INFECTIONS WITH *EHRlichia canis* AND *Borrelia burgdorferi* IN A DOG*

INFEKCIJE BAKTERIJAMA *EHRlichia canis* I *BORrelia burgdorferi* KOD JEDNOG PSA

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A clinical case of *Ehrlichia canis* and *Borrelia burgdorferi* infections in a 5-year-old male German Shepherd is described. Clinical, serological, necropsy and histopathological examinations supporting the diagnosis have been performed.

Key words: *Ehrlichia canis*, *Borrelia burgdorferi*, dog, necropsy findings, histopathological findings

Introduction / *Uvod*

Canine monocytic ehrlichiosis (CME) is a tick-borne disease, caused by *Ehrlichia canis*. Ehrlichiae are Gram negative, obligate intracellular bacteria with a size of 0.5-1.5 µm. A principal vector of their distribution is the brown dog tick *Rhipicephalus sanguineus* [1]. Recently published data from 2006 are unquestionably proving the zoonotic character of the disease, observed among people in Venezuela [2].

CME is nosogeographically related to Africa, Asia, the Middle East, Europe, North and South America. The data for its prevalence in the Mediterranean basin are as follow: Italy 13.5%, Greece 21.8%-41.2%, France 46.4%, Israel 63%, Spain 23-66.7%, Tunisia 72%, Turkey 74% [3, 4, 5, 6, 7, 8, 9, 10, 11, 12].

In Bulgaria, *Ehrlichia canis* was both clinically and serologically detected for the first time by Tsachev in a kennel near Plovdiv [13], and later retrospectively, in many regions in South and North Bulgaria – Stara Zagora, Yambol, Plovdiv, Burgas, Blagoevgrad, Silistra, Varna. Veliko Tarnovo, Pleven, Montana and Rousse [14,15].

Lyme borreliosis (LB) is a zoonotic tick-borne, naturally focal, multisystemic disease with dermatological, neurological, cardiovascular, arthropathic...
and other manifestations. Ticks are vectors of the infection, and the principal transmissor is *Ixodes ricinus*. This tick species is widely distributed in Bulgaria and according to the data of Hristova et al., 2003 [16] it is up to 20% infected with LB agents.

The etiological agent of borreliosis is *Borrelia burgdorferi* sensu lato complex. On the basis of DNA/DNA hybridization, ribotyping and 16S ribosomal sequencing of isolates, the strains *Borrelia burgdorferi* sensu stricto, *B. afzelii* and *B. garinii* have been specified.

At present, borreliosis in humans and dogs is highly prevalent all over the world, including in Bulgaria. For 1990-2002, 3261 cases of diseased people in 26 regions have been reported [17]. For the region of Stara Zagora, the clinically recorded cases for 1998-2002 are 75 [18].

The scientific reports about the incidence of LB among dogs in Bulgaria are very few – only three. The first one describes a clinical case of erythematous form in an 8-year-old Boxer [19]. The authors isolated the causative agent, performed an antibiogram and treated the dog with amoxicillin and consequently – with doxycycline and sulphonamides. The second report was a seroepidemiological one and comprised a population of 106 dogs, 499 cows and 512 sheep from 13 regions, endemic for human LB. The results showed the highest seroprevalence in sheep (56.25%) followed by cows (54.31%) and the lowest in dogs – 22.6% [20]. In a study of 16 dogs using *ELISA*, Martinov et al., 2006 [21], observed a significant result only in one dog whereas by indirect immunofluorescence – only 2 seropositive out of 46.

### Materials and methods / Materijal i metode ispitivanja

In March 2005, a 5-year old, 30-kg German Shepherd named Buck, was referred to a clinic in Stara Zagora. The state of the dog was poor – enhanced heart rate, body temperature of 39.9 °C, adynamia, weakness, depression, apathy, tachypnea, dehydratation, anorexia, myalgia, slightly enlarged popliteal lymph nodes, pale mucous coats and swelled hindlimbs. A 2-day therapy with glucose, vitamin C, novocaine, degan, dexamethasone and lincospectin was applied. No blood laboratory analysis was done.

Due to the lack of remission by the 3rd day, euthanasia was performed according to the owner’s will. From the history it became clear that the dog lived in the settlement of Dve Mogili, a region of Stara Zagora, together with 2 other dogs. One year ago it was vaccinated against canine distemper, hepatitis, parvovirus, leptospirosis and rabies. Several times, ticks were observed on the dog’s skin.

