamensis. The reactive pattern of parasites isolated from humans in Ecuador was very similar to that of L. b. panamensis reference strain. From the result obtained, the present human strains (MHOM/EC/87/G05, G06 and G07) were identified as L. b. panamensis.

The reactive patterns of three strains from wild mammals for

Table 1. A comparison of the radioimmune binding assay* results and human strain isolated from Ecuador

Stock code#	Species	Monoclonal	
		B3	B4
MHOM/BR/74/PP75##	L. d. chagasi	0.9	1.0
MHOM/BZ/82/BEL21##	L. m. mexicana	1.8	0.7
MHOM/BR/73/M2269##	L. m. amazonensis	1.9	0.9
MHOM/BR/75/M4147##	L. b. guyanensis	1.2	1.3
MHOM/BR/75/M2903##	L. b. braziliensis	70.5	0.9
MHOM/PA/71/LS94##	L. b. panamensis	26.0	9.0
MHOM/EC/87/G05	L. b. panamensis	12.7	13.2
MHOM/EC/87/G06	L. b. panamensis	15.7	14.9
MHOM/EC/87/G07	L. b. panamensis	32.9	30.3

* Results shown express the ratio cpm bound monoclonal anti-

** From hybridoma clones: B3, VI-4D10-D12; B4, VI-2A5-A4; 3E6-B11; B18, XIV-2A5-A10; B19, XLIV-5A2-B9.

\# Recommended stock code: host/country of origin/year of iso-

International reference strain (WHO, 1984).

employing monoclonal antibodies with Leishmania reference strains

antibodies**					
B7	B11	B12	B16	B18	B19
0.6	1.4	1.8	0.7	1.2	1.2
1.8	0.8	1.4	1.0	0.8	1.0
1.6	1.4	0.7	1.4	0.9	0.6
11.5	0.8	1.4	1.4	1.2	16.5
2.8	1.8	30.5	27.0	26.5	2.7
10.4	7.8	13.0	1.0	1.2	2.0
14.5	12.6	8.5	1.6	2.0	3.0
5.5	10.4	8.3	1.3	2.0	3.0
39.6	27.8	10.0	1.0	0.8	2.3

bodies/cpm bound control.

B7, VI-2A4-E3; B11, VI-5G3-F3; B12, XIII-3H6-A12; B16, XIII-

lation/original code (see WHO, 1984).

Table 2. A comparison of the radioimmune binding assay* results bodies with reference strains and stocks isolated from

Stock code#	Species	Monoclonal		
		M2	M3	M4
MHOM/BR/74/PP75##	L. d. chagasi	0.4	0.4	0.5
MHOM/BR/75/M2903##	L. b. brazilienis	2.0	1.3	1.6
MHOM/PA/71/LS94##	L. b. panamensis	1.5	0.9	1.2
MHOM/BR/75/M4147##	L. b. guyanensis	1.2	1.8	0.8
MNYC/BZ/62/M379##	L. m. mexicana	1.7	1.8	1.9
MORY/PA/68/GML3##	L. m. aristidesi	16.9	0.7	5.4
MHOM/BR/75/M2269##	L. m. amazonensis	34.5	22.6	16.8
MTAM/EC/87/G04	L. m. amazonensis	33.8	12.6	9.6
MPOT/EC/87/G03	L. m. amazonensis	31.5	11.3	9.4
MSCI/EC/87/G02	L. m. amazonensis	18.1	4.4	3.0

* Results shown express the ratio cpm bound monoclonal anti-

** From hybridoma clones: M2, IX-2H7-E10; M3, IX-5H9-C10; M4, 4D8-E3; M9, XLV-2B5-H7; M11, XLV-1D11-E11; D3, LXXVIII-B19, XLIV-5A2-B9.

\# Recommended stock code: host/country of origin/year of iso-

International Leishmania reference strains (WHO, 1984).

employing New World Leishmania species specific monoclonal anti-

wild animals in Ecuador

antibodies**									
M6	M7	M8	M9	M11	D3	B11	B16	B18	B19
2.3	0.7	1.2	0.4	1.2	13.0	1.0	1.0	0.7	0.6
3.0	2.4	2.0	1.3	1.3	1.4	1.2	27.8	34.5	2.0
0.9	1.4	0.9	0.9	1.7	0.5	12.2	2.5	1.0	1.2
1.2	1.0	2.0	2.0	2.8	0.7	1.0	1.0	1.0	13.2
27.5	26.3	32.9	4.9	6.6	1.1	1.5	2.0	1.4	1.7
5.7	2.9	4.9	21.8	27.7	0.5	1.0	1.0	2.3	1.0
4.3	6.8	4.2	26.0	34.1	1.2	1.8	1.3	1.4	1.5
8.3	9.9	10.1	25.6	37.9	1.7	1.4	1.2	1.0	1.2
8.6	11.2	11.3	25.4	31.8	1.2	1.5	1.2	1.1	1.3
3.4	4.7	4.1	10.7	19.0	1.5	1.5	1.4	1.2	1.2

bodies/cpm bound control.

IX-1F9-D8; M6, LXVIII-4C7-B8; M7, LXVIII-1D7-B8; M8, LXVIII-1F2-A2; B11, VI-5G3-F3; B16, XIII-3E6-B11; B18, XIV-2A5-A10;

lation/original code (see WHO, 1984).

thirteen monoclonal antibodies, M2, M3, M4, M6, M7, M8, M9, M11, D3, B11, B16, B18 and B19, were compared to those of seven WHO recommended reference strains (Table 2). The reactive pattern of parasites from Potos flavus (MPOT/EC/87/G03) and Tamandua tetradactyla (MTAM/EC/87/G04) was similar to that of the reference strain of L. m. amazonensis (MHOM/BR/75/M2269). The reactivity of parasites from Sciurus vulgaris (MSCI/EC/87/G02) was not so strong in comparison with that from other two wild mammals, i.e., T. tetradactyla and P. flavus. The reactivity of M3, however, was positive for this isolate. Therefore, the parasite from S. vulgaris was also considered to be L. m. amazonensis, but not L. m. aristidesi.

Discussion

A variety of molecular and biochemical methods have been used to characterize and identify Leishmania parasites. Currently, isoenzyme electrophoresis (zymodeme analysis) is commonly used to identify Leishmania parasites at species and/or subspecies levels. Monoclonal antibody binding technique is also one of the new procedures developed for parasite characterization and identification (McMahon-Pratt and David, 1981). The high specificity of some of these monoclonal antibodies permits Leishmania parasite identifications, and provides evidence for the stability of intrinsic molecular characters of the parasite. Further, the results of parasite identification using monoclonal antibodies parallel to those with isoenzyme electrophoresis and kinetoplast DNA (Momen et al., 1983; Lopes et al., 1984; Grimaldi et al.,

1987).

In the current study Leishmania isolates from humans and wild mammals were characterized and identified based on the result of radioimmune binding assay employing monoclonal antibodies. Three isolates from humans were identified as L. b. panamensis. On the other hand, the parasites from three different wild mammals were identified as L. m. amazonensis.

It is known that L. b. panamensis is distributed to Central American coutries and Colombia in South America (Grimaldi et al., 1987). In this study, the parasites identified as L. b. panamensis were isolated from humans living in different areas of the Pacific coast regions in the north-western Ecuador near Colombia.

Hashiguchi et al. (1985) isolated Leishmania parasites from three species of mammals, i.e., Choloepus hoffmani didactylus, Sciurus granatensis and Potos flavus caught in Naranjal, Department of Guayas, Ecuador. For the species identification, however, these isolates are still in characterization. In this study, L. m. amazonensis was isolated from three wild mammals, T. tetradactyla, P. flavus and S. vulgaris in human leishmaniasis-endemic areas of the Pacific coast regions.

There was no great distance between the two endemic areas where the present two Leishmania species, panamensis and amazonensis, were isolated. In these regions, therefore, the two species of Leishmania might be principal causative agents of leishmaniasis.

The current study is the first trial to characterize Leishmania organisms isolated from humans and wild mammals in Ecuador.

The detailed information requires further investigations. In order to further elucidate features of causative agents of Ecuadorian leishmaniasis, assays for isolates from different endemic areas are currently in progress, using monoclonal antibodies, isoenzymes and k-DNA probes. The results will be published elsewhere.

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Chapter 5

VECTOR ENTOMOLOGY

1. Natural infections of sand flies with *Leishmania* promastigotes

Abstract. To obtain more information on the vector sand flies of the New World leishmaniasis in Ecuador, man-biting flies were collected using human bait and examined for *Leishmania* infection. A total of 1,107 anthropophilic sand flies of seven species, i.e., *Lutzomyia hartmanni*, *gomezi*, *trapidoi*, *panamensis*, *shannoni*, *serrana* and *dysponeta*, were dissected. The first two species showed positive for *Leishmania* promastigotes; *Lu. gomezi* was newly-recorded as an Ecuadorian leishmaniasis vector. In all the infected sand flies, parasite infections were principally located in the hindgut, suggesting that the organisms observed belonged to the *L. braziliensis* complex. The last species, *Lu. dysponeta*, was collected in Ecuador for the first time using human bait.

Sand flies collected from leishmaniasis-endemic areas of Ecuador have been taxonomically examined and described by several investigators (Rodriguez, 1950, 1952, 1953a,b,c, 1956; Arzube, 1960; Young and Rogers, 1984). In 1982, we started field research on the transmission of Ecuadorian leishmaniasis, with special reference to the vectors and reservoirs. Two anthropophilic species of the genus *Lutzomyia*, *Lu. trapidoi* and *hartmanni*, were found to be naturally-infected with promastigotes of *Leishmania* in endemic areas of Ecuador, and were considered as possible principal vectors of Ecuadorian leishmaniasis (Hashiguchi *et al.*, 1985a, b).

In this paper we present additional data from work performed

during 1986 and 1987, including the first record in Ecuador of a Leishmania infection in Lu. gomezi, together with further records of infected Lu. hartmanni from another endemic area.

Materials and Methods

Study sites

Sand fly examinations for natural infections with Leishmania promastigotes were made at 3 localities of the endemic areas in the Pacific coast region, i.e., Selva Alegre, Department of Esmeraldas, Pajan, Department of Manabi and Mocache, Department of Los Rios, and at 1 locality in the Andean slope, Echeandia, Department of Bolivar (Fig. 1). In all the collecting sites, capture of anthropophilic sand flies was performed using human bait, during the hours from 18:30 to 23:30. Collecting sites in the study area were selected on the basis of information from local inhabitants in each area, who knew sand flies (manta blanca in Spanish) and had experience of sand fly biting in and around their houses.

Sand fly collection

We divided ourselves into several groups of two persons each, consisting of 1 human bait and 1 collector. Human baits who agreed to participate in our study were chosen from inhabitants of each endemic area; the human bait sat with trousers rolled up to the knee, among dense vegetation, while the collector aspirated the sand flies using a capture tube, as soon as they alit on the former's skin (Plate 1A). Sand flies were kept in the tube and taken back to the laboratory for dissection (Plate 1B).

Figure 1. Outline map of the Republic of Ecuador, showing 4 study sites, 1. Selva Aregre, Department of Esmeraldas, 2. Pajan, Department of Manabi, 3. Mocache, Department of Los Rios and 4. Echeandia, Department of Bolivar, and also showing two main cities, Q. Quito, the capital of the country, and G. Guayaquil where the Instituto Nacional de Higiene y Medicina Tropical is situated and all the laboratory works was carried out there.

Dissection of sand flies

All the sand flies were dissected using the method reported previously by Hashiguchi et al. (1985a). The gut of each sand fly was isolated and placed on a slide (Plate 1C), and covered with a cover slip, and then examined microscopically using x400 and x1,000 magnifications. Spermathecae and cibarial armatures of the sand flies dissected were also observed for species identification. Positive flies with promastigotes (Plate 1D) were separated and their gut contents were inoculated into golden hamsters.

Results

A total of 7 man-biting species of sand flies, i.e., Lu. trapidoi, panamensis, hartmanni, gomezi, shannoni, serrana and dysponeta, were captured, the last of which had not previously been captured by us using human bait. The results of the sand fly dissections are shown in Table 1. Among 1,107 sand flies dissected, 2 (0.7%) of the 305 Lu. gomezi and 3 (1.9%) of the 161 Lu. hartmanni, revealed positive for promastigotes (specimens obtained in Mocache, Los Rios and Echeandia, Bolivar, respectively). Parasites had not been found in Lu. gomezi from Ecuadorian leishmaniasis-endemic areas prior to this study. Parasites were mainly observed in the hindgut of all infected sand flies.

Discussion

In our previous study, we found natural infections of Leishmania promastigotes in Lu. trapidoi and Lu. hartmanni and incri-

Explanations for the Plate

Plate 1. Fly collections and dissections. A, a pair of sand fly collection team consisting of two persons, 1 human bait (right: volunteer) and 1 collector (left: Mr. Roberto Sud, an experienced technician). B, a sand fly kept in collecting tube. C. Sand fly gut isolated: c, cardia; mg, midgut; s, stomach; ht, hind triangle; hg, hindgut; Mt, Malpighian tubules. D. promastigotes (arrows) in hindgut of a sandfly collected.

See illustration page, 75

Plate 1

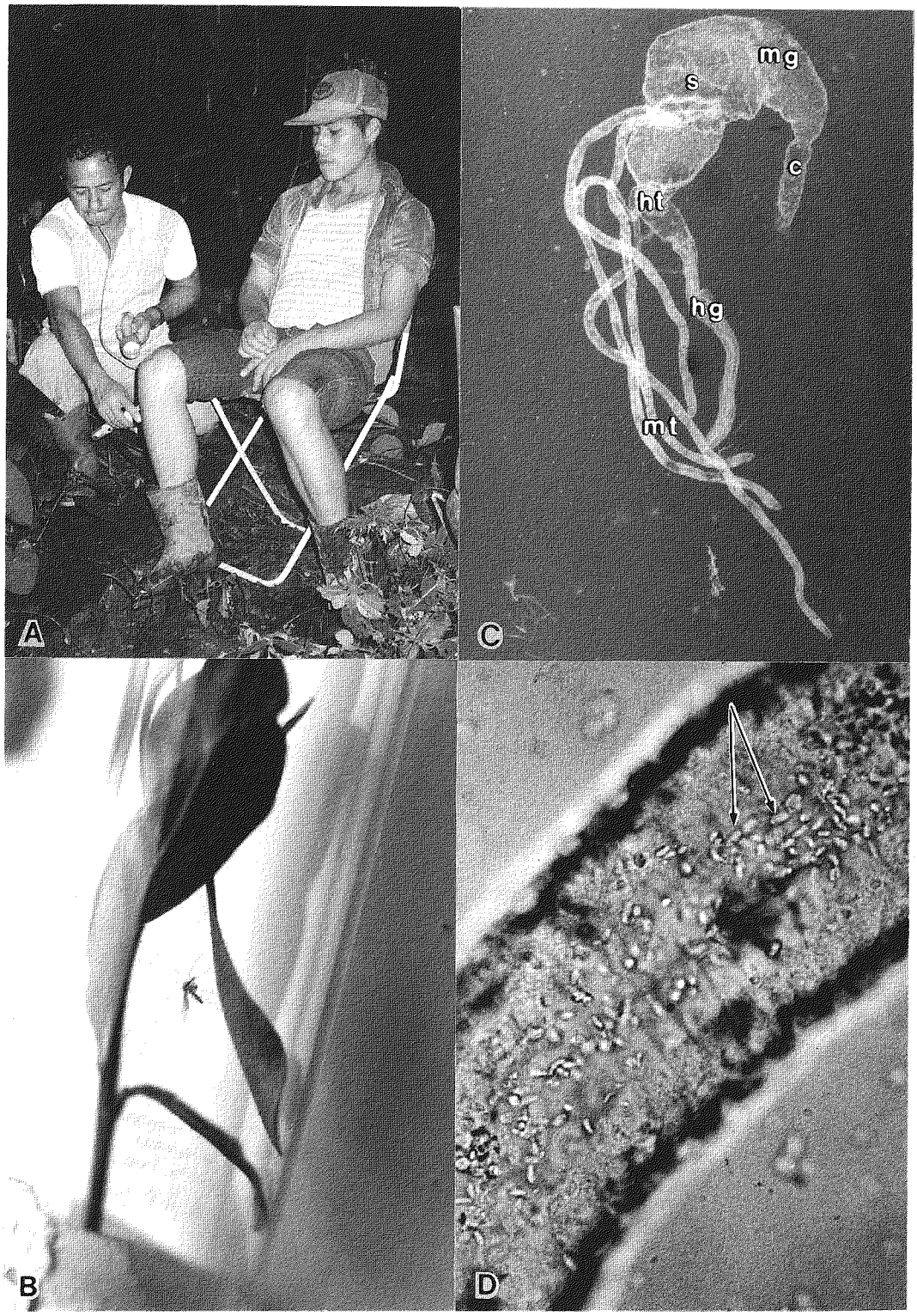

Table 1. Natural infections with leishmanial promastigotes in 7 man-biting sand fly species collected in 4 different endemic areas of Ecuador

Locality	Altitudes (m)*	Sand fly species	No. dissected	No. positives (%)
S.Alegre	70	Lu. trapidoi	52	0
		Lu. panamensis	59	0
		Lu. hartmanni	85	0
Pajan	20	Lu. gomezi	23	0
		Lu. shannoni	18	0
		Lu. serrana	8	0
Mocache	12	Lu. gomezi	305	2 (0.7)
		Lu. trapidoi	60	0
Echeandia	600	Lu. trapidoi	276	0
		Lu. hartmanni	161	3 (1.9)
		Lu. dysponeta	60	0
Total			1,107	5 (0.5)

* Altitudes above sea level.

minated these two species as the probable main vectors of leishmaniasis in Ecuador (Hashiguchi et al., 1985a). In this study, more specimens of Lu. hartmanni were found to be positive for promastigotes, together with one other species, Lu. gomezi in leishmaniasis-endemic areas of Ecuador. No infections have been found to date in three other man-biting species present in our study area, i.e., Lu. panamensis, Lu. shannoni and Lu. serrana. Species identification of the promastigotes isolated awaits the results of hamster inoculations and isoenzyme characterization. However, it is likely that the parasite belonged to the Leishmania braziliensis complex, from its biological characteristics, such as prevalence of hindgut infections. In the present study, Lu. dysponeta was added to our list of proven Ecuadorian anthropophilic sand flies. The Ecuadorian sand fly fauna contains several species of Lutzomyia which are proven or suspected vectors of leishmaniasis in other countries (see Chapter 2); no information is available on the anthropophilic behaviour of Lu. dysponeta and further study of this species is necessary.

