Preventive Veterinary Medicine, 13 (1992) 173–178
Elsevier Science Publishers B.V., Amsterdam

Seroprevalence of canine leishmaniasis around Cáceres, Spain

C.G. Nieto[a], I. Navarrete[a], M. Habela[a] and S. Hernández-Rodríguez[b]

[a]*Parasitology and Parasitic Diseases, Department of Animal Health and Medicine, Faculty of Veterinary Sciences, 10071 Cáceres, Spain*

[b]*Parasitology and Parasitic Diseases, Department of Animal Health and Medicine, Faculty of Veterinary Sciences, 14005 Córdoba, Spain*

(Accepted 17 January 1992)

ABSTRACT

Nieto, C.G., Navarrete, I., Habela, M. and Hernández-Rodríguez, S., 1992. Seroprevalence of canine leishmaniasis around Cáceres, Spain. *Prev. Vet. Med.*, 13: 173–178.

A random epidemiological examination for canine leishmaniasis was conducted in 381 dogs of all types in the city of Cáceres. The indirect immunofluorescent antibody test (IFA) was carried out using promastigotes of *Leishmania infantum* as antigen. Of the 381 sera analyzed, 54 (14%) samples were positive for anti-*Leishmania* antibodies (titer 1/80 or greater) with one important focus (30%) of leishmaniasis in the province.

INTRODUCTION

Sánchez Botija (1936) reported the first canine leishmaniasis infection in Spain. *Leishmania infantum* is spread over almost all the Iberian peninsula (prevalence of 5–10%, depending on the province) (Botet et al., 1987; Castillo Hernandez et al., 1985; Reyes-Magaña et al., 1985). The non-uniform geographical distribution of infection might be influenced by numerous ecological factors (such as climate and topography) which determine the presence of the transmitter *Phlebotomus*. This paper reports the results of a survey carried out on the canine population of urban and outlying areas of Cáceres (Spain), and the mountainous area (Sierra de la Mosca) surrounding the city.

Correspondence to: C.G. Nieto, Parasitology and Parasitic Diseases, Department of Animal Health and Medicine, Faculty of Veterinary Sciences, 10071 Cáceres, Spain.

MATERIAL AND METHODS

Randomized serum samples were taken from 381 dogs of the city of Cáceres and the adjacent mountain area, Sierra de la Mosca. The type of selection which took place was a probability sampling i.e. a deliberate and random-type sampling based on some approximate knowledge of the canine population in the town — 1086 dogs according to official data from the Health Council. The 381 sera were taken from dogs chosen at random from different areas of the town and its outlying areas. This random and stratified sampling of the 381 dogs may be considered as being representative (Thrusfield, 1986). The city is in central western Spain near the border with Portugal, 350–450 m above sea level. The inner part of the town and the suburbs were studied.

Venous blood samples (3–5 ml) were taken. Serum was obtained by centrifugation and kept at −80°C prior to use. The prevalence was analyzed according to variables of age, sex and use. The sampling period was from March 1988 to April 1989.

The antigen used was a suspension of *L. infantum* promastigotes derived from an indigenous canine isolate (code LEM 2002) corresponding to zymogen 1, and obtained by mass culture in semi-defined medium 199 enriched with 2% synthetic bovine fetal serum.

An indirect immunofluorescent antibody test (IFA) was used to test the serum. An anti-dog IgG conjugate from dogs marked with fluorescein isothiocyanate was used at a dilution of 1/160 in phosphate buffer (pH 7.2).

Samples were analyzed at dilutions ranging from 1/40 to 1/5120; positive titers were 1/80. Positive and negative controls were tested for each series of samples.

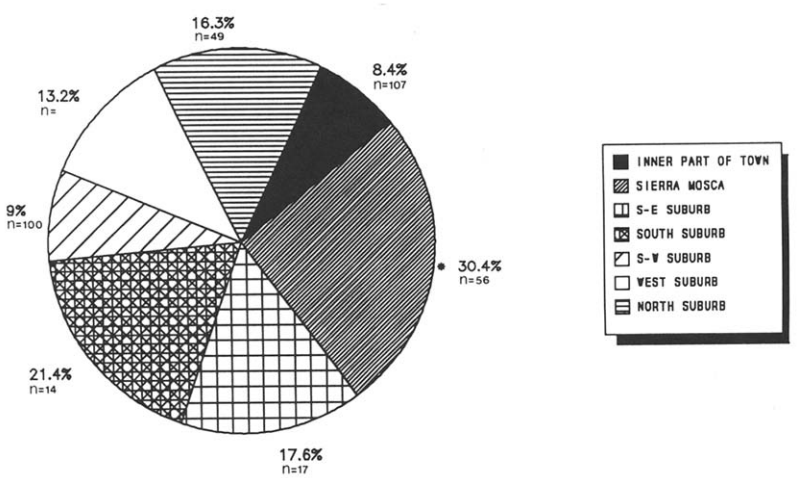

Fig. 1. Prevalence of positive canine leishmaniasis titers in the town of Cáceres, Spain. *Significant differences ($P<0.01$) in relation to the other areas.

