# Serological survey of Canine leishmaniasis in Southwestern Region of Kosovo

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*Veterinaria Italiana* 2020, **56** (1), 47-50. doi: 10.12834/VetIt.1345.7407.5
Accepted: 19.02.2018 | Available on line: 24.04.2020

**Keywords**
Canine leishmaniasis,
ELISA,
*Leishmania infantum,*
Kosovo,
Stray dogs.

**Summary**
Canine leishmaniasis caused by *Leishmania infantum* is endemic illness in Mediterranean countries. The disease represents an important public health issue in Kosovo which reported several cases in previous years. In this study we performed a clinical and serological surveillance of canine leishmaniasis in dogs within the Southwestern Region of Kosovo. Blood samples were collected from stray dogs in four municipalities including Prizren, Gjakova, Rahovec and Deçan. A total of 125 samples were collected between April/September 2016, and antibodies of *Leishmania infantum* were detected by competitive enzyme-linked immunosorbent assay (ELISA IgG). Out of 125 serum samples, 23 dogs (18.4%, 95%CI 12.6-26.1) tested positive for *Leishmania infantum*. Three of the 23 positive dogs (13%) showed typical clinical signs of Canine leishmaniasis including skin and ocular lesions, decreased appetite, lameness, diarrhea, lethargy and progressive weight loss. The present study confirmed that Leishmaniasis is endemic in the southwestern part of Kosovo and emphasized the need for establishing a stronger surveillance and control.

## Introduction

Leishmaniasis is a vector-borne disease caused by flagellated protozoans of the genus *Leishmania* (Ready 2010). Canine leishmaniasis (CanL) due to *Leishmania infantum* is an endemic zoonosis in the Mediterranean basin, whose dogs are the main reservoir (Miranda *et al.* 2008). *L. infantum* is transmitted by blood-sucking phlebotomine sand flies (Miro *et al.* 2012). *Phlebotomus* spp. (Syn. *Lutzomyia* spp.) is the primary vector, domestic dogs, rodents, sloths, and opossums play the role of reservoir hosts (Pigott *et al.* 2014). *L. infantum* infection in dogs is characterized by a chronic subclinical infection (Esteve *et al.* 2015). Some dogs may present with one or few clinical symptoms and others with multiple clinical manifestations, which are called polysymptomatic dogs (Saridomichelakis 2009). Clinical signs in dogs include generalized lymphadenomegaly, hepatomegaly, splenomegaly, fever, diarrhea, lethargy, and progressive weight loss (Solano-Gallego *et al.* 2012). Furthermore, the majority of dogs shows skin lesions. Common biochemical abnormalities include hyperproteinemia with hypergammaglobulinemia and hypoalbuminemia (Naucke and Lorentz 2012). Age seems to be a risk factor that influences the development of the disease. Clinical disease has two peaks, one in young dogs, between 2 and 4 -years old, then again in older dogs, older than 7 years of age (Sykes and Greene 2012).

The most useful diagnostic approache for investigation of infection in sick and clinically healthy infected dogs is the detection of specific antibodies by several serological techniques and the demonstration of the DNA of the parasite in tissues by molecular techniques (Solano-Gallego *et al.* 2009). Currently, the data regarding CanL in Kosovo is scarce.

In this study, we aimed at determining the seroprevalence of CanL in stray dogs in the southwestern region of Kosovo.

## Materials and methods

The study was conducted between April/September 2016 in the shelter 'StrayCoCo' located in Nagavc, Rahovec. Some municipalities in Kosovo have indeed established shelters for the control of stray

dogs through a catch-neuter-release project. Blood samples were collected from 125 stray dogs that were captured in four municipalities including Prizren, Gjakove, Rahovec and Decan. A total volume of 5 ml blood sample was collected from each dog by cephalic venipuncture and sera were separated at the laboratory and kept at - 20 °C until analysis. Age estimation and clinical visit of the dogs were performed at the moment of blood sampling, and then during their stay in the shelter. The dogs showing skin and ocular lesions, decreased appetite, diarrhea, lethargy and lameness were identified as suspected cases for CanL. A serodiagnosis was conducted by detecting specific