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2. A Laboratory Trial of Sand Fly Rearing in Ecuador

Abstract. A laboratory colony of the sand fly Lu. trapidoi was established, because of the importance of this species in the transmission of leishmaniasis in Ecuador. A large number of wild-caught female flies obtained from human bait collections was transported to the laboratory in special containers. Male insects captured from diurnal resting sites were also used for establishment of the laboratory colony. Both males and females were placed in a special cage for blood engorgement and copulation. The gravid females were kept in rearing bottles inside insulated polystyrene containers. Following oviposition by 50 of the females, eggs were left in the same containers to allow larvae to hatch and continue development. Larvae were fed with powdered, dehydrated rabbit feces. Numbers of insects were monitored at each developmental stage, and a total of 1,022 eggs, 706 larvae, 510 pupae and 498 adults were obtained from this first trial.

In any insect-transmitted disease, eradication or control activities depend on detailed knowledge of the transmission process, requiring information of aspects such as the ecology and physiology of the vector species. Appropriate control measures may then be selected based on the information on the most easily-controlled parts of the vector's life cycle.

Phlebotomine sand flies of the genus Lutzomyia (DIPTERA: PSYCHODIDAE) are among the most important vectors of tropical arthropozoonoses. These insects transmit American leishmaniasis, which is widely distributed in Ecuador; the first human case was described in 1920 by Valenzuela (Rodriguez, 1974). We recently began to investigate transmission of the disease, and incriminated two Lutzomyia species for the first time as vectors of Leishmania (Hashiguchi et al., 1985a) in Ecuador. We also studied several aspects of their man-biting behavior in the endemic area (Hashiguchi et al., 1985b).

In intensive investigations of leishmaniasis epidemiologiy it is necessary to understand certain details of the biology of phlebotomine sand flies, particularly their ecology, physiology, genetic characteristics and vector capacity. Information on these aspects can be used in the planning of future control measures. It is nevertheless difficult to study these features in the natural habitat of the insects. The alternative is to develop artificial rearing of sand flies in the laboratory, providing generations of non-infected insects which can be manipulated for experimental studies.

Hertig and Johnson (1961), Johnson and Hertig (1961) and Hertig (1964) reared several New World sand fly species in the laboratory. The WHO has stressed the importance of establishing laboratory colonies for sand flies and disseminating information on rearing methods (WHO, 1977). Killick - Kendrick (1978), Beach et al. (1983), Young et al. (1981) and Endris et al. (1982) have modified artificial rearing methods for production of various species of sand flies in the laboratory. Our present technique for sand fly rearing involves minor modifications, pertinent to the biological requirements of Ecuadorian Lutzomyia species.

Materials and Methods

Feeding cages

The feeding cage consisted of a plastic packing box, of dimensions 56 x 36 x 28 cm; the original lid was replaced by a transparent plexiglass panel which formed the front face of the cage. Two holes (12 cm each in diameter) were made in the lowest

third of the panel, and gauze sleeves fitted to allow access to the sand flies within the cage and prevent their escape. A ventilation window of 35 x 10 cm was made in the upper third of the panel and covered with gauze.

The floor and inside back wall of the cage were covered with a layer of plaster of Paris (1 cm in depth), which allowed humidity to be maintained and provided a resting surface for the insects. On both side walls, small holes (2 cm) with cross-cut latex diaphragms (made from surgical gloves) were also made to allow introduction of aspirators for collection of insects. To observe sand flies in darkness, we used a fluorescent lamp (cold light) covered with a red acrylic panel (Fig. 1A). Our field research experience suggests that sand flies cannot see red light and behave as if they were in complete darkness when illuminated by a lamp of this kind. Observations of natural behavior can therefore be made during the night.

Capture tubes

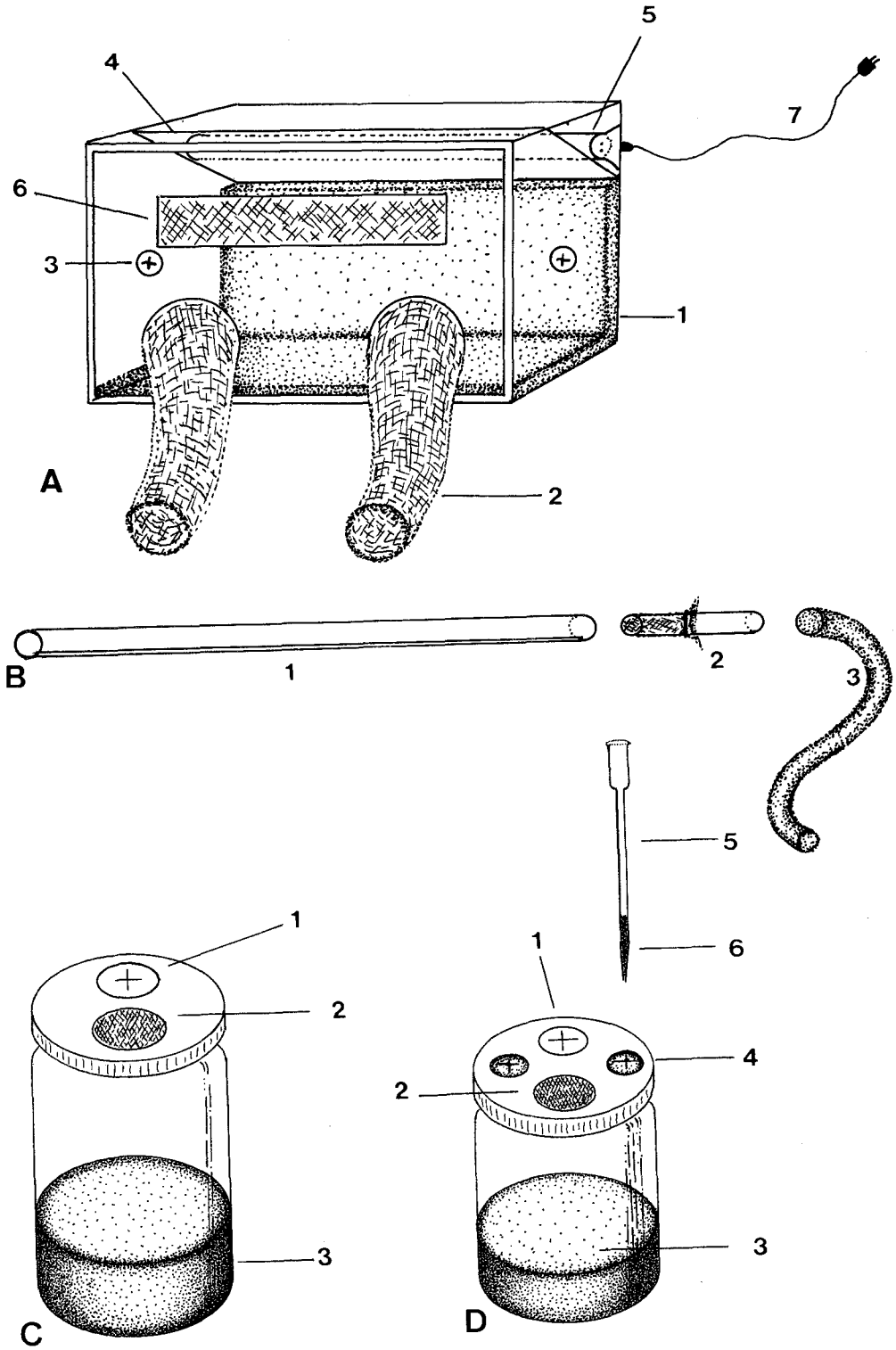
The capture tube consists of a glass tube (8 mm in diameter) and a rubber tube (50 cm long) with a gauze protector at the joint to prevent passing of the insects into the collector's mouth. The glass tube should be longer than 20 cm, so that insects can be kept in the tube for a few seconds without suction. It is necessary to have two types of capture tubes, one for human bait collections, the other for collection inside of the feeding cage. All capture tubes should be kept completely clean and dry (Fig. 1B).

Transporting (carrying) bottles

Explanations for the Figure

Figure 1. A: Feeding cage for sand fly rearing. 1, plaster of Paris layer (1 cm in depth); 2, gauze sleeves for handling; 3, diaphragms made of latex surgical gloves, to allow introduction of suction tube; 4, red acrylic panel; 5, fluorescent lamp (cold light); 6, ventilation window covered with gauze; 7, connector. B: Capture tube for sand fly collection or handling. 1, glass tube; 2, gauze protector to prevent passage of sand flies; 3. rubber tube. C: Transporting (carrying) bottle. 1, latex cross-cut diaphragm to allow introduction of capture tube; 2, ventilation window covered with gauze; 3, wet plaster of Paris. D: Rearing bottle. 1, latex cross-cut diaphragm to allow introduction of capture tube; 2, ventilation window covered with gauze; 3, wet plaster of Paris; 4, latex cross-cut diaphragm to allow introduction of a feeding pipette; 5, feeding pipette; 6, water-honey solution (1:1) as sugar source.

See illustrations page, 83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These are wide-mouthed transparent plastic (polystyrene) bottles of capacity 400 ml, with a plastic cap. Wet plaster of Paris is placed in the bottom and left to dry for 24 hours. Two holes (1.5 cm in diameter) are cut into the cap, to be covered with gauze and latex cross-cut diaphragms respectively. To make the diaphragm a piece of a latex surgical glove is cut and glued inside and outside one of the holes. Both pieces of latex should be cross-cut to ensure adequate sealing against sand fly escape. The capture tube passes through this hole to release flies into the bottle. The other hole is covered with gauze and serves as a ventilation window for the flies (Fig. 1C).

Rearing bottles

The rearing bottle is similar to the carrying bottle with a minor difference; it has a capacity of 300 ml and has two extra holes (2 mm in diameter), for insertion of feeding pipettes. The base should be covered with plaster of Paris, as in the other bottles (Fig. 1D).

Thermic boxes

Any polystyrene container, for example those used for storing food and drink on camping trips, will serve as a thermic box to hold transporting and rearing bottles in the field.

Results

Preliminary observations on laboratory rearing of sand flies

In order to rear sand flies in the laboratory, a stock of wild-caught female sand flies must be obtained. In our study we decided to colonize Lutzomyia trapidoi, which is probably the

main vector of leishmaniasis in Ecuador (Hashiguchi et al., 1985a). From past experiences on leishmaniasis research in Ecuador, we knew this species to be prevalent in the humid forest of Ocaña, Department of Cañar, an Andean province of Ecuador (350 m above sea level) and our colony was founded from specimens obtained at this site.

Only female Lu. trapidoi bite men and wild animals to obtain blood, a necessary protein source for oviposition. We therefore used human bait to capture the sand flies and obtained a large number of them in a few hours of collecting (Plate 1A). In order to capture female sand flies, the collector had to wait until they alit on the skin of human bait; they were then gently aspirated and introduced from the capture tube into the transporting bottle. The plaster of Paris in the base of the bottle had been wetted a few hours earlier with 6 to 7 drops of fresh water to maintain an adequate humid atmosphere.

Fifty to one hundred sand flies could be easily kept in a single transporting bottle. Bottles containing flies were kept in a thermic box with a damp cloth to maintain the constant humidity necessary for survival of the insects; this has to be about 80%. A number of male Lu. trapidoi, were also collected at the same site from their resting sites in the surrounding forest. Males collected were kept in transporting bottles and placed in the thermic box. It was necessary to obtain male insects because female sand flies may copulate before or after a blood meal, prior to oviposition. A total of 150 female and 15 male sand flies were taken back to the laboratory in Guayaquil and used to

found the colony.

Immediately an arrival at the laboratory, transporting bottles were opened inside the feeding cage, the plaster of Paris of which had been moistened with several drops of fresh water spread on the surface of the floor and back wall of the cage. A hamster was then anesthetized with Ketalar (0.5 ml of ketamine hydrochloride) and presented to female sand flies as a blood source (Plate 1B). Apple slices were also placed in the cage as a sugar source for both males and females. The hamster was laid on its back with legs up to expose them better to the sand flies, since the feet and other hairless extremities are easiest for flies to feed on. The red light inside the cage had to be turned on to allow the observation of copulating and feeding behavior of sand flies, without disturbing either activity. Photographs can also be taken during the observation period.

It was advisable to leave the flies to rest in the feeding cage for 2 or 3 hours before and after the blood meal, since some females (Plate 1C) copulate before engorgement, and others afterward. The copulation pattern of 150 females is shown in Table 1. Sugar also had to be provided before and after copulation and engorgement. For this purpose, some small pieces of apple were placed inside the cage.

Each gravid female was captured using the suction tubes and placed in a rearing bottle until oviposition occurred. Plastic pipettes were introduced through the hole cut for this purpose in the cap of each rearing bottle, and filled with 1:1 solution of water and honey (Fig. 1D-6). The water-honey solution provided

Table 1. Copulation (Cp) pattern of 150 female Lu. trapidoi* in the feeding cage, in relation to sugar meal (Sm) and blood meal (Bm) intake (7 hours observation)

Order of activity**			Approximate %
1	2	3	
Sm	Cp	Bm	25
Sm	Bm	Cp	13
Cp	Bm	Sm	50
Cp	Sm	Bm	10
Bm	Cp	Sm	2
Bm	Sm	Cp	0

* All the sand flies were collected before engorgement, using human bait in nature.

** The order of activity could be dependent on circumstances such as the order of sand fly contact with a hamster (blood meal) and apple pieces (sugar meal) in the present small-sized feeding cage, rather than on choice.

the flies with sugar which was indispensable for oviposition. In nature, female sand flies presumably take sugar from wild plants or insect honeydew, before or after engorgement of blood meal, before depositing their eggs. Males appear to subsist on sugar alone. Most female sand flies died after the first ovipositon, a frequent problem in sand fly rearing in the laboratory (Kapur and Mutinga, 1981; Mutinga and Odhiambo, 1986). A few survivors of the oviposition were reintroduced into the feeding cage to engorge again on hamster blood and to copulate with males, that were continuously kept in the cage and fed on apple slices. Dead flies which had oviposited were removed daily.

Eggs were left in the rearing bottles until the larvae emerged. Plaster of Paris in the bottle had been previously moistened with some drops of fresh water. Bottles were checked every day to keep humidity levels and to determine hatching time of the eggs. After hatching, larvae were counted and fed on powdered dried rabbit feces, a small amount of which was spread on the surface of plaster of Paris as soon as larvae were detected (Endris et al., 1982). Fungus which grew in the humid conditions of the rearing bottle also served as larval food.

Although a considerable number of larvae died, before pupation, most reached this stage, and all rearing bottles which contained pupae were placed in the feeding cage without the caps, allowing newly-emerged sand flies to escape from the bottle and pass into the feeding cage, where they could be fed on apples and allowed to engorge on a hamster when ready. Rearing bottles containing gravid females and subsequently eggs should be kept at

all times in the thermic box and provided with suitable humidity (80%), obtained by placing wet paper or cloth into the box. Similarly, the plaster of Paris in the feeding cage must be checked daily to maintain suitable humidity. Some drops of water should be added if necessary. Checking of the rearing bottle was always done under a stereomicroscope to observe the presence of eggs, larvae and pupae, as well as fungi and other surface contaminants of the plaster of Paris.

Observations on the 1st generation of laboratory reared flies

Fifty of the field-captured females were chosen for this purpose. As soon as these had engorged with hamster blood, and been allowed to rest or copulate in the feeding cage for 2 to 3 hours, the females were removed and placed into individual rearing bottles. Gravid females (Plate 2A) began ovipositing 4 or 5 days after feeding on blood (Plate 2B,C), and all had oviposited by the end of the sixth day, producing a total of 1,022 eggs, distributed among the 50 rearing bottles. Plaster of Paris in the bottles was moistened again and eggs allowed to incubate, the bottles being placed inside the thermic box. Most eggs hatched 15 days after oviposition. A total of 706 1st instar larvae were obtained, measuring an average of 250 μ in length (N = 200) (Plate 2D). Larvae molted 3 times before pupation, and 510 pupae were obtained 20 days after hatching. Many pupae lay on the surface of the plaster of Paris, while others were affixed to the walls and under the cap of the bottles (Plate 2E). About 6 weeks after oviposition, 498 F_1 adults eclosed in the rearing bottles. This represented the first generation of laboratory-reared sand

flies in Ecuador (Plate 2F). The recently-eclosed adults fed immediately on apple pieces left in the feeding cage.

Comments

A ready supply of insects free of the infection studied is indispensable to any research program on the transmission mechanisms of an arthropozoonosis. Such insects can be used in carefully-controlled transmission studies in the laboratory, elucidating features of the disease which might be inapparent in the natural situation, and aiding in the formulation of control programs.

No laboratory colonies of Ecuadorian sand flies have been available to date. Our current success in sand fly rearing in the laboratory could provide important information on leishmaniasis transmission in Ecuador. Laboratory colonies of sand flies could be used to study the following points; 1) vector potential, 2) life cycle, 3) physiology, 4) experimental infection with different strains of Leishmania, 5) biting and transmission index, 6) genetic studies, 7) insecticide resistance, 8) relationship of sand flies with other micro-organisms, 9) biological control studies.

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Explanations for the Plates

Plate 1. A: A sand fly (arrow) on the skin of human bait. B: A hamster (arrow) inside the feeding box, anaesthetized with Ketalar for sand fly feeding (blood meal). C: A sand fly (arrow), engorging on leg of a hamster.

Plate 2. A: A laboratory-mated and gravid sand fly. B: A gravid sand fly in the act of depositing an egg. C: Deposited eggs observed under stereo-microscope. D: A first instar larva with two very long bristles at the posterior end, moving on the plaster of Paris in the rearing bottle. E: A pupa on the plaster of Paris in the rearing bottle. F: Adult of the 1st generation of a laboratory-reared sand fly.

