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## **Leishmaniasis in Tuscany (Italy): VI. Canine leishmaniasis in the focus of Monte Argentario (Grosseto)**

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### **Summary**

The results of a survey of canine leishmaniasis in the coastal focus of Monte Argentario (Tuscany, Italy) are reported. Of the 171 dogs examined, 41 (23.9%) were positive in the fluorescent antibody test (FAT), but only 12 (7.0%) were positive by microscopic observation of lymphatic ganglia. Of the 41 FAT positive dogs, 17 (10.0%) showed signs of the disease. The evolution of leishmaniasis over one year from the first examination showed that 88.0% of the dogs with signs died of the disease, and 36.0% of the asymptomatic dogs became patent or died; 52.0% of the asymptomatic dogs had apparently recovered. The prevalence of the disease, the geographical, age, sex and race distribution of cases, the evolution of leishmaniasis in dogs and FAT positivity are discussed.

*Key words:* *Leishmania donovani*; canine leishmaniasis; fluorescent antibody test.

### **Introduction**

Canine leishmaniasis, reported in Italy early in this century (Basile, 1910), has been shown to be present in most of the country, but with a higher occurrence in the central and southern regions (Pampiglione and Bettini, 1981). Seganti and Palombelli (1955) reported that, in the area of Monte Argentario, the local veterinarian was said to have diagnosed clinically cases of canine leishmaniasis during an outbreak of infantile leishmaniasis.

In a recent short note, Gradoni et al. (1980) reported two foci of canine leishmaniasis from the province of Grosseto: in the inland focus at Baccinello (Scansano) 3 out of 103 dogs were found to be infected and two of these were

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asymptomatic<sup>1</sup>; in the coastal focus of Monte Argentario, the prevalence was some 10 fold higher. Patent human cases, on the other hand, have in recent years been absent from this focus, a single case being reported in 1973 (Bettini et al., 1977) following those reported in the post-war period (Seganti and Palombelli, 1955). This indicated that *Leishmania* was surviving in animal reservoir(s).

In the present note we report on the results of a thorough survey of canine leishmaniasis in March–June of 1979 and 1980, and on environmental characteristics in the Monte Argentario area.

## Methods

The survey was based on the clinical examination of the dogs as well as on the following laboratory findings: microscopic observation of biopsy material from lymphatic ganglia, the isolation of the parasite by inoculation into hamster of biopsy material and fluorescent antibody test (FAT).

Both clinical examination and specimen collection were carried out locally by visiting all farms and peripheral districts of towns; a history of the dogs examined was also obtained from the owners.

### *Clinical examination*

Notes were taken on the general condition of the dog as well as on the condition of the skin, nails, lymph nodes, and spleen size. All information was transferred to nosographic cards.

The FAT positive dogs were classified as follows:

- asymptomatic cases (0): complete absence of signs referable to *Leishmania* infection;
- oligosymptomatic cases (+): adenopathy, decrease in weight, slight splenomegaly;
- patent cases (++): cutaneous alterations (desquamation, hair opaque), keratoconjunctivitis, hypertrophy of claws, distinct splenomegaly;
- severe patent cases (+++): the dogs look extremely weak and in pain, with depilation and ulcers.

### *Specimen collection*

Blood (4–5 ml) was taken from the radial or jugular vein, the serum separated and stored at –30° C. Hypertrophic ganglia were punctured according to the technique described by Lanotte et al. (1974), and the material obtained was partly smeared on slides and stained with Giemsa's stain, and partly diluted with saline and injected i. p. into hamsters for parasite isolation.

### *Fluorescent antibody test (FAT)*

The technique employed was that of Oddo and Cascio (1963), improved by Quilici et al. (1968). Promastigotes of an isolate originating from a dog from the province of Grosseto (Gradoni et al., 1980) were used as antigen. Conjugated gammaglobulins, donated by the Istituto Zooprofilattico Sperimentale di Perugia (Italy), were used at a dilution of 1/15. Sera were assayed routinely at a dilution of 1/20–1/80, and when the greatest dilution was positive, the test was repeated at even greater dilutions, until the extinction value was reached.

<sup>1</sup> Though the terms "asymptomatic" and "oligosymptomatic" should be confined to humans, they are also commonly employed for animals in the place of the terms "without signs" and "with few signs", respectively (Hull, 1955; Adler, 1964; Rioux et al., 1972; Ranque, 1977).

