

Dog leishmaniasis in Slovenia: a probable creation of the first enzootic focus - a case report

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ABSTRACT

Leishmaniasis is a vector-borne disease caused by protozoal parasites of the genus *Leishmania*. Parasites are transmitted by Phlebotominae flies (Diptera, Psychodidae). *Leishmania infantum* is the most important species in Europe. Humans, as well as animals, can become infected. The parasite produces a visceral form of the disease that can end in death if not treated properly. Dogs are considered the main reservoir for human infection. Traditional endemic regions in Europe are the countries of the Mediterranean basin. Slovenia is considered non-endemic. The article reports a case of canine leishmaniasis (CanL) in a Slovenian dog. This particular case was a 5 year old Brandlbracke bitch, born close to the Croatian border, but which had never crossed the Slovenian state borders. She was a regular outdoor sleeper. Beside considerable loss of weight, seborrhoeic and alopecic skin with crusts on the legs and head were noticed. Laboratory tests revealed hyperproteinaemia, hypoalbuminaemia, leucocytosis, anaemia, and thrombocytosis. An immunofluorescence antibody titre test revealed 1: >160 antibody titre against *L. infantum*. Clinical improvement was achieved following therapy with allopurinol. Real-time PCR performed on conjunctival swab and blood specimens 1.5 years post therapy was negative. Since Phlebotominae flies were found in the south of the country recently, and infection by other routes in this case seems unlikely, this dog was most probably infected by sand fly bites. The possibility that CanL could be spread endemically in Slovenia therefore exists.

Key words: leishmaniasis; CanL; *L. infantum*; sand flies; Phlebotominae

Introduction

Canine leishmaniasis (CanL) is a vector-borne disease, caused by protozoal parasites of the genus *Leishmania*. Parasites are transmitted by Phlebotominae flies (Diptera, Psychodidae). Historically, these insects are called sand flies because of the sandy colour of their body. Sand flies are small, mostly nocturnal insects. The adults feed on nectar and other plant-derived sugars. However, the females, in addition, feed on blood required for

maturing eggs. Although many mammals, (humans, dogs, cats, horses, rabbits, foxes, jackals and rodents) may serve as hosts, dogs are considered the main reservoir of the disease (ALVAR et al., 2004). *L. infantum* is currently the predominant causative agent of visceral leishmaniasis in people and dogs in Mediterranean region. Through sand fly bites, leishmanial parasites are inoculated into the skin. They infect phagocytes and multiply in them. In the

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phagocytes, parasites are transported to the regional lymph nodes, and from there, parasites may be disseminated by infected phagocytes into different tissues all over the body. This happens in cases of inadequate immune response. Nevertheless, the majority of infected dogs (80%) remain asymptomatic. It depends on the capability of the cellular (Th1-dominated) immune response and parasite load whether *L. infantum* infection remains asymptomatic or leads to a severe, potentially fatal disease in dogs (ALVAR et al., 1994)

Dogs with the clinically developed disease predominantly show the following symptoms: loss of body mass, intermittently elevated body temperature, lymphadenopathy, exfoliative dermatitis with onychogryphosis, epistaxis, uveitis and polyuria / polydipsia. Laboratory tests usually reveal non-regenerative anaemia, hyperproteinaemia, hypoalbuminaemia, proteinuria and uraemia (ALVAR et al., 1994).

Case presentation

In autumn 2015 a questionnaire survey was conducted using the mailing list of the Slovenian Veterinary Chamber, involving the 103 Slovenian practices dealing with small animals at that time. The survey included a few simple questions such as "have you ever been involved in the diagnostics and / or treatment of CanL case?" and "have you ever seen a CanL case in an animal born in Slovenia that has never left the country?". Amongst the answers which mainly reported infected dogs rescued from Spain and Portugal, data were found about the bitch reported in this article as the first known CanL-infected Slovenian dog that has never left the country.