See illustrations pages, 93-94

Plate 1

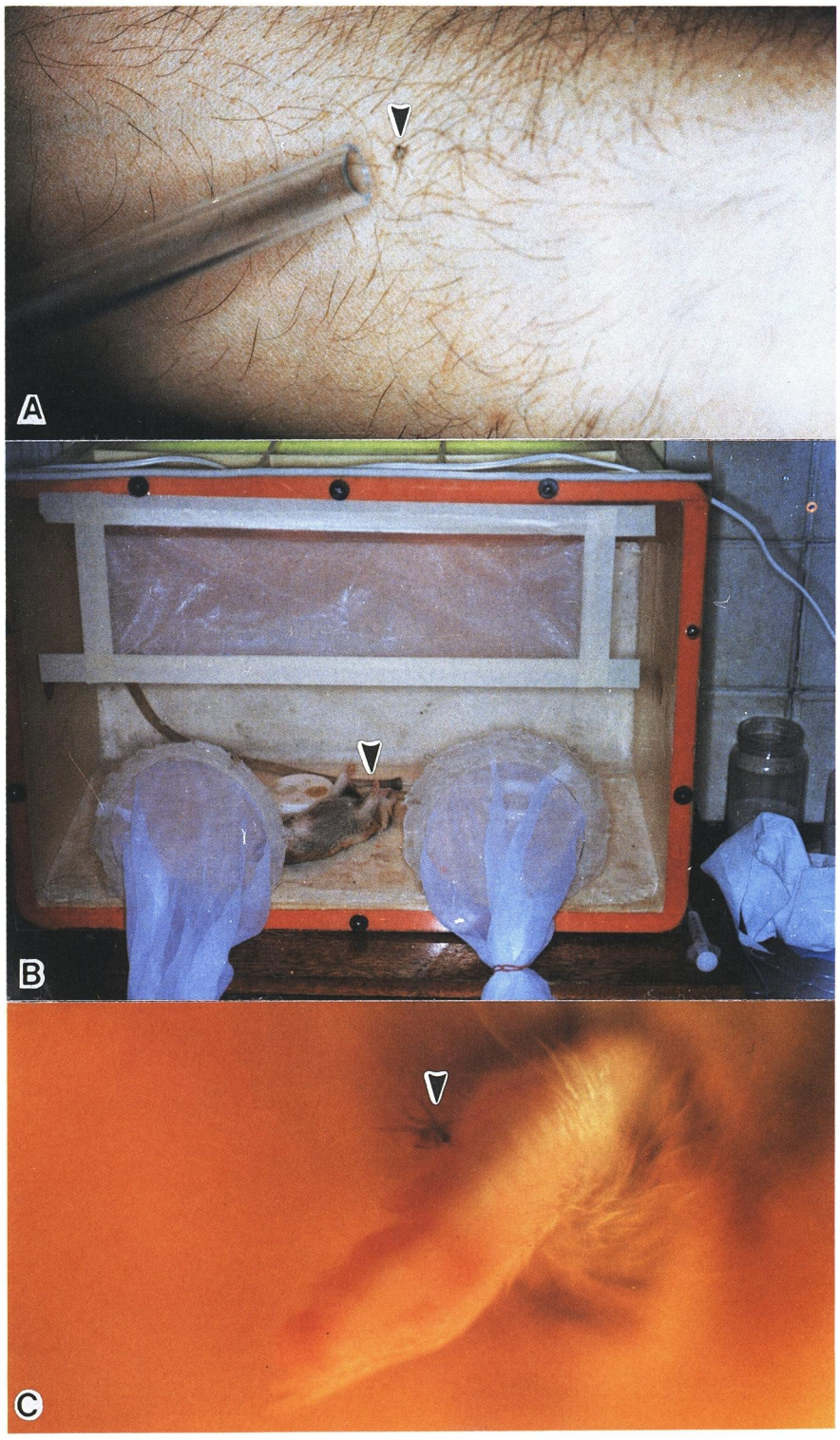
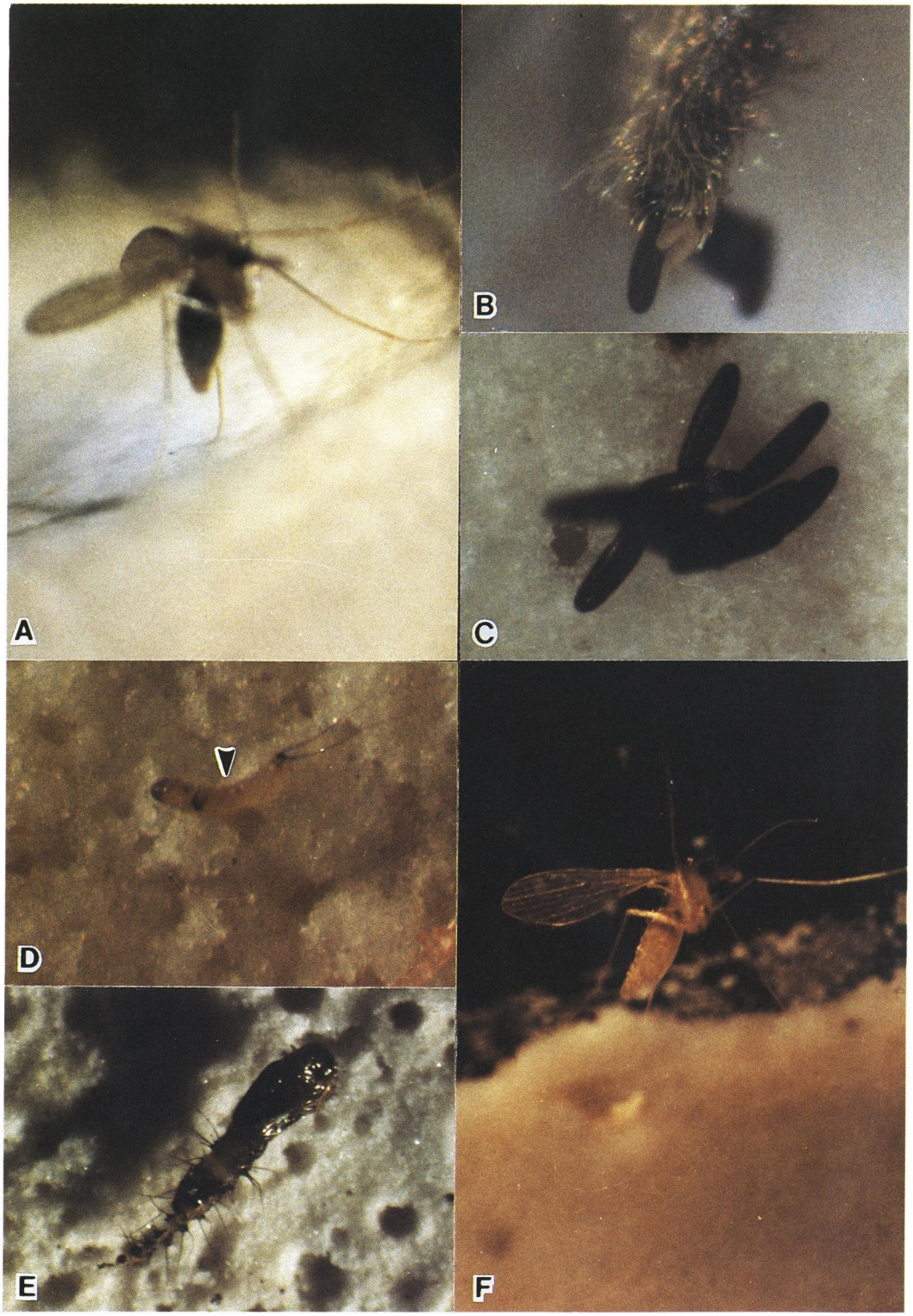


Plate 2



Chapter 6

IMMUNOLOGY

1. Evaluation of skin test and ELISA in the screening of leishmaniasis

Abstract. The present study was designed to evaluate skin test and ELISA as diagnostic tools in the screening of Ecuadorian cutaneous and mucocutaneous leishmaniasis. The antigen for skin test was prepared from ruptured promastigotes of Leishmania braziliensis. A preliminary trial of skin testing using this antigen was conducted on 63 subjects with active dermal lesions, together with ELISA. The skin test and ELISA positive rates were significantly high in the parasite (amastigote) positive cases, demonstrating high sensitivity and specificity against leishmaniasis patients. An epidemiological survey in Selva Alegre, Esmeraldas, revealed that among 115 inhabitants 38 (10 active and 28 healed cases) were positive for the clinical signs; of these subjects 33 (86.8%) showed positive reactions against skin test and/or ELISA. The present skin test could detect leishmanial patients with lesions on the usually unexposed areas of the body. It was otherwise difficult to find such patients, especially among younger school children by routine physical examination and interview. Based on the results obtained, therefore, we concluded that the present skin testing antigen and ELISA were very useful for the screening of leishmaniasis in the endemic areas of Ecuador.

American cutaneous or mucocutaneous leishmaniasis is known to occur in most provinces of Ecuador. Little information has however been available on the epidemiological features of the disease in the country (Hashiguchi et al., 1984). This dearth of epidemiological information has partly been due to the lack of a reliable diagnostic tool in field studies. Recently, Reed et al. (1986) reported that a soluble leishmanial antigen prepared from ruptured Leishmania donovani chagasi promastigotes was highly

sensitive and specific for the diagnosis of American visceral leishmaniasis in patients. In order to obtain a better understanding of the epidemiology of leishmaniasis in Ecuador we therefore designed a preliminary examination procedure to evaluate a similarly-prepared skin testing antigen. For comparative purposes enzyme-linked immunosorbent assay (ELISA) was also performed on the subjects examined. The present paper deals with the finding that the soluble antigen prepared from L. braziliensis promastigotes can be readily employed in skin testing for the screening of cutaneous and mucocutaneous leishmaniasis in the endemic areas of Ecuador.

Materials and Methods

Study areas and subjects

The present study was carried out during July and August (dry season) of 1986 in Ecuador. Preliminary examinations using skin test and ELISA were made on 63 subjects with active dermal lesions who visited the Instituto Nacional de Higiene y Medicina Tropical (INHMT), Guayaquil, and rural health centers and hospitals in several endemic areas of Ecuador. An epidemiological survey was conducted on 115 inhabitants in Selva Alegre, Esmeraldas, Ecuador, by performing skin tests and ELISA. Thirty-four school children in Gramalote Chico, Los Rios, were also examined by skin testing alone. Thus a total of 212 subjects were tested by leishmanial skin test and/or ELISA in this study.

ELISA

L. b. braziliensis (MHOM/BR/75/M 2904) was cultured with the

medium described by Pan (1984). The harvested promastigotes were washed five times with a balanced salt solution, and were ruptured by ultrasonic treatment in 0.05M carbonate bicarbonate buffer (pH 9.6). The homogenate was centrifuged at 10,000 x g for 30 min at 4 C. The supernatant was used as antigen. The ELISA procedure was done according to the method described by Mimori et al.(1987). All the serum samples tested were diluted 1:10. Spectrophotometer absorbance values of more than 0.25 at 500 nm were employed as criteria for evaluating positive serum, since the absorbances in tested control sera from 66 healthy individuals in Ecuador were always less than 0.25.

Skin test

Principally, a soluble antigen used for skin testing in this study was prepared by the method of Reed et al. (1986). Leishmanial promastigotes were harvested and washed five times with balanced salt solution. After the final washing the parasite-containing pellet was resuspended in 5 volumes of distilled water, and a freeze-thawing procedure with acetone-dry ice and tepid water was repeated 10 times. The disrupted parasites were diluted in PBS and centrifuged at 10,000 x g for 30 min at 4 C. The supernatant was adjusted to 25 μg protein concentration per ml before filtration through sterile 0.45 μ millipore filter. The antigen solution was injected intradermally in 0.1 ml in the flexor surface of the forearm. Induration size of more than 5 mm at the site 48 hours after injection was considered to be a positive reaction based on the criteria employed by Reed et al. (1986).

Smear specimens

Smear samples were taken from the margins of ulcerated lesions, and then stained with Giemsa or Wright solutions.

Culture of lesion aspirates or biopsy materials

Saline aspirates or biopsies from cutaneous and mucocutaneous lesions were cultured in Pan's medium. The culture materials were taken with a 27-gauge needle method (Hendricks and Wright, 1979), and/or a Holth-type sclerocorneal punch.

Results

As a preliminary examination, an evaluation of skin test (ST) and ELISA was made on 63 subjects with active dermal lesions who visited INHMT, rural health centers and hospitals in Ecuador (Table 1). Of these, 45.5% (30/63) were positive for leishmanial amastigotes in smear specimens. The skin test and ELISA positive rates were significantly high in the parasite positive group compared with the parasite negative one ($p<0.1$ in ST, $p<0.05$ in ELISA, and $p<0.05$ in the both tests). However, a relatively high positive rate for skin test (73.7%) and ELISA (75.9%) was observed in the parasite negatives. This high rate suggests that a considerable number of true leishmanial patients may be included in those parasite negatives. Nineteen (70.4%) out of 27 subjects examined for both the skin test and ELISA, proved to show positive reaction against the two tests. Such data indicate that the present skin test and ELISA are very suitable as immunodiagnostic tools in the screening of Ecuadorian leishmaniasis.

Using these diagnostic tools, an epidemiological survey was

Table 1. Results of a preliminary evaluation of skin test and ELISA in 63 subjects with active cutaneous (leishmanial) lesions in Ecuador

Smear specimens	No. examined	% positive rates per examinees*		
		skin test	ELISA	skin test and ELISA
+	30	93.3 (14/15)	92.3 (24/26)	90.9 (10/11)
-	33	73.7 (14/19)	75.9 (22/29)	56.3 (9/16)
Total	63	82.4 (28/34)	83.6 (46/55)	70.4 (19/27)

* Examinees were not the same numbers in each test, mainly because of follow-up difficulties.

Table 2. Subjects with cutaneous leishmanial signs in Selva Alegre, Esmeraldas, Ecuador

Sex	No. examined	No. with leishmanial signs			
		active	healed	total	%
Male	46	7	14	21	45.7
Female	69	3	14	17	24.6
Total (%)	115	10	28	38	(33.0)

conducted in Selva Alegre, Esmeraldas (Table 2). Of 115 subjects examined dermatologically for leishmanial ulcers, nodules and scars, 10 active and 24 of 28 healed cases were discovered from physical examinations and interviews prior to the skin testing. The remaining 4 of the healed cases were detected by re-examination after these had shown skin test-positive results. These infections had not been noticed from the original physical examinations. All the leishmanial scars in healed cases were observed on the usually-unexposed areas of the body. The survey was repeated in another endemic area, Gramalote Chico, Los Rios, and typical leishmanial scars were found and confirmed as such in six out of eight skin test positives among 34 examinees.

In Selva Alegre, a parasitological examination was done on seven out of 10 subjects with active dermal lesions. Only one patient tested positive for leishmanial amastigotes, and he also showed positive responses to skin test and ELISA. The skin test and ELISA positive rates among these subjects were 80.0% (8/10) and 66.7% (6/9) respectively. In the subjects with healed dermal lesions, the positive skin test and ELISA rates were 79.2% (19/24) and 71.4% (20/28) respectively. Correlation between clinical signs and immunodiagnostic results is shown in Table 3. Twenty subjects positive for both tests had either active or healed lesions. It was found that five subjects with those signs were negative for both the skin test and ELISA, while eight ones without active or healed lesions were positive for one of the two tests. The results for 105 subjects from Selva Alegre, 33 with and 72 without active or healed lesions, who received both skin

Table 3. Correlation between clinical diagnosis and immunodiagnosis in 115 inhabitants in Selva Alegre, Esmeraldas, Ecuador, using skin test and ELISA

Reaction to		No. with signs			No. without signs	Total
skin test	ELISA	active	healed	total		
+	+	5	15	20		20
+	-	2	4	6	2	8
-	+	1	1	2	6	8
-	-	1	4	5	64	69
+	ND*	1		1		1
-	ND				4	4
ND	+		4	4		4
ND	-				1	1
Total		10	28	38	77	115

* Not done.

tests and ELISA were as follows: both positive, 19.0%; both negative, 65.7%; and positive against one of the tests, 7.5%.

Frequency distribution of the induration size of skin test in the subjects with active or healed dermal lesions is shown in Table 4. Average induration size was 15.7 ± 7.7 in active cases and 20.0 ± 5.4 in healed ones. The size was significantly different between both cases ($p<0.05$), showing a strong skin test reaction in the healed one. No significant difference was found in the induration size between the patients with active and healed cutaneous lesions, when the size was arranged by sex or age.

Table 5 summarizes the results of clinical, parasitological and immunological examinations of 212 examinees in the current study. Coincidence rates between the both tests among 59 subjects who had clinical signs and received the two tests were as follows: positive and negative for the two tests, 66.1% (39/59) and 8.5% (5/59), respectively; and positive against one of the two, 13.5% (8/59) in skin test and 11.9% (7/59) in ELISA. Thus, with a few exceptions a close agreement between dermal clinical signs and immunodiagnosis was seen.

