Preliminary study of the effects of preventive measures on the prevalence of Canine Leishmaniosis in a recently established focus in northern Italy

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Summary
Canine Leishmaniosis is endemic in Mediterranean areas, with a well-documented northward spread. The mass use of preventive measures against sandfly bites (collar and spot-on formulations) was tested in a small focus recently established in an isolated hilly area of north-eastern Italy (Colli Euganei). In 2006 and 2007, a total of 449 dogs living in the southern part of Colli Euganei were screened against *Leishmania infantum* using an immunofluorescence antibody test (IFAT), and 31 (6.9%) were seropositive. A risk factor analysis clearly described the focus as limited to a small village named Calaone. In 2010, 63 animals from Calaone were sampled and their owners interviewed to verify the effectiveness of the preventive measures. According to what reported by owners, dogs started to be protected in 2006 (66.7% dogs protected), and protection rate incremented (around 90%) during the subsequent years. The seroprevalence value (4.2%) of the youngest age class (<5 years) was significantly lower than other classes, demonstrating that animals born after 2006 had low probabilities of getting infected. Besides, seroprevalence value referred only to dogs living in Calaone was 32.4% (23/71) in 2006-2007 and 20.6% (13/63) in 2010, showing a decreasing trend. Although still preliminary, the results show high sensitization of dog owners and suggest that the mass use of collars and spot-on acts positively in reducing the circulation of *L. infantum*.

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Introduction

Leishmania infantum is considered an important and emerging zoonotic pathogen (17). It is the etiologic agent of Canine Leishmaniosis (CanL) and of cutaneous and visceral zoonotic Human Leishmaniasis (HumL) in Mediterranean areas. Canine Leishmaniosis is endemic along the Mediterranean coast, where its prevalence varies widely (1, 8, 18).

A northward spread of CanL in Italy is well documented (11) and there is a high risk of emergence in other parts of Europe further north (17). The massive use of sandfly bite preventive measures (e.g. deltamethrin- and imidacloprid 10%/flumethrin 4.5%-impregnated collars, and imidacloprid 10%/permethrin 50% in spot-on formulation) has been shown to be one of the most effective systems in preventing CanL in dogs and reducing its incidence in endemic and hyper-endemic areas (6, 10, 14, 16). Although this approach has been extensively promoted also in newly established foci to control the spread of the parasite, no field studies have documented its effectiveness so far.

Many new autochthonous foci of CanL in northern Italy have been described since the 90s (15). Among these, a small focus was first suspected and then confirmed in a small village in the southern part of Colli Euganei, an isolated hilly area in the central part of the Veneto Region, north-eastern Italy. This study summarizes a 5-year surveillance activity and presents the preliminary results of a mass use of

sandfly bite preventive measures in controlling, and possibly eradicating, CanL from the area.

Material and methods

Study area

The first part of the study investigated the diffusion of *L. infantum* in southern Colli Euganei (Figure 1). The area presents unique climatic and environmental characteristics, which substantially differ from the surrounding plain. In particular, southern slopes are characterized by a Mediterranean climate.

The second part of the study was limited to Calaone, which is a small village located in Baone municipality, southern part of Colli Euganei (45°14'58"N-11°39'54"E). Calaone is the only village of the municipality located mostly 100 m above sea level (a.s.l.), with an average altitude of 223 m a.s.l. (range 74-377 m a.s.l.), and a predominant southern exposition. According to the local veterinary service office (ULSS 17) the registered Calaone dog population in 2010 consisted of 119 animals, with an estimated 5% of unregistered dogs.

Field sampling

The first suspicion of the presence of *L. infantum* in the area was based on the report of one autochthonous case of CanL and on the presence of *Phlebotomus perniciosus* in Calaone village, documented in 2005 (3). The spread of CanL in the southern area of Colli Euganei was assessed in 2006 and 2007, when 245 and 229 dogs, respectively, were screened by serological tests (25 dogs were tested both years). Dog owners in Baone and surrounding hilly municipalities were invited to test their animals during a one-day sampling campaign organized on a free basis in the late May 2006 and in June 2007. Since the first 2006 campaign, dog owners had been invited to use preventive measures (collars or spot-on formulation) during the June-October period. Furthermore, an informative meeting was organized in October 2006 in Baone, aimed at increasing the public awareness of the disease and its prevention.

A new specific epidemiological survey, including queries about dog owners' use of preventive measures, was designed in 2010 to verify the effectiveness of the intervention promoted in Calaone village. A total of 63 animals were sampled and their owners interviewed on the use of preventive measures against sandfly bites during the previous years.

Laboratory analyses

Blood samples were analysed using an immunofluorescence antibody test (IFAT) according to OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (13). Immunofluorescence antibody test serum titres ≥1:40 were considered positive and indicative of CanL exposure, i.e. a contact with an infected vector in the previous summer season. Whenever possible, aspirates from lymph nodes were collected from dogs positive at serological analysis, and cytological smears were stained using Diff-Quick® kit (Medion Diagnostics International Inc., Miami, FL, USA).