## Area of the epidemiological survey

The area extends over the whole promontory of Monte Argentario (Grosseto), a fossil island connected to the main land, in geologically recent times, by two low sandy dunes (that of Giannella on the north and that of Feniglia on the south) which enclose the lagoon of Orbetello. The surface of this area is of about 61 km<sup>2</sup> and its maximum height 635 m. The climate is typically Mediterranean, with scarce rainfall mostly during spring and fall. The territory is mostly covered with *macchia mediterranea*, the few plains are cultivated, and rare vineyards and olive groves are found on the hills. Four “bioclimatic plains” can be identified in the area: a littoral plain with halophilic and lagoon type vegetation; an inferior Mediterranean plain typified by *Quercus ilex*, whose southern part is also characterized by the midged palm (*Chaemerops humilis*); a superior Mediterranean plain typified by *Q. pubescens*; a mountain plain typified by *Castanea vulgaris*.

The greatest part of the human population of about 11,000 inhabitants lives in the towns of Porto Santo Stefano and Porto Ercole. The rest of the population (about 3000) lives on farms scattered in the plains, along the coast and in the valleys. The inland territories are practically uninhabited. The total population increases considerably during the tourist season.

The areas surrounding the two towns are densely populated by man and resident dogs, unlike along the north-eastern coast which takes in the narrow strip of land bordering the Orbetello lagoon and the two main plains (Le Piane and Terra Rossa). On the south-western coast, characterized by a steep and high bank, there are only a few tourist hamlets and scattered farms with a relatively low number of dogs.

The total canine population is about 250 dogs distributed from sea level to about 350 m. A few stray feral dogs live in areas next to rubbish dumps. In summer, due to the high number of tourists, the canine population increases considerably.

In the general area of Monte Argentario, wild mammals are abundant (foxes, black rats, etc.), and their role in the ecology of leishmaniasis is being studied.

The probable vector of leishmaniasis in Monte Argentario is *Phlebotomus perniciosus* which is the predominant local species (Corradetti and Neri, 1955; Maroli and Bettini, 1977; Biocca et al., 1977).

## Results

### *Prevalence of infected dogs*

The data referring to the prevalence of dogs with leishmaniasis in the area surveyed are reported on Table 1. Out of about 250 dogs present in the area, 171 have been examined: 17 (10%) showed signs of leishmaniasis, but in only 12 of

Table 1. Prevalence of canine leishmaniasis in Monte Argentario focus\*

Dogs in the focus .....	~250
Dogs examined .....	171
Patent and oligosymptomatic dogs .....	17 (10.0%)
Cases confirmed by finding of parasites .....	12 (7.0%)
FAT positive dogs .....	41 (23.9%)
<i>Leishmania</i> isolates .....	7

\* Percentages should be referred to the total of dogs examined.

