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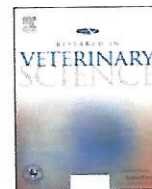
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## A survey on canine leishmaniasis and phlebotomine sand flies in central Italy

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## ABSTRACT

Zoonotic visceral leishmaniasis (ZVL) is a vector-transmitted zoonosis caused by the parasitic protozoan *Leishmania infantum*. Bloodsucking sand flies of the subfamily Phlebotominae are the obligatory insect hosts, and the dog is the only domestic reservoir.

This study reports data from a survey of canine infection and sand fly phlebotomine monitoring in the province of Perugia in central Italy. The overall seroprevalence in a total of 100 dogs tested was 8% (95% confidence interval: 3.8–15.6%). Data analysis revealed that serological positivity was statistically associated with age ( $p$ -value = 0.03) and the area where dogs lived. Standard blacklight traps employed for sampling *Culicoides* midges in bluetongue disease surveillance were used in phlebotomine monitoring. A total of 5698 sand flies were collected and the two species, *Leishmania* competent vectors, were identified, *Phlebotomus perfiliewi* (50%) and *Phlebotomus perniciosus* (30%).

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Visceral leishmaniasis (VL) is one of the most important parasitic diseases worldwide. The dog is considered the reservoir host for *Leishmania infantum*, and phlebotomine sand flies are the biological vector. Traditionally, in Italy only the coastal areas and islands of the Tyrrhenian and Ionian seas have been considered to be stable endemic foci of canine leishmaniasis (Bettini and Gradoni, 1986). However, VL incidence has been increasing in humans and dogs (Maroli et al., 2008), and new foci of canine leishmaniasis and the presence of competent sand fly vectors have also been reported in northern regions of the country where autochthonous cases had not previously been reported (Ferroglio et al., 2005; Maroli et al., 2008.). The objectives of the study were to determine the prevalence of anti-*Leishmania* antibodies among dogs from the province of Perugia and to investigate possible risk factors. In addition, results are reported of an entomological investigation aimed at studying the presence of phlebotomine sand flies in the same zone.

The study area is the municipality of Perugia, which is divided in four districts (Perugia, Assisi, Marsciano and Castiglione del Lago) and located in central Italy, between 42°42' and 42°48' latitudes north, and 13°6' and 11°59' longitudes east. The altitude varies from 197 to 300 m a. s. l. and the area is 2495 km<sup>2</sup>.

Local canine registry offices were used to randomly select 100 owned dogs (sample size based on an expected seroprevalence

50%, confidence interval 95% and precision of 10%), more than 2 years old.

Indirect fluorescent antibody testing (IFAT), according to Mancianti and Meciani (1988) was used to detect anti-*Leishmania* IgG antibodies; samples with clear-cut fluorescence when diluted at 1:80 were considered positive. Dog serum samples were monthly collected between August 2005 and February 2007.

A questionnaire was used to collect information regarding age, gender, breed, activities, lifestyle, living with other dogs, travel outside of Umbria in the last 2 years, and district of origin of dogs.

Five sites (Marsciano; Castiglione del Lago; two sites in Perugia, A and B; Assisi) were monitored for sand flies using standard blacklight traps, and specimens were collected weekly during August–October 2005 and May–October 2006. Phlebotomines were sorted from other insects under a binocular stereoscope, counted and stored in alcohol prior to their identification (Theodor, 1958; Léger et al., 1983).

Univariate and logistic regression were performed with Stata software, 9.1. Statistical significance was considered at  $p \leq 0.05$ . The 95% confidence intervals (95% CI) of seroprevalence rates were calculated.

Of 100 dog samples, 52 were males and most of them were pure breeds (66%), lived outdoors (86%), had contact with other dogs (63%) and had not travelled outside of Umbria in the last 2 years (85%). The age of the dogs ranged between 2 and more than 10 years.

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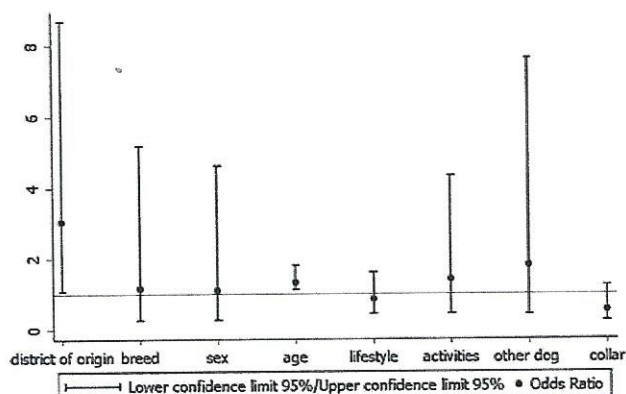


Fig. 1. Estimates of the logistic regression model investigating the risk of leishmaniasis in dogs in Umbria (Italy). The serological positivity for *Leishmania* antibodies (yes/no) represented the dependent variable and dog's data represented the independent variables. The 95% confidence intervals (95% CI) of seroprevalence rates were calculated.