Discussion

The present study was carried out to evaluate two immunodiagnostic tools, skin test and ELISA, in the screening of cutaneous leishmaniasis in the endemic areas of Ecuador. In the epidemiological survey in endemic areas, a presumptive diagnosis is usually made on the basis of clinical diagnosis and immunodia-

Table 4. Frequency distribution of induration size of leishmanial skin test in subjects with active and healed cutaneous (leishmanial) lesions

Induration size (mm)	Total no. examined	Active lesions		Healed lesions	
		No.	%	No.	%
<5	1	1	2.7		
5-10	11	9	24.3	2	8.0
11-15	10	10	27.0		
16-20	18	7	18.9	11	44.0
21-25	16	6	16.2	10	40.0
26-30	2	2	5.4		
>31	4	2	5.4	2	8.0

Table 5. Summary of the results of clinical, parasitological and immunological examinations in 212 subjects in Ecuador

Reaction to		No. with signs					No. without signs	Total
skin test	ELISA	active			healed	total		
		smear specimens						
		+	-	ND*				
+	+	11	12	1	15	39		39
+	-		4		4	8	2	10
-	+	1	5	1		7	6	13
-	-			1	4	5	64	69
Sub-total		12	21	3	23	59	72	131
+	ND	4	4		6	14	2	16
ND	+	13	8		6	27		27
-	ND						30	30
ND	-	2	5			7	1	8
Sub-total		19	17		12	48	33	81
Total		31	38	3	35	107	105	212

* Not done.

gnoses, Montenegro skin test and ELISA, in addition to parasitological diagnosis from the lesions (Mayrink et al., 1979; Werner and Barreto, 1981). A definitive diagnosis of cutaneous or mucocutaneous leishmaniasis requires demonstration of the ethiological agent from the lesion materials. However, the visual detection of protozoans in tissue samples and the isolation of parasites from active lesions by culture methods are often difficult (Weigle et al., 1987). Weigle et al. (1987) recommended two diagnostic procedures based on their studies, i.e., dermal scraping smears for immediate diagnosis, and the taking of aspirates for a definitive parasitological diagnosis of cutaneous lesions. In the present survey, however, parasite positives were observed in only 30 (47.6%) of 63 subjects demonstrating active lesions by these methods (Table 1). Difficulty was also encountered in isolating parasites from the lesions.

In this study, the skin test positive rate was 81.8% (36/44) for examinees with active lesions (Table 5). In the parasite-positives cases, furthermore, all but one of the subjects proved to be positive for the skin test. On the other hand, the positive rate in subjects without clinical signs was only 3.8% (4/104). It is well recognized that the immunological methods, especially skin test and ELISA, are useful tools for diagnosis of New World cutaneous leishmaniasis (Bray, 1980). The reliability of these tests has however been questioned, mainly due to the problem of cross-reactivity with various other species within the family Trypanosomatidae, as well as with other microorganisms such as mycobacteria (Mauel and Behin, 1982). We have no data

with regard to cross reactivity of the present L. braziliensis antigen preparation. Reed et al. (1986), however, reported that their similarly-prepared L. donovani chagasi soluble antigen produced no positive responses in either normal test controls, tuberculosis and schistosomiasis patients, and less than 5% positive responses in persons with Chagas' disease. From their information and our results, it is assumed that the present soluble antigen also shows a lesser degree of cross reactivity.

Diagnosis using skin test and ELISA is commonly used against New World cutaneous leishmaniasis. However, the standardization of skin test antigen has not yet been done sufficiently to have a satisfactory result. In this paper, we evaluated the soluble extract obtained by the method of Reed et al. (1986) from ruptured L. braziliensis promastigotes, as a skin test antigen. Results obtained showed that the extract was highly sensitive and specific against Ecuadorian cutaneous and mucocutaneous leishmaniasis patients. It was concluded that use of the present skin test, together with ELISA, would be useful in diagnosis for patients with active and healed leishmanial lesions in the areas endemic for the disease.

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2. Examination of leishmaniasis reservoir hosts using counter immunoelectrophoresis

Abstract. The present examination was designed to detect Leishmania antigens in the tissues of wild mammals caught in the areas endemic for leishmaniasis in Ecuador. The antigen demonstration was performed by counter immunoelectrophoresis (CIE). Precipitin lines were observed between anti-L. braziliensis serum and liver extract (antigen) obtained from 3 Didelphis marsupialis, 2 Caluromys lanatus and 1 Choloepus hoffmani didactylus. Precipitin lines were also detected between the antiserum and spleen extracts of one specimen of C. lanatus which had yielded a positive CIE test of liver extract. These three species of arboreal mammals were considered probable reservoir hosts of leishmaniasis in Ecuador; D. marsupialis and C. lanatus, were recorded as reservoir hosts of the disease in the country for the first time.

In the field of parasitology, counter immunoelectrophoresis (CIE) has been mainly used in the detection of antibodies associated with a variety of infectious diseases, such as leishmaniasis (Abdalla, 1977), amebiasis (Krupp, 1974), malaria (Bidwell and Voller, 1975), trypanosomiasis (Aguilar-Torres et al., 1976), hydatid disease (Pinon, 1976), and schistosomiasis (Hwu et al., 1978). The technique has also been extensively used for the detection of hepatitis-associated antigens. The present study was designed to evaluate the CIE in the detection of leishmanial antigens in tissues obtained from wild-caught mammals in leishmaniasis-endemic areas of Ecuador.

Materials and Methods

Antisera

In order to obtain a hyper-immune serum against Leishmania antigens, rabbits were immunized several times with cell homoge-

nates of L. b. braziliensis (MHOM/BR/75/M2904) promastigotes in the presence of Freund's complete or incomplete adjuvant. Rabbits were bled on days 14 after the last injection and the sera were pooled and stored at -20 C until ready for use.

Animals examined and antigen extracts used

Four species of wild-caught mammals belonging to 4 genera, 6 Diderphis marsupialis, 5 Caluromys lanatus, 3 Choloepus hoffmani didactylus and 1 Bradypus variegatus ephippiger, were examined for the presence of leishmanial tissue antigens in the present investigation. The liver and spleen from these animals were triturated with glass homogenizer before adjusting with Tris barbital buffer containing 1% Triton-X 100 (pH 8.0, I=0.02) to a final concentration of 10% (W/V). The homogenated suspension was further treated by sonication procedures for 2 min at several intervals and centrifuged at 10,000 x g for 10 min. The supernatant was used as antigen for the CIE test. The following materials were used for checking the cross-reactions: as serum components, the sera of the present wild-caught mammals, bovines and humans; as tissue components, the liver and spleen extracts of laboratory animals; and as parasite components, the epimastigote extracts of Trypanosoma cruzi (Thulahuen strain).

Counter immunoelectrophoresis (CIE)

CIE was performed at room temperature (25-27 C). The glass plate, 10 x 5 cm, was covered with 7.5 ml of 1% Special agar-Noble (Difco) in Tris barbital buffer pH 8.6 (ionic strength, 0.02). The wells on the anode side were filled with 30 µl antisera and the wells on the cathode side with 10 µl of antigen

solution. CIE was carried out for 35 min at 8 volts/cm and the slides read immediately after electrophoresis.

Results

Fourteen of the 15 mammals examined were captured in a leishmaniasis endemic area, i.e., Echeandea, Department of Bolivar, Ecuador; the remaining one was collected from Selva Alegre, Esmeraldas. Prior to the examinations, cross-reactions against anti-L. b. braziliensis serum used were tested using the above mentioned materials. No precipitin lines were observed in the CIE test.

In the tissue extracts obtained from six out of 14 wild-caught mammals in Echeandea, precipitin lines were observed around the center area between the two wells. The results obtained are shown in Table 1 and Fig. 1. In one specimen (No. 7 in Table 1) of C. lanatus, precipitin lines against anti-L. b. braziliensis serum were detected in the tissue extracts obtained from both the liver and spleen. In the other animals, the precipitin lines were observed only in the liver extracts.

Isolation of parasites from these animals was attempted by culture procedures using homogenized samples from the liver and spleen, or by injecting the samples into the nose and foot pads of golden hamsters. In the latter case, the animals were autopsied about 1 month later, and homogenized materials from the injection sites were inoculated into culture medium. In both trials, however, no Leishmania positive animals were detected, probably because of culture contamination and/or too short term

Table 1. Results of counter immunoelectrophoresis in the detection of *Leishmania* antigens from organs of wild mammals caught in leishmaniasis-endemic areas of Ecuador

No.	Mammalian species	Detection of precipitin lines
1	*D. marsupialis*	positive (liver)
2	*D. marsupialis*	positive (liver)
3*	*D. marsupialis*	negative
4	*D. marsupialis*	negative
5	*D. marsupialis*	positive (liver)
6	*D. marsupialis*	negative
7	*C. lanatus*	positive (liver and spleen)
8	*C. lanatus*	negative
9	*C. lanatus*	negative
10	*C. lanatus*	negative
11	*C. lanatus*	positive
12	*Ch. h. didactylus*	negative
13	*Ch. h. didactylus*	positive (liver)
14	*Ch. h. didactylus*	negative
15**	*Br. v. ephippiger*	negative

* *Trypanosoma* sp. (presumably *rangeli*) was observed in the culture materials of the animal (No. 3).

** Only this animal (No. 15) was from Selva Alegre, Esmeraldas, the others (No. 1 through No. 14) from Echeandea, Bolivar.

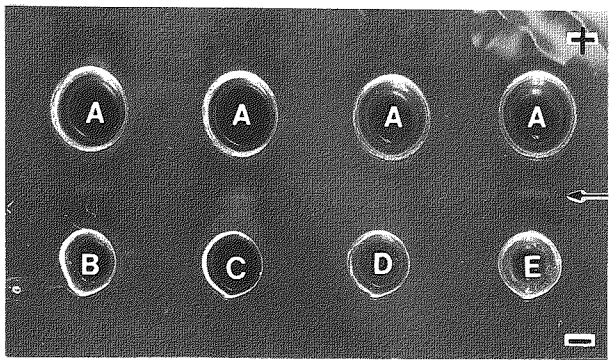

Figure 1. Counter immunoelectrophoresis to detect leishmanial antigens. Electrophoresis was carried out for 35 min at 8 volts/cm. A: rabbit-anti L. b. braziliensis serum, B: liver extracts obtained from animal No. 1 in Table 1, C: liver extracts from animal No. 7, D: liver extracts from animal No. 3, E: L. b. braziliensis promastigote soluble antigens. Arrow shows the location of precipitin lines between anti-serum (A) and liver extracts (B, C, D & E).

(only 1 month infection) for lesion development in hamsters. Trypanosoma sp. (presumably rangeli) epimastigotes were isolated from the hamster injected with liver and spleen materials of D. marsupialis. No precipitin lines were observed in the CIE test with the subjects from this Trypanosoma-positive animal (No. 3 in Table 1).

Discussion

New World leishmaniasis is widely distributed in Central and South America, where it is a considerable health hazard (Molyneux and Ashford, 1983). For a better understanding of the epidemiological features of the disease in these areas, it is necessary to investigate the development of infections not only in human residents but also in reservoir hosts and vector sand flies.

Carrera (1953) suspected several species including dogs, cats, horses, agoutis (Dasyprocta puncutata) and opossums (D. marsupialis) to be reservoirs of leishmaniasis in Ecuador. His assumptions were however only based on his observations of the ecological situation in the endemic area where he worked and not from any reservoir examination. Recently, Hashiguchi et al. (1985) performed a reservoir host survey in Ecuador and reported three species of arboreal mammals, i.e., Sciurus granatensis, Ch. h. didactylus and Potos flavus to be reservoir hosts. This mainly involved the use of laborious, somewhat insensitive parasitological procedures, such as examination of tissue smear specimens and culture materials in the field. Future studies of leishmaniasis epidemiology urgently require more sensitive and

specific immunological methods to detect true animal leishmaniasis cases in the endemic areas.

Among more specific serological tests used in the diagnosis of leishmaniasis, different gel precipitation techniques have been employed in the diagnosis of human and canine leishmaniasis, using homologous as well as heterologous antigens (Mauel and Behin, 1982). However, no information on the detection of leishmanial antigens in organs or circulating antigens has been reported. The present study attempted to detect leishmanial antigens in wild-caught mammals using immunoelectrophoretic methods.

Precipitin lines were detected in the tissue extracts from six out of 15 wild mammals examined. The anti-Leishmania serum used in this study did not react with T. cruzi epimastigote extracts. Moreover, the tissue extracts from D. marsupialis naturally-infected with Trypanosoma sp. did not cross-react with the present antiserum.

From the results obtained by using the CIE technique, we concluded that three species of arboreal mammals, D. marsupialis, C. lanatus and Ch. h. didactylus, probably play an important role as reservoirs in the areas endemic for leishmaniasis in Ecuador.

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Chapter 7

EPIDEMIOLOGY

1. Andean Leishmaniasis in Ecuador

Abstract. An autochthonous Andean leishmaniasis was reported for the first time in the highlands of south-western Ecuador (Canton Paute, Department of Azuay), near the Peruvian frontier. Two subjects with active lesions positive for Leishmania amastigotes were observed in Paute. They had not visited other leishmaniasis-endemic areas such as the Pacific coastal and Amazonian region. Sixty-one (10.6%) of 577 school children in Paute showed leishmanial scars on the face (95.1%) and upper extremities (4.9%). In a rural hospital, a total of 26 cases positive for amastigotes were diagnosed during the period from 1984 to 1986; all the patients were aged between 6 months and 2 years except for one adult male. None of the school children and hospital cases had visited any other leishmaniasis-endemic areas. Only one sand fly species, Lutzomyia peruensis, was recorded from collection in the present study area, suggesting that this was the probable natural vector, although no Leishmania promastigotes were observed in any of the 51 flies dissected. Because of depauperate mammalian fauna of the area, it was considered that dogs would be the most probable reservoir hosts, although a few opossums and mice were also caught during the study.

Although American cutaneous and mucocutaneous leishmaniases have been reported from most of the provinces in Ecuador, there are few accounts available which detail autochthonous foci of the disease in the Andean highlands of the country. To our knowledge, no information is available on the distribution of Andean leishmaniasis or uta, which is considered to be caused by Leishmania braziliensis peruviana at the moment. In Peru, it is well known that uta exists at altitudes of between 600 m to 3,000 m above sea level in valleys of the Andes (Lumbreras and Guerra, 1985).

In the course of our detailed study of Ecuadorian leishmani-

asis, a brief review was made of the leishmaniasis cases diagnosed from 1975 to 1986 in the National Institute of Health and Tropical Medicine, Guayaquil (see Chapter 7-3). In this review, we found a few cases from some provinces in the Andean highlands of south-western Ecuador, close to the Peruvian border. Some of these subjects had apparently contracted the disease during short trips to the Pacific coastal or Amazonian regions, while the remainder had never visited these areas. With regard to the latter cases, we strongly suspect the existence of an autochthonous Andean leishmaniasis in the Ecuadorian areas near the Peruvian frontier. We therefore made a preliminary survey of highland leishmaniasis in the area, collecting data in rural health centers and hospitals, from the epidemiological examinations of school children and from interviews and/or diagnosis made in house to house visits. The current paper deals with parasitologically proven autochthonous cases of Andean leishmaniasis, and also with the epidemiological features of the endemic areas, including the involvement of sand fly and mammalian species.

Materials and Methods

The study areas (Fig. 1)

The epidemiological examination was mainly carried out in and around the Canton Paute, Department of Azuay, located at 2° 46' south latitude and 78°45' west longitude, 2,300 m to 2,500 m above sea level in mid Andes (Plate 1A). Sand fly and mammalian collections were also made in two small villages, Challuabamba and Nulti near the Canton Paute (Plate 1B). In these study areas

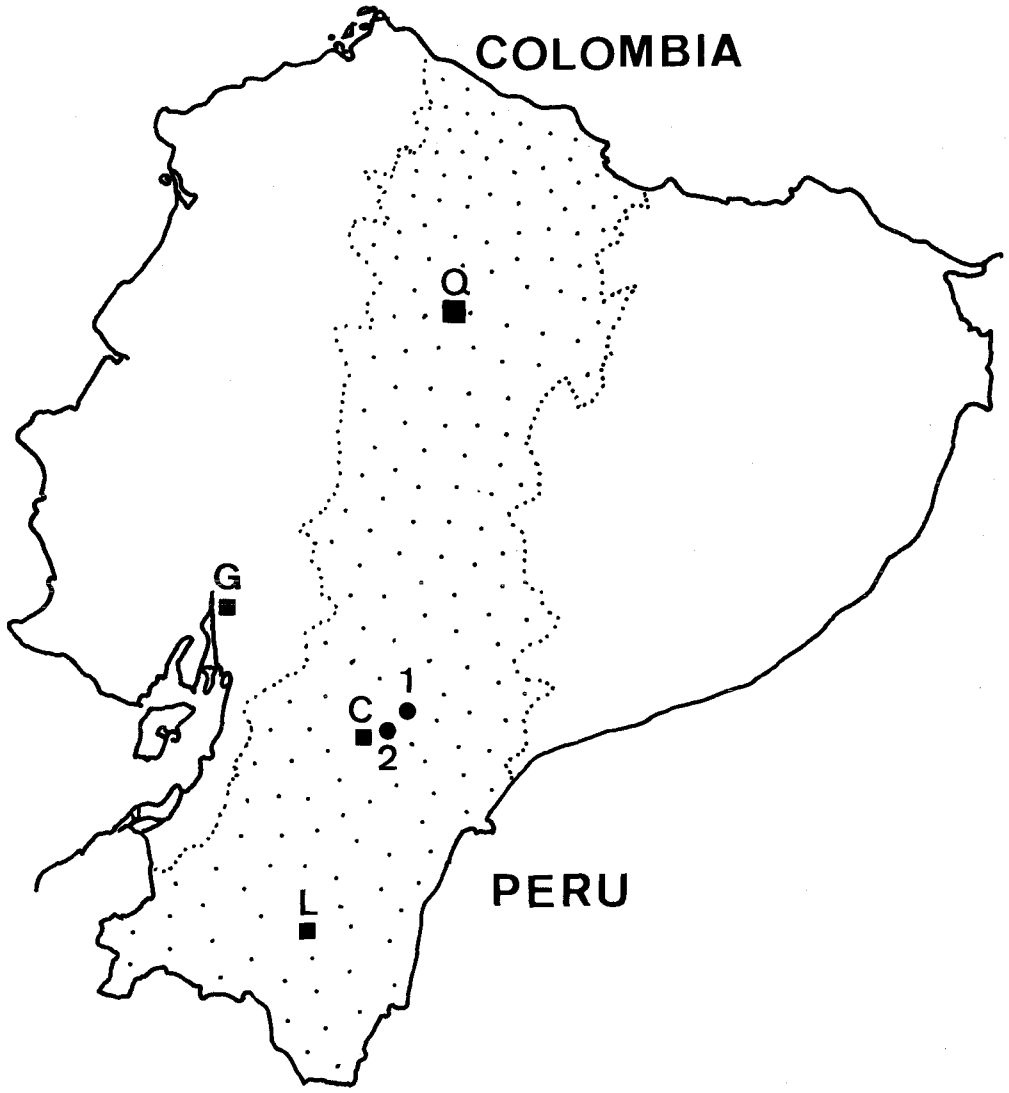


Figure 1. Outline map of the Republic of Ecuador, showing study areas: 1, Paute; 2, Challuabamba and Nulti. Q, Quito; G, Guayaquil; C, Cuenca; L, Loja. Shaded areas lie 1,000 m or more above sea level.

the vegetation is very sparse and is represented by a typically Alpine flora. This is interspersed with scattered human dwellings and cultivated fields, especially on the outskirts of the Canton and the villages (Plate 1C).

