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Case Report

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CASE REPORT OF CANINE CO-INFECTION WITH *LEISHMANIA INFANTUM* AND *EHRLICHIA CANIS*

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ABSTRACT

Canine leishmaniasis (CanL) due to *Leishmania infantum* and canine monocytic ehrilichiosis (CME) due to *Ehrlichia canis* are common diseases with zoonotic potential in the Mediterranean area. Their prevalence in R. Macedonia as a neighboring Mediterranean county is expected. In both diseases similar clinical symptoms can be manifested in dogs such as: lethargy, anorexia, weight loss, epistaxis, fever, pale mucous membranes, enlarged lymph nodes, splenomegaly, ocular signs. This case report present an atypical case of 11 year old female Samoyed with starting single clinical symptom epistaxys. Initial diagnostic procedures revealed the presence only of CanL, which was diagnosed using indirect immunofluorescence method and ELISA. First laboratory findings showed normal hematological and renal profiles. Dog was put on a treatment with Allopurinol (20mg/kg, p/o) for at least 9 months. Termination of the therapy after 6 months brought a numerous clinical symptoms involving weakness, dehydration, pale mucous membranes lost pupilar reflex, uremic breath and biochemical parameters revealed a renal failure. Using a commercial ELISA kit *Ehrlichia canis* as a co-infection was diagnosed. Most probably the second infectious agent was induced in the past 6 months, causing more severe pathological effects than CanL infection alone.

Key words: *L. infantum, Ehrichia canis, dog, IFAT, ELISA,*

INTRODUCTION

Leishmaniasis in dogs is a serious protozoan zoonosis that infects millions worldwide. Dogs and human get infected by leishmania promastigotes induced by bite of infected sand fly *Phlebotomus spp*. Promastigotes multiply into the macrophages as intracellular amastigotes. Clinical signs may appear months or years after the infection (10). Visceral leishmaniasis or Kala-azar is a life-threatening disease that has medical, social and economic importance in the endemic areas. Dogs are the main reservoir for *Leishmania infantum*. The clinical findings are very different which impedes the diagnosis. Some dogs show clinical signs while others remain asymptomatic carriers and are infective for sand fly. There is no breed or age predisposition, although infection hasn't been found in dogs younger than 5 months. Infected animals are usually affected with lymphadenopathy, anemia, desquamated and ulcerative skin lesions and glomerulonephritis with proteinuria. Ocular lesions like blepharitis, uveitis, corneal edema or chorioretinitis may also be present. Unspecific symptoms may appear often, which makes the disease hard to diagnose (1). Renal lesions are often present in dogs with leishmaniasis. Membrane-proliferative glomerulonephritis appears because of circulating immune complex deposit, which leads to proteinuria. These lesions may progress

to chronic renal failure or nephritic syndrome. Renal insufficiency is one of the classic general symptoms that follow leishmaniasis, but are rarely the only manifestation of the disease (1; 4). Due to the capacity of the kidneys it is estimated that over 75% of the tissue should be destroyed before the appearance of serum creatinine and urea elevations (12).

Canine monocytic ehrlichiosis (CME) is a diseases in dogs caused by rickettsia *Ehrlichia canis*. *Rhipicephalus sanguineus*, known as a brown dog tick, traditionally found in southern Europe represent a main vector of the canine ehrlichiosis. (17). When the infected tick ingests a blood meal, the infective agent is inoculated on the feeding site through the salivary secretions. The course of ehrlichiosis in infected dog can be divided into three phases: acute, subclinical and chronic (18). In all three phases a wide variety of clinical signs can be manifested in dogs (6). It is proven by Schouls, L.M. *et al.* (1999) that the same tick species can be a vector for several pathogens and co-infection with different infectious agent by individual ticks can occur. The common clinical signs between leishmaniasis and ehrlichiosis that can mislead to a correct diagnosis are : weakness, lethargy, anorexia, chronic weight loss, epistaxis, fever, pale mucous membranes, enlarged lymph nodes, splenomegaly, ocular signs. Furtheremore L. infantum kDNA in salivary glands of R. sanguineus ticks has been reported (3). Hence, it is very crucial to run several diagnostic test for detection of different pathogens and perform detailed laboratory analyses before defining the final diagnosis, especially in cases where ticks were registered on dogs.