Statistical analyses

Considering all dogs sampled in 2006 and 2007 (n = 449; dogs tested both years were considered only once), a risk-factor analysis was performed to evaluate seroprevalence differences among sex, life style (companion animals; hunting dogs/watchdogs), age class (<5; 5-7; >7 years), municipality of origin (Arquà Petrarca; Baone; Cinto Euganeo), and altitude (plain: under 100 m a.s.l.; hill: above

Figure 1. *Study area.* Boundaries of municipalities involved in the study (thick white lines) and of Colli Euganei area (thin white lines).

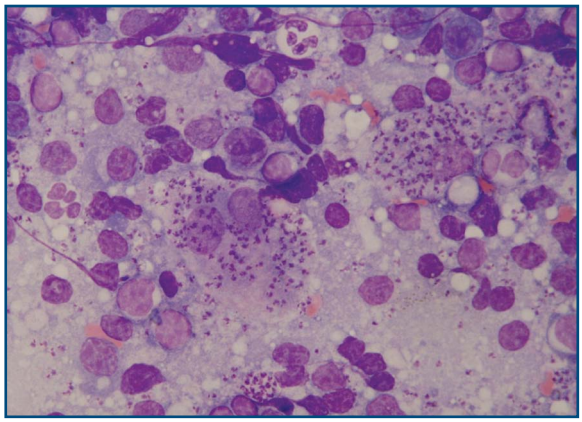

Figure 2. *Amastigotes in a positive lymph node aspirate collected from a dog living in Calaone stained using Diff-Quick® kit.*

100 m a.s.l.). Sleeping outside at night, living with other dogs, and travelling to endemic areas were also investigated as possible risk factors. Differences in seroprevalence were first evaluated using univariate statistic, specifically Pearson's Chi-squared test or, when appropriate, Fisher's exact test. Statistical analyses were also performed considering positive animals with titres ≥1:160, which is indicative of CanL infection (5). Moreover, the dataset of dogs was analysed by means of multivariate logistic regression model (9) to evaluate potential risk factors associated with *L. infantum* seroprevalence at different cut-off (1:40 or 1:160). Statistical analysis was performed using PASW Statistic 18 (SPSS Inc.).

Differences in seroprevalence among age classes (<5; 5-7; >7 years) of the dogs sampled in 2010 in Calaone village (n = 63) were evaluated using Pearson's Chi-squared test. Seroprevalence obtained in 2010 was compared to the values referred only to Calaone dogs and obtained from the dataset of 2006-2007 samples.

Results

Preliminary survey (years 2006-2007)

A total of 31 (6.9%) out of the 449 dogs tested were seropositive (≥1:40). Nearly all positive dogs (n = 29) were from Baone municipality, and 24 lived in Calaone village. Only one animal had history of travelling to an endemic area.

Most of the dog owners were not willing to let their dogs being checked by means of lymph nodes aspirates. Thus, only 4 dogs were sampled and 2 resulted positive, respectively with 1:320 and 1:160 titres at IFAT (Figure 2).

The results of univariate analysis are presented in Table I, whereas logistic regression model results are shown in Table II. Dogs living in hilly areas and particularly in Baone municipality and kept outside at night showed higher probability to be exposed to an infected sandfly bite.

Epidemiological investigation in Calaone (year 2010)

Dog owners interviewed during the 2010 campaign reported that they had started using collars or spot-on formulations or both since 2006. At the beginning 66.7% (30/45) dogs resulted to be protected from sandfly bites. The percentage increased in subsequent years: 90.0% (45/50) in 2007, 91.1% (51/56) in 2008, and 88.9% (56/63) in 2009.

In 2010, 13 (20.6%) dogs, out of 63 investigated, resulted positive. Figure 3 shows the differences in age class seroprevalence values in 2010. The <5 age class presents a value (4.2%) significantly lower

Table I. L. infantum *seroprevalence based on epidemiological data and significant statistical differences (years 2006-2007).*

Factor		N	Exposure (1:40 cut-off)		Infection (1:160 cut-off)	
			Pos	Prev (%)	Pos	Prev (%)
Municipality	Arquà Petrarca	48	0	0.0	0	0.0
	Baone	242	29	12.0**	20	8.3**
	Cinto Euganeo	111	1	0.9	0	0.0
Altitude	Plain	273	6	2.2	2	0.7
	Hill	174	25	14.4**	18	10.3**
Night outdoor	No	131	6	4.6	1	0.8
	Yes	316	25	7.9	19	6.0*
Age class (years)	<5	185	12	6.5	9	4.9
	5-7	123	10	8.1	5	4.1
	>7	133	9	6.8	6	4.5
Sex	Male	246	16	6.5	10	4.1
	Female	199	15	7.5	10	5.0
Life style	Companion animals	278	17	6.1	10	3.6
	Hunting dogs/watchdogs	94	9	9.6	6	6.4
Living with other dogs	No	208	11	5.3	9	4.3
	Yes	238	20	8.4	11	4.6
Travels to endemic areas	No	413	30	7.3	20	4.8
	Yes	32	1	3.1	0	0.0

