



SHORT COMMUNICATION

Canine Leptospirosis Cases and Molecular Screening for *Leptospira interrogans* Infection

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ABSTRACT

A molecular survey for pathogenic *Leptospira* spp. in kennelled dogs in Sicily, Italy, showed up to 16% of positive results. A fatal leptospirosis case was confirmed in a stray dog in the city of Palermo in May 2012. The dog, a mixed breed male, was recovered for therapy but died within an hour. Inflammation was present in the liver and kidneys. Haematoxylin-eosin staining of the kidney tissue revealed interstitial nephritis indicative of leptospirosis. The meninges were congested with some icteric shades. Several organs and prepuce swabs showed positive reaction by PCR specific for pathogenic *Leptospira* spp. The serum analysis by MAT (microscopic agglutination test) showed positive results for *Leptospira* serovar Canicola with a titre of 1:400. Additionally, five clinical cases of canine leptospirosis were suspected over a two years period (2011-2012) in the province of Palermo, in contrast to the six in a previous 5 years period. An increased concern of the veterinarians on leptospirosis in pet dogs should be suggested.

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INTRODUCTION

Leptospirosis is probably, the most common bacterial zoonosis in the world, caused by more than 200 different serotypes of the spirochete *Leptospira* spp. The infection of dogs results in a disease with highly variable symptomatology as in human beings. Some dogs display mild or no signs of disease, whereas others develop severe illness or death (Van de Maele *et al.*, 2008; Saleem *et al.*, 2012). The epidemiological picture in dog's population is based mainly on serology obtained by the microscopic agglutination test (MAT), which is considered the gold standard.

In Europe, high MAT titers from 1:400 to 1:800 are considered useful to identify the serovar strains responsible for recent clinical infections and to exclude cross-reactions (Ellis, 2010). In contrast, very low titers to serovars Icterohaemorrhagiae or Canicola could be a result of vaccination, and low titers to other strains could result from cross-reactions in the MAT assay (André-Fontaine, 2006). To prevent the disease, vaccinations are suggested for companion dogs. Vaccine composition is based on the locally predominant circulating serovars that might differ in various regions of the world (Koizumi *et al.*, 2013). The serovar Canicola is maintained by dogs and has no other known maintenance host but its

seroprevalence is decreasing in many European countries. This has been attributed to the use of vaccines containing serovars Canicola and Icterohaemorrhagiae for half a century (André-Fontaine, 2006). However, serological evidence of exposure in dogs, and some clinical cases related to both serovars, is still reported across Europe; new pathogenic serovars can become predominant and, new vaccines are requested with the inclusion of other serovars such as Bratislava and Grippityphosa (Ellis, 2010). In Italy, a previous serological analysis in kennelled dogs showed that the most common infecting serovars found, were Bratislava and Grippityphosa (Scanziani *et al.*, 2002). In this paper, a case of canine fatal leptospirosis in the city of Palermo caused by *L. interrogans* serovar Canicola and a molecular survey for pathogenic *Leptospira* spp. on stray dogs of kennels, in Sicily, are reported.

MATERIALS AND METHODS

Clinical case: A male dog of mixed breed was occasionally seen in the same areas of a hill above the city of Palermo for many months, until it showed clinical signs. The animal vomited, felt very weak and had jaundice in the eyes and mucous membranes. The clinical signs suggested an acute leptospirosis; the dog was

recovered for a therapeutic intervention, but it died within one hour. Anatomical pathology and necropsy observations were made on internal organs.

Haematoxylin-eosin (H&E) staining was performed on the organs after 4 days of fixation in 10% buffered solution of formalin and paraffin embedding. Serology was performed by MAT against a panel of the common strains circulating in Italy and PCR in organ samples was performed with the following primers targeting the rRNA locus 16S-For, 5'-AGGGAAAAATAAGCAGCGATGTG-3' and 16S-Rev, 5'-ATCCACTCCATGTCAAGCC-3' as described (D'Andrea *et al.*, 2012).

Sampling and molecular analysis: Molecular survey in kennelled stray dogs was performed in five kennels in Sicily by prepuce and vaginal swabs sampling over a 12 months period from April 2009 to March 2010. Vaginal and prepuce swabs sampling was performed on at least 10% of the animals normally hosted in the kennels: A total of 85 females and 98 males of approximately 3 years old were chosen for the sampling.