MATERIALS AND METHODS

Case history

Our patient was a dog, 11 year old Samoyed, weighing 25kg.The origin of the dog was from town Stip in East part of R. Macedonia, historically known as Kala azar district. The dog has been kept in yard and it was in a good body condition. Prior diseases connected with parasitic infections were not present. The owner gave information about occasional appearance of heavy nose bleeding (epistaxis). Any other clinical signs were not noticed. The dog was never protected with any repellent during the season of insects.

Clinical observation and diagnosis

First physical examination revealed normal temperature, respiration and pulse and good quality of the coat. Submandibular and popliteal lymph nodes were enlarged with normal temperature and no pain. The clinical signs and medical history didn't give enough information for diagnosis, so additional examinations were made, such as hematological and biochemical analyses. Based on a single clinical symptom epystaxis a doubt was placed for *L. infantum* and *Ehrlichia canis*, hence a few diagnostic methods were performed. Serum sample was tested for *Leishmania*-specific antibodies by an indirect fluorescent-antibody test (IFAT) according to recommendation from OIE (5) (Figure 1). Samples showing fluorescence with serum dilutions equal or higher than 1:80 were regarded as positive. Antibody detection against CanL was also performed using an indirect ELISA (INGENASA). For the detection of *E. canis* antibodies a commercial ELISA kit (SNAP® 4Dx®; IDEXX Laboratories, Inc. U.S.A.), was performed twice, after the first clinical examination and 6 months after. Both ELISA test were performed according to the manufacturer's instruction listed in the product package insert.

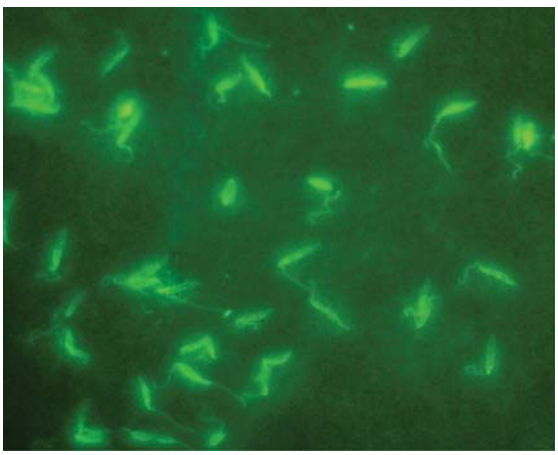

Figure 1. *Leishmania*-specific antibodies by an indirect fluorescent-antibody test (IFAT)

Treatment

Most common therapy approach in dogs diagnose with CanL is using pentavalent antimonials. Besides the reduction of the parasites they create resistance, so a parallel treatment with allopurinol is necessary to prevent relapse (9). Allopurinol is an oral medication (hypoxanthine) which is

metabolized by the parasites of leishmania and produces inactive analog inosine that incorporates into parasite's RNA and produces false protein synthesis. It is used in dogs independently or in combination with pentavalent antimonials, because of its limited toxicity, efficiency and low price (2). The most common doses are 10-30 mg/kg daily, divided in two doses every 12 hours, in a long period of time. Side effects are rare. Some of the combined therapeutic protocols are given in Table 1. Due to the availability of medications, Allopurinol (Zyloprim; GSK, 100mg) therapy (20 mg/kg, p/o 9-12 months) was chosen, with recommendation for controls every 3 months.