** $p<0.01$; * $p<0.05$.

Table II. *Results of multivariate risk-factor analysis (years 2006-2007).*

Factor	P value	Odds Ratio	95% C.I. for O.R.	
			Lower	Upper
Baone municipality	<0.001	8.718	3.181	23.890
Hilly area	<0.001	4.225	2.468	7.232
Night outdoor[a]	0.026	3.332	1.158	9.592

[a] Only dogs positive at IFAT >1:160.

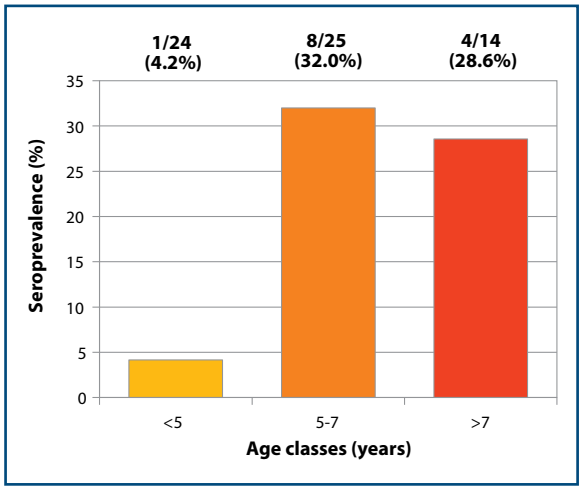

Figure 3. *Seroprevalence for* L. infantum *in different age classes (year 2010).*

($p < 0.05$) than other classes. In 2006-2007, 71 dogs out of the 449 sampled were from Calaone village, and 23 (32.4%) of these resulted positive at IFAT, at least at one sampling. The reduction of prevalence between 2006-2007 and 2010 was 11.8%.

Discussion

The screening of dogs in 2006-2007 clearly demonstrated that a CanL focus has established in the southern part of Colli Euganei area. Provenances from a hilly area and from Baone municipality were identified as the two major risk factors, so confirming Calaone village as the centre of the focus. The lack of differences in seroprevalence values among age classes is indicative of a newly established infection in which old dogs have the same probability of getting infected as young animals. The CanL focus was therefore described as limited to Calaone village and recently established.

Dogs spending the night outdoor resulted at higher risk when using the 1:160 cut-off. This result suggests that also dogs kept inside during the night may have occasional contacts with infected sandflies, but sleeping outside is confirmed to be an important risk factor for the development of the infection and consequently of the disease (4, 12).

Although the results of the epidemiological study conducted in 2010 to evaluate focus progression are still preliminary and affected by the limited size of the dog population sampled, they are encouraging.

Data on the use of sandfly bite preventive measures suggest that dog owners have been highly sensitized to the disease and are aware of the appropriate measures to control the infection.

The mass use of collars and spot-on products seems to act positively, considering the decreasing trend of the seroprevalence and the reduced number of seropositive dogs among young animals. In fact, seroprevalence in Calaone was reduced by one third in about three years. Furthermore, considering only animals sampled in 2010, the seroprevalence value of the youngest age class (up to 4 years old) is significantly lower than other classes, demonstrating that animals born after 2006, when the mass use of preventive measures started in the village, had clearly lower probabilities of being exposed to the parasite than older animals (Figure 3).

This focus, along with the numerous new foci of CanL detected in northern Italy (11, 14), confirm the expansion of *L. infantum* infection in this part of the country, which must now be considered endemic, at least in most of its hilly areas with Mediterranean climate. At present, only one autochthonous case of HumL has been documented in north-eastern Italy (7), but subclinical cases and the underestimation of HumL cases in official reports can conceal a higher circulation of the parasite in the human population, as it has been found in north-western Italy (2). The presence of areas with high prevalence and the geographic spread of the parasite among dog populations suggest that the risk to the human population may increase in the near future.

Conclusions

The study describes the identification of a new autochthonous focus of CanL in a small village of a hilly area of northern Italy, previously considered free from the parasite. A prompt intervention to promote the use of sandfly bite preventive measures was implemented to stop the spread of the parasite and showed to be able to reduce the seroprevalence among dogs in the area. The results confirm that active CanL monitoring is a useful tool in alerting and preparing public health services, and that any action aimed at preventing the spread of CanL also contributes to lowering the risk for the human population.

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