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Leishmaniasis in Tuscany (Italy) (**)

SUMMARY. — Research work on leishmaniasis in Tuscany is being reviewed. The geographic and temporal distribution of human visceral and cutaneous cases is reported. Leishmanin skin reaction of human population has shown a positivity as high as 30% in some foci. The suspected vectors are *Phlebotomus perniciosus* and *P. perfiliewi*.

Leishmania was isolated from the fox and from the black rat. The role of these wild species as *Leishmania* reservoirs is, however, still to be proved.

Let me first thank the illustrious Accademia dei XL and the Istituto Italo-Africano for the opportunity given to me of informing this distinguished audience on our present research work.

Ecology is of paramount importance in the study of diseases whose cycles include one or more vertebrate hosts. Information in this field is essential for prevention and control of such diseases. One of these is leishmaniasis, a complex of infections caused by a protozoan, occurring in temperate and tropical regions of all continents except Australia and Antartica. It shows diverse manifestations, from an innocuous cutaneous sore to a severe, often fatal, visceral form. The incriminated vectors are different species of sandflies. Several vertebrates, beside man, have been indicated as being the parasite hosts: dogs and canids mainly for visceral leishmaniasis, rodents principally for cutaneous leishmaniasis. Human infection is an accidental occurrence, a side line of the cycle, not involved in the transmission, except, as far as we know, in India and North East Kenya where the vector apparently transmits visceral leishmaniasis from man to man (a true anthroponosis).

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Visceral and cutaneous leishmaniasis are distributed in Italy from the Riviera down to Sicily, Sardinia and minor islands. Many epidemiological aspects of the disease in our country are still obscure:

a) Up to present, biochemical identification of the Italian parasites has not been carried out;

b) It is still to be proved that the incriminated vectors are the only ones responsible for the transmission;

c) The reservoir of cutaneous leishmaniasis is still unknown, and for visceral leishmaniasis there may be reservoirs others than the dog.

To elucidate some of these points, a research project, sponsored by the Ministry of Health, has been started by our Institute. (The graduate personnel