The subjects

The children of two schools in Paute were dermatologically examined for cutaneous ulcers, nodules and scars. Such examinations were also performed by house to house visits in and around the area, in order to detect leishmaniasis cases, especially in children of pre-school age. Where the subjects showed active dermal lesions, thin smears were made on slides for microscopical examination. Intradermal skin tests (Montenegro tests) were performed on patients by the injection of 0.1 ml leishmanial antigen (Reed et al., 1986). In addition, the subjects who had already been diagnosed at Hospital Cantonal Paute were reconfirmed by examination of their smear specimens kept at the hospital.

The sand fly and mammalian collections

All sand fly collections were made between 18:00 and 23:00 during the dry season of July to August 1986 in the vicinity of Paute and Challuabamba. In most of the collections, two persons took part, one as human bait and the other as the collector. The human bait sat on the ground with rolled-up sleeves and trousers, and the collector aspirated sand flies with a collecting tube immediately they alit on his exposed skin. Sand flies caught were preserved for identification in the laboratory. All the flies were individually dissected by the method of Hashiguchi et al. (1985). Wild mammals such as opossums and mice were caught

by live trap around human dwellings. The biopsy and blood materials from these animals (4 opossums and 2 mice) were used for *in vitro* cultivation and immunological assays, respectively, together with skin and blood samples from 71 domestic dogs. The results of the reservoir host examinations will be reported elsewhere.

Characterization of Leishmania *isolates from patients*

Biopsy materials from the margins of ulcerous lesions were homogenized and then inoculated into culture media and golden hamsters. The isolates obtained were enzymatically characterized by starch gel electrophoresis (Miles *et al.*, 1980). Only glucose phosphate isomelase (GPI) was used as an enzyme marker in the present study. The isolates were compared with reference strains of the genus *Leishmania*, i.e., *L. b. braziliensis* (MHOM/BR/75/M2904), *L. b. panamensis* (MHOM/PA/71/LS94), *L. b. guyanensis* (MHOM/BR/75/M4147), *L. m. mexicana* (MHOM/BZ/82/BEL21), *L. m. amazonensis* (MHOM/BR/73/M2269), *L. m. garnhami* (MHOM/VE/76/JAP78), *L. m. pifanoi* (MHOM/VE/57/LL1) and *L. d. chagasi* (MHOM/BR/74/M2682). The detailed characterization of the present isolates obtained will also be published elsewhere (see Chapter 4-3).

Results

Parasitologically proven two active cases from Paute

Following a program of house to house visits, the following 2 out of 10 subjects with dermal lesions were found to be positive for leishmanial infections.

Case 1. A 4-year-old male (J.P.V.C) with an ulcer of dimensions 3x5 mm on the upper arm, not showing lymphadenopathy (Plate 1D). The lesion had appeared 5 months before as a small papule and then gradually increased in size. The patient showed a positive Montenegro skin test and his biopsy materials showed abundant small-sized amastigotes. He was born in Florida, U.S.A. and arrived at the present site with his parents (owners of a farm) 2 years ago, since which he had never visited other leishmaniasis-endemic areas such as Pacific coastal and Amazonian regions. The subject received no treatment until the present examination. A search for potential vectors and reservoir hosts around the patient's house (Plate 1C) and sand flies and opossums captured. The family also owned raised 4 dogs and considerable numbers of dogs were also seen around the neighbouring area.

Case 2. A 6-year-old female (C.J.T.) with a facial ulcer (15x10 mm) on the right cheek (Plate 1E). About 1 year and 2 months before, she had been bitten by an insect while in Paute, and a lesion evolved gradually from this bite. The subject had received insufficient intramuscular injections with antimonial meglumine (Gulcantime) 2 months before the present examination. However, she was still positive for the intradermal reaction by skin test and showed amastigotes in smear specimens. The patient lives in Cuenca city near the Canton Paute, but formerly visited the Canton every weekend and stayed there for several days. She had never visited other leishmaniasis-endemic areas.

Examinations of school children in Paute

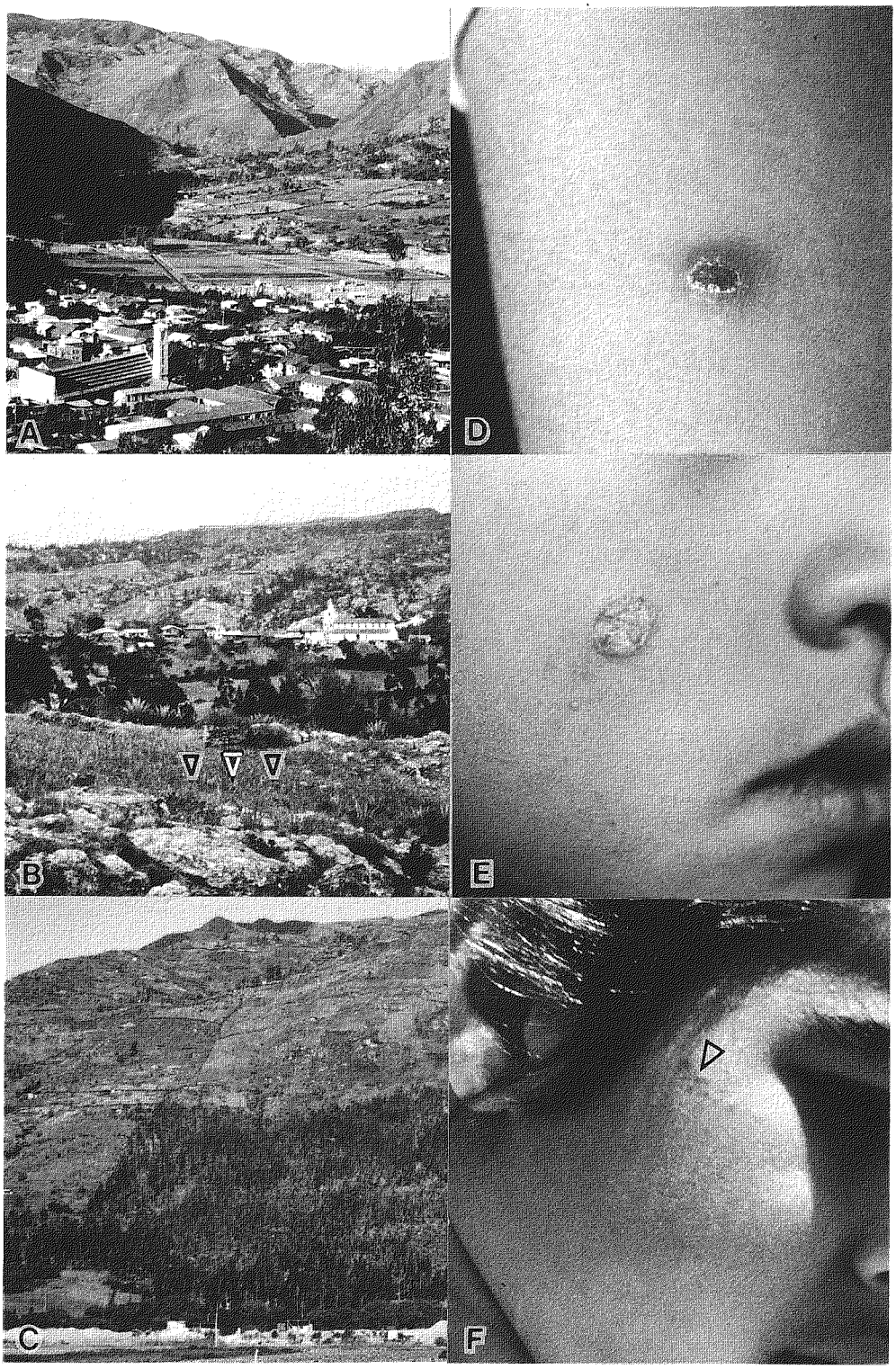
In cutaneous Andean leishmaniasis, it is well known that the

Explanations for the Plate

Plate 1. Ecological features of Andean leishmaniasis and the active (ulcer) and cured (scar) lesions. A. central part and outskirts of the Canton Paute. B. an area of Challuabamba with a typical alpine flora with shrubs (arrows) where the sand fly, Lutzomyia peruensis, was captured. C. landscape around the Case 1 patient's (J.P.V.C.) house, showing patches with the eucalyptuses planted in alpine flora. D. an ulcer positive for amastigotes on the upper arm of a 4-year-old male (Case 1), after about 5 months of ulceration. E. a facial ulcer of a 6-year-old female (Case 2) positive for the parasite, after about 14 months of ulceration. F. a typical scar of cutaneous Andean leishmaniasis on the face of a 8-year-old female (sister of Case 1 patient). She had suffered from the disease 18 months ago (being positive for amastigotes during the active phase), and had received curative chemotherapy 5 months ago.

See illustrations page, 123

Plate 1



lesion leaves a depressed scar which shows characteristic radial striations after healing (Plate 1F). In the survey, well-experienced physicians examined school children for on these scars. The results obtained are arranged by age and sex as shown in Table 1. Of 577 subjects examined dermatologically, 61 (10.6%) revealed typical leishmaniasis scars on the face (58; 95.1%) and upper extremities (3; 4.9%), while no active case was observed in any subject. The average infection rate was significantly high ($0.001<p<0.01$, $x^2=10.05$) in females compared with that in males. This tendency was most marked in the 11 - 14 age group, but the reason for this is not clear. It was from interviews that the majority of subjects had contracted the disease when they were under school age. None had visited other leishmaniasis-endemic areas in the past.

Parasitologically proven cases diagnosed at a rural hospital in Paute

During the period from 1984 to 1986, a total of 26 leishmaniasis cases were diagnosed at a rural hospital, Hospital Cantonal Paute, viz., 12 cases (5 males and 7 females) in 1984, 11 (5 and 6) in 1985 and 3 (3 males only) in 1986. They were all between 6 months and 2 years of age except for an adult male of 45 years. All the lesions were observed on the faces of subjects and gave positive diagnoses for Leishmania amastigotes. No registration was available in the hospital before 1983. All the patients recorded had lived and contracted the disease in and around Paute.

Table 1. Results of the examination of school children for leishmanial lesions in Paute, arranged by age and sex

Age	Male		Female		Location of lesions*	
	No. examined	No.(%) positive	No. examined	No.(%) positive	Face	Upper-limbs
6-7	31	5(16.1)	40	2(5.0)	7	0
8	74	6(8.1)	38	4(10.5)	9	1
9	100	5(5.0)	26	3(11.5)	8	0
10	69	5(7.2)	35	2(5.7)	7	0
11	64	5(7.8)	23	10(43.5)	13	2
12-14	58	5(8.6)	19	9(47.4)	14	0
Total	396	31(7.8)	181	30(16.6)	58	3

* All the lesions were observed as single or multiple leishmanial scars (n=95, lesion size: 4.5±3.14 mm in diameter).

Characterization of Leishmania isolate from a patient

Gulcose phosphate isomelase (GPI), an enzyme of the isolate from a patient (Case 2) with an active lesion, was electrophoretically characterized as shown in Fig. 2. The GPI revealed a completely different mobility and band pattern from 8 reference strains of the genus Leishmania.

Sand fly species collected

A total of 51 sand flies were collected by 15 collecting pairs (collector and human bait) over 5 nights (18:00-23:00) at Paute and Challuabamba during the period from July to August 1986 (dry season). All the sand flies collected were identified as Lutzomyia peruensis based on the characteristics of their spermathecae and cibaria. All were dissected and found to be negative for Leishmania promastigotes.

Discussion

The current study reports an autochthonous endemicity of Andean leishmaniasis for the first time from the highlands of Ecuador. In the area there were two active cases positive for Leishmania amastigotes, 61 (10.6%) school children with leishmanial scars and 26 cases diagnosed in a rural hospital. These figures seemed to indicate a rather low endemicity of the disease on the Ecuadorian Andes. In Peru, 2,000 cases of uta are reported annually to the Ministry of Health, a figure which is probably an underestimate of the actual number of cases (Lumbreras and Guerra, 1985). Furthermore, in Peru, this form of the disease is endemic on the western slopes of the Andes and in many inter-

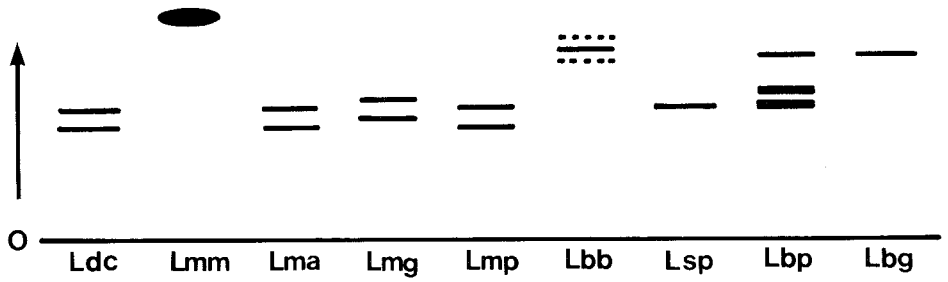


Figure 2. Diagrammatic representation of the electrophoretic patterns of glucose phosphate isomelase (GPI) obtained from promastigotes of Leishmania spp. Ldc, L. d. chagasi; Lmm, L. m. mexicana; Lma, L. m. amazonensis; Lmg, L. m. garnhami; Lmp, L. m. pifanoi; Lbb, L. b. braziliensis; Lsp, Leishmania isolated from a patient with Andean leishmaniasis in Paute, Ecuador; Lbp, L. b. panamensis; Lbg, L. b. guyanensis.

Andean valleys (Herrer, 1957), and has shown an increased number of the cases in recent years (Herrer et al., 1980).

The causative agent of Andean leishmaniasis in Peru has been believed as L. b. peruviana based mainly on clinico-geographical grounds. The species has long been thought as a subspecies of L. braziliensis or of L. mexicana (Lainson et al., 1979; Kreutzer et al., 1983). Recently, however, Romero et al. (1987) made a characterization of Leishmania isolated from Andean patients in Peru, using cellulose acetate electrophoresis, k-DNA hybridization and monoclonal antibody binding, and reported that the Andean isolates are subspecies of the L. braziliensis complex, with characteristics surprisingly similar to those of L. b. braziliensis. McMahon-Pratt et al. (1982), moreover, failed to detect any difference between the Andean isolates and the subspecies L. b. braziliensis by monoclonal antibodies that had been previously shown to provide a highly sensitive procedure to distinguish the latter subspecies from other subspecies of the L. braziliensis complex.

On the other hand, the present Andean isolate in Ecuador revealed a different pattern of isoenzyme profiles by starch gel electrophoresis from other reference strains including L. b. braziliensis, although only GPI was used as an enzyme marker. However, species identification of this Andean isolate requires further investigations. The detailed examination is still in progress, using monoclonal antibody bindings, isoenzymes and DNA probes. The results will be reported elsewhere. As to the employment of only one enzyme (GPI) profile, Beach and Mebrahtu (1985)

pointed out that only one enzyme, GPI, if properly produced, could identify Kenyan leishmanial isolates (3 Leishmania species) from the same geographical region.

In this study no comparison of isoenzyme profiles was made between the Andean isolates from Ecuadorian and Peruvian patients, because no reference strain of L. b. peruviana was available at the time. It was therefore uncertain whether these Leishmania from the two countries were taxonomically identical or not. According to Romero et al. (1987), there exists in the Peruvian Andes, isolate which is not a member of either the L. braziliensis or L. mexicana complexes; the organism was originally isolated from the sand fly Lu. peruensis, and causes typical skin lesions in hamsters, but no isolates have been obtained from human patients in neighbouring areas. Further investigations should be performed on Andean leishmaniases in both countries, in order to obtain additional information on the taxonomic status and pathogenicity of the causative organisms. In Ecuador, little characterization of the causative agent of leishmaniasis had been done prior to the present study.

Only one species, Lu. peruensis, was collected in the study area, suggesting a possible role in transmission of Andean leishmaniasis in Ecuador, though none of 51 flies dissected were positive for Leishmania promastigotes. This species has already been incriminated as the vector of Andean leishmaniasis in Peru (Herrer, 1982). The limited numbers of sand fly collected in this study might be due to seasonal population variation of this species in the area during the dry season. During the wet season,

on the other hand, considerable numbers of sand flies are seen, even in the central part of the Canton Paute (Dr. Mendez, personal communication). Dogs are the most likely reservoir hosts although opossums and mice should also be considered, because the mammalian fauna of the area is otherwise very poor around there.

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2. Bacterial flora isolated from two types of leishmanial ulcer in Ecuador

Abstract. Bacterial flora from distinct two types of highland and lowland cutaneous leishmaniasis ulcers were examined in an attempt to elucidate the effect of concomitant infections on the development of the leishmanial skin manifestations. A total of 51 leishmanial ulcers, 11 samples from highland and 40 from lowland were examined; 46 (90.2%) were positive for microorganisms. Bacterial flora isolated were aerobics: Staphylococcus, Enterococcus, Mycobacterium and gram-negative rods (GNR); anaerobics: Peptococcus, Peptostreptococcus, Fusobacterium and Bacteroides; and yeast. The bacterial isolation rate was 81.8% for highland, and 92.5% for lowland. Histological examination showed inflammatory cell infiltrations throughout the dermis in the highland specimens, but restricted to deep dermis in the lowland ones. Only highland specimens showed heavily parasitized reticuloendothelial cells with leishmanial organisms.