Eight dogs were positive for anti-*Leishmania* antibodies (prevalence 8.0%, 95% CI: 3.8–15.6) showed serological titres higher than 1:160 and were asymptomatic. Four dogs were males; five were pure breeds, four lived with other dogs; six lived outdoors; five were used in sporting activities five were over 10 years of age, and three were between 2 and 5 years. Five lived in the district of Castiglione del Lago, two in Perugia, one in Marsciano; no positive dogs were found in the district of Assisi; None of the positive dogs had travelled outside Umbria in the last 2 years.

Serological positivity was statistically associated with age and with the district of residence (bivariate analysis, Fig. 1) with the highest prevalence observed in dogs 10 years of age or more and in dogs from Castiglione del Lago. In the final main-effect model of the multivariate analysis age and district of origin of dogs remained significant risk factors.

Only four sites monitored were positive for sand flies (Marsciano, Castiglione del Lago, and the two sites in Perugia). An overall total of 5698 phlebotomine specimens were collected, with 93% captured from a blacklight trap in Castiglione del Lago.

The number of sand fly specimens collected and the percentage of species identified at each site are shown in Table 1.

Our results indicate that the seroprevalence in Umbria is less than that of the seroprevalence found in southern Italy (Moreno and Alvar, 2002) but higher than that of northern Italy (Ferroglio et al., 2005), with regard to old age as a predisposing factor for the development of leishmaniasis, which has also been reported in other studies, one interpretation is that the immune system of the dogs declines with age; in addition, concomitant infections, which are more likely to be found in older dogs, may influence sus-

ceptibility to disease, furthermore, in endemic areas, older dogs have been more exposed to the infection over time.

With regard to the district of origin of dogs, small numbers complicate further statistical analyses. Nonetheless, in the district Castiglione del Lago, where seroprevalence was statistically different from that of the other areas, we collected over 5000 sand flies, while the other traps were at least 25-fold less successful. In the district of Assisi where no positive dogs were found, no sand flies were captured. Higher density of the vector sand fly favors disease transmission. Indeed, over the study period, three of six foci of human visceral leishmaniasis in all of Umbria were located in Castiglione del Lago.

As previous field studies have revealed, gender does not appear to be a risk factor (França-Silva et al., 2003). We did not find any significant difference within lifestyle classes and activity groups, even though dogs which live outside, such as sporting and guard dogs, are likely to have more contact with sand flies.

The data presented here highlight that the blacklight was attractive for sand flies, and thus UV light traps can be a useful tool for monitoring their presence. In fact UV light traps have rarely been used in sand fly collections (Bosnić et al., 2006), their specific use being for *Culicoides* sp. catches (Torina et al., 2004). Data from sand fly studies carried out in Italy showed that *Phlebotomus perniciosus* and *P. perfiliewi* were the prevalent species incriminated as vectors in Italy for transmitting *L. infantum* (Maroli and Khoury, 1998) and that *P. perniciosus* is known to be abundant along the coastal regions of Italy and inland (Bongiorno et al., 2003; Rossi et al., 2008).

In summary, results from this study confirming the presence of phlebotomine sand flies in the study area and that all the infected dogs never left the region may have important implications for the epidemiology of human leishmaniasis in the same areas. Indeed there is strong evidence for the occurrence of human cases in areas where the prevalence rate in dogs is high (Oliveira et al., 2001) as demonstrated by the fact that the risk for VL was twice as high if there were dogs in the domestic setting than if they were absent (Costa et al., 1999).

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Table 1

Abundance of the two *Leishmania* competent vectors recorded in each collecting site of Perugia province, central Italy.

| Collecting site | No. of specimens | <i>Phlebotomus perniciosus</i> |      | <i>Phlebotomus perfiliewi</i> |      | Other species <sup>a</sup> |      |
|-----------------|------------------|--------------------------------|------|-------------------------------|------|----------------------------|------|
|                 |                  | No.                            | %    | No.                           | %    | No.                        | %    |
| Marsciano       | 16               | 5                              | 31.2 | 8                             | 50.0 | 3                          | 18.8 |
| C. del Lago     | 5329             | 1599                           | 30.0 | 2664                          | 50.0 | 1066                       | 20.0 |
| Perugia A       | 168              | 50                             | 29.8 | 84                            | 50.0 | 34                         | 20.2 |
| Perugia B       | 185              | 57                             | 30.8 | 92                            | 49.7 | 36                         | 19.5 |
| Assisi          | 0                | 0                              | –    | 0                             | –    | 0                          | –    |
| Total           | 5698             | 1711                           | 30.0 | 2848                          | 50.0 | 1139                       | 20.0 |

<sup>a</sup> *Phlebotomus mascitti*, *Phlebotomus papatasi*, *Sergentomyia minuta*.

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