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STUDIES ON VISCERAL LEISHMANIASIS IN VENEZUELA

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SUMMARY

The Authors list all cases of visceral leishmaniasis recorded in Venezuela until 1960, along with the principal data concerning each case. They point out that the available information on kala-azar in Venezuela claimed for additional investigations to elucidate problems not yet studied, chiefly those related to its epidemiology (geographic distribution, transmission and animal reservoirs).

Since October 1960, the Authors are carrying out studies which include the search of human cases and of animal reservoirs and the capture of sandflies. In a survey of canine kala-azar, 112 dogs were examined and 12 (10.7%) were positive for leishmaniae, probably *L. donovani*. The Authors show that, according to their own observations and to those of other workers, kala-azar in Venezuela seems to be similar to the Mediterranean type. They finally point out that in areas where the disease is found, *Phlebotomus longipalpis* is frequently encountered.

INTRODUCTION

Visceral leishmaniasis is known in Venezuela since 1941, when MARTÍNEZ & PONS [14] reported the first human case found in the State of Guárico (Las Mercedes). These authors emphasize the need for more investigations on that disease, which might be missed on account of the possibility of being confused with Chaga's disease and malaria, common in that part of the country. POTENZA & ANDUZE [20] described the second case from the Puerto de Ciudad Bolívar, diagnosed by histological examination of liver material obtained by viscerotomy. FRANCO [10] studied the third case, a girl eight years old, from Yagua, State of Carabobo. MISLE PEÑA, RON PEDRIQUE & HENRÍQUEZ [15] published a case from the State of Aragua. BARNOLA & POTENZA [2] presented three more autochthonous cases, one from Los Chaguaramitos (State of Guárico), another from Macuto (Federal District) and a third from Mamo (Federal District). A case from Madrid (Spain) was described in the same paper.

Subsequently, PIFANO [17] referred that in Venezuela, until 1954, kala-azar had been found in the following places: States of Aragua, Bolívar, Carabobo, Cojedes, Guárico, Lara and the Federal District. LIZARRAGA & GULACSY [12] reported six new cases, all in children: three from the State of Cojedes, two from the State of Carabobo and one from the State of Guárico. LIMA GÓMEZ, WUANI & CASTILLO [11] observed an adult in-

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TABLE I

Published cases of kala-azar in Venezuela

No.	Date	Age	Sex	State of origin	Diagnosis	Treatment	Author
1	1941	20 y.	M.	Guárico (Las Mercedes)	Splenic puncture	Neostibosan	Martínez and Pons
2	1942	10 y.	F.	Bolívar (Las Bombitas)	Viscerotomy	—	Potenza and Anduze
3	1945	8 y.	F.	Carabobo (Yagua)	Bone marrow puncture	Emetic (good results)	Franco Palacios
4	1946	18 y.	M.	Aragua	Splenic puncture and autopsy	—	Misle Peña, Ron Pedrique and Henríquez
5	1950	3 y.	F.	Federal District (Mamo)	Bone marrow puncture	Emetic (good results)	Barnola and Potenza
6	1950	22 m.	F.	Federal District (Macuto)	Autopsy	—	Idem
7	1950	3 y.	F.	Guárico (Los Chaguaramitos)	Autopsy	—	Idem
8	1955	7 y.	M.	Cojedes (Potrero Lincero)	Splenic puncture	Emetic and anthiomaline (good results)	Lizarraga and Gulacsy
9	1955	7 y.	F.	Cojedes (La Quesera)	Bone marrow puncture	Emetic. Death (cardiopathia)	Idem
10	1955	21 m.	F.	Cojedes (Tinaquillo)	Smear of peripheric blood	Death before 24 hours of hospitalization	Idem
11	1955	11 y.	F.	Carabobo (Mariara)	Splenic puncture	Emetic, anthiomaline and solustibosan (good results)	Idem
12	1955	2 y.	F.	Carabobo (Paya)	Bone marrow puncture	Anthiomaline and solustibosan (good results)	Idem

TABLE I (*continued*)

No.	Date	Age	Sex	State of origin	Diagnosis	Treatment	Author
13	1955	1 y.	M.	Guárico (El Totumo)	Bone marrow puncture	Anthiomaline. Death (toxicosis)	Idem
14	1956	22 y.	M.	Lara (Curarigua)	Splenic puncture	Solustibosan (good results)	Lima Gómez, Wuani and Castillo
15	1958	15 y.	M.	Carabobo (Belén)	Bone marrow puncture	Emetic (good results)	Pineda
16	1958	13 m.	F.	Guárico (S. José de Tiznados)	Bone marrow puncture	Neo-antimoson. Death (complications)	Pineda
17	1959	28 y.	M.	Falcón (Pueblo Nuevo)	Bone marrow puncture, spleen material	—	Bemerqui and Pirela
18	1960	2 y.	M.	Zulia (Campo Barúa)	Sternal biopsy; splenic puncture	Glucantime. Death (complications)	Núñez Montiel and Mayz Vallenilla