Table 1. Recommended therapy protocols

Medicine	Doses and applications	Duration of therapy
Alopurinol	10-30 mg 2x24 p/o	9-12 months
Amfotericin B	0,5-0,8 mg/kg 2 x per week Total dose 6-12 mg/kg	creatinin controle once a week.
Meglumin antimonat + **Alopurinol**	50 mg/kg; 10-30 mg 2x24 p/o	20-40 days 9-12months
Amfotericin B+ **Alopurinol**	0,5-0,8 mg/kg; 10-30 mg 2x24 p/o	9-12 months creatinin control

RESULTS

The haematological and biochemical results after the first clinical examination given in the Table 2 and 3 revealed only light leucocitosis. All other results were normal.

Table 2. Hematology analysis

Parameter	*Reference*	*Result*
WBC	6 – 17 * 10^9/L	17,7
RBC	5,5 – 8,5 * 10^{12}g/l	6,75
Hgb	12 – 18 *10g/l	13,2
PCV	37 – 55 %	34,2
MCV	60 – 77 fl	51,4
MCH	19,5 – 24,5 pg	19,6
MCHC	32 – 36 g/dl	38
Platelets	2 – 9 *10^{11}/l	3,6

Table 3. Biochemical analysis

Biochemical analysis			
Parameter	Unit	Reference	Result
ALT	U/l	8,02-57,3	15
GGT	U/l	1,0-9,7	3
AST	U/l	8,9 – 48,5	29,1
Creatinine	μmol/L	*44,3-138,4*	106,7
Urea	mmol /L	*3,1-9,2*	7,9
Glucose	mmol /L	*3,4-6,0*	3.96
Total protein	g/L	55,0-75,0	62,3
Albumins	g/L	26,0-40,0	27,4
BUN *(blood urea nitrogen)*	mmol/L	0-0.12	0.02
Ca	mmol /L	2,2 - 3	1,3
Phosphates	mmol /L	*1 - 2*	1,19

Case monitoring

Because of the distance, the owner wasn't able to bring the dog for controls. After 6 months, therapy was ended by the owners, without prior consultation. Week after the therapy was ended weakness and reduced appetite were noticed. The symptoms after short period of time elevated to low coordination in space, probably blindness, incontinent urination and complete loss of appetite. By our recommendation, the patient was delivered for further examinations. During the clinical examination weakness, dehydration, pale mucous membranes and uremic breath were noticed, pupilar reflex was lost, eye balls were drawn into the orbit. Palpation of the lymph nodes revealed enlargement of the submandibular lymph nodes. Body temperature was 36, 6°C, the pulse was barely sensed, shallow breathing was registered. The patient was kept in the clinic's stationary. Biochemical serum analyses were made and enormously elevated serum creatinin and urea, elevation of the total serum proteins and decreased albumins were noticed. These parameters, especially the levels of creatinin and urea indicated that the glomerular filtration was disturbed. Depression of serum albumins and increased activity of serum GGT, gave us indication of early stages of hepatic damage. Elevation of the total serum proteins indicates nephrotoxic condition in the organism. The results are given in table 4.

Table 4. Biochemical analysis 6 month after the initial examination

Biochemical analysis			
Parameter	Units	Reference	Result
ALT	U/l	8,02-57,3	54.0
GGT	U/l	1,0-9,7	11.4
Creatinine	μmol/L	*44,3-138,4*	673.5
Urea	mmol /L	*3,1-9,2*	54.35
Glucose	mmol /L	*3,4-6,0*	3.96
Total proteins	g/L	55,0-75,0	106.4
Albumins	g/L	26,0-40,0	21.4
BUN	mmol/L	0-0.12	0.02

Indirect immunofluorescent-antibody test (IFAT) was regarded as positive since the titter of the examined serum was 1:320. The other serological method performed with ELISA test confirm the positivity for *L.infantum*, because it resulted with an optical density (0,525) higher than the cut off value.

The commercial ELISA test for detection of the presence of *E. canis* antibodies was negative after the first clinical examination and positive after six months, respectively after the second examination.

