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CANINE LEISHMANIASIS IN THE SALENTO PENINSULA OF APULIA, ITALY: A PRELIMINARY REPORT

SUMMARY

Canine leishmaniasis is endemic to the Mediterranean area. In Italy this zoonosis is distributed over a large portion of the Country. We studied a representative sample of 638 dogs of the canine population subjected to routine veterinary check in Salento peninsula (Apulia, Italy) where the exact entity of the zoonosis is currently unknown. Amastigote's indirect immunofluorescence (IFAT) and electrophoresis of serum proteins were used as specific and non-specific diagnostic tests, respectively. In addition, lymph node and bone marrow aspirates were examined by light microscopy after May-Grunwald Giemsa or Diff Quick staining, to confirm the suspected pathology. Results demonstrated that about 13% of dogs were affected by leishmaniasis. This prevalence value, significantly higher than that reported in a previous study conducted twenty years ago, strongly suggests that leishmaniasis is endemic in Salento.

RIASSUNTO

La leishmaniosi canina è una zoonosi endemica dell'area mediterranea. In Italia questa zoonosi è presente nella maggior parte del paese, ma nel Salento (Puglia, Italia) non è nota la distribuzione. In questo studio, un campione rappresentativo composto di 638 cani sottoposti a controllo veterinario periodico è stato monitorato per valutare l'entità della zoonosi nel Salento. Come prove diagnostiche specifiche e non specifiche sono state utilizzate rispettivamente l'immunofluorescenza indiretta (IFAT) e l'elettroforesi delle proteine del siero. In più, sono stati esaminati degli ago-aspirati dei linfonodi e del midollo osseo mediante microscopia ottica dopo colorazione May-Grunwald Giemsa e Diff Quick, per confermare la patologia sospetta. I risultati hanno dimostrato che circa il 13% dei cani era affetto da leishmaniosi canina. Il valore di prevalenza riscontrato è significativamente più alto di quello riportato in uno studio precedentemente condotto venti anni fa, il che suggerisce fortemente che la leishmaniosi canina è endemica nel Salento.

INTRODUCTION

Canine leishmaniasis is a protozoan disease that is widely spread over the Mediterranean area. The life cycle of parasitic *Leishmania* is characterized by the alternation of two phases that occur in two distinct hosts: an invertebrate (promastigote stage) and a vertebrate (amastigote stage). The parasite is transmitted by the bite of sand fly, and various species of the genus *Phlebotomus* (Diptera), have been identified in Italy (Maroli *et al*., 1994). The dog represents the main reservoir for this zoonosis (Abranches *et al*., 1991) but humans can be affected as well (Moreno and Alvar, 2002). In Italy this pathology is endemic in several regions (Gramiccia *et al*., 1987; Brandonisio *et al*., 1992; Orndoff *et al*., 2000; Pedonese *et al*., 2000) and *Leishmania infantum* is the major responsible species. Human infection occasionally occurs in those regions where this canine disease is endemic (Mancianti *et al*., 1986; Gradoni *et al*., 1993; Cascio *et al*., 2002). Recently, canine leishmaniasis foci have been reported in Northern Italy (Maroli *et al*., 1995; Ferroglio *et al*., 2000). In Southern Italy, this disease has been reported in the Apulia region where studies have shown the presence of the zoonosis in the Gargano promontory (Brandonisio *et al*., 1992) and in the Salento peninsula (Maroli *et al*., 1983).

MATERIAL METHODS

The aim of this study was to survey the distribution of canine leishmaniasis in the Salento area 20 years after the Maroli *et al*.'s work. The investigation was performed in the districts of Galatina, Maglie, and Parabita (Fig. 1). The study area is characterized by high mean levels of dampness all year round in spite of the typical Mediterranean climate, and by the presence of a channel, the "Asso", collecting surface waters from all three districts.

Over a two-year survey period (April 2003-April 2005) a sample of 638 domestic dogs (341 males and 297 females), subjected to routine check in veterinary surgeries, was analyzed. The dogs were screened by blood sampling for specific and non-specific clinical tests (Palma and Gutierrez, 1991).

Biochemical (non-specific) tests were: protein electrophoresis, azotemia, creatininemia, glutamic-oxalacetic acid amino transferase (GOT) and glutamic-pyruvic acid amino transferase (GPT) levels, (Ciaramella *et al*., 1997). A Cobas-Mira analyzer (ABX S.p.A.) was used for biochemical assays. Amastigote's indirect immunofluorescence (IFAT) (Bio-Merieux) was used as a specific serological test (Fernandez-Perez *et al*., 1999). In addition lymph node and bone marrow aspirates were examined by light microscopy after May-Grunwald Giemsa (Sigma) or Diff Quick (Dade Behring) staining to confirm the suspected pathology. We found the Diff Quick faster and easier to use than May-Grunwald Giemsa, giving also better staining of the parasites (Fig. 2).