TABLE II

Cases only referred by Pifano [17]

No.	Date	Age	Sex	State of origin	Diagnosis	Treatment	Author
19	1952	20 y.	...	Lara (El Tranquero)	Bone marrow puncture	—	Zapata and Figueroa
20	1953	8 y.	M.	Lara (Las Casitas)	Bone marrow and hepatic puncture	—	Zapata and Zubillaga
21	1953	1½ y.	M.	Lara (Monte-Cristo)	Bone marrow puncture	—	Alvarez and Zapata

fected in the State of Lara. PINEDA [18] described two new cases from the State of Guárico and BEMERQUI & PIRELA [4] published one from the State of Falcón. Finally NÚÑEZ MONTIEL & MAYZ VALLENILLA [16] reported the case of a boy two years old, from the State of Zulia.

In Table I we list the 18 autochthonous cases of kala-azar published in Venezuela, from 1941 to 1960, and give the principal data concerning each (date, age, sex, origin, methods of diagnosis, treatment, author). In Table II three more cases are listed, which were not the subject of detailed publications, but are referred in the paper of PIFANO [17]. So, according to the Venezuelan bibliography, until 1960, twenty-one human cases of autochthonous visceral leishmaniasis were known. Their distribution by State is as follows:

Guárico	4
Carabobo	4
Lara	4
Cojedes	3
Federal District	2
Bolívar	1
Aragua	1
Falcón	1
Zulia	1

The finding of new cases, practically every year since 1941, indicated that the disease deserved more attention. So we started studies on the subject, chiefly with the purpose of clarifying its actual importance in Venezuela.

The data on the geographical distribution of kala-azar in Venezuela, which we could get from previous publications, made it advisable to start our studies in one of the three States where its incidence seemed to be higher, and of those it was in Guárico that we found adequate facilities. Therefore, it was in the Distrito Roscio, of this State, that we started our surveys in October 1960. In this first paper we present the program of our studies and the first results obtained. These studies aim to clarify some hitherto unsolved problems on the epidemiology of the disease in Venezuela, such as its geographical distribution, non-human reservoirs and transmission.

Our investigations are being undertaken in different villages (pueblos, caseríos), mostly in Distrito Roscio, and include: 1) search for human cases; 2) search for non-human reservoirs; 3) capture of sandflies; 4) taxonomy and biology of sandflies, and 5) search for natural infection among these insects and observations on their experimental infection.

SURVEY ON CANINE KALA-AZAR

The importance of the domestic dog as a reservoir of *L. donovani* in other endemic regions of kala-azar is well known.

In South America, recent studies in various endemic foci in Brazil have emphasized the importance of that animal in the maintenance of the endemy (DEANE [9], BRENER [5], ALENCAR [1]).

So, adequate examination of these animals has been one of our principal aims.

At first we looked for a method which would permit the examination of a maximum number of dogs in a short time, without danger of death or with the minimum possible risk, in order to avoid their owners' objections, because, to the "campesino", the dog is not only a pet, but also a very useful animal in watching over his "rancho" (house), his "conuco" (plantation), and following him in his hunting expeditions.

In our experience, the best way to reach this end is to submit the animal to a general anesthesia before proceeding to the examinations. In field conditions, this anesthesia may be easily performed by using and intraperitoneal injection of nembutal (sodium pentobarbital), in a dosis of, approximately, 30 mg per kilogram of body weight. We use to dissolve the drug in sterile saline to a concentration of 25 mg per milliliter.

In an adequate card the animal is identified as to name, sex, color, name and address of the owner, and the results of a clinical examination, particularly as to suspicious cutaneous lesions which are duly recorded.

To examine the dogs of a "caserío" (village), we usually assemble most of them in

Figure — Anaesthetized dogs in a yard.

one place (for instance, the yard of a countryman's house, as shown in Figure) and proceed to the general anesthesia of all the animals. The dogs fall asleep within a few minutes to half an hour. The anesthesia lasts from about 30 minutes to 4 hours.

During this interval, one or various persons, consecutively or simultaneously, can proceed to the examinations. After trying different procedures, we selected the following ones:

1) *Bone marrow puncture* — At the beginning, the puncture was made at the level of the sternum. But, as the sternal puncture of dogs presents some difficulties, we replaced it, with good results, by the tibial puncture, performed at the level of the upper extremity of this bone, approximately one finger below the femoro-tibial articulation (CABASSU [6]), using a needle provided with a mandrel, the type employed for sternal puncture in human adults. The bone marrow material is aspirated with a 20 ml syringe, previously washed in sterile saline, to avoid precocious coagulation of blood, which would prevent the making of good smears.

2) *Hepatic puncture* — This is made according to the classical technique used by the authors who have studied canine kala-azar, as described by DEANE [9].