This particular case was a 5 year old Austrian Black and Tan Hound bitch (Brandlbracke) named Ala. Ala was born in Kostelo (a small village close to the Croatian border). She had travelled to the Slovenian coast and to the eastern part of Slovenia, but had never crossed the state borders. When two years old she had a litter of two puppies that were killed soon after birth by another bitch living in the household. Ala was neutered a few months later. At the time of presentation, Ala was an active hunting dog. She was a regular outdoor sleeper. In autumn

2013 her owner noticed skin changes. The skin was seborrhoeic and alopecic, with crusts on the legs and head. Oedematous nodules were observed on the legs and a serous and purulent discharge was leaking from some of the nodules. Beside the skin changes she had lost 6 kg in quite a short period of time. In January 2014, Ala was presented to the local veterinarian. Little improvement was achieved by initial antibiotic treatment (enrofloxacin, medicated shampoos). Her basic biochemistry profile was in the normal range (urea 4.2 mmol/L, creatinine 31 µmol/L, AST 0.57 IU/L, ALT 0.39 IU/L, AP 1.54 IU/L), but total blood proteins were found high, while blood albumins were low (Table 1).

Table 1. Total proteins, albumins and differential blood count of the bitch before treatment

| Measurement | Value | Unit | Reference range |
|--------------------|-------------|------------|-------------------|
| S-Proteins | 117 | g/L | 65-80 |
| S-Albumin | 19 | g/L | 32-55 |
| Neutrophils | 67.0 | % | 40.0-75.0 |
| Lymphocytes | 8.0 | % | 20.0-50.0 |
| Monocytes | 14.0 | % | 2.0-10.0 |
| Eosinophils | 5.0 | % | 1.0-6.0 |
| Basophils | | % | 0.0-2.0 |
| S-IgG | 2.13 | g/L | 6.36-17.01 |
| S-IgA | <0.24 | g/L | 0.80-4.36 |
| S-IgM | 0.81 | g/L | 0.36-1.42 |

Complete blood count showed leucocytosis, anaemia, and thrombocytosis (Table 2). Differential blood count showed lymphopenia with monocytosis and low IgG numbers (Table 1). An immunofluorescence antibody titre test (IFAT; BioMerieux, with a cut-off dilution 1:64) revealed end dilution of 1 : >160 antibody titre against *L. infantum*.

Clinical improvement was achieved following therapy with allopurinol in a daily dose of 20 mg/kg. Ala gained her weight back, and after clinical improvement treatment by the local veterinarian was ended. At the time of the photographs enclosed (winter 2016) the bitch had been off the allopurinol treatment for 1.5 years, without any reappearance of symptoms of systemic disease (Fig. 1-3).

Table 2. Complete blood count of the bitch before treatment

| Measurement | Value | Unit | Reference range |
|---------------------|--------------|-------------------------|------------------|
| Leucocytes | 12.6 | 10⁹/L | 4.0-10.0 |
| Erithrocytes | 4.31 | 10 ¹² /L | 4.2-6.3 |
| Hemoglobin | 112 | g/L | 120-180 |
| Hematocrit | 0.321 | 1 | 0.37-0.54 |
| MCV | 74 | fL | 81-94 |
| MCH | 25.9 | pg | 26-32 |
| MCHC | 348 | g/L | 310-350 |
| RDW | 10.7 | % | 11.6-13.7 |
| Thrombocytes | 363 | 10⁹/L | 140-340 |
| MPV | 7.9 | fL | 7.4-10.4 |

Altered values are written in bold



Fig. 2. The bitch in the winter of 2016, a year and a half after cessation of treatment



Fig. 1. The bitch in the winter of 2016, a year and a half after cessation of treatment



Fig. 3. A close-up of the skin photographed in the winter of 2016, a year and a half after cessation of treatment

However, the owner had been observing seasonal seborrhoeic skin changes every winter with regular spring ameliorations. Skin changes are also visible on the photographs. Cytology examination of swab smears from the lesions revealed no *Leishmania* parasites, but showed degenerate neutrophils with phagocytosis of cocci. Real-time PCR, performed on conjunctival swab and blood specimens at this point was negative. Antibiotic treatment was suggested to treat the pyoderma. Unfortunately, no laboratory follow-up was done.