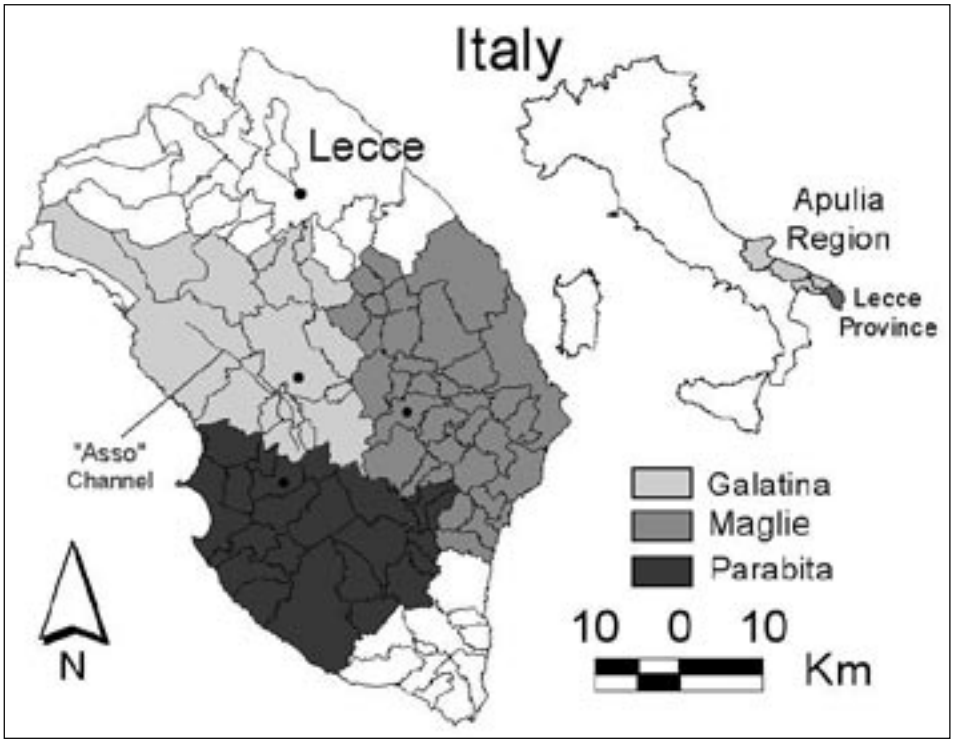

Fig. 1 - Study area for the two-year survey. The area is divided in three districts: Galatina, Maglie and Parabita. The "Asso" channel is also indicated.