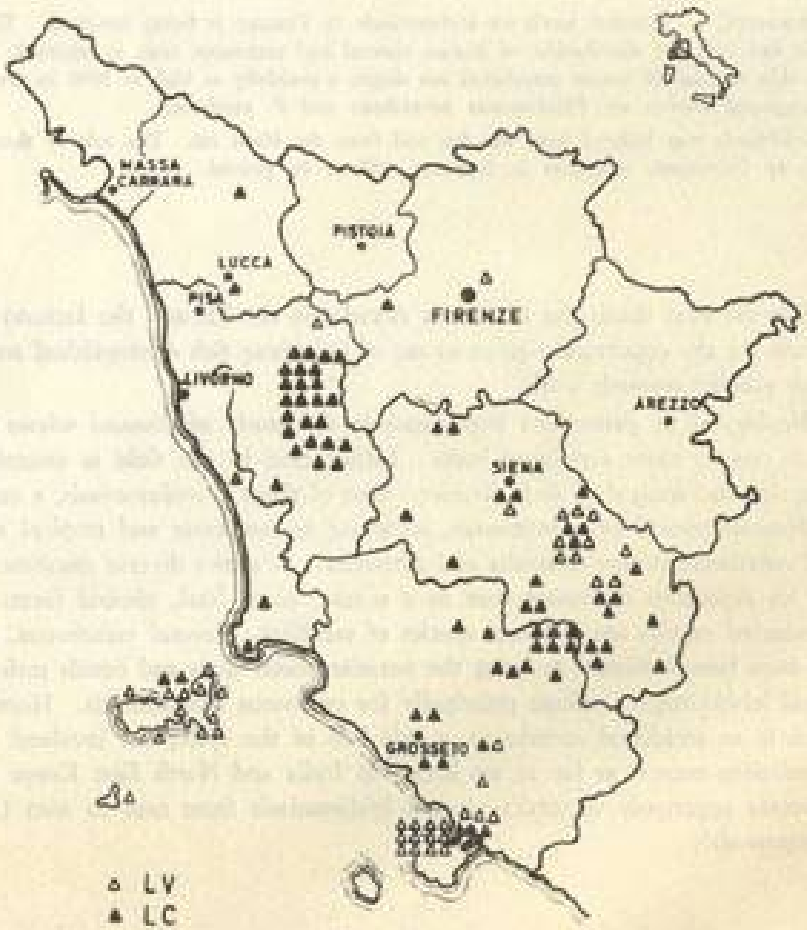

Fig. 1. — Geographical distribution of VL and CL cases reported in Tuscany from 1933. (Bettini, Maroli and Gradoni, to be published).

involved in the project at the Institute is the following: S. Bettini, project leader; M. Maroli, entomologist; L. Gradoni, parasitologist; E. Pozio, zoologist; M. Gramiccia, microbiologist; A. Pitini, histologist).

The project aims are:

a) Setting up a cryobank of isolates to be biochemically identified and maintained at −180°C in liquid nitrogen for further research;

b) Studying the epidemiology of the disease in Tuscany where both visceral and cutaneous leishmaniasis are present (incidentally, very rarely the two diseases coexist).

On the map (fig. 1) are indicated all visceral and cutaneous leishmaniasis cases reported in Tuscany from 1933. Note the patchy distribution of both forms, which is a characteristic of the disease. The temporal distribution of patent cases of both forms (fig. 2) shows a low endemicity of the disease, which strongly suggests the presence of a vertebrate host, other than man, where the parasite may survive.

It would, however, be incorrect to state that *Leishmania* infection in man is a rare occurrence. An investigation carried out in some foci of Tuscany has revealed that the positivity of the human population to the skin test with leishmanin was as high as 30%, the frequency increasing with age (fig. 3), suggesting that in the last 60 years there has been a steady probability for the parasite to

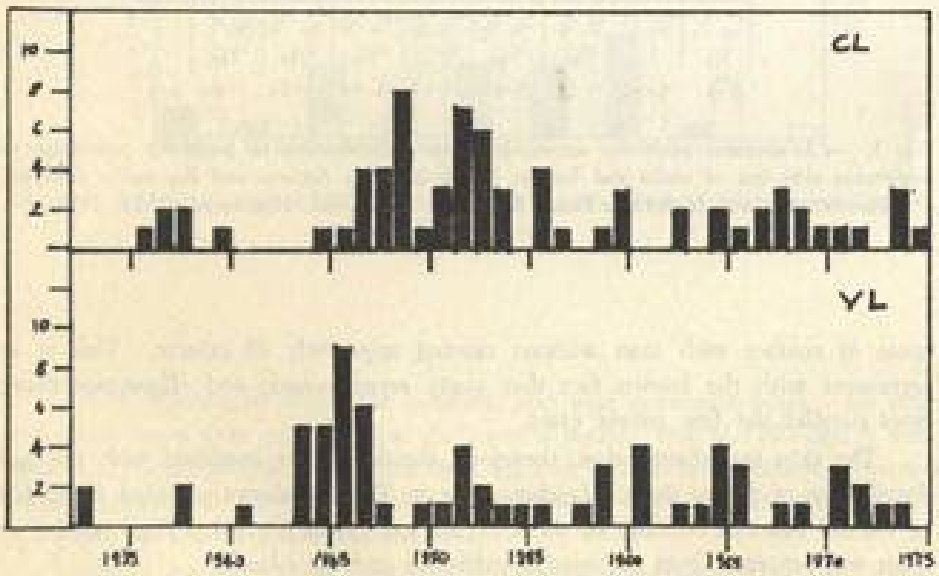

Fig. 2. — Temporal distribution of CL and VL cases reported in Tuscany from 1933. (Bettini, Maroli and Gradoni, to be published).