It has been well documented that clinical features of cutaneous leishmaniasis tend to differ between endemic regions in Latin America. Although various clinical pictures reflect the different species or subspecies of Leishmania, the genetically determined host responces to the parasite and the modification by environmental factors should also be considered. In Ecuador, the extensive epidemic of cutaneous and mucocutaneous leishmaniasis caused by the agents, Leishmania spp., occurs in the low land of the bilateral regions in the Andes mountains. Besides, sporadic cases of Andean leishmaniasis or "uta" have been recently found in mid-Andean regions of south-western Ecuador near the border of Peru (see Chapter 7-1). This type of lesion develops single or few painless skin ulcer, and usually heal spontaneously in a short term. The causative agent is considered to be L. b. peruviana at the moment. In the present study, bacterial flora from

highland and lowland cutaneous leishmaniasis ulcers were determined in an attempt to delineate the effect of concomitant bacterial infections on the development of these distinct skin manifestations.

Materials and Methods

This study was done in three endemic foci for leishmaniasis, namely, Paute, Department of Azuay, Puerto Quito, Department of Los Rios, and Selva Alegre, Department of Esmeraldas. Paute is located at 2,300 to 2,500 m above sea level in the south-western Ecuador where Andean leishmaniasis have newly been recognized. On the other hand, both Puerto Quito and Selva Alegre are in the tropical forests in the north-western lowlands. Cutaneous and mucocutaneous leishmaniases are endemic in these areas. For this study, 40 patients with lowland leishmaniasis and 11 ones with highland (Andean) leishmaniasis were examined. Leishmaniasis was diagnosed using parasitological (impression smear from the margin of the ulcerous lesions) and/or immunological (immediate type hypersensitivity reaction by skin test) examinations.

To isolate microorganisms from the skin ulcer lesion, specimen swabs were taken using cotton-tipped applicators. After treated with sterile saline solution, the surface around the ulcer margin was swabbed using two applicators. One swab was placed in media tubes used to culture aerobic organisms (EIKEN Swab No. 1, Eiken Chemical Co., Ltd.), while the other swab was placed in media tubes used to culture anaerobic organisms (KENKI PORTER, Clinical Supply Co., Ltd.). The culture tubes were

refrigerated immediately and transported to the laboratory. Subsequent laboratory procedures, such as isolation and identification of organisms were conducted at the Departamento de Bacteriologia, Instituto Nacional de Higiene y Medicina Tropical, Guayaquil, Ecuador.

Results and Discussion

Bacterial examinations were done on a total of 51 leishmanial ulcers; 11 samples from highland patients with Andean leishmaniasis, and 40 from lowland ones with another type of the disease. Microorganisms were detected in 46 (90.2%) of 51 cultures. Of the 46 positive cultures, 38 (82.6%) contained multiple organisms. One patient, a 14-year-old male, living in Esmeraldas, had five organisms (Mycobacterium, Serratia rubidaea, Peptococcus, Bacteroides and yeast) in his arm ulcer. The microorganisms isolated from the leishmanial ulcer are summarized in Table 1. Aerobic bacterial isolates included Staphylococcus which was isolated from 22 cases; 4 isolates of coagulase negative (S. epidermides) and 18 isolates of coagulase positive (S. aureus). Peptococcus was the most common microorganism in anaerobic group (66.7%).

The isolation rate was analyzed in relation to the two types of leishmanial ulcer, highland and lowland leishmaniasis (Table 2). The isolation rate was 81.1% for highland, and 92.5% for lowland. There were no distinct difference in the prevalence rate of Staphylococcus or the anaerobic group. However, apparent difference was observed in the prevalence rate of gram-negative

Table 1. Bacterial flora isolated from leishmanial ulcer in Ecuador

Bacterial species		No. of cases
Aerobic	Staphylococcus	22
	Enterococcus	2
	Mycobacterium	15
	Gram-negative rods (GNR)	16
Anaerobic	Peptococcus	34
	Peptostreptococcus	4
	Fusobacterium	2
	Bacteroides	3
Yeast		11

Table 2. Bacterial flora isolated from two types of leishmaniasis from Ecuador

Types of disease	No. examined	No. (%) positives	No. (%) isolated*					
			Staph.	Enter.	Mycob.	GNR	Anaer.	Yeast
Highland	11	9 (81.8)	6 (54.5)	0	2 (18.2)	2 (18.2)	8 (72.7)	0
Lowland	40	37 (92.5)	16 (40.0)	2 (5.0)	13 (32.5)	15 (37.5)	30 (75.0)	11 (27.5)

* Staph: Staphylococcus, Enter: Enterococcus, Mycob: Mycobacterium, GNR: gram-negative rods, Anaer: anaerobic group.

rod (GNR), Mycobacterium, and yeast between two groups. The species of GNR obtained in this study are listed in Table 3. This group was mainly composed of enterobacteria such as Escherichia, Serratia, Klebsiella and Enterobacter. Also non-fermentating Acinetobacter and Alcaligenes were isolated.

Histological examination showed inflammatory cell infiltrations composed primarily of small lymphocytes throughout the dermis in the highland specimens, but restricted to deep dermis in the lowland ones. Moreover, only specimens from the highland showed heavily parasitized reticuloendothelial cells with leishmanial organisms.

The subjects were divided into two groups according to their age (less than 19 years, and 20 years or older), and the isolation rate of the groups was analyzed (Table 4). The prevalence rate was slightly higher in the younger than in the older age group, particularly in GNR (43.2% in younger age group, and 7.1% in older age group).

This study indicates that there is a difference in bacterial flora and histology between the highland (Andean) and lowland types of leishmaniasis. In Ecuador, the lowland type of the disease is associated with L. braziliensis complex and L. mexicana complex, while the causative agent of Andean type remained still unknown (see Chapters 4-3 and 7-1). Although distinct differences in the clinical picture between the two types may be due to the virulence of each subspecies of parasite, modification by concomitant bacterial infections may possibly play a role in the development of the clinical manifestations. One should note

Table 3. Gram-negative rods isolated from leishmanial ulcer in Ecuador

Species isolated	No. isolated
Escherichia coli	3
Serratia rubidaea	1
Klebsiella rhinoscleromatis	2
Enterobacter aerogenes	3
E. cloacae	1
Proteus mirabilis	1
P. rettgeri	1
Acinetobacter calcoaceticus	3
Alcaligenes faecalis	1

Table 4. Bacterial flora isolated from leishmanial ulcers in relation to subject's age

Age group	No. examined	No.(%) positives	No. (%) isolated*					
			Staph.	Enter.	Mycob.	GNR	Anaer.	Yeast
<19	37	35 (94.6)	15 (40.5)	2 (5.4)	13 (35.1)	16 (43.2)	28 (75.7)	9 (24.3)
>20	14	11 (78.6)	7 (50.0)	0	2 (14.3)	1 (7.1)	10 (71.4)	2 (14.3)

* Staph: *Staphylococcus*, Enter: *Enterococcus*, Mycob: *Mycobacterium*, GNR: gram-negative rods, Anaer: anaerobic group.

that the concomitant infections with GNR occurred more frequently in lowland leishmaniasis than in highland (Andean) type. However, it is not clear whether GNR is associated with the development of the leishmanial ulcer. Recent reports show that the skin infections accounted for approximately one-third of the nosocomial infections among surgical patients, and that gram-negative organisms were more prevalent than gram-positive organisms (Brenner and Bryan, 1981; Bryan et al., 1983). These infections could be associated with patient's underlying immunosuppressive condition, since infections with Leishmania strongly induce immunosuppression in experimental animals and humans (Turk and Bryceson, 1971). Thus comparative studies assessing the patient's immune status, should be performed, particularly the local immune status around the ulcer of highland and lowland leishmaniasis.

There is a constant and well-defined bacterial flora on normal skin, even thoguh it is particularly apt to contain unspecified microorganisms because of the constant exposure to the environment. The higher prevalence of Mycobacterium (acid-fast bacilli) or yeast was associated with the cutaneous leishmaniasis ulcers. There are many species of yeast or yeast-like organisms found in human disease. Candida albicans is the most common agents, and Sporathrix schenckii produce the open, ulcerating "chancroid" type lesion in subcutaneous tissue. Recovery of Mycobacterium sp. from skin lesions appears to be variable. Thus the significance of concomitant infections with these microorganisms is still unclear and requires further study.

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3. Parasitologically-proven retrospective cases diagnosed in INHMT

Abstract. In order to obtain general information on the epidemiological features of leishmaniasis and its endemic areas in Ecuador, a review was made on a total of 672 microscopically-confirmed cases recorded during the period from 1975 to 1986 at the outpatient facility of the Instituto Nacional de Higiene y Medicina Tropical (INHMT), Guayaquil, Ecuador. All the cases reviewed were positive for Leishmania amastigotes, and those cases in which the organisms were not demonstrated were excluded in the present study. A great majority (around 60%) of the total cases had been diagnosed in 1984-5, indicating an abnormal occurrence of the disease perhaps related to heavy rainfall caused by unusual movement of the El Niño Current during those years in Ecuador. In the geographical distribution of the present cases, more than 80% of the total were from the littoral regions bordering the Pacific Ocean, with a few others, from the Amazonian region and the Andean slope or highlands. The cases diagnosed had largely occurred during the wet season (October to April). In reviewing the cases in relation to age or sex of the subjects, a pronounced peak was found between 16 and 25 of age in both sexes. Mucosal (espundia) and ear (chiclero's ear) leishmaniasis revealed no geographically-limited distribution and the former clinical form was also frequent in the 21-25 age group with a lower frequency of cases in older age groups.

American cutaneous and mucosal leishmaniases in Ecuador constitute a permanent threat to human health in most area of the country, lowland and highland, and on both sides of the Andes. Little information is currently available on epidemiological features, vectors, reservoirs and etiological agents of the disease, though the first record of cutaneous leishmaniasis in Ecuador dates back to that made in 1920 by Valenzuela (Rodriguez, 1974). Two clinical forms of the disease have been reported in this country: cutaneous and mucosal leishmaniases in most of the endemic areas, and visceral leishmaniasis (not yet parasitologic-

ally proven) in the Amazonian tropical forest and in the Department of Esmeraldas in the Pacific coastal region. To obtain further information on the epidemiology of leishmaniasis in Ecuador, we initiated investigations on the infection of human residents, reservoir hosts (wild mammals) and vector sand flies in different endemic areas. The present paper deals with microscopically-diagnosed cases of leishmaniasis at the outpatient facility of the National Institute of Health and Tropical Medicine, Guayaquil, Ecuador, during the period from 1975 to 1986. Although the data analyzed here cannot reveal the true incidence and prevalence of leishmaniasis in Ecuador, they do offer some rough information on the distribution and seasonal occurrence of the disease in the country.

Materials and Methods

Subjects

All the subjects examined came from different endemic areas, i.e., the Departments of Esmeraldas, Pichincha, Manabi, Los Rios, Guayas, Cotopaxi, Bolivar, Cañar, Azuay, El Oro, Loja, Napo and Pastaza (Fig.1). All received differential diagnosis of leishmaniasis at the outpatient facility in the Instituto Nacional de Higiene y Medicina Tropical (INHMT). The Institute is located in Guayaquil, the major city (population, ca. 1,000,000) of a Pacific coastal plateau region in south-western Ecuador. One of the main activities of the National Institute is to provide differen tial diagnoses against various parasitological, bacterial, viral and fungal infections. In general, when physicians in rural and

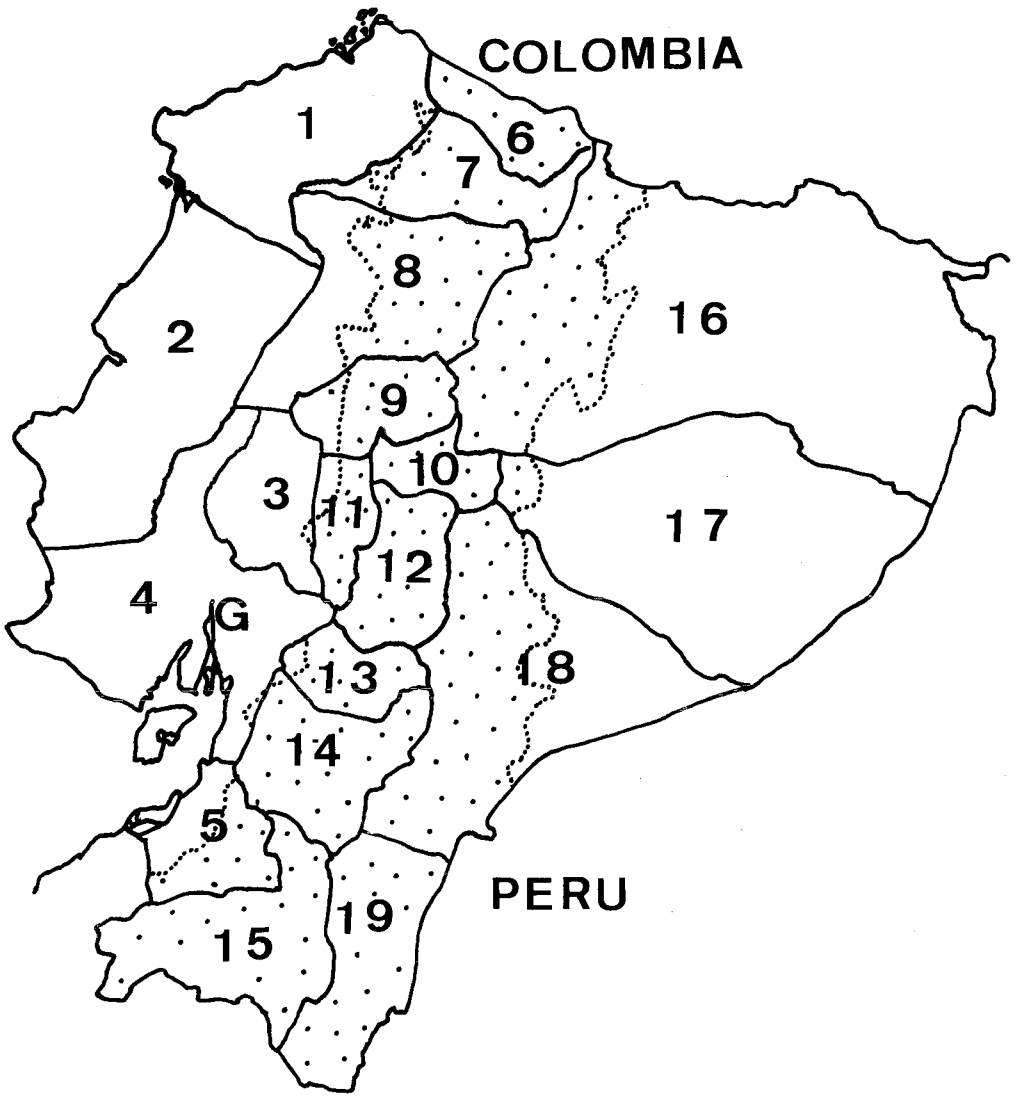

Figure 1. Outline map of the Republic of Ecuador, showing each Department and Guayaquil city (G) where Instituto Nacional de Higiene y Medicina Tropical is located. 1. Esmeraldas, 2. Manabi, 3. Los Rios, 4. Guayas, 5. El Oro, 6. Carchi, 7. Imbabura, 8. Pichincha, 9. Cotopaxi, 10. Tungurahua, 11. Bolivar, 12. Chimborazo, 13. Cañar, 14. Azuay, 15. Loja, 16. Napo, 17. Pastaza, 18. Morona Santiago, 19. Zamora Chinchipe. Shaded area shows 1,000 m or over above sea level.

urban hospitals or health centers preliminary diagnosed patients with dermal or mucosal lesions as having leishmaniasis, the examinees were recommended to have differential diagnosis at the Institute. Thus suspected cases came from almost all the leishmaniasis-endemic areas of Ecuador to Guayaquil. In our studies made at the outpatient facility of the Institute, questionnaires were prepared to record the residence and occupation of each person, history of the disease and leishmanial lesions (location, type, number and onset), treatment, and other features.

Microscopical examinations of biopsy materials

Biopsy specimens were taken from the margins of ulcerous or nodular lesions using a surgical knife. They were then smeared onto a slide glass, making a thin film. After drying the materials at room temperature, they were stained with Wright's staining solution and then examined by experienced microscopists at magnifications of x400 or xl,000. All the smear specimens which were judged as positive for Leishmania amastigotes by the microscopist were thoroughly checked by parasitologists (V.V.C. or E.A.G.L.). We reviewed 672 such cases seen at the outpatient facility of the Institute between 1975 and 1986. We excluded those cases in which amastigotes were not demonstrated microscopically, unless otherwise specified.

Geographical and climatic situation

Ecuador is situated in north-western South America, between 1°21' N Latitude, 78°44' W Longitude and 5°0' S Latitude, 78°55' W Longitude (Teran, 1984). The country bordered by the Pacific Ocean and is divided by the Andes into three geographical re-

gions, i.e., Pacific coastal, Amazonian lowlands and Andean highland. In Ecuador ecological features such as temperature, relative humidity, annual precipitation, vegetation and fauna, are quite variable within each region, and are dependent mainly on the altitude above sea level. The climate of the Pacific coastal and sub-Andean regions of the country may be broadly divided into two seasons, the hot wet season (October to April) and cool dry season (May to September) in the Pacific coastal and sub-Andean regions, while rainfall is recorded in the Amazonian region throughout the year.

Results

Cases diagnosed between 1975 and 1986 in INHMT

Between 1975 and 1986, a total of 1,429 persons with dermal or mucosal lesions visited the outpatient facility of the INHMT, 672 (47.0%) of which were positive for leishmanial amastigotes in impression smears. The yearly case numbers positive for the parasites were 5 in 1975, 6 in 1976, 18 in 1977, 20 in 1978, 13 in 1979, 36 in 1980, 44 in 1981, 38 in 1982, 17 in 1983, 195 in 1984, 201 in 1985 and 79 in 1986. The ratios of these leishmaniasis cases per total persons examined dermatologically in INHMT ranged from 11.4% to 70.1% during the 12 years. It is most noticeable that the majority of positive cases occurred between 1984 (195 cases) and 1985 (201 cases), i.e., 396 (58.9%) leishmaniasis cases in total. Following this abnormal occurrence of cases in the two years, 79 persons were diagnosed as leishmaniasis patients during the period from January to August of 1986.