**Table I.** *Results of tested samples for CanL in stray dogs in four municipalities of Southwestern Kosovo, 2016.*

| Municipality | | Prizren | Gjakova | Rahovec | Deçan | Total |
|---|---|---|---|---|---|---|
| No. of samples | | 60 | 17 | 35 | 13 | 125 |
| Age | 1-3 years | 38 | 11 | 20 | 8 | 84 |
| | 3-5 years | 22 | 6 | 15 | 5 | 41 |
| ELISA positive | | 4 pos. (1-3 y) | 1 pos. (1-3 y) | 2 pos. (1-3 y) | 0 pos. (1-3 y) | 7 pos. (1-3 y) |
| | | 9 pos. (3-5 y) | 2 pos. (3-5 y) | 4 pos. (3-5 y) | 1 pos. (3-5 y) | 16 pos. (3-5 y) |
| ELISA negative | | 34 neg. (1-3 y) | 10 neg. (1-3 y) | 18 neg. (1-3 y) | 8 neg. (1-3 y) | 70 neg. (1-3 y) |
| | | 13 neg. (3-5 y) | 4 neg. (3-5 y) | 11 neg. (3-5 y) | 4 neg. (3-5 y) | 32 neg. (3-5 y) |
| Prevalence (%) | | 21.6% | 17.6% | 17.1% | 7.6% | 18.4% |
| 95%CI | | 13.1-33.6 | 6.1-41.0 | 8.1-32.6 | 1.3-33.3 | 12.6-26.1 |