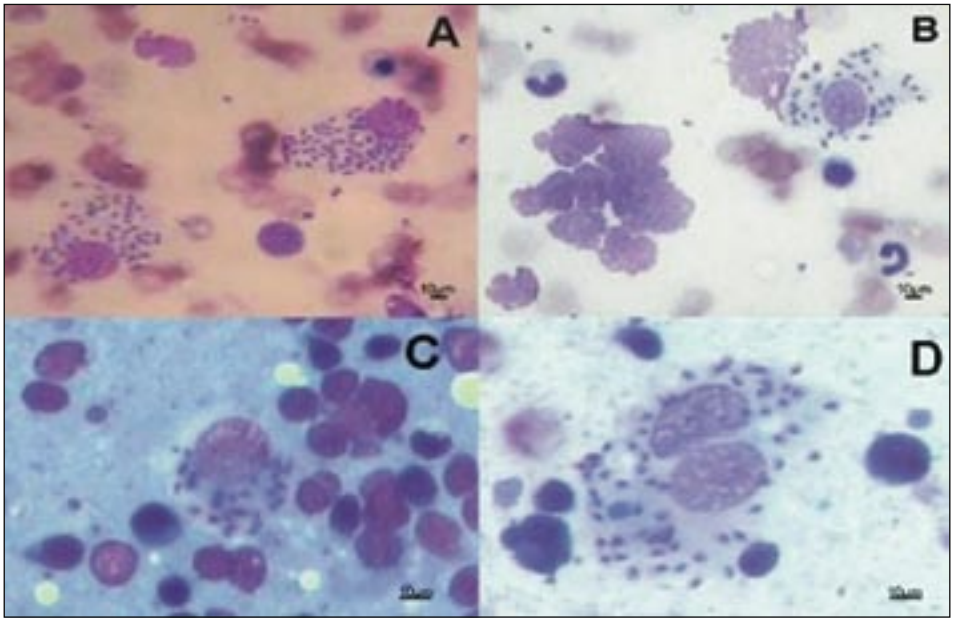

Fig. 2 - Light microscopy of biopsies showing macrophages with parasites in the cytoplasm and in the intercellular spaces. May-Grunwald Giemsa (A) or Diff Quick (B) staining of bone marrow aspirates. Bone marrow aspirates were collected by myelo-centesis of sternum using a "needle gun". After staining, samples were analyzed by an optical microscope (Eclipse 600E Nikon) equipped with 63X lens in oil-immersion after placing a cover-slide and fixing with Eukitt (Kindler GmbH and Co). May-Grunwald Giemsa (C) or Diff Quick (D) staining of lymph node aspirates. Lymph node aspirates were obtained from popliteal or pre-scapular lymph node after surgical preparation. After staining, samples were analyzed by an optical microscope with 100X lens in oil-immersion.

All standard procedures comply to the International Guiding Principles for Biomedical Research Involving Animals and Italian Law n° 281/1991 for the care and health of pet company.

RESULTS

Using these diagnostic tools, 83 out of the 638 dogs tested positive to canine leishmaniasis during the first year (Tab. 1). Eleven dogs were asymptomatic and 72 exhibited two or more clinical symptoms of leishmaniasis (CIARAMELLA *et al.*, 1997). Fifty-one dogs were affected by cutaneous signs, and 21 dogs showed visceral symptoms. Out of this latter group, three animals suffered from hepatic and 8 from renal damage. Six dogs were affected by joint disease, four of them with hepatic and renal damage. Blood samples from infected dogs showed an electrophoretic layout typical of infection (CIARAMELLA *et al.*, 1997) with hypoalbuminaemia and increase in γ- and β-globulins. Among symptomatic dogs, 71 had an increase in azotemia and 12 an increase in creatininemia. GOT levels for 21 dogs were found to have physiologically abnormal values, while GPT levels were abnormal only in 7 animals. IFAT test was positive in the infected dogs with antibody titres equal or higher than 1/80.

Diagnostic tests demonstrated the presence of leishmaniasis in 13% of the canine population (Tab. 1). To explore the spatial variability for the zoonosis in the study area, for each district relative percentages of infected dogs were determined (Galatina18%, Maglie 7%, Parabita 12%) (Tab. 1).

Tab. 1 - Prevalence of canine leishmaniasis in 1983 and in 2004.

District	Controlled dogs	Positive dogs
[a] 2004 Parabita	212	25 (12%)
[a] 2004 Galatina	264	47 (18%)
[a] 2004 Maglie	162	11 (7%)
[a] Total 2004	638	83 (13%)
[b] 1983 Parabita	41	1 (2.5%)

[a]Data obtained in our first year (2004) survey period. - [b]Data reported in the 1983 survey by MAROLI *et al.*

The prevalence values during the first year were confirmed by new cases during the second year within the same population. Seventy-eight new cases (6 asymptomatic) were notified with a similar relative distribution in the three districts.

Results demonstrate a statistical difference in prevalence (13%) of canine leishmaniasis for the study area with respect to prevalence previously found by MAROLI *et al*. (1983) of 2.5% at a *P* value of 0.0268 (Fisher's exact test; SOKAL and ROHLF, 1995). The same does not hold, statistically speaking, when considering only the data from the Parabita district: the null hypothesis of equality of frequencies can not be rejected because of a *P* value of 0.052, that is slightly higher than the type one error assumed of 0.05. But when the small sample size and the narrow time window used by MAROLI *et al*. (1983) are considered, our result could be interpreted as a first hint of a possible change in frequencies between 1983 and 2004.

A spatial component in the frequency variability among districts was found (3 by 2 contingency table chi-square test with a *P* value of 0.004), with frequencies for Galatina and Parabita higher than those for Maglie. This variability could be related to the presence of a possible gradient of the zoonosis promoted by local environmental differences (the "Asso" channel is placed in the Galatina district), thereby supporting the idea of the Galatina district as a possible focus of leishmaniasis.

Finally it is important to underline that MAROLI *et al*.'s work (1983) has considered only two short time windows (June and September 1982), when the insect vector(s) was supposed to be present. Our study was carried out over a two-year period hypothesizing an increased persistence of the vector(s). Identification of *Phlebotomus* species that are now prevalent and characterization of the leishmania zymodeme(s) will help to define the epidemiology of the zoonosis in Salento.

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