The dry swabs were put in 1 ml of TE (10mM Tris pH 7.5, 1 mM EDTA pH 8) and left from 1 to 24 hours at 4°C. Then 200 µL of the suspension was used for DNA extraction. DNA was extracted by Sigma genomic DNA kit following manufacturer's instructions and PCR was performed as described above.

RESULTS AND DISCUSSION

Clinical findings in the stray dog: The estimate age of the dog was 3 years. The anatomic-pathological examination showed icterus in the external mucosal and sub-mucosal areas and external appearance was typical for canine leptospirosis (Khan *et al.*, 2010).

Upon the abdominal cavity opening, a generalized icterus and hemorrhages of intestine and pericardium were observed. The liver volume resulted increased and, the kidneys appeared highly congested; a severe inflammation of the liver and of the cortical areas of the kidneys was present. The meninges were also highly congested with some icteric shades. All these signs were compatible with an infection of *L. interrogans* sensu lato.

Laboratory examination: PCR for the pathogenic *Leptospira* spp. on urine, liver, kidneys, brain, lungs samples and prepuce swabs were all positive. However, isolation from urine and kidney gave negative results. Haematoxylin-eosin staining of the kidneys revealed a picture of interstitial nephritis indicative of leptospirosis. The serum analysis by MAT showed positive results for *L. interrogans* serovar Canicola only, with a titre of 1:400.

Dogs are usually the reservoir host for the serovar Canicola and might develop a chronic carrier state for it, although the incidence for this is still unknown (Goldstein, 2010). The serotype has been included in vaccines for dogs for many years and for this reason its prevalence is declining in Europe, although clinical cases are still being reported (Ellis, 2010). Many other serovars are involved in canine leptospirosis in Italy (Mastrorilli *et al.*, 2007), and other European countries in the last decades, but the occurrence of fatal canine leptospirosis due to the Canicola serovar suggests that this serovar

should always be included in new multivalent vaccine formula (Ellis, 2010).

In a period of two years (2011-2012), five cases of canine leptospirosis death occurred in the province of Palermo, and were reported to Zooprofilactic Institute of Sicily, in contrast to the six cases that were reported during the previous five-year period (2005-2010). We do not know if this is due to a real increase in the incidence of canine leptospirosis, or simply reflects an increased focus on the disease. However, since environmental factors in Sicily are favourable for the transmission of the infection, probably veterinarians should be more aware on follow-up and vaccination of pet dogs for leptospirosis. All past fatal cases in companion dogs were only clinically suspected by the private veterinarians and not officially reported. No confirmation by laboratory tests had been performed on the animal samples, since the cases were referred by the owners of the dogs, weeks after the death of their pets. In some cases the fatal canine leptospirosis had been reported, a few weeks after the death of the dog, only when family members had developed symptoms of fever or flu and the analysis of PCR for pathogenic *Leptospira* on their blood samples were required.

Molecular screenings in kennels: The molecular screening of females showed 10 positives out of 85 (11.76%). In males 16 positive dogs were found out of 98 (16.32%). None of them had clinical evidence of leptospirosis. The positive dogs did not show any clinical sign at least in one year follow-up.

The PCR screening in kennelled dogs in Sicily revealed high prevalence of *Leptospira* spp. among stray dogs. In the island, the climate has been changing in the last decades with increases in average temperatures and rain fall; heavy rain episodes occur all year around with water flooding in the streets of many cities such as Palermo. Additionally, problems related to the garbage collection might contribute to the expansion of the rodent population infected with pathogenic *Leptospira* spp. A relation between waste collection problems, rodent population and human leptospirosis has been reported in France (Socolovschi *et al.*, 2011). Urinary shedding of spirochetes in urban streets can be the source of infection for several stray animals such as dogs and cats. In the city of Palermo, a population of 8-10 thousands of stray dogs is estimated by the local municipal authorities although the canine registry to calculate the real number is still in progress. Many of these stray dogs can be exposed to *Leptospira* infection because of problems in waste collection and flooding of the streets during rainstorms.

Conclusion: The molecular surveys on prepuce and vaginal swabs of the stray dogs in kennels revealed that urinary shedding of pathogenic *Leptospira* spp. can represent an additional risk for its zoonotic transmission in urban settings. The clinical case caused by *L. interrogans* serovar Canicola suggests that although the prevalence for this serovar is declining in Europe, it should always be included in vaccine composition to prevent dog leptospirosis. Additionally, in the presence of environmental factors (climate change, waste collection

problems, heavy rain episodes) that are highly favorable for the spread of the infection, the veterinarians should be more aware on vaccination and follow up of pet dogs for leptospirosis.

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