Discussion

The majority of *Leishmania*-infected dogs never show the disease clinically. The incidence rate in endemic regions is usually less than 10%, while seroprevalence can be as high as 90% (SOLANO-GALLEGO et al., 2011). In endemic regions, about 20% of infected dogs never show symptoms but eliminate parasites before they could be disseminated from the lymph nodes all over the body. Another 40% of infected dogs do not show symptoms either but they are still carriers and therefore infectious to sand flies. Only 40% of infected dogs develop the disease clinically (FERRER et al., 1995). Recent studies show that two of the major risk factors for CanL are an age of 2 years or more, and sleeping mostly outdoors (CORTES et al., 2012). Those were also risk factors in our case.

Infected dogs may show skin changes that can be present in about 60% of symptomatic dogs (FERRER et al., 1995). Dogs become anorexic by antigen-antibody complex overload - producing chronic kidney disease. Kidney failure is the main cause of death in CanL (BOURDEAU et al., 2014). Dogs that have developed a visceral form of disease look tired, weak, and depressed. These are the consequences of anaemia, muscle atrophy, and poliartropathy. Lymphadenopathy is also very common. Lymphadenopathy can be localized but in the majority of cases it is generalised. Blood loss (often in the form of epistaxis) is also common and it is the second most common cause of death in dogs (PETANIDES et al., 2008). Skin inflammation, depression, and weight loss were also present in our case. Anaemia, hyperproteinaemia, and hypoalbuminaemia were noticed in laboratory parameter deviations. The laboratory test results

described in our case are common in CanL (BOURDEAU et al., 2014).

Before treatment, PCR was not performed in our dog. Treatment was established on the basis of the clinical picture and positive IFAT test. Two years after the clinical course of disease, real time PCR for *Leishmania spp.* was performed. It revealed negative results. Real time PCR is the best test to be performed during the follow-up period. The negative results in our case could be a consequence of parasite elimination by the immune system or a lack of test sensitivity. PCR sensitivity varies considerably according to the type and number of different samples evaluated, the target DNA copy numbers, and the target DNA sequence (PALTRINIERI et al., 2016; SOLANO-GALLEGO et al., 2017). In our case, blood and conjunctival swabs were sampled. According to the literature, bone marrow, lymph node, spleen, and skin allow the most sensitive detection of *Leishmania* DNA, with PCR of bone marrow currently considered as the gold standard for diagnosis of CanL. Whole blood PCR is less sensitive (SOLANO-GALLEGO et al., 2017). In our case, whole blood and conjunctival swabs were used for PCR. The major problem in obtaining bone marrow for PCR is the invasiveness of the procedure and its considerable cost. Nevertheless, PCR sensitivity using non-invasively obtained conjunctival swabs can be 78.4%, when testing dogs in a non-endemic area (GEISWEID et al., 2013).

The photographs of the bitch presented in this article were taken in winter 2016. According to the owner's description, Ala had been showing seasonal deteriorations of skin condition every winter, with following ameliorations every spring. These features could most probably be ascribed to an allergic disease. When symptoms of the disease appear and spontaneously disappear without any treatment, they are usually provoked by seasonal allergens. Allergens that atopic dogs are most commonly sensitized to in our experience are food storage mites and house dust mites, followed by grass pollens. Pyoderma, also proved in Ala, is a common feature in allergic disease (KOTNIK, 2007). Unfortunately, no allergic tests were performed in our case, so any further discussion on these symptoms remains speculation.

Various infection routes have been proved in dogs (the placental route, by mating, by blood transfusion, and by infected fly bites) (SOLANO-GALLEGO et al., 2011). Other routes of infection than via fly bites seem unlikely in our CanL case. Vertical transmission seems unlikely, since Ala's mother was born in Slovenia and never travelled out of the country. According to the owner, she was never seriously ill nor was her mating male, Ala's father. Both dogs were generally healthy, the mother died when old and father was euthanized after a serious hunting injury. Horizontal transmission from Ala's mate seems unlikely since the male was of Slovenian origin, never travelled out of the country and was healthy at the time of mating (data obtained by phone interview). Ala had never left the country, regularly slept outdoors and never had a blood transfusion. Therefore it is very likely that Ala was infected by a Phlebotomine fly bite.