Prior to the euthanasia of Buck, blood for analysis was obtained in an EDTA-containing vacutainer and separately, in another vacutainer for blood serum separation. The other two dogs living in the same yard were also sampled. The following analyses in the blood serum were performed:
Monocytic ehrlichiosis, Lyme disease and dirofilariosis / Monocitna erlihiosa, lajmska bolest i dirofilarioza. ELISA /IDEXX Snap® 3DXTM Test, USA was used. The result is qualitatively visualized (+/-), without detecting the antibody load. The diagnostic value of the used test was very high – specificity for all three diseases – 100%; sensitivity: for E. canis – 99%, for dirofilariosis – 98%; for B. burgdorferi – 92% [22, 23, 24].

Ehrlichiosis / Erlihioza. An indirect immunofluorescence technique /IFA/ was used [25], that is still a gold standard for ehrlichiosis [26]. A formal inactivated suspension (2.10^6/ml) of cells infected with E. canis (Synbiotics Europe, France) and monospecific rabbit anti-canine FITC- labeled IgG (Sigma, Germany) was used as the antigen. Serum working dilutions of 1:100 to 1:1600 were used, and the result was detected on an immunofluorescent microscope LOMO (x 200) (Fig. 1). Titres higher than 1/100 were accepted as significant [27].

Leptospirosis / Leptospiroza. An analysis for the presence of leptospirosis was done using the reaction of microagglutination-lysis (RMAL). Taking into consideration the current etiological image of the disease [28], apart from the conventional L. canicola and L. icterohaemorhagiae, L. pomona and L. tarassovi were also included (Regional Diagnostic and Research Veterinary Institute, Stara Zagora). Titres of 1/100 were accepted as significant [29].

Blood smears were prepared from the EDTA blood and stained according to Romanovski-Giems.

After the euthanasia, a gross pathology examination was done and samples for histological study were obtained. The materials included mesenterial lymph nodes, spleen, stomach, ileum, urinary bladder and brain. Imprint preparations were prepared from lymph nodes and stained with Hemacolor®. The histological specimens were fixed in 10 % neutral formalin and embedded in paraffin.
cut on a paraffin microtome with a thickness of cross sections of 4 µm, and stained with haematoxylin/eosin (H/E).

**Results / Rezultati**

**Serological results / Serološki rezultati.** The ELISA testing of Buck showed the following results: (+) for ehrlichiosis, (+) for Lyme disease and (−) for dirofilariosis. The possibility to determine quantitatively the antibody titre in ehrlichiosis induced us to perform an IFA test, that yielded a titre of 1:800.

The testing for leptospirosis to *L. canicola*, *L. icterohaemorragiae*, *L. pomona* and *L. tarasovi* was negative (−) at 1:50. It was performed because leptospirosis was suspected on the basis of the observed weak icterus and the necroses of the tongue.

Of the other two dogs that lived together with Buck, one reacted positively for LB, whereas the other was LB-negative. Both dogs were negative for ehrlichiosis, dirofilariosis and leptospirosis.

The blood smears obtained from Buck and the other two dogs were negative for babesiosis and haemobartonellosis.

**Gross pathology findings / Ukupni patološki nalazi.** The inspection of the body revealed icteric visible mucous coats and hindlimb swellings. The peripheral lymph nodes were slightly enlarged. After dissection of body cavities, a marked haemorrhagic diathesis was observed. The haemorrhages (petechiae and ecchymoses) were primarily located on gastrointestinal, genitourinary and cardiac mucosae (Fig. 2). On the tongue’s borders, symmetrical necroses were observed (Fig. 3). The lungs were oedematous, and the chest cavity – filled with about 0.5 L yellow-reddish fluid. The bronchial and mediastinal lymph nodes were

![Figure 2. The haemorrhages (petechiae and ecchymoses) on epicardiac mucosae](image-url)
slightly enlarged and reddened. The same alterations, but more severe, were observed in the mesenterial lymph nodes (Fig. 4). The spleen was enlarged and showed macroscopic signs of chronic venous hyperaemia (Fig. 5). The kidneys were significantly enlarged and spattered with spot-like haemorrhages (Fig. 6). The urinary bladder was with a distended wall and its mucous coat was with multiple petechial haemorrhages. After dissection of the skull, a marked venous hyperaemia of cerebral blood vessels was revealed.

Figure 3. Tongue’s borders - symmetrical necroses
*Slika 3. Rubovi jezika – simetrične nekroze*

Figure 4. Enlargement and reddening of mesenterial lymph nodes (Lymphadenitis simplex)
*Slika 4. Uvećanje i crvenilo mezenterijalnih limfnih žleza (limfadenitis simpleks)*
Histopathological findings / Histopatološki nalazi. The histological examination of the kidneys, liver and lymph nodes, showed a proliferation of lymphoreticular and plasmatic cells. In the spleen, multiple necrotic foci and siderocytes were observed. In the gastric and intestinal propria, epithelial cell desquamation, haemorrhages and infiltration with neutrophils were present. In the liver, centrolobular necroses and Kupffer cell activation were revealed. In the kidneys, haemorrhages, degeneration of epithelial cells of renal tubules and proliferation into renal glomerules, mainly from plasmatic cells were shown. Haemor-
rhages and mononuclear proliferation were also observed in the propria of the urinary bladder. Some areas of the lymph nodes and the spleen were necrotic. The brain hemispheres revealed vascular hyperaemia, oedema and lymphocytic-plasmatic proliferations.