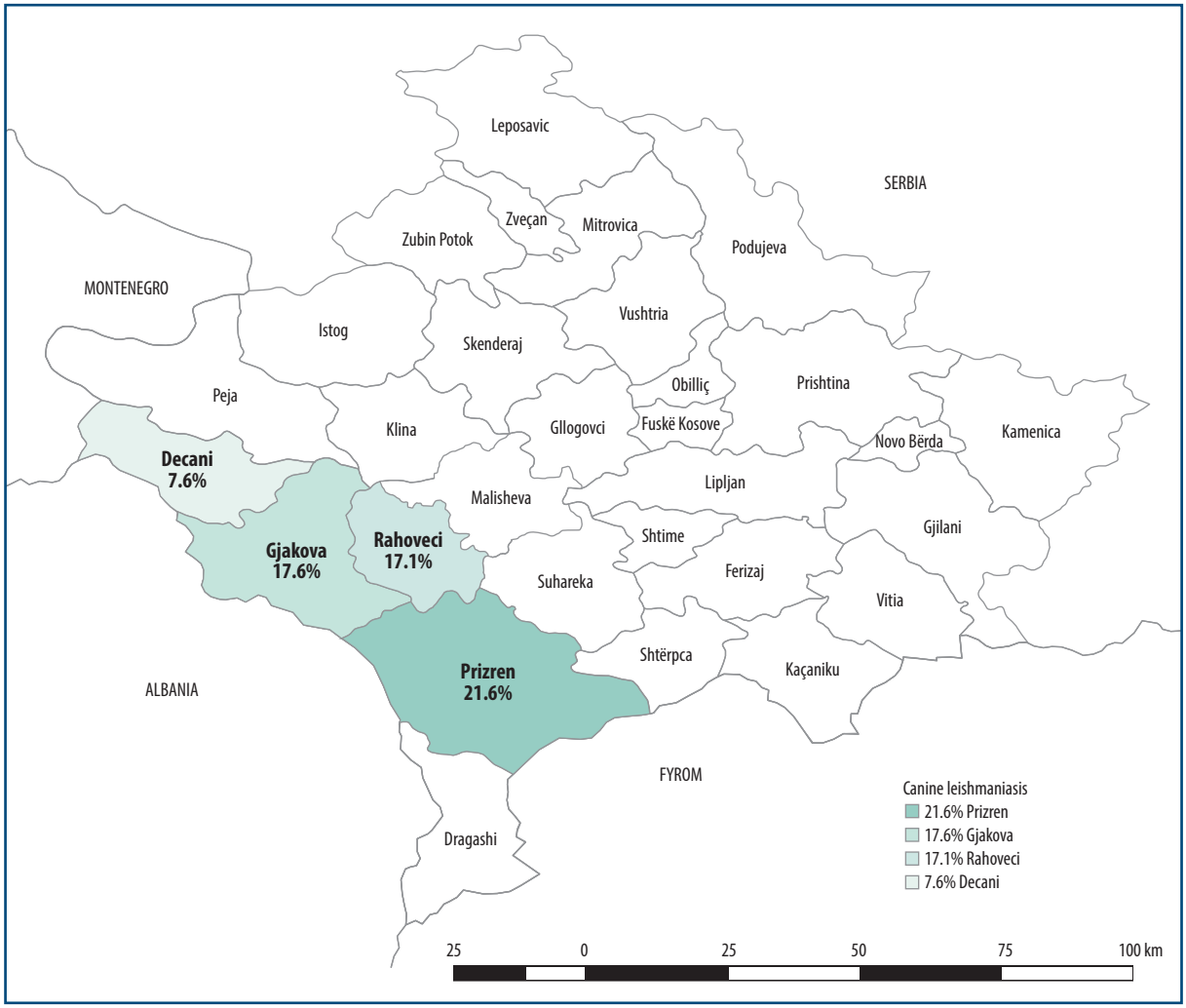


**Figure 1.** *Prevalence rate of CanL in the Southwestern Region of Kosovo.*


*Veterinaria Italiana* 2020, **56** (1), 47-50. doi: 10.12834/VetIt.1345.7407.5

antibodies against *L. infantum* using competitive enzyme-linked immunosorbent assay (ELISA IgG), according to manufacturer instructions (Nova Tec Immundiagnostica GmbH, Germany), at the Institute of Veterinary and Agriculture in Kosovo.

## Results

Antibodies of CanL were detected in 23/125 stray dogs representing the 18.4% (95% CI 12.6-26.1) of tested samples.

The highest number of positive samples were detected in the Prizren municipality. After correction of sensitivity (95%) and specificity (96%) of the test, the highest estimated true prevalence was 21.6% (95%CI 13.1-33.6) in Prizren (13 out of 60 samples), followed by Gjakova 17.6% (95%CI 6.1-41.0) (3 out of 17 samples), Rahovec 17.1% (95%CI 8.1-32.6) (6 out of 35 samples) and Deçan 7.6% (95%CI 1.3-33.3) (1 out of 13 samples). In three out of 23 positive dogs (13%), clinical signs of CanL were observed. Suspicion of CanL was made through observation of dogs skin and ocular lesions, decreased appetite, lameness, diarrhea, lethargy and progressive weight loss. Dogs between 3 and 5 years represented the 70% of the CanL positive dogs (16/23). The remaining 30% (7/23 dogs) were dogs with age between 1 to 3 years old. Results and origin of study regions are showed in Table I and Figure 1, respectively.

## Discussion

CanL is a major zoonotic disease, endemic in tropical and subtropical countries and fatal in humans and dogs. The first surveillance of CanL in Kosovo was done in 2008, when the percentage of CanL was 3.3% positive (Lazri *et al.* 2008).

Although the limitations of the sample size, the results of this survey confirm the high prevalence of CanL in stray dogs of Kosovo. The infection rate of CanL was higher in municipalities in the southwestern region of Kosovo, bordering Albania. This area includes Prizren (21.6%), Gjakova (17.6%), Rahovec (17.1%) and Deçan (7.6%) (Alten 2014, unpublished data). The competent vectors of CanL *Phlebotomus* spp. were identified in the different municipalities of Kosovo, and found that they are especially high in abundance of *Phlebotomus* (Lar.) *major s.l.* that were found in southwestern part of the country.

The present study highlighted the need for increasing control capacity of CanL in Kosovo. Reasonably, further studies are warranted in order to establish the prevalence of the diseases in other areas of Kosovo as well as a deep entomological survey.

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