

**LEISHMANIASIS IN TUSCANY (ITALY) :**  
**VIII — Human population response to leishmanin**  
**in the focus of Monte Argentario (Grosseto)**  
**and epidemiological evaluation**

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**SUMMARY.** A skin test survey carried out in the towns of Porto St. Stefano and Porto Ercole showed a total positivity rate of 15.3 %. The progressive increase of positivity according to age was not uniform but similar for the two towns since the values concerning the last two age groups did not follow the general trend of the graph but were lower than expected. The possible causes of this phenomenon have been discussed. The absence of overt visceral leishmaniasis cases in recent years and the concomitant high prevalence of canine leishmaniasis have been considered in the light of the occurrence of infectious diseases and the increase of the nutritional levels in the local human population.

**Leishmaniose en Toscane (Italie) : VIII — Réponse à la leishmanine par la population humaine du foyer de Monte Argentario (Grosseto) et évaluation épidémiologique.**

**RÉSUMÉ.** Une enquête par intradermoréaction à la leishmanine réalisée dans les deux petites villes de P. St. Stefano et P. Ercole a montré un taux de positivité de 15,3 %. La hausse de positivité en fonction de l'âge n'était pas uniforme mais similaire pour les deux villages puisque les valeurs concernant les tranches d'âges les plus hautes n'ont pas suivi la courbe mais étaient plus basses que attendues. Les causes possibles de ce phénomène ont été discutées. L'absence dans les années récentes de cas cliniques de leishmaniose viscérale et la concomitante prévalence élevée de leishmaniose canine ont été considérés à la lueur de la présence de maladies infectieuses et de la hausse des niveaux nutritifs parmi la population humaine locale.

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### Introduction

Human visceral leishmaniasis has been declining remarkably during recent years in Italy. On the other hand, canine leishmaniasis is generally still present at high levels in former VL foci. This is the case of the focus of Monte Argentario, a coastal Commune of the province of Grosseto, Tuscany, where work on sandflies (Maroli

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Accepté le 26 Mai 1983.

and Bettini, 1977), on canine leishmaniasis (Gradoni *et al.*, 1980 ; Pozio *et al.*, 1981a) and on wild *Leishmania* reservoirs (Pozio *et al.*, 1981b), had been previously carried out.

In the present note are given the results of a leishmanin skin test (LST) survey on the human population of this area, performed during July-August 1981.

### Area involved

M. Argentario is a fossil island, geologically distinct from the inland territories of the province of Grosseto, thus its flora and fauna are peculiar of this area (Pozio *et al.*, 1981a). Its surface is of about 61 km<sup>2</sup> and its maximum height 635 m. The majority of the human population lives in the two towns of Porto Santo Stefano, with about 8,000 inhabitants, and Porto Ercole, with about 3,000 ; the remaining 3,000 inhabitants live scattered in farm houses, mainly along the coast. This area is one of the most populated of the province of Grosseto, according to the 1971 census. The total number of inhabitants has not changed considerably during the last 35 years (10,039 in 1936, 10,692 in 1951, 11,569 in 1961 and 13,676 in 1971) (Istat, 1977).

### Human VL cases

In the last 40 years, 16 VL clinical cases have been reported from this territory ; 15 of them during the post-war period (Seganti and Palombelli, 1955) and one in 1973 (Bettini *et al.*, 1977). All cases lived in the town of P. St. Stefano and its surroundings. During pre-war time, the limited knowledge of this disease and high frequency of malaria cases in the Grosseto province might have influenced the number of VL cases reported.

### Canine leishmaniasis

A survey on canine leishmaniasis (Gradoni *et al.*, 1980 ; Pozio *et al.*, 1981a) has been carried out in this territory over a two year period (1979-80) ; 171 dogs (out of a population of about 250) have been examined, 23,9 % were positive in the immunofluorescence antibody test (IFAT) and 59 % of them displayed no signs of the disease. The infected dogs were distributed over the whole territory, inside and around the two towns, as well as in the scattered farms. The highest frequency of IFAT positive subjects (69.3 %) was observed in the south-western area. A few feral dogs were found, thriving close to garbage dumps, others living in packs in the "bush". The canine population increases considerably during summer, in connection with the high number of tourists.