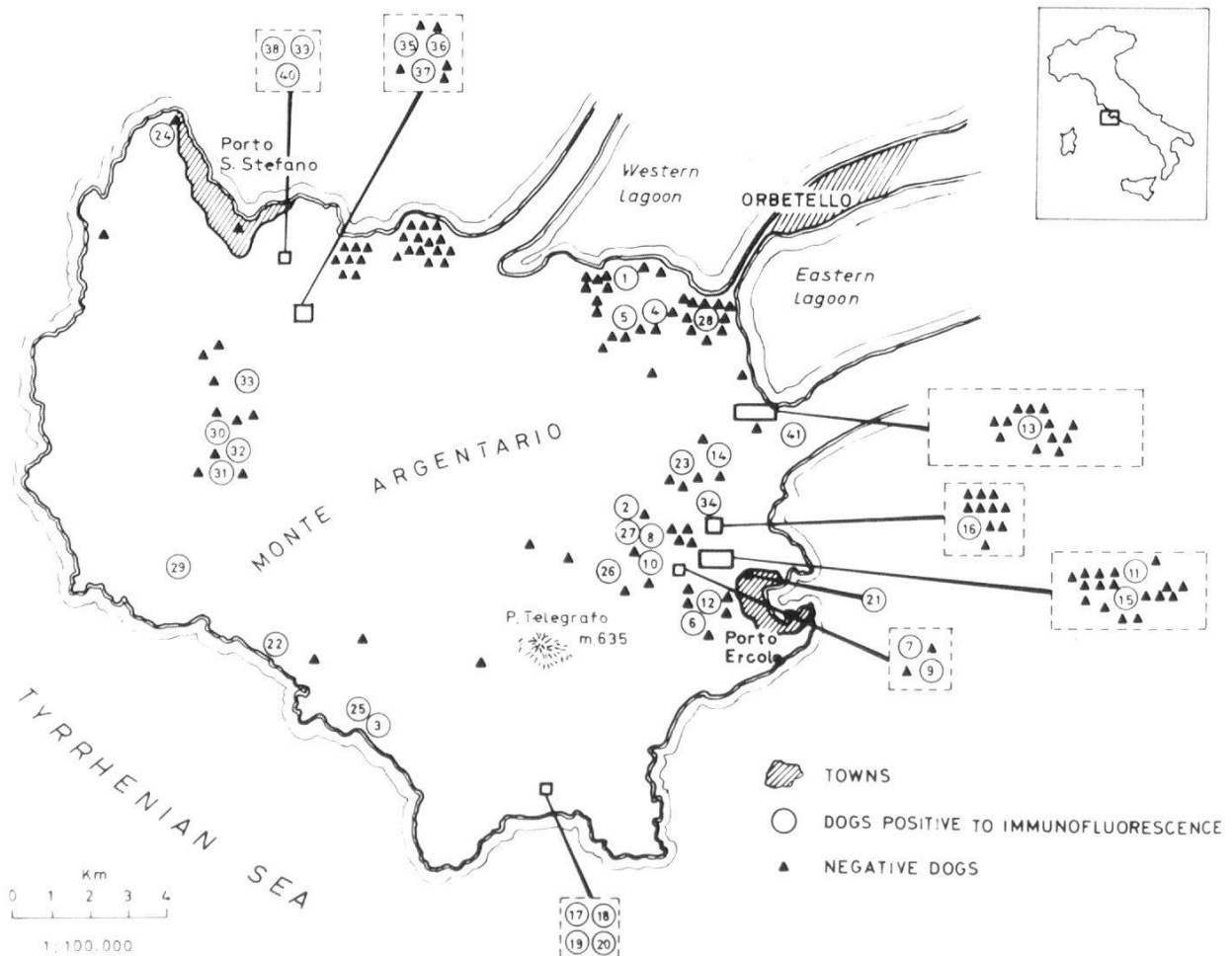


Fig. 1. Geographical distribution of FAT positive, and negative, dogs (circled numbers correspond to order number of positive cases list).

them (7.0% of the total) was the microscopic observation of lymphatic ganglion biopsy material positive. From these 12 dogs, 7 *Leishmania* isolates were obtained. Sera of 41 dogs (23.9%) showed a positive FAT. All patent cases belonged in this group of FAT-positive dogs.