No remarkably high rate of the occurrence was recognized between 1975 and 1983, number of cases ranging from 5 to 44.

Geographical distribution of the cases

Based on the leishmaniasis cases diagnosed in INHMT, the distribution of patients was geographically analyzed as shown in Table 1. Of the total positives (672) for Leishmania amastigotes, 239 (43.0%) subjects came from the Department of Los Rios, suggesting that the disease was most prevalent in this area. In other Departments, the numbers of leishmaniasis cases were 129 (19.2%) in Esmeraldas, 99 (14.7%) in Guayas and 37 (5.5%) in Manabi. Thus, the majority of cases diagnosed in INHMT originated from the Departments in the Pacific coastal regions. A few cases were also found in areas of the Andean slope, such as the Departments of Pichincha, El Oro, Bolivar, Cañar and Cotopaxi, and also in two Departments of Andean highlands, Azuay and Loja. In the Amazonian regions of Ecuador, only 13 cases in total were reported from two Departments, Napo and Pastaza. The low number of recorded cases from these Amazonian regions might be due to their greater distance from Guayaquil, where the Institute is located.

Seasonal occurrence of the cases

Seasonal occurrence of leishmaniasis cases is depicted in Fig. 2, based on the cases diagnosed in INHMT. In these leishmaniasis cases the exact onset time was not known, but according to the interview records it was found that almost all of the cases were diagnosed within two months of the onset of the disease. The availabe data therefore only give a rough idea of the

Table 1. Leishmaniasis cases, 672 in total, positive for Leishmania amastigotes diagnosed in INHMT, Guayaquil, Ecuador from different areas of the country during the period from 1975 to 1986

Departments and villages	No.of cases (%)*	Departments and villages	No.of cases (%)*
1.Los Rios	239 (43.0)	Tachina	2
Quevedo	92	San Miguel	1
Ventanas	60	Rio Verde	1
Montalvo	35	Others	104
Catarama	22	3.Guayas	99 (14.7)
Quinsaloma	16	Naranjal	21
Urdaneta	10	El Triunfo	18
Babahoyo	9	Bucay	11
El Empalme	9	Balao	11
Mocache	8	Milagro	7
Gramalote	7	Daule	6
Vinces	4	Duran	5
Zapotal	3	Manglarto	5
Sta. Lucia	2	Balzar	4
Palenque	1	Tenguel	2
Others	11	Samborondon	2
2.Esmeraldas	129 (19.2)	Sta. Elena	2
Quininde	10	San Carlos	1
San Lorenzo	5	Pascualez	1
Muisne	3	Taura	1
Borbon	2	Others	2

Table 1. (continued)

4.Manabi	37	(5.5)	7.Bolivar	24	(3.6)
Ricaurte	24		Caluma	11	
Calceta	4		Echeandia	3	
Conventa	2		Salampe	1	
Sta. Ana	1		Guaranda	1	
El Carmen	1		Las Naves	1	
Portoviejo	1		Others	7	
Flavio Alfaro	1		8.Cañar	13	(1.9)
Pajan	1		Troncal	7	
Rio Chico	1		Cochancay	5	
24 de Mayo	1		Suscal	1	
5.Pichincha	31	(4.6)	9.Napo	11	(1.6)
Sto. Domingo	26		Tena	3	
LOs Bancos	2		Lago Agrio	3	
Others	3		Coca	2	
6.El Oro	25	(3.7)	Others	3	
Zaruma	7		10.Cotopaxi	8	(1.2)
Piñas	4		Moraspungo	1	
Sta. Rosa	2		Jesus Gran Poder	1	
Machala	1		Others	6	
Pto. Bolivar	1		11.Azuay	3	(0.04)
Portovelo	1		Cuenca	3	
Huaquillas	1		12.Pastaza	2	(0.03)
Pasaje	1		Puyo	2	
Others	7				

* % per total 672 microscopically confirmed cases.

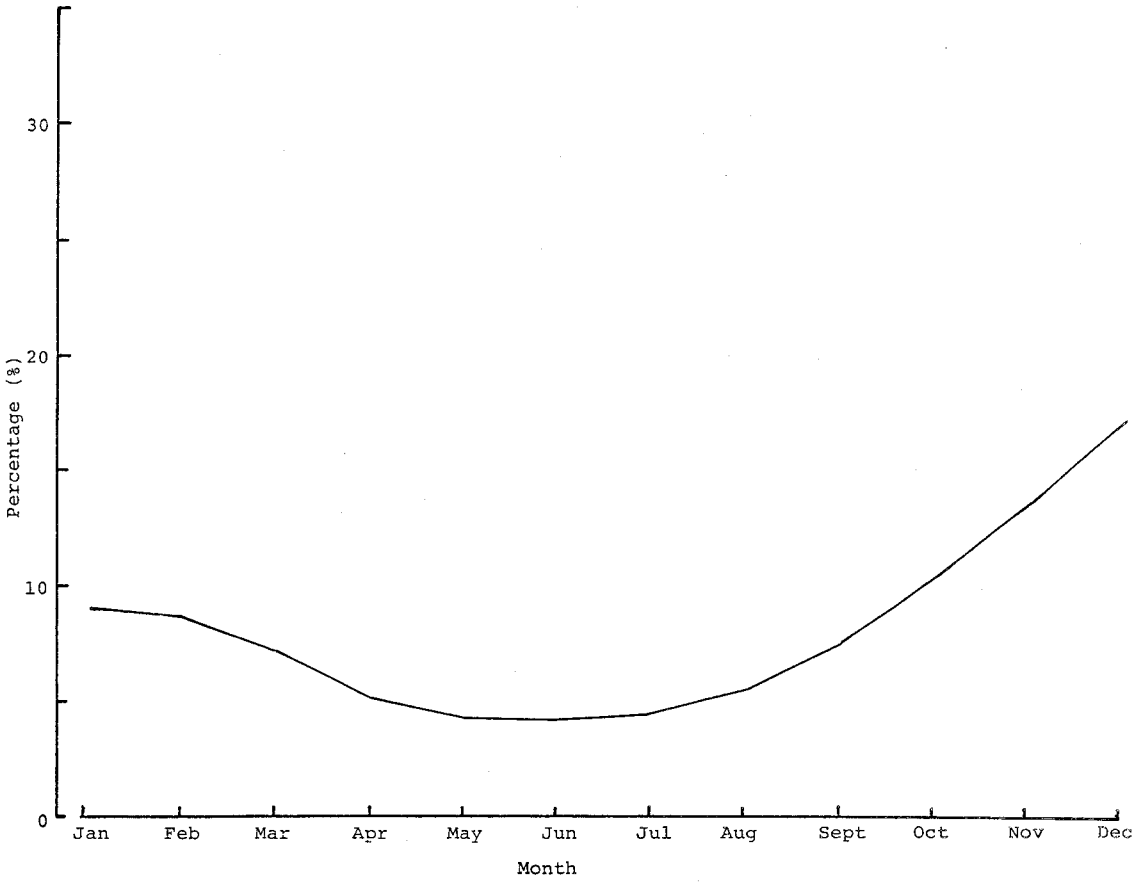

Figure 2. Seasonal occurrence of leishmaniasis cases depicted based on 531 cases diagnosed in INHMT in the years, 1977 (n=18), 1978 (n=20), 1980 (n=36), 1981 (n=44), 1983 (n=17), 1984 (n=195) and 1985 (n=201).

true seasonal occurrence of leishmaniasis in Ecuador. Complete records of the cases in the Institute throughout the year were available from 1977, 1978, 1980, 1981, 1983, 1984 and 1985, but not for the remaining years. The results indicated that the occurrence of cases was quite variable among the years, with a relatively higher number during the wet season (October to April).

Age distribution of the cases

Frequency distribution of active leishmaniasis cases diagnosed in INHMT is arranged by age and sex of the subjects in Fig. 3. The results showed that there was greatest number of the cases among people less than 35 years old, with a pronounced peak between 16 and 25 in both sexes. The number of cases diagnosed was less frequent in persons of age 36 or over.

Anatomical distribution of leishmanial lesions

The subjects of this study from whom Leishmania were demonstrated showed a single or multiple cutaneous and mucosal lesions on their body surface. A total of 693 such lesions were located on the face, ear, neck, upper or lower extremities, shoulder, back, abdomen and thorax (Table 2). The majority (75%) of the total lesions observed was found in the upper or lower extremities, while relatively few lesions (15%) were situated on the face. The main affected areas on the face were cheek (cutaneous) and nose including nasal septum (mucosal). Twenty-eight (4%) of lesions were found on the ears of patients.

Geographical distribution of mucosal and ear leishmaniasis

It is worthwhile to examine the geographical distribution of

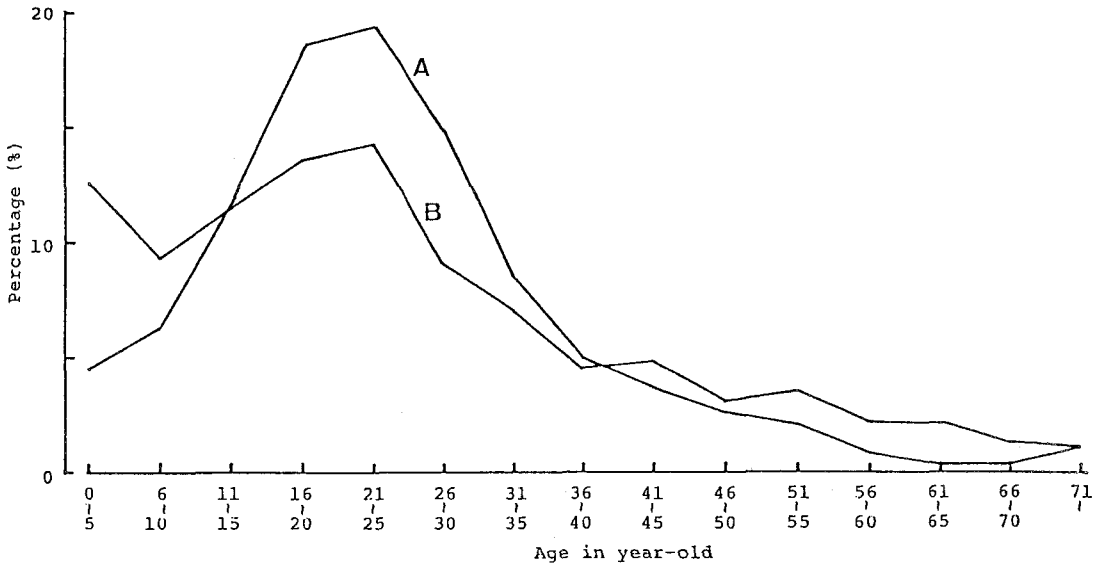

Figure 3. Age distribution of 672 leishmaniasis cases diagnosed in INHMT between 1975 and 1986. A. male, B. female.

Table 2. Number and location of leishmanial lesions on the anatomical body areas of the patients diagnosed in INHMT during the period from 1975 to 1986

Anatomical body areas	No. of lesions	%
Face		
cheek	48	7.07
front	5	0.72
chin	2	0.29
eye-lid*	3	0.43
nose (nasal septum)*	44	6.35
lip*	4	0.58
Ear	28	4.04
Neck	6	0.87
Upper extremities	286	41.27
Lower extremities	236	34.06
Shoulder	12	1.73
Back	14	2.02
Abdomen	1	0.14
Thorax	3	0.43
Total	693	100.00

* Mucocutaneous leishmaniasis cases, 51 in numbers and 7.4% per total lesions observed.

mucosal (espundia) and ear (chiclero's ear) leishmaniasis in Ecuador, because these two clinical forms might be caused by different species of the genus Leishmania, from the L. braziliensis and L. mexicana complexes, respectively. Based on this hypothesis, we reviewed the geographical distribution of 48 subjects with lesions on the nose and lip, and of 26 ones with lesions on the ear (Fig. 4). The results revealed that there was no geographically-limited distribution of these two clinical forms, and that both occurred throughout the country.

Age distribution of mucosal and ear leishmaniasis

In order to know the relationship between the occurrence of mucosal or ear leishmaniasis and the onset time, age distribution of the subjects positive for Leishmania amastigotes was examined. Mucosal leishmaniasis was frequently found in the age-groups between 6 and 35 years old with a peak at 21-25 years of age, while ear forms of the disease were seen in cases aged between 11 and 40 ages, with a peak incidence at around 21-35 years old. Age distribution of these two clinical forms closely corresponded to that of the total cases, as shown in Fig. 3, suggesting that there was no trend for mucosal leishmaniasis to occur more frequently in higher age groups. This clinical form has generally been considered to occur in persons as a result of metastasis some months or years after appearance and healing of cutaneous lesions.

Discussion

Among 1,429 subjects with dermal or mucosal lesions, who visited INHMT for differential diagnosis of leishmaniasis between

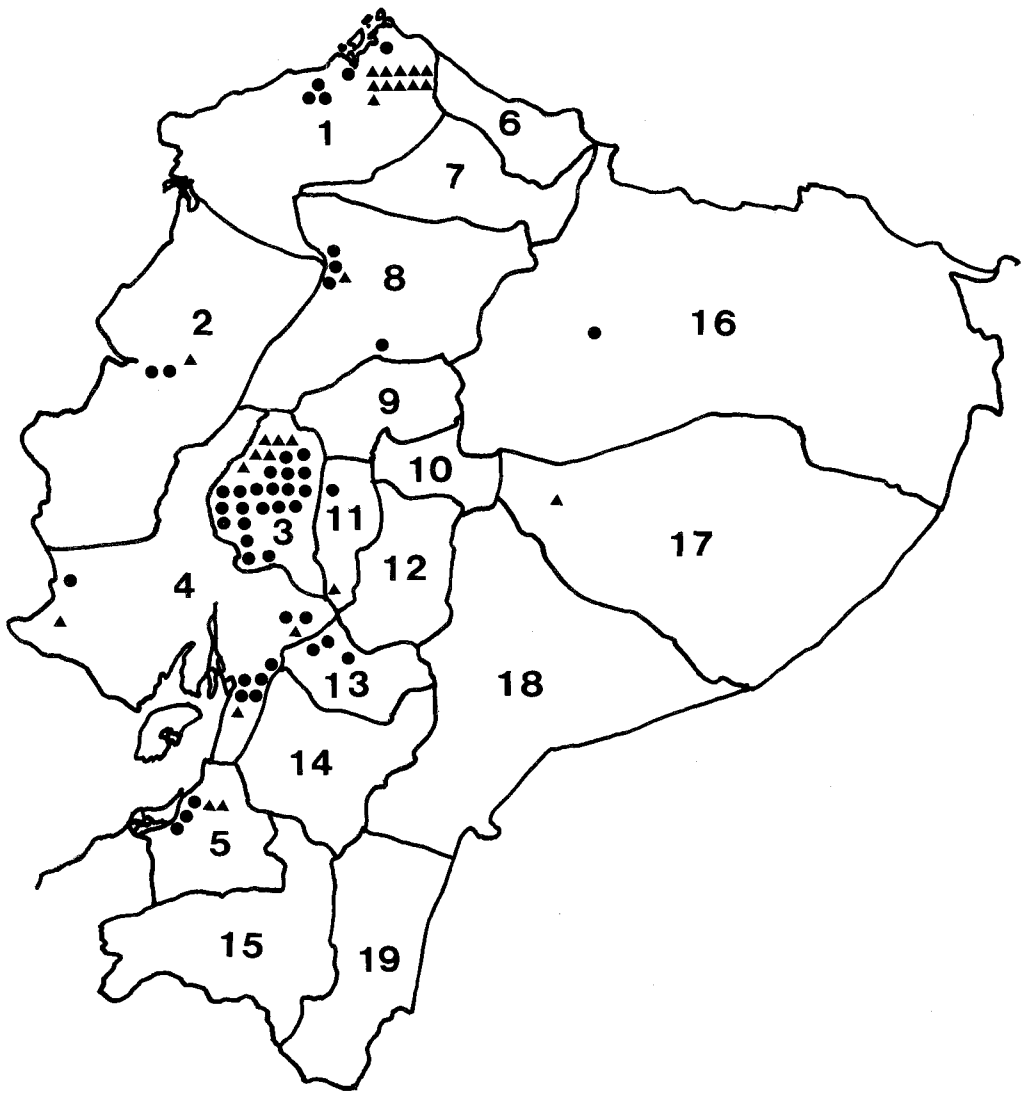

Figure 4. Geographical distribution of patients with mucosal (●) and ear (▲) lesions in different Departments of Ecuador; 48 cases in the former and 26 in the latter. 1 to 19: see Fig. 1.

1975 and 1986, a total of 672 (47.0%) revealed a positive diagnosis for Leishmania amastigotes. These numbers were considered to be rather lower than the actual rates of infection, since impression smears alone had been employed as a diagnostic tool. This method is too insensitive to detect a large number of true leishmaniasis-positive cases, and the number positive cases could be increased by the use of other diagnostic tools, such as skin test and other immunological methods.

In a review of the positive cases diagnosed at INHMT, about 60% of the total had occurred during only two years, 1984 and 1985. This abnormally high occurrence might have been caused by heavy rainfall which was caused by unusual movement of the El Niño Current during that period in Ecuador. This climatic factor could have affected the annual pattern of leishmaniasis transmission by providing a favorable environment for vectors of the disease. Similar considerations of the influence of rainfall were also made on the spreading of onchocerciasis in Ecuador, although the breeding sites are completely different between the two diseases vector insects, Lutzomyia and Simulium. The editorial of Parasitology Today (vol. 2, p. 131, 1986) commented that "in 1982-84 the coastal regions of Ecuador suffered a marked change in climate due to changes in the Pacific ocean current known as El Niño; during these years, rainfall occurred over nine months of the year, causing flooding and displacement of many families; the rise in insect populations associated with the flooding may have contributed to the increased intensity of transmission". On the contrary, excessively heavy rain in Panama

during the 1981 wet season appeared to depress sand fly populations there (Dr. Alexander, personal communication).