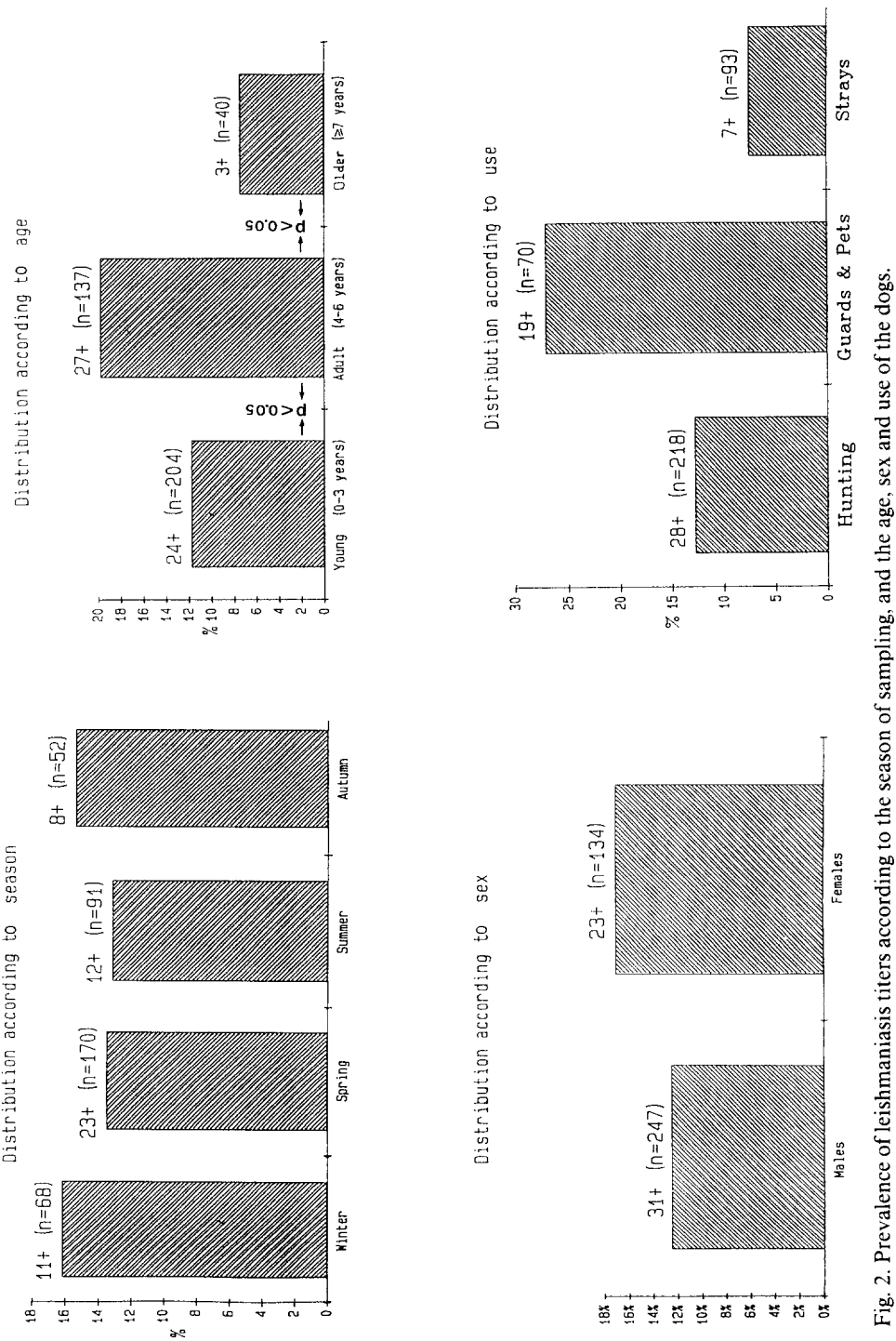

Fig. 2. Prevalence of leishmaniasis titers according to the season of sampling, and the age, sex and use of the dogs.

Direct diagnosis by observation of *Leishmania* amastigotes was made on seropositive dogs by suction of the popliteal and/or prescapular lymph node (following the method of Catarsini (1980) or once necropsy was completed (by microscopic observation of Giemsa-stained spleen and lymph node samples).

The χ^2 test was used for statistical analysis of the age, sex, use and season (from spring 1988 to winter 1989) data.

RESULTS

Of the 381 dogs tested, 54 (14%) were positive for titers of 1/80 and greater. These results were heterogeneously distributed over urban and suburban zones of the city (Fig. 1). The greatest prevalence was 17 cases (30%) from the Sierra de la Mosca. In different outlying districts, 28 dogs were diagnosed, yielding 12.8% positivity.

The statistical analysis (χ^2 test) performed on each of the areas sampled reveals important significant differences which should be taken into account when defining areas with a high risk of infection (Fig. 1).

No significant differences (Fig. 2) were found for sex and season, although older animals (aged 7 years and above) showed reduced seroprevalence. When functional use was analyzed, guard dogs and pets revealed a high percentage of infection (27.1%) compared to hunting dogs (12.8%) or strays (7.5%).