Due to the poor general condition of the patient and owner's approval human euthanasia with Dorminal 20% (200mg pentobarbital sodium, Alfasan Holland) was undertaken.

DISCUSSION

Generally visceral leishmaniasis in dogs has similar symptoms to that in people including: irregular fever, pale mucous membranes and progressive weight loss to cachexia. Hypertrophy of the mononuclear-fagocitic systemis which is often present leads to splenomegalia, hepatomegalia and generalized adenopathy. The most common signs are related to skin lesions as local or diffuse lost of hair, isolate ulcerations of the snout and legs, dermatitis, as well as purulent conjunctivitis and keratitis, apathy, diarrhea and vomiting (15). According to the same authors no-regenerative anemia is an often finding. A.Blavier et al. (2001) found that membrane-proliferative glomerulo-nephritis appears as consequence of the circulating immune-complex deposits, which later results in proteinuria. These lesions can progress to chronic renal insufficiency or nephritic syndrome. Renal insufficiency is one of the classic general symptoms that fallow leishmaniasis, bur rarely are the only manifestation of the disease (1). According to the research done by Soares, M.J.V. et al. (2005) it was noticed that the concentrations of serum creatinin and urea are elevated in 5, 88% from the examined dogs with leishmaniasis. In 61, 76% from the group and 17, 65% from the control group membrane-proliferative glomerulo-nephritis was present. They concluded that kidneys are one of the first organs impacted by the parasite, but due to absence of clinical signs these changes are revealed much later. This case of leishmaniasis was very atypical, since at the beginning of the appearance of the disease only one clinical symptom such as epistaxis was present. This clinical symptom is not present only in leishmaniosis, since platelet-related bleeding, such as petechiae and echymoses of the skin and mucous membranes and epistaxis are common findings in erlihiosis (16). Also many clinical symptoms and laboratory findings may be common for this two diseases. According to the latter findings in our case, the major pathogenicity was in the kidneys. Similar case of renal insufficiency due to leishmanisasis was presented in a Pit bull dog in Brazil (7). Exceptions were additional signs of alopecia, hepatic and spleen enlargement. Popliteal and prescapular lymph nodes were enlarged, while in our case enlargement was noticed in the submandibular and popliteal lymph nodes. Since renal insufficiency can also be one of the symptoms in canine erlichiosis, we considered that crucial role in progression of the disease might be the co-infection with erlichiosis, termination of the therapy, as well as the age of the dog. In dogs co-infection with vector born diseases occurs in endemic area and that could partially explain variations in clinical presentation, pathogenicity and response to therapy (17). In our case we could not estimate when the secondary infection with *E.canis* happened, hence it was hard to conclude what was the role of this agent on the fast relapse of the disease, neither it's role in pathological findings of the kidneys. Discontinuation of the therapy with Allopurinol undoubtedly had a major impact on the course of the disease. Allopurinol can be discontinued only when the presence of complete physical and clinicopathological recovery will be evaluated by a thorough physical examination, complete blood count, full biochemistry panel and urinalysis. A marked decrease of antibody levels (to negative or borderline by a quantitative serological assay) should also be considered (8). Based on pervious investigations, leishmaniasis is a disease which is hard to diagnose in time because of the long asymptomatic period, unspecific clinical signs that can faulty lead to other diseases. Treatment of the disease is very long and it can be challenging for the owners of the dog, so they have to be prepared for what follows. It is important to mention the fast relapse of the disease in case of early termination or impropriate application of the therapy protocol. In conclusion, the present case study shows that dogs in R. Macedonia can be infected with more than one vector-borne pathogens, some of which can be zoonosis. Co-infections can be hard for diagnosis and consequently might lead to more severe clinical symptoms and pathogenicity. Therefore , prophylactic measures, such as the use of ectoparasiticides against insects, must be put in place in order to protect dogs and limit the risk of zoonotic potential.

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