3) *Skin biopsy of the ear* — After cutting the hairs, a fragment of skin approximately 1 cm^2 is cut from the tip of the ear; the blood from the piece is dried with filter paper; by apposition a smear is made on a slide, with the cut surface on the fragment. The hemorrhage that supervenes at the level of the biopsed ear may be checked by an incandescent platinum loop. It seems necessary to emphasize that these hemorrhages are more conspicuous when the animal presents lesions peculiar to the disease (nodules, edema, infiltration). The fragment of tissue used for the smear is afterwards put in formalin solution (10%) for posterior histopathological examination.

4) *Thick blood films* — At the beginning we made the thick film by collecting blood flowing from the surface of the biopsed ear. As conspicuous hemorrhages hinder the making of a good smear, we now use the following procedure: before cutting the ear is pricked at its tip with the teeth of

a Kocher's or another forceps provided with teeth; pressing the ear at this place, drops of blood flow and may be easily collected on a slide. Of course, thick blood films are made in order to look for possible infections with *Trypanosoma cruzi* or *T. rangeli.*

All the bone marrow, skin and liver smears, were fixed by methanol and stained by Giemsa. The thick blood films were directly stained. The biopsed ear fragment was included in paraffin and the sections stained by hematoxylin-eosin.

Each smear was examined systematically for 10 minutes; when negative, other smears of the same material were examined for another five minutes.

The positive dogs were transferred to the laboratory for observation.

From some of the positive dogs, bone marrow material was planted in modified N.N.N. medium, and definite development of leptomonal forms was seen in three instances, indicating that we were dealing with a parasite of genus *Leishmania.* More details on the cultures obtained from these dogs will be given in another note (TORREALBA *et al.*[21]).

Up to now 112 dogs were examined and 12 of them (10.7%) were positive for leishmaniae, probably *Leishmania donovani.*

On Table III we include the 12 positive dogs and the methods of examination, in order to compare the results obtained by each method.

TABLE III

Parasites in bone marrow liver and skin smears of dogs with kala-azar

Dog No.	Positivity in:		
	Bone marrow	Liver	Skin (smear of ear)
42	+	+	—
61	+	—	—
79	+	—	—
83	+	+	+
86	+	+	—
87	+	+	+
89	+	—	+
91	+	+	—
100	+	—	+
101	+	—	+
107	+	+	+
110	—	—	+
Total positives	11	6	7
Percent positives	91.6	50.0	58.3

HUMAN CASES

During our surveys, from October to December 1960, six new human cases of kala-azar were studied (BARRIOS *et al.*[3]): four were children below 5 years of age; one was a girl 10 years old and one was an adult. As regards age distribution, kala-azar in Venezuela is similar to the type observed in the Mediterranean, as shown by our own few observations and those previously published by other workers (see Tables I and II).

SANDFLIES

Various captures have been made in the areas where we found human and canine kala-azar. The insects collected are being studied. It seems interesting, however, to point out that in those areas, *Phlebotomus longipalpis* is frequently found. As this species is related with the transmission of kala-azar in other regions of South America (CHAGAS, CASTRO & FERREIRA[7]; CHAGAS, FERREIRA, DEANE *et al.*[8]; MANGABEIRA[13]; PONDÉ, MANGABEIRA & JANSEN[19]; DEANE[9]) it will be the subject of detailed studies to be presented in future notes.

RESUMO

Após um histórico dos estudos realizados sôbre o calazar na Venezuela até 1960, os autores apresentam o programa das pesquisas que vêm efetuando no país a partir de outubro daquele ano e que compreendem a procura de casos humanos e de reservatórios animais e capturas de flebótomos.

Descrevem um inquérito de calazar canino, em que examinaram 112 cães, encontrando 12 (10,7%) positivos para leishmânias, provàvelmente de *L. donovani*. Segundo suas próprias investigações e as de outros pesquisadores, o calazar na Venezuela parece com o que ocorre no Mediterrâneo. Chamam, por fim, a atenção para o encontro freqüente, nas zonas endêmicas venezuelanas, do *Phlebotomus longipalpis*, espécie relacionada com a transmissão do calazar em outros focos da região neotropical.

RESUMEN

En esta nota los autores hacen primero un histórico de los estudios sobre el Kala-azar en Venezuela, desde 1941 hasta 1960. En seguida presentan el programa de estudios que vienen desarrollando desde Octubre de 1960: búsqueda de casos humanos; búsqueda de reservorios extra humanos; capturas de flebotomos para diferentes investigaciones. Relatan una encuesta sobre Kala-azar canino, en que observaron 112 perros con el encuentro de 12 positivos (10,7%) para formas de leishmania, probablemente de la *L. donovani*. Según sus propias investigaciones y las de otros observadores, el Kala-azar en Venezuela se parece al que ocurre en el Mediterráneo. Finalmente, llaman la atención para el encuentro frecuente en las zonas endémicas, de *Phlebotomus longipalpis*, que, en ciertos focos de Kala-azar de la Región Neotropical, se ha mostrado tener relación con la transmisión de esta enfermedad.

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