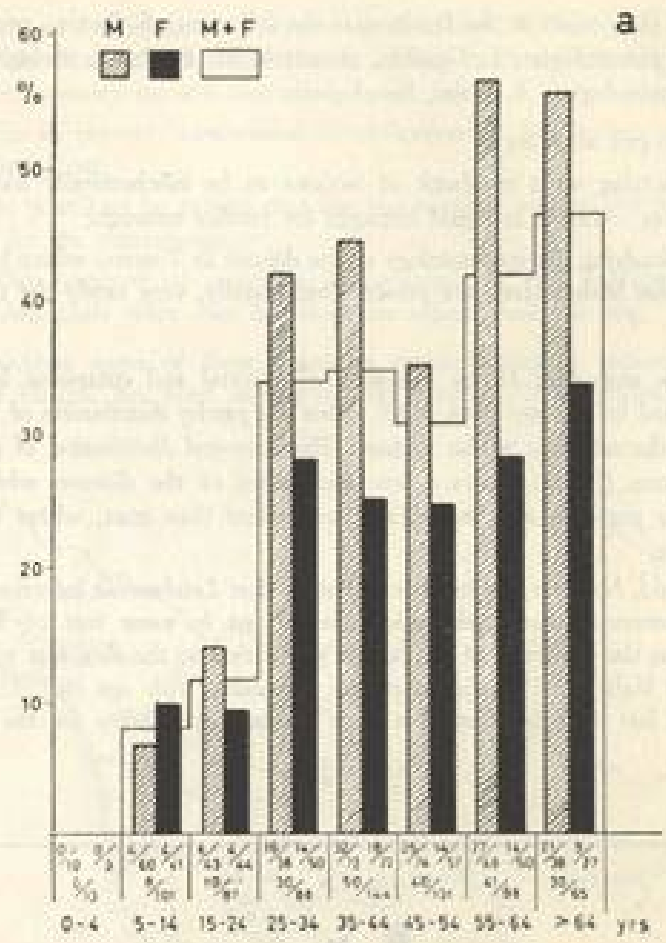

Fig. 3. — Leishmanin positivity according to age: distribution of positivity percentage to leishmanin skin test of males and females in the localities Asciano and Baccinello. (Bettini, Pampiglione and Maroli, «Trans. Roy. Soc. Trop. Med. Hyg.», *71*, 73-79, 1977).

come in contact with man without causing apparently ill effects. This is in agreement with the known fact that many asymptomatic and oligosymptomatic cases parallel the few patent cases.

The skin test distribution, therefore, should not be confused with the age distribution of patent cases. Leishmaniasis in Tuscany shows a higher frequency of visceral cases in children of the 1-2 year age group (fig. 4). The majority of them was reported from the coastal territories and the islands.

Let us see now which is the vector distribution and prevalence in the province of Grosseto, where about 11.000 female sandflies were collected during

one season (fig. 5). *Phlebotomus perniciosus*, suspected vector of visceral leishmaniasis, is present mainly on the coast; *P. perfiliewi*, suspected vector of cutaneous leishmaniasis, on the hills, but also on the intermediate territory where visceral leishmaniasis has been transmitted to man. The problem of *Leishmania* transmission is, therefore, still open.