Seven *Leishmania* isolates from dogs of M. Argentario have shown, on enzyme typing, to be identical to typical *L. infantum* (Gramiccia *et al.*, 1982).

## Wild reservoirs

Wild mammals, in particular the red fox, *Vulpes vulpes*, are abundant in the area. In one locality, in the surroundings of P. Ercole, the black rat, *Rattus rattus*, has been found infected (Pozio *et al.*, 1981b), the enzyme typing of the isolate showing it to be identical to typical *L. infantum* (Gramiccia *et al.*, 1982). In Tuscany, *R. rattus* has been proved experimentally to play the role of wild reservoir of VL (Gradoni *et al.*, 1983). Search for *Leishmania* has not been carried out in any other wild mammal species of M. Argentario.

In two other areas, one at about 40 km (Baccinello, Grosseto) and the other at 5 km from M. Argentario (Orbetello Scalo), a fox and two dogs have been found infected with a variant of *L. infantum* (Bettini *et al.*, 1980 ; Gramiccia *et al.*, 1982).

## Vectors

*Phlebotomus perniciosus*, the predominant species, is highly suspected of being the local vector of VL (Corradetti and Neri, 1955 ; Maroli and Bettini, 1977 ; Biocca *et al.*, 1977). Only a few samples of *P. perfiliewi* have been caught in the area (Maroli, personal communication). Specimens of *P. perniciosus*, trapped in a farm near P. Ercole and fed on a naturally infected dog and on *R. rattus* experimentally infected with *L. infantum*, have become infected at a frequency rate of 53 % and 21-25 % respectively (Maroli *et al.*, 1980 ; Gradoni *et al.*, 1983). *P. perniciosus* has been readily caught on human bait (Maroli *et al.*, in press). Its attraction to *R. rattus* in nature has been demonstrated (Gradoni *et al.*, 1983).

## Materials and methods

The leishmanin used in the present survey was obtained from Dr. D. A. Evans of the Department of Protozoology, London School of Hygiene and Tropical Medicine. It was prepared from a dog isolate, originating from the province of Grosseto (Gradoni *et al.*, 1980), which, later, on enzyme typing showed to be a variant of *L. infantum* (Gramiccia *et al.*, 1982). This antigen, used as control on six volunteers that had recovered from overt VL infections, gave an intense skin reaction. The strength of the leishmanin was of  $10^7$  organisms/ml. The leishmanin specificity for leishmaniasis was demonstrated by several authors, as reported by Pampiglione *et al.* (1975). The skin test procedure and the reading of the reaction, were carried out according to the method described by Manson-Bahr (1961) and Pampiglione *et al.* (1975). To avoid "observer variation", reading of skin reaction was carried out by only one of us (S.B.).

Authorization for performing LST on the population of M. Argentario was obtained from the Local Health Unit (USL No. 29) and from Regional authorities. A

poster explaining the purpose of the survey and the principles of skin testing was distributed to schools, out-patients' clinics and public buildings. The local county doctors and other physicians were informed in detail of the survey's aim and procedures. The planning of the operations in the elementary, primary and secondary schools was done by the two local school inspectors. Testing on youngsters was performed in school buildings, that on adults and infants in the office of one of the authors (S.M.) at P. Ercole and in the " Poliambulatorio " of P. St. Stefano.

The survey involved population samples of all ages living either inside the two towns or in the surroundings. A total of 1,818 people, 750 from P. Ercole and 1,068 from P. St. Stefano, have been examined. In the periphery of the two towns, there is no clear cut boundary between urban and rural dwellings, the formers being often surrounded by gardens. In the rural section, fowls and rabbits are often the only farm animals present, but sporadic farms with horses, pigs or cattle may also be found.

Every effort has been made to avoid systematic errors in the planning of the survey. Nevertheless, the consistency of the age groups above 55 years in P. St. Stefano could not be avoided to be inferior to that of the other age groups. In P. Ercole, on the other hand, all age groups were homogeneous. The nonresponse of P. St. Stefano's target group segment could not be overcome even by carrying out a second skin survey preceded by an intense propaganda. It appears therefore that our inquiry had raised little interest in the majority of adults of P. St. Stefano, even though it had been "sponsored by organizations of great prestige (national and local health authorities) in the eyes of the persons surveyed" (Mausner and Bahn, 1974). Two facts are indeed difficult to explain; a) the majority of adults nonparticipants to the survey did authorize their children to be tested in school, and b) a similar adult nonresponse was not observed in the town of P. Ercole.