The region where Ala was living has a mild microclimate and is close to the Croatian border. In this region, the presence of important leishmania Phlebotomine fly vectors were recently proved (IVOVIĆ et al., 2015). Amongst the five collected species that were found in the region, two of them - *P. neglectus* and *P. perniciosus* - are well known and proven vectors of *L. infantum* in the Mediterranean region (DEPAQUIT et al., 2010). Due to their limited flight range (< 1 km), transmission of leishmaniosis within endemic areas is often intermittent, with characteristically small and separate foci close to the reservoir host habitats (JACOBSON et al., 2003). Sand flies seeking blood meals generally advance upwind in short flights, close to the ground, guided by host-derived cues, such as CO₂, temperature, and humidity gradients (KILLICK-KENDRICK, 1999). Due to rapid climatic changes, *P. neglectus* has shown rapid aerial expansion from the traditional endemic regions of the Balkans, and was recently found as far north as near Budapest (Hungary) (FARKAS et al., 2011). Spread of sand flies to Slovenia (IVOVIĆ et al., 2015) most probably occurred from the well-known endemic region of Dalmatia (Croatia), where human leishmaniosis cases were diagnosed as early as in 1930, and the *L. infantum* seroprevalence ratio in dogs was found to be as

high as 42.85% (ŽIVIČNJAK et al., 2005). Recent data on seropositivity of the human population resident in Istria near the southern Slovenian border (ŠIŠKO-KRALJEVIĆ et al., 2013), together with import of infected dogs from endemic countries (KOTNIK and IVOVIĆ, 2017), and the confirmed presence of sand flies in the region (IVOVIĆ et al., 2015) represent a threat for the creation of the first enzootic focus in Slovenia. A large seroprevalence study was therefore recently started in Slovenia, supported by financial sources from the World Health Organisation.

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SAŽETAK

Lišmanioza je bolest koja se prenosi vektorima, a uzrokuje ju protozoalni parazit iz roda *Leishmania*. Parazite prenose muhe Phlebotominae (Diptera, Psychodidae). *Leishmania infantum* najvažnija je vrsta u Europi, kojom se mogu invadirati i ljudi i životinje. Parazit uzrokuje visceralni oblik bolesti koji može završiti smrću ako se odgovarajuće ne liječi. Psi se smatraju glavnim rezervoarom za invaziju u ljudi. Tradicionalne endemijske regije u Europi jesu zemlje koje se nalaze u Mediteranskom bazenu. Slovenija se ne smatra endemijskim područjem. Članak prikazuje slučaj lišmanioze psa (CanL) iz Slovenije. Radi se o pet godina staroj kuji pasmine austrijski ravnolaki gonič (Brandlbracke) okoćenoj u Sloveniji blizu hrvatske granice, i koja nikad nije napuštala Sloveniju. Kuja je redovito boravila vani. Osim znatnoga gubitka tjelesne mase, uočena je seboroična i alopecična koža s krastama na ekstremitetima i glavi. Laboratorijski testovi pokazali su hiperproteinemiju, hipoalbuminemiju, leukocitozu, anemiju i trombocitozu. Titar protutijela Imunofluorescencijom utvrđen je titar protutijela u vrijednosti 1 : > 160 za *L. infantum*. Liječenje alopurinolom dovelo je do kliničkog poboljšanja. Lančana reakcija polimerazom u stvarnom vremenu učinjena iz obriska konjunktiva i uzorka krvi 1,5 godina poslije liječenja bila je negativna. Iako je u novije vrijeme dokazano da insekata iz skupine Phlebotominae ima na jugu zemlje i invazija drugim putem ne čini se vjerojatnom, kuja je najvjerojatnije invadirana ubodom pješćane mušice, stoga postoji mogućnost endemijskog širenja lišmanioze psa u Sloveniji.

Ključne riječi: lišmanioza; CanL; *L. infantum*; pješćana mušica; Phlebotominae