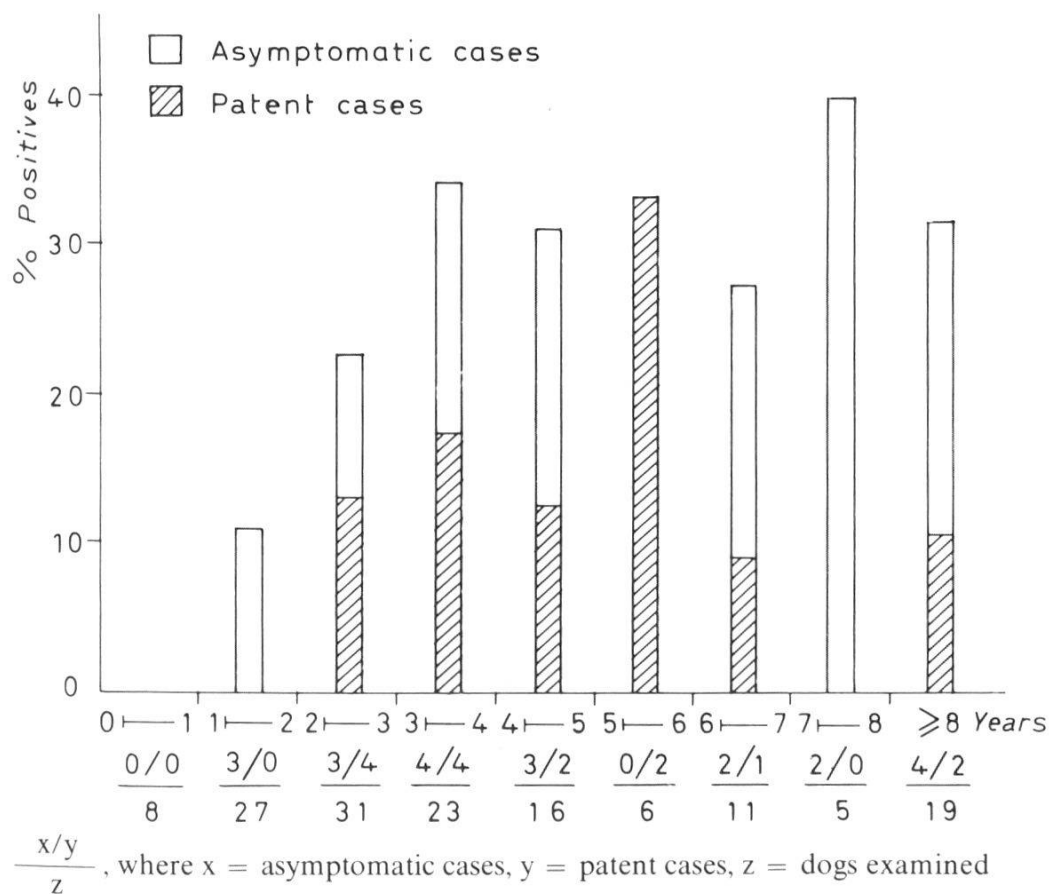


Fig. 2. Frequency distribution of positive dogs according to age.

### Geographical distribution of cases

The highest frequency of FAT positive cases (Fig. 1) was found where the number of dogs per unit area was lower, i.e. in the south-western area, where 9 (69.3%) out of 13 dogs examined were found positive. In both the areas surrounding P. Ercole and P. S. Stefano, where the number of resident dogs was higher, the frequency of dog positive in the FAT was much lower, 16 out of 63 (25.4%) and 10 out 27 (37.0%), respectively. Even lower was the positivity of the north-eastern strip of coastal land where, out of 68 dogs examined, only 6 (8.8%) were found positive.

### Age, sex and race

The age distribution of dogs positive in the FAT (Fig. 2) shows no cases in the first year of age and an increase of frequency up to the third year. In the older classes the proportion remains constant. Patent cases of leishmaniasis are present in almost all classes of age.

Among the dogs found positive in the FAT, the males are 68.3%; this frequency is not significantly different from the ratio of males (66.6%) among all subjects examined.

The majority of dogs in the area under study belongs to local races used mainly for boar hunting. There seems to be no correlation between race and *Leishmania* infection.