## Results and discussion

As shown in *Table I*, the subjects positive to LST in P. Ercole were 99, out of 750 examined (13.2 %), and 180 out of 1,068 (16.8 %) in P. St. Stefano; the total positivity was of 15.3 %. In partial areas of M. Argentario, as alongside Via dei Molini on the outskirts of P. Ercole, the positivity to LST was higher than the general positivity of the whole area; 18 subjects out 92 examined (19.5 %) were positive.

TABLE I. — Subjects examined and positivity to LST of males and females from P. Ercole and P. St. Stefano.

	P. Ercole			P. St. Stefano			P.E. + P.St.S.
	♂♂	♀♀	♂♂+♀♀	♂♂	♀♀	♂♂+♀♀	♂♂+♀♀
Examined	375	375	750	471	597	1,068	1,818
Positivity	56 (14.9 %)	43 (11.5 %)	99 (13.2 %)	76 (16.1 %)	104 (17.4 %)	180 (16.8 %)	279 (15.3 %)

The total positivity to LST in the territory of M. Argentario (15.3 %) is of the same order of that found in Catania (Sicily) and nearby villages (16.2 %), a well known endemic focus of Mediterranean Kala-azar (Pampiglione *et al.*, 1975), but it is lower than in some inland foci of the provinces of Grosseto and Siena, namely the hamlet of Baccinello (about 400 inhabitants) and the town of Asciano (about 6,200) where it was found to be of about 30 % (Bettini *et al.*, 1977) and much lower than in some south-west Ethiopian foci where the total positivity ran over 70 % (Fuller *et al.*, 1979). The total positivity to LST should, however, be carefully interpreted since some restricted foci of a given area may show much higher values than the area average, as observed in Emilia-Romagna, Eastern Sicily (Pampiglione *et al.*, 1975) and Tuscany (province of Siena) (Bettini *et al.*, 1977). A similar, but less intense, phenomenon can also be found along Via dei Molini in P. Ercole, as already mentioned. The LST positivity frequencies showed, in the two towns, a progressive increase according to age and were remarkably similar in their trend (*fig. 1*).

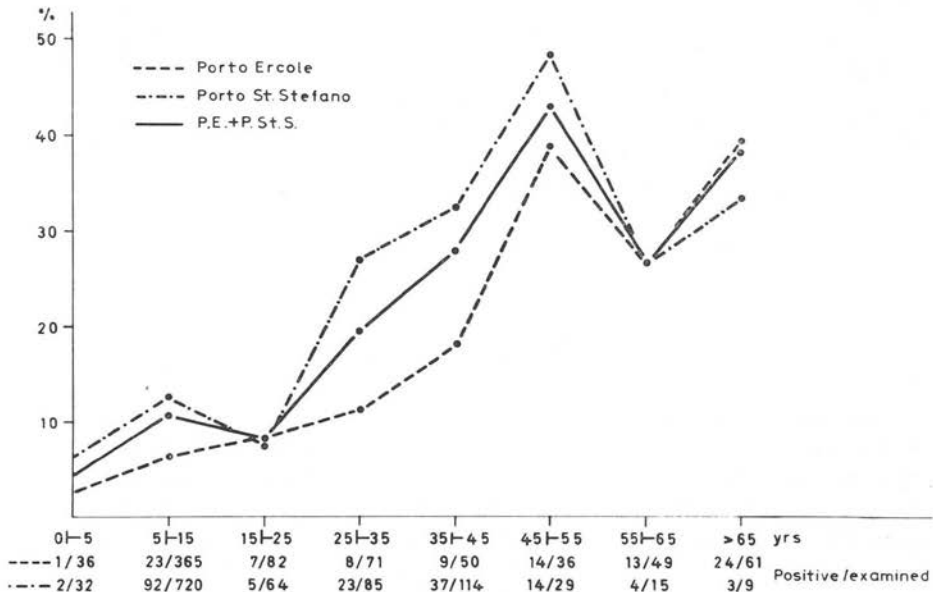


FIG. 1. — Response to leishmanin, according to age, of the human population of P. St. Stefano and P. Ercole (1,068 and 750 subjects respectively).