These distinct differences are, however, based on epidemiological and not physiopathological grounds since these guard dogs and pets live in a zone of high epidemiological risk (namely in or around the endemic focus of the Sierra de la Mosca), while the hunting dogs live grouped in kennels located in the suburban area and are only brought out to hunt in the winter (as is traditional in Spain). During these months, the vectors are not active.

Titers obtained by IFA mainly showed carriers to be in a state of immunity in which the antibody concentration had leveled off or was in decline, and most animals examined revealed a rich pattern of visceral, cutaneous or mixed symptoms.

DISCUSSION

In our experience, the confirmation of 83% of the IFA-positive dogs (45/54) by microscopic observation of biopsy material from lymphatic ganglia and smears from spleen have allowed us to show the utility of the IFA (however, we have not attempted histologic confirmation in seronegative dogs). Furthermore, the dilution 1/80 is consistent with the findings of Lanotte et al. (1974) who reported that the test is highly sensitive ($99 \pm 1\%$) and specific (98%) which is in accordance with Badaro et al. (1983).

In any case, the possible error of sensitivity found in seropositives at a di-

lution of 1/40 would motivate a slight increment in the parasitation index that would corroborate even more the endemic situation of canine leishmaniasis in Cáceres.

Cross-reactions with human IFA tests have been reported with trypanosomiasis, tuberculosis and some autoimmune diseases (Badaro et al., 1983; Dunan and Toga, 1988). Dunan and Toga (1988) did not detect cross-reactions with *Babesia canis* or *Ehrlichia canis* in the dog.

The prevalence observed in Cáceres (14.2%) was somewhat higher than those reported for other Spanish provinces: 8.5% in Zaragoza (Castillo Hernandez et al., 1985) and 7% in Granada (Reyes-Magaña et al., 1985).

The biotopes existing around the Sierra of Cáceres are ideal conditions for the insect transmitter, *Phlebotomus*: mountainous slopes (300–700 m above sea level), houses in ruins, abundance of vegetation, organic matter of animal origin (stables, kennels, etc.), together with high canine density, all combine to make it an area of high leishmaniasis risk.

Results obtained for different areas of Cáceres show high levels (ranging from 12.8 to 15.96%) of canine leishmaniasis in built-up areas and outlying districts, respectively. The authors believe that these figures should lead to an increased awareness and vigilance on the part of the various health authorities to prevent zoonotic infection.

ACKNOWLEDGMENTS

The authors wish to express their profound gratitude to the Regional Government of Extremadura for its approval of the research project which financed the survey of the canine population. They are also deeply grateful to Prof. J.A. Rioux for the enzymatic standardization of *Leishmania* strains in the Medical Ecology Laboratory of the University of Montpellier (Service d'Identification enzymatique de *Leishmania*). We are also grateful to Mr. M. Gómez-Blázquez for his technical assistance.

REFERENCES

Badaro, R., Reed, S.G. and Carvalho, E.M., 1983. Immunofluorescent antibody test in American visceral leishmaniasis: sensitivity and specificity of different morphological forms of two *Leishmania* species. Am. J. Trop. Med. Hyg., 32: 480–484.

Botet, S., Serra, T. Portus, M., Mora, R. and Gallego, M., 1987. Incidencia de la leishmaniosis en el área de Barcelona. Rev. Ibér. Parasitol., Vol. Extr., 51–54.

Castillo Hernandez, J.A. Sanchez Acedo, C., Gutierrez Galindo, J.F., Lucientes Curdi, J., Estrada Peña, A. and Galmes Femenias, M., 1985. Estudio epidemiológico de la leishmaniosis canina en la provincia de Zaragoza. Abstracts IV Congr. Nac. Parasitol., 9–13 July 1985, Tenerife, Spain, page 31.

Catarsini, O., 1980. Diagnosi e terapia della leishmaniosi del cane. Oviet. Vet., 2: 31–35.

Dunan, S. and Toga, I., 1988. Immunologie et leishmaniose. Prat. Méd. Chir. Anim. Com., 23: 81–83.

Lanotte, G., Rioux, J.A., Croset, H. and Vollhardt, Y., 1974. Ecologie des leishmanioses dans le sud de la France. 7. Dépistage de l'enzootie canine par les méthodes immunosérologiques. Ann. Parasitol. Hum. Comp., 49: 41–62.

Reyes-Magaña, A., Gonzalez Castro, J., Valero Lopez, A., Morillas Marquez, F. and Benavides Delgado, I., 1985. Encuesta sobre leishmaniosis canina en la provincia de Granada. Abstracts IV Congr. Nac. Parasitol., 9–13 July 1985, Tenerife, Spain, page 141.

Sánchez Botija, C., 1936. Leishmaniasis canina. Algunas observaciones sobre los caracteres generales del foco endémico de Madrid. Trab. Inst. Biol. Anim., 2: 70–87.

Thrusfield, M., 1986. Veterinary Epidemiology. Butterworths, London, pp. 153–165.