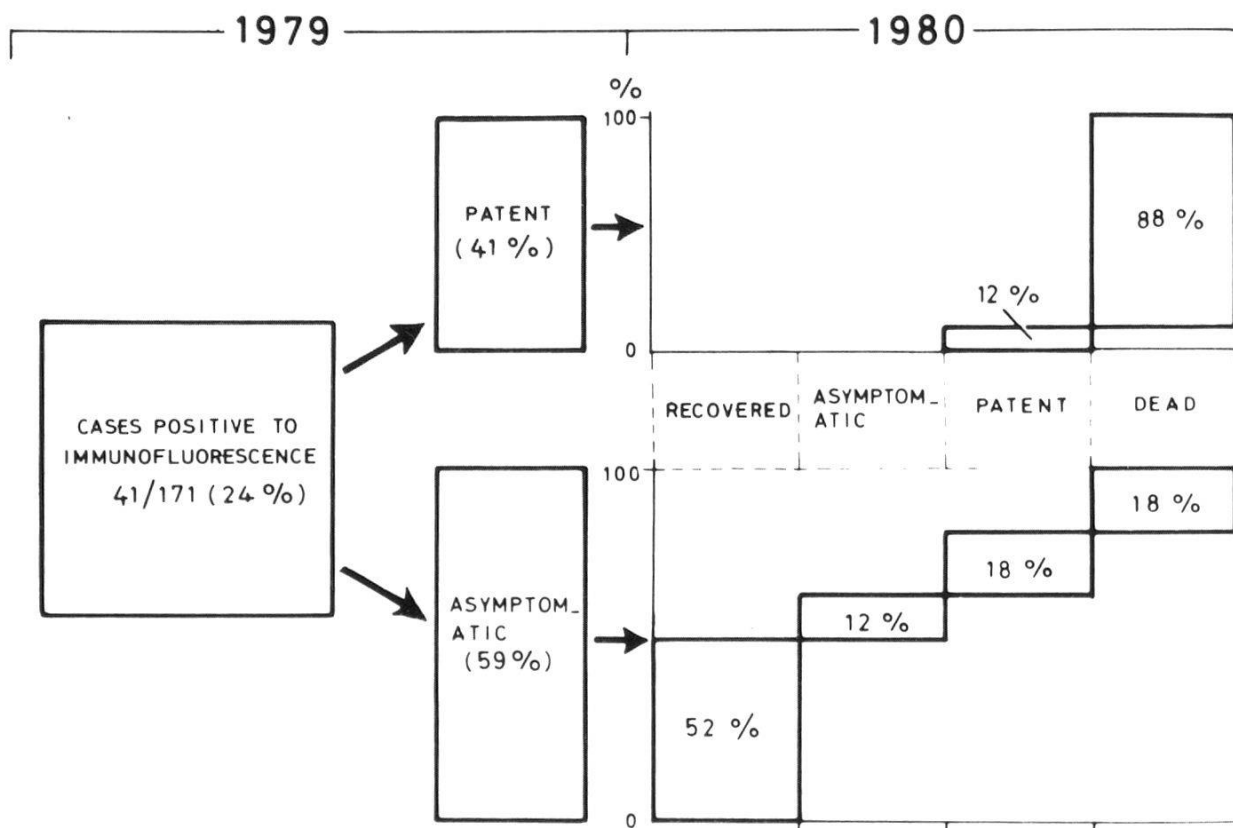


Fig. 3. Evolution of infection as recorded from Monte Argentario canine population, two examinations one year apart.

### *Evolution of the disease*

Reliable data on the evolution of the disease could be obtained only in 34 out of the 41 dogs found positive in the FAT.

How the disease evolved in one year following the first examination, is shown on Fig. 3, where 88% of the patent cases diagnosed in the first survey died probably of leishmaniasis, while the remaining 12% still showed evident signs of the disease<sup>2</sup>. The evolution of asymptomatic cases is more complex: 52% of them had apparently recovered (disappearance of serum specific antibodies), 12% continued to be positive in the FAT even though no signs of the disease had appeared; 18% displayed an evolution toward patency, and the remaining 18% had died as a consequence of severe leishmaniasis. In each of these groups all ages were represented.

### *Correlation between antibody titre, signs and microscopical examination*

On Table 2 is shown the relationship between the antibody titre obtained in the FAT, the presence of signs and the direct observation of the parasite in ganglion preparations. The data were obtained from thorough and repeated examinations of 41 dogs.

<sup>2</sup> Following the first survey, some of the patent cases were treated with several courses of Glucan-time; none, however, apparently recovered following treatment.



Table 2. Correlation between antibody titres, signs and microscopical examination of FAT positive dogs\*

Fluorescent antibody titres	Asymptomatic and patent cases**				Cases positive by microscopical examination
	0	+	++	+++	
1/20 .....	1	1	0	0	0
1/40 .....	2	3	0	0	1
1/80 .....	15	2	0	0	1
1/160 .....	5	0	2	0	1
1/320 .....	1	0	2	0	1
1/640 .....	1	0	5	4	8
1/1280 .....	0	0	1	2	3
Total	25	6	10	6	15

\* Data were obtained from thorough examinations carried out once, or twice, on 41 dogs.

\*\* See text.

Only 15 out of 48 (31.9%) examinations positive in the FAT were also positive by direct microscopic examination.

A general correlation exists between increase of titre, severity of the signs and positivity of microscopical examination. On the other hand, some of the asymptomatic cases, showed high titres up to 1/640 (the highest titre found was 1/1280), and some of the oligosymptomatic cases, positive on microscopical examination, showed low titres (1/40 and 1/80).

## Discussion

Our survey has shown a high prevalence (23.9%) of canine leishmaniasis in the focus of Monte Argentario, compared to the figures recently reported in the Mediterranean area by other authors who have also used serological techniques, e.g. 6.2% by Dedet et al. (1973) in Tunisia; 4.4% by Lanotte et al. (1974) in the Cevennes, France; 10.2% by Bucci et al. (1975) in the promontory of Gargano, Italy; 1.5% by Houin et al. (1977) in an area near Tours, France and 7.5% by Colella et al. (1979) in the province of Matera, Italy. However, most of the above authors report only average frequencies which refer to several foci distributed over large areas, while in our survey the area under study is rather small (61 km<sup>2</sup>).