The highest value, nearly 50 %, was reached by the 45-55 age group of P. St. Stefano. However, the increase of positivity frequency was not uniform, since in both towns the values concerning the 55-65 and > 65 age groups did not follow the trend of the increasing frequency of the younger age groups, but were lower than expected.

The progressive increase of positivity to LST according to age in an endemic VL focus is justified by the lifelong skin reactivity of a "stable" population which has come in contact with the parasite (Pampiglione *et al.*, 1976).

The general trend of positivity observed in the territory of M. Argentario shows a slope similar to those obtained from other endemic foci (Pampiglione *et al.*, 1975; Bettini *et al.*, 1977). However, the lower positivity frequencies of the last two age groups (55-65 and > 65 years) of both towns could be interpreted by accepting one or all of the following conditions: 1) the last two age groups correspond to the population fraction which during the last war had left the territory and had been therefore less exposed to the infection; 2) the extension of sandfly breeding was reduced because of a decrease of stabled animals inside and in the surroundings of towns; 3) the domestic use of insecticides decreased the parasite/man contacts during a well defined period.

As for the first group of age (0-5 years), the 5% positivity observed indicates an active transmission of parasites. A similar phenomenon was not shown in other inland foci of Tuscany (Bettini *et al.*, 1977).

As reported by Gardoni *et al.* (1980), the overall positivity of dogs to IFAT in M. Argentario was of about 24%. This figure is extremely high if compared with the prevalence of dog infections in Baccinello/Asciano (about 3%) (Gradoni *et al.*, 1980). On the other hand, human positivity to LST of M. Argentario (about 15%) is half that of Baccinello/Asciano (about 30%). Nevertheless, a correlation between canine and human infections exists also in M. Argentario territory as in other VL foci of the country. This is proved by a higher LST positivity along Via dei Molini of P. Ercole which is paralleled by a higher prevalence of infected dogs.

The recent drastic decrease of human VL cases and the still high prevalence of canine leishmaniasis in the M. Argentario focus are similar to analogous situations in other Italian areas recently surveyed, as in Sicily (Mansueto, 1982) and Sardinia (Bettini *et al.*, in preparation).

It is the current opinion that the disappearance of overt human VL cases might have been caused by a decrease of transmission as a consequence of reduction of sandfly density. In M. Argentario, dog to dog transmission is, however, high enough to maintain a canine infection prevalence of about 24%. On the other hand, there is no reason to doubt that *P. perniciosus*, which is the only potential vector present in the area, is responsible also for the 15%, and in some sites even 19%, positivity rate to LST. Therefore, it is justifiable to consider the local *P. perniciosus* population both zoophilic and anthrophilic. This condition has been observed in other regions where this sandfly species is a suspected vector of VL (Adler and Theodor, 1931, 1935; Fraga de Azevedo, 1948; Parrot and Donatien, 1952; Rioux and Golvan, 1969; Ready and Ready, 1981). The anthrophilic habit of *P. perniciosus* in the M. Argentario area has also been directly proved by Maroli *et al.* (in preparation) who have observed a biting rate on man of 19 bites/hour (21.30-12.30 hrs, in July).

The data and considerations reported above, however, do not explain the gradual and constant reduction of symptomatic cases of Kala-azar, a phenomenon which has

been observed in the majority of Mediterranean foci, and should therefore share with them one or more causes.

Old authors were aware of the fact that infantile Kala-azar was a severe disease confined to "very poor" rural communities (Basile, 1910). Poverty, in those days, meant inadequate hygienic conditions and malnutrition; thus the population natural resistance was low and infectious diseases were more frequent and severe.