In the geographical distribution of the cases, more than 80% of the total were found in the Departments of the littoral regions bordering the Pacific Ocean. Most of the case reports reported in the literature were also from these regions (Heinert, 1924; Valenzuela, 1931; Leon, 1951; Carrera, 1953; Rodriguez and Aviles, 1953; Zerega, 1961; Rodriguez, 1969; Calero and De Coronel, 1981; Hashiguchi et al., 1984), with a few cases were from the Amazonian regions (Carrera, 1945; Amunarriz, 1982) where communications and the medical care system are generally inadequate. In the current study only 13 cases positive for amastigotes of Leishmania were from the latter regions. However, Amunarriz (1982) recently observed 22 patients from the Amazonian regions with cutaneous lesions and 10 with mucosal ones, who were hospitalized for treatment, suggesting that leishmaniasis might be more prevalent than supposed. In the Andean highland of Ecuador, a total of 4 cases were parasitologically positive in subjects from Cuenca (2,541 m above sea level), Department of Azuay, and Loja (2,135 m), Department of Loja. It is uncertain whether these recorded cases were autochthonous in these areas in a manner similar to that of uta in the Peruvian Andes.

An increase in the number of leishmaniasis cases was recognized during the wet seasons, though the occurrence was slightly variable with years. This trend suggested again that rainfall was an important factor in leishmaniasis transmission in the endemic areas of Ecuador. This finding would be important in the timing

of future preventative or control measures.

Age distribution of leishmaniasis cases diagnosed in INHMT revealed a pronounced peak between 16 and 25 of ages in both sexes. This differed somewhat with the results of a previous epidemiological survey, carried out by the present workers in a newly established settlement in Ecuador, which reported that there is no marked age and sex difference in infection rates (Hashiguchi et al., 1984). No age and sex difference of leishmaniasis infection was also recognized when the homesteads were established in areas with active foci of cutaneous leishmaniasis in Panama (Herrer and Christensen, 1976). Moreover, Pessoa (1961) had already pointed out that the American leishmaniasis could attack any persons regardless to age, sex and race, and also that its prevalence depend on the occupation of subjects, on the distance between human habitations and forest areas. Taking these facts into consideration, the results obtained here might indicate a somewhat different pattern of age distribution from the actual pattern in endemic areas. This might be explained by the fact that only cases with active lesions from which amastigotes could be obtained were recorded at INHMT, ignoring already-healed subjects with leishmanial scars. Moreover, a low rate of active cases in examinees aged 36 or over might indicate an acquisition of total lasting immunity in the susceptible population. Recovery from any leishmanial skin lesion has generally been thought to impart a firm and life-long immunity to reinfection (Lainson and Shaw, 1978).

In the anatomical distribution of leishmanial lesions, the

great majority was located in arms (41%) and legs (34%), suggesting these to be the prefered biting sites of vector sand flies. Rodriguez and Aviles (1953) recorded the location of 38 cutaneous lesions in 29 patients from the Pacific coastal region of Ecuador; 65.8% in arms and legs, 31.6% in face and neck, 2.6% in thorax. In the present study, 15% of the total lesions were found on the face, 7% of them on the nose and lip as mucosal leishmaniasis. In the Amazonian region of Ecuador, on the other hand, 60% of the total examinees had lesions on the legs, with only 14%, on the arms (Amunarriz, 1982).

A review of mucosal (espundia) and ear (chiclero's ear) leishmaniasis, showed no limited geographical distribution for these forms in Ecuador. Furthermore, there was no tendency for espundia to occur in older age-groups (36 or over) of the present subjects. With regard to the appearance of mucosal lesions, Hyneman (1971) stated that mucous attack could occur seven or more years after the original cutaneous lesion had healed. Walton et al. (1973) followed the medical histories of patients with leishmanial infections, and found that the characteristic mucous lesions of espundia were observed in these patients 11, 18, 19 and 24 years after the original infection. Based on these findings, it seemed quite reasonable to assume that the subjects with espundia would be found among older age groups in the present study, but such a tendency was not apparent. This discrepancy should be investigated in future follow-up studies of Ecuadorian leishmaniasis patients.

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Maria C. de Aroca
Yoshihisa Hashiguchi

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Chapter 8

SUMMARY

In the present text, results of field studies on several aspects of leishmaniasis epidemiology in Ecuador are presented. These aspects include parasite isolation and characterization, detection of natural infections of sand flies and mammalian hosts with Leishmania, and evaluation of immunological tools in the epidemiological survey. In addition, current knowledge of Ecuadorian leishmaniasis and its endemicity were reviewed. The following points were extracted from each chapter of this text.

Leishmaniasis investigations in Ecuador

Prior to 1982 the principal leishmaniasis research activity in Ecuador was limited to case reports and/or the treatment of patients in medical centers or hospitals, although some studies of vector entomology had been done by several investigators. Thereafter, transmission studies were initiated by the present workers, who detected natural infections of sand flies and wild mammals with leishmanial parasites in endemic areas. According to the articles published in Ecuador to date, there may be three or four clinical forms of the disease: cutaneous cases (CL), ca. 93% of the total; mucocutaneous (MCL), ca. 6 or 7%; and visceral (VL) and diffuse cutaneous ones (DCL). The last two forms have not yet been parasitologically proven in the country. Analysis of the data accumulated in medical institutions revealed that the

disease had a country-wide distribution in Ecuador.

Ecology of areas endemic for leishmaniasis

The Andes divide the country into three natural regions: the Pacific coast including the Andean slope, the Andean and the Amazonian region. The majority of leishmaniasis cases reported was from the Pacific coast, followed by the Amazon. A few cases were also observed in the Andean highland or the mid-Andes. In the text, ecological features of each region relating to the mammalian and sand fly fauna, are taken into special consideration in discussion of disease transmission.

Parasite isolation and their characterization

We have isolated eight stocks, five from humans and three from wild mammals, in the present study. Identifications based on results of serodeme typing using monoclonal antibodies revealed that three of the five from humans are L. b. panamensis (MHOM/EC/87/G05, MHOM/EC/87/G06 and MHOM/EC/87/G07) and all three from wild mammals are L. m. amazonensis (MSCI/EC/87/G02, MPOT/EC /87/G03 and MTAM/EC/87/G04). The remaining stocks from humans require further investigation until they are fully characterized. Results of this will be reported elsewhere.

Natural infections of sand flies and wild mammals

One species of Lutzomyia, Lu. gomezi, was added to the list of Ecuadorian leishmaniasis vectors, in addition to the two known vector species, trapidoi and hartmanni. With regard to reservoir

hosts, one species, Tamandua tetradactyla, was newly implicated. Of three other mammal species, Potos flavus, Sciurus vulgaris and Choloepus h. didactylus, which had already been listed as leishmaniasis reservoirs, the first two mammalian species were also positive for leishmanial parasites in the current study. A search for leishmaniasis reservoir hosts was also made by the immunological method using counter immunoelectrophoresis (CIE) in this study. The CIE technique revealed that the tissue extracts (antigen) of three arboreal species, Didelphis marsupialis, Caluromys lanatus and Choloepus h. didactylus, reacted immunologically with anti-leishmanial serum, producing precipitin lines. In the first two mammalian species, no natural infections with leishmanial parasites have parasitologically been observed. It was, however, suggested that these immunologically positive mammals play an important role as reservoirs of the desease in endemic areas of Ecuador.

Immunological diagnosis of the disease

The present immunological tools, skin test and ELISA, were highly sensitive and specific for cutaneous and mucocutaneous leishmaniasis in Ecuador. From the results obtained, it was concluded that these diagnostic method could be very useful in screening of the disease in epidemiological surveys.

Epidemiological findings

Andean leishmaniasis (uta) in Ecuador was first described from the mid-Andes (2,300 to 2,500 m above sea level). The

suspected sand fly vector is Lu. peruensis, which was the only species collected during our field survey. No Leishmania-positive fly was found among 51 specimens dissected. In order to clarify epidemiological features such as human, reservoir and vector infections in this mid-Andes endemic area, a further investigation will be conducted by the present workers. Bacterial flora was isolated from highland and lowland leishmanial ulcers, in an attempt to determine the effect of bacterial concomitant infection on the development of the distinct skin manifestations. The prevalence rate of Gram-negative rods, but not Gram-positive cocci or anaerobic bacilli was apparently different between two types of ulcer, occurring in 18.2% of highland as opposed to 37.5% of lowland infections. Gram-negative rods were composed of such enterobacteria as Escherichia, Serratia, Klebsiella and Enterobacter. Histological examination showed inflammatory cell infiltrations mostly composed of small lymphocytes throughout the dermis in highland ulcers, while those from lowland cases restricted to the deep dermis. When the parasitologically-proven prospective leishmaniasis cases were reviewed, the most important period for transmission of the disease in Ecuador was considered to be during the rainy season, from October to April.

Most of the findings presented here can be considered as preliminary results of the investigation. Based on these basic data obtained, however, we hope to further elucidate the epidemiological features of leishmaniasis in the New World, with particular reference to Ecuador, in future studies.

Yoshihisa Hashiguchi

Chapter 9

APPENDIX

Abstract of Related Papers Published

Japanese Journal of Parasitology,
33 (5), 1984, pp. 393-401

1. An Epidemiological Study of Leishmaniasis in a Plantation "Cooperativa 23 de Febrero" Newly Established in Ecuador

Yoshihisa Hashiguchi, Vicenta Vera De Coronel and Eduardo A. Gomez L.

Abstract. An epidemiological study was performed on leishmaniasis in September 1982, in a plantation "Cooperativa 23 de Febrero" newly established in the region of Andean slope in Ecuador. The first immigration of inhabitants in this plantaion started from August, 1977. Fifteen (15.8%) of the 95 inhabitants examined were diagnosed as positive for leishmaniasis with ulcers (active leishmanial lesions) on the skin. During the period between 1977 and 1982, a total of 57 (60.0%) of 95 examinees have suffered from the disease. Regardless to age and sex, leishmanial infections occurred almost evenly. The result indicated that the transmission of leishmaniasis had been occurring in a wide range of working and housing areas in the plantation. In most of the active patients, the onset occurred in July or August. The length of time between immigration and the onset of leishmaniasis ranged from 3 to 59 months, mostly 9 to 36 months in those with active leishmanial lesions. A large number of leishmanial lesions were located on the upper parts of the body exposed.

American Journal of Tropical Medicine and Hygiene,
34 (3), 1985, pp. 440-446

2. Natural Infections with Promastigotes in Man-biting Species of Sand Flies in Leishmaniasis-endemic Areas of Ecuador

Yoshihisa Hashiguchi, Eduardo A. Gomez L.,
Vicenta Vera De Coronel, Tatsuyuki Mimori
and Masato Kawabata

Abstract. In order to determine the vectors of leishmaniasis in Ecuador, 1,054 man-biting sand flies from the Department of Cañar were dissected and examined for promastigotes. There were 2 man-biting species, Lu. trapidoi and Lu. hartmanni in this endemic area of the disease. The infection rates were 7.7% in the former and 3.9% in the latter species, demonstrating the different rates in various localities and altitudes of the study areas. There was an association between infection rates and the time of day, suggesting some connection with biting activity of sand fly species. In collections using human bait at 7 study areas in 5 Departments, 6 man-biting species were recognized, indicating different dominant species in each area. It was assumed that the dominant species would play an important role as the principal vector of leishmaniasis in each endemic area. As to species determination of the present Leishmania promastigotes, suffice it to say that the parasites are Leishmania sp., presumably L. braziliensis s.l., until the isolates have been typed.

Annals of Tropical Medicine and Parasitology,
79 (5), 1985, pp. 533-538

3. Biting Activity of Two Anthropophilic Species of Sandflies, Lutzomyia, in an Endemic Area of Leishmaniasis in Ecuador

Yoshihisa Hashiguchi, Eduardo A. Gomez L.,
Vicenta Vera De Coronel, Tatsuyuki Mimori
and Masato Kawabata

Abstract. The biting patterns of Lutzomyia trapidoi and Lu. hartmanni, vectors of leishmaniasis, were studied using a human bait in an endemic area on the Pacific slope of the Andes in Ecuador. The results suggest that Lu. trapidoi is primarily an early biter at dusk, with the first peak at 20:00-21:00 hours and the second at 03:00-04:00 hours; and that Lu. hartmanni bites more constantly throughout the night, with a pronounced peak between 23:00 and 24:00 hours. The biting activity, however, shows a marked variation at each site and between different collections at the same site. The activity and the biting places on man are discussed in relation to human infection with leishmaniasis in the area and the location of lesions on patients.

Transactions of the Royal Society of Tropical Medicine and Hygiene, 79 (1), 1985, pp. 120-121

4. *Leishmania* Isolated from Wild Mammals Caught in Endemic Areas of Leishmaniasis in Ecuador

Yoshihisa Hashiguchi, Eduardo A. Gomez L., Vicenta Vera De Coronel, Tatsuyuki Mimori and Masato Kawabata

Abstract. In total, the following 48 wild mammals were caught and examined for *Leishmania* infections in the two localities, Naranjal (N) and Ocaña (O): *Didelphis marsupialis*, nine in N and five in O; *Tamandua tetradactyla*, one and nil; *Choloepus hoffmani didactylus*, one and nil; *Sylvilagus braziliensis*, one and nil; *Dasypus novemcinctus*, one and one; *Sciurus granatensis*, four and one; *Rattus espinosus*, six and nil; *R. rattus*, one and nil; *Coendou bicolor*, two and nil; *Agouti paca*, two and nil; *Dasyprocta punctata*, two and nil; *Potos flavus*, eleven and nil. Of these animals, only three were positive for the parasite, namely, one *Choloepus hoffmani didactylus*, one of four *Sciurus granatensis* and one of 11 *Potos flavus* from Naranjal. Only cultures from the liver of these three animals were positive for *Leishmania*, those from the spleens being negative. In the light of future planning of control measures of the disease in Ecuador, it is thought to be important to make a search for the reservoir hosts in endemic areas. To determine the principal host in this country, however, more detailed such a work should be performed.

Japanese Journal of Tropical Medicine and Hygiene,
13 (3), 1985, pp. 205-2453

5. A Review of Leishmaniasis in the New World with Special Reference to its Transmission Mode and Epidemiology

Yoshihisa Hashiguchi

Abstract. Leishmaniasis is a widespread protozoan disease in the New World from southern US at the north to northern Argentina at the south. The disease is principally divided into three forms, i.e., cutaneous, mucocutaneous and visceral leishmaniasis, mainly based on the clinical manifestations in patients and on the species of the causative agents, Leishmania. The leishmaniases are well known as a considerable public health problem in endemic areas of the disease in the New World, except for Canada, Chile and Urguay where no such a disease occurs. In this review, an attempt was made to understand a global situation of the epidemiology of the New World leishmaniasis, laying an emphasis on the pick-up of known endemic areas, vectors and reservoir hosts of different species of the genus Leishmania in each country. From the information published hitherto, it was found that an intensive leishmaniasis research has been made in Central and South American countries, such as Belize, Panama, Venezuela and Brazil. The study, however, was poorly done in many other countries of the New World, without limiting endemic areas or deciding

vectors and reservoir hosts of the disease. In the present text, the author emphasized on a future research importance of epidemiological characteristics including the transmission mode of New World leishmaniases, in order to search for suitable control measures in each endemic area of different countries. Most of the transmission of leishmaniasis in the New World have been found in dense tropical rain forests with various species of Leishmania, sand flies and mammals. In such circumstances of endemic areas of leishmaniasis in the New World, the difficulty of the prophylaxis and control has frequently been pointed out by several investigators. At the present situation of leishmaniasis research without a suitable vaccine and sufficient epidemiological data, ones have commented that the only control measure for New World leishmaniasis is to remove all the inhabitants of communities from regions at risk of the disease, or to perform throughly deforestrations around dwelling areas or working places. Past trials of several control measures, such as the spraying of insecticides, destruction of reservoir hosts, application of some vaccines and etc., were also briefly reviewed in the text. (in Japanese with English summary)

Japanese Journal of Tropical Medicne and Hygiene,
15 (1), 1987, pp. 7-15

6. Leishmaniasis in Different Altitudes on Andean Slope of Ecuador

Yoshihisa Hashiguchi, Eduardo A. Gomez L.,
Vicenta Vera De Coronel, Tatsuyuki Mimori
and Masato Kawabata

Abstract. An epidemiological survey was performed in a leishmaniasis-endemic area along highway which was established about 15 years ago on the Andean slope of Ecuador; the area ranged from 300 m to 1,500 m above sea level. In general survey, 64 (14.3%) of the 446 subjects examined were positive for leishmanial signs. In order to know leishmanial infections in relation to the altitudes of dwelling sites of subjects, analysis was made on 224 children with 5 to 15 years of age. At 4 different sites with 500 m, 1,000 m, 1,300 m and 1,500 m above sea level, the infection rates of the subjects from the individual sites were 17.4, 18.8, 5.6 and 8.8%, respectively. A statistically significant difference was recognized between the altitudes, 500-1,000 m and 1,300-1,500 m ($0.01<p<0.05$, $\chi^2 = 5.314$), but not between 500 m and 1,000 m and between 1,300 m and 1,500 m. Leishmanial infections of the children who came from forest and highway areas were compared in each altitude. But no significant difference was found between forest and highway dwellers at any study sites.

Annals of Tropical Medicine and Parasitology,
81 (5), 1987, in press

7. The Relationship between Severity of Ulcerated Lesions and Immune Responses in the Early Stage of Cutaneous Leishmaniasis in Ecuador

Tatsuyuki Mimori, Yoshihisa Hashiguchi,
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and Vicenta Vera De Coronel

Abstract. The relationship was examined between the severity of ulcerated lesions and immune responses in 19 Ecuadorian patients in the early stages of New World cutaneous leishmaniasis. As an immunological assay, the humoral immune response was assessed by enzyme-linked immunosorbent assay (ELISA) and the cell-mediated response by delayed type skin test for leishmanial antigen (leishmanin test). There was a statistically significant correlation ($r = 0.61$, $p<0.01$) between the total area of ulcerated lesions and the reciprocal titre of ELISA in identical subjects. However, no significant difference was observed in the ELISA titre between patients with a single lesion and those with multiple lesions ($x^2 = 7.06$, $df = 5$, $p>0.01$). These results suggest that the severity of ulcerated lesions relates to the activation of both the humoral and cell-mediated immune systems in the early stage of New World cutaneous leishmaniasis.