The proximity of dogs to the sea shore or to the lagoon seems to be the only factor, among the many examined, which limits the distribution of cases in extra-urban environments. This may be related to low density of *P. perniciosus*, the probable vector, which does not breed in a strictly halophilic environment as represented by the littoral plain (Rioux et al., 1969). The higher prevalence of dogs with leishmaniasis (69.3%) has been found in the south-western coastal



strip corresponding to the less inhabited area of Monte Argentario. In such an area, the presence of isolated cases of canine leishmaniasis, a few kilometers distant the one from the other, could lead to the hypothesis that the parasite may survive in other reservoirs. Such an hypothesis is supported by the isolation of *Leishmania* from *Rattus rattus* trapped in Monte Argentario (Pozio et al., 1981) and from *R. rattus* and *Vulpes vulpes* in the focus of Baccinello (Grosseto) (Bettini et al., 1980). The role of each of these vertebrate species in the ecology of leishmaniasis has not as yet been established. It has been observed, however, that *Leishmania* isolates from the above species and from the local dogs, are quite homogeneous and belong to the group characterised by *L. donovani* (Dr. D. Evans, personal communication), as do the Italian human isolates.

Considering the high prevalence of canine leishmaniasis in this area, the negative results of the FAT carried out in March–June in dogs below one year of age which had lived during the whole summer in that area, cannot be easily explained. This phenomenon is not in agreement with what has been observed in Mediterranean human leishmaniasis foci, with apparently high transmission levels, where the incidence of the infections in the lower groups of age is particularly high (Bettini et al., 1981). The hypothesis could be advanced that in the dog the parasite does not induce an efficient immunity but survives in the host in an unstable equilibrium, and that such an equilibrium could be altered by a decrease of the host resistance (intercurrent diseases, alimentary deficiency, old age, etc.).

It has been reported recently that, following immunosuppressive therapy, a patent state of visceral leishmaniasis was induced in a man who had contracted the disease many years before (Ma, 1979). Sanchis et al. (1976) reported differences of resistance to the disease in dogs experimentally infected.

Some of the less resistant dogs could therefore develop the disease, while the more resistant ones could recover, as has occurred in the 52.0% of our asymptomatic cases.

The high frequency of asymptomatic dogs, which had been reported by Adler and Theodor (1932, 1935) in Mediterranean endemic foci and recently by Lanotte et al. (1979) in France, would represent therefore either a pre-patent phase of acute or chronic viscerocutaneous infections, or infections where a parasite-host equilibrium has been reached, and maintained for long periods and which may eventually evolve toward recovery. This phenomenon was observed by us both in young and adult dogs. These non-infective cases (Rioux et al., 1972) are obviously of great epidemiological importance, since they represent a latent reservoir which may evolve toward infective cases, i.e. with parasites in the skin (Adler and Theodor, 1932).

Our results obtained with the FAT are not in agreement with those of other authors who have employed the same technique. For instance, we have considered the serum dilution 1/40 as the threshold titre, i.e. the lowest concentration corresponding to a positive microscopical diagnosis. Low threshold titres were

also reported by Bucci et al. (1975) and Colella et al. (1979) (titre 1/10) and by Lanotte et al. (1974) (titre 1/20). Others (Kerbœuf, 1974; Ranque et al., 1974; Dunan, 1978), on the other hand, considered as specific a threshold dilution of 1/100. Dunan (1978) believes that lower titres may reveal cross reactions or a weak immunological response or tolerance to the parasite. A similar consideration could be made also in the case of the highest titre, which in our case reached a dilution of 1/1280; Bucci et al. (1975) reported 1/320 as the highest titre, while Lanotte et al. (1974) values reached 1/81,920 and Dunan (1978) 1/12,800.

Since the various workers employed conjugated antisera at differing concentrations, it is difficult to compare their results. A standardization of FAT to be applied to the diagnosis of canine leishmaniasis would therefore be desirable, as also reported by Rowe (1977).

To this epizootiological picture of canine leishmaniasis, in a restricted and zoogeographically well delimited focus, there is a corresponding hypoendemic focus of human leishmaniasis where a low number of infantile cases have occurred in the post-war period (Seganti and Palombelli, 1955), one being focused in 1973 (Bettini et al., 1977). Among the causes which might be considered responsible of this epidemiological situation are: a) the zoophilic tendency of *P. perniciosus* (Fraga de Azevedo, 1948; Parrot and Donatien, 1952); b) an improvement of the sanitary conditions and, consequently, an increase of overall resistance especially during childhood, and a decrease in sandflies and consequently in the number of parasite-man contacts; c) or a rise of protective immunity in human population due to periodic contacts with a low number of parasites unable to develop in the host (Pampiglione et al., 1974; Bettini et al., 1981).

Finally, it should be pointed out that dogs which are temporarily brought into the area of Monte Argentario by tourists during summer may become infected and may disseminate the disease in other regions and indeed a few cases have been diagnosed in our Laboratory in Rome.

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