There is no doubt that some infections depress cell-mediated immunity, thus preparing the terrain for the onset of other infectious diseases. The association LV-malaria had long ago been observed in Sardinia; 14 out of 24 leishmaniotic patients had malaria before (Manai, 1932). Though in the past malaria was endemic in most of the province of Grosseto, no active transmission has ever been observed in M. Argentario's territory since it lacked vectors' breeding places. From 1942 to 1976 only 8 malaria cases (probably imported) have been reported from this area (in 1944-45). *Figure 2* shows the yearly incidence of infectious diseases, and, in parallel, that of VL, from 1942 to 1976, as reported by the Commune of M. Argentario. However, no correlation can be observed between the occurrence of VL and that of any of the

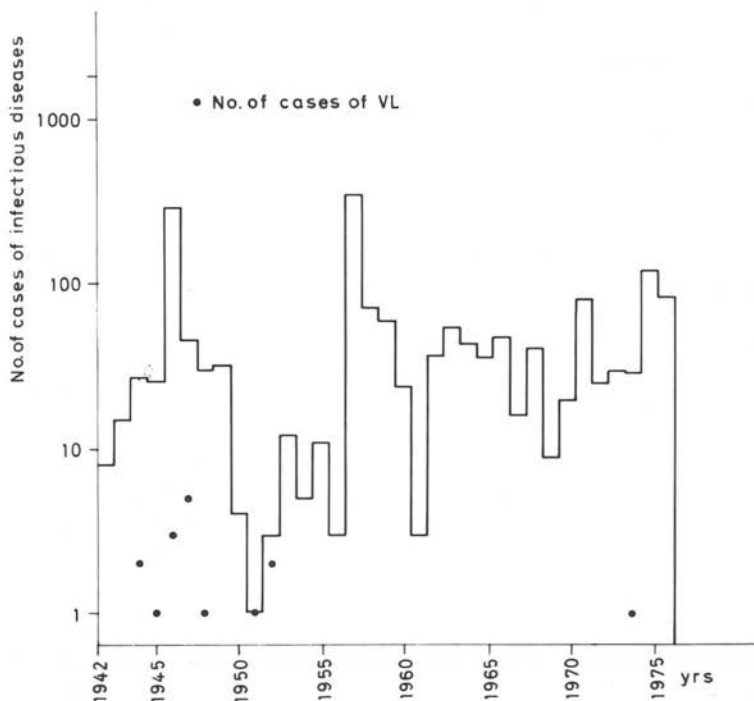


FIG. 2. — Yearly incidence of human infectious diseases in the Commune of M. Argentario from 1942 to 1976. In 1946, preminently typhoid fever (227 cases); in 1957, influenza (346 cases); in 1958, 1963 and 1978, measles (47, 40 and 72 cases respectively); in 1959, epidemic parotitis (25 cases); in 1966 and 1971, viral epatitis (40 and 57 cases respectively).



common epidemic diseases during this said period (influenza, epidemic parotitis, typhoid fever, diphtheria, measles, viral hepatitis).

Malnutrition, as well, may induce an immunodeficient state. Interference with the host's protein metabolism and reduction of antibody synthesis was shown to lower both innate and acquired resistance to *L. donovani* in the white mouse (Actor, 1960). Frequent cases of extreme malnutrition due to qualitative and/or quantitative lack of nutrients, as reported during the war and pre-war periods, are seldom observed in present times in Italy. From 1938 to these days the average consumption of animal proteins per inhabitant has doubled, and the consumption of fruits, vegetables, citrus fruits, etc. has more than doubled (Istat, 1968; data from Ministry of Agriculture). The uneven distribution of wealth, a social trait of past times, should also be taken into account.

Improvement of nutrition quantity and quality, a phenomenon common to Italy and other Mediterranean countries in the post-war period, should therefore not be underestimated, since it might be one of the factors linked to the decrease of overt VL cases in the area under study. The correlation between nutrition level and Kala-azar should be further investigated especially in countries where malnutrition and endemic Kala-azar are still present, e.g. in India, as early suggested by Napier (1954).

On the other hand, the constant contact of human population with the parasite, as shown by LST positivity, is probably a good warrant against the occurrence of epidemic episodes.

ACKNOWLEDGEMENTS : We are indebted to the school authorities of the Commune of M. Argentario for rendering feasible the present survey in the school age population. Testing in schools was possible thanks to the valuable collaboration of Mrs. N. Fanciulli and Mrs. A. Papini, school nurses of P. St. Stefano and P. Ercole respectively. We wish to thank Dr. E. Finizio, Provincial Doctor of Grosseto, for providing us with the data on infectious diseases. Thanks are also due to Mrs. L. Mugnai for keeping records of the people examined at P. Ercole. We are also grateful to Dr. Golia for the use of the Poliambulatorio at P. St. Stefano.

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