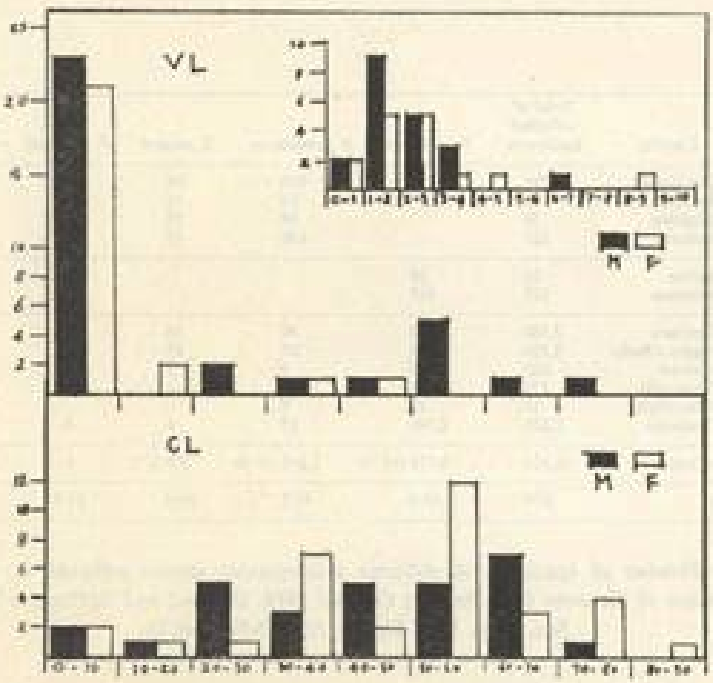

Fig. 4. — Age distribution of VL and CL cases in Tuscany. (Bettini, Maroli and Gradoni, to be published).

A few words now about the domestic and wild vertebrate hosts. All dogs of a single focus were examined and their serum tested. Out of 110 dogs, 3 were positive (2 asymptomatic, 1 showing clear signs of visceral leishmaniasis recovered without treatment). Asymptomatic leishmaniasis in dogs may be important by increasing the risk of transmission to man.

A search for *Leishmania* from wild vertebrates in the same focus gave encouraging results. More than four hundred specimens belonging to 12 species of Insectivora, Rodentia and Carnivora were trapped and spleen homogenates inocu-

lated into hamsters, a laboratory animal highly susceptible to the disease. *Leishmania* was thus isolated from three black rats and one fox. Spleen material of the infected hamsters was then inoculated into agar-blood medium for *in vitro* cultures. Parasite growth showed peculiar characterictics. Rapid growth of canids cultures, slow for rodent cultures. At present, one strain from man, seven from dogs, one from fox and three from rodents have been isolated and partly cultured in tubes.

Station No.	Locality	Total of collected specimens	*P. perfiliewi*	*P. pernicieusi*	*S. minuta*	*P. papatasi*	*P. mascittii*
1	Cala Galera	1,004		910	94		
2	Convento Frati	15		12	1		2
3	Campone	34		19	15		
4	Lividonia	221		178	43		
5	Parrina	16	16				
6	Polveresa	107	107				
7	Cumpena	2,780	2,753	10	16		1
8	Poggio Abechi	3,334	3,218	29	87		
9	Pianetto	103	96	5	2		
10	S. Antonio	372	366	6			
11	Mille Miglia	25	16	9			
12	Ciperuino	2,821	2,799	15	1	6	
Totals (% of total)		11,032	9,571 (86·7)	1,193 (10·8)	259 (2·3)	6	3
% of males		57·0	53·8	81·1	44·6	83·3	0·0

Fig. 5. — Number of specimens of different phlebotomine species collected in 12 stations of the Province of Grosseto from June to October 1975. (MAROLI and BETTINI, « Trans. Roy. Soc. Trop. Med. Hyg. », *71*, 315-321, 1977).

So far, the only identified isolates (identification by Dr. Chance of Liverpool) are those from the fox and dogs, all of which are similar to *L. infantum* occurring in the Mediterranean area. The fox, therefore, should be highly suspected, as it is in France, to represent a wild reservoir, the chain link with the dog.

While waiting for the identification of the isolates, three possible explanations exist:

a) the parasite may belong to a cutaneous leishmaniasis strain which has visceralized in rats, as it was observed in Iraq;

b) it could be a wild rat strain, as the one isolated from *Tatera robusta* in North East Kenya;

c) the isolates could be similar to a human visceral strain selected in *Rattus rattus*, a naturally resistant species, with difficulty to adapt itself to the artificial medium.

Any hypothesis on rodents being possible reservoirs of human leishmaniasis is, therefore, still premature.