**Discussion / Diskusija**

Cases of mixed infection with zoonotic agents in the dog, especially with vector-borne ones, have been present for a long time, but are very few [30]. Data from Israel showed 73% co-infections with more than three transmissive agents: *Babesia canis*, *Babesia gibsoni*, *Ehrlichia canis*, *Rickettsia conorii*, *Borrelia burgdorferi*, *Bartonella visonii* [7]. It was established that 85% of dogs with hepatozoonosis were seropositive for *E. canis* as well; there is also evidence for cases of co-infections with *Ehrlichia platys*, *Borrelia burgdorferi* and *Leishmania infantum* [31, 32, 34]. Studies on leptospirosis and leishmaniasis performed in Greece, also have shown mixed infections – occult and clinical [29]. The latest information reports a certain increase in the prevalence of vector-borne infections [34].

The percentage of ixodid ticks, infected with Borrelia and Ehrlichia is relatively low, varying from <1% to 6% [35]. Experimental studies in mice showed that these agents were transmitted independently one from the other [36]. The Lyme borreliosis and the human granulocytic ehrlichiosis is a rarely seen combination – they accounted for 3-15% of tick-borne infections in Connecticut and Wisconsin [37]. The serological data from the *ELISA* and IFA tests showed an infection with *E. canis* and *Borrelia burgdorferi*, that could be prior or current.

It is known that 50-90% of animals, seropositive for LB, are asymptomatic. Less than 5% manifest clinical signs of the disease. During the acute phase, fever, lethargy, lymphadenopathy, lameness, are most commonly exhibited. In our patient, no lameness was observed.

The clinical signs in ehrlichiosis is more various, but the fever, depression, anorexia, weight loss, pale mucosae, hindlimb swellings, are all accompanying signs [38]. Ecchymoses on mucous coats could also be present and were observed by us as well. The region of origin of Buck and the other two dogs (Stara Zagora region) was with a proved 15% seroprevalence of *E. canis* [14].

According to most investigators, the most important gross pathology finding in ehrlichiosis was the enlargement of the spleen and the liver, the gastrointestinal and genitourinary haemorrhages and pulmonary oedema [33, 39, 40, 41, 42]. The same changes could be observed in LB, but very frequently, arthritis, uveitis, myocarditis could also be present; and in cases of generalized infection – dermatitis as well [23, 43, 44, 45]. The petechial bleedings in kidneys, hepatic necroses and lymph nodes’ and splenic enlargements were more characteristic for the acute form of leptospirosis, whereas the uraemic syndrome was more typical for the chronic course of the disease due to the developing interstitial nephritis [46, 47]. The similar pathoanatomical and histopathological findings in these dis-
eases makes their exact diagnostics difficult, thus necessitating the use of other laboratory diagnostic methods.

Our clinical, epizootological, serological, pathoanatomical and histopathological data allowed us to diagnose monocytic ehrlichiosis. The serological results showed also a *Borrelia burgdorferi* infection. It could not be said for sure whether it was current or prior, because no attempts at isolation or PCR were performed. We assume that in this case, a carrier was more likely present, because *Borrelia burgdorferi* antibodies were also present in one of the two dogs that lived together with the patient, without clinical signs of the disease.

Tick-borne infections in both men and animals are endemic for many regions of the world, including Bulgaria. The continuously appearing new data showed that these diseases are widely distributed all over the world and because of this, are systematically and thoroughly studied. The correct identification of the detailed etiological study of their reservoirs and vectors, would result in a more successful control of their prevalence.

References / Literatura

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INFEKCIJE BAKTERIJAMA *Ehrlichia canis* i *Borrelia burgdorferi* KOD JEDNOG PSA

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Opisan je klinički slučaj infekcije bakterijama *Ehrlichia canis* i *Borrelia burgdorferi* kod 5-godišnjeg Nemačkog ovčara. Izvršena su klinička, serološka i histopatološka ispitivanja, kao i nekropsija koji potvrđuju dijagnozu.

Ključne reči: *Ehrlichia canis, Borrelia burgdorferi*, pas, nalazi nekropsije, histopatološki nalazi

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ИНФЕКЦИИ БАКТЕРИЯМИ *Ehrlichia canis* и *Borrelia burgdorferi* У ОДНОЙ СОБАКИ

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Описан клинический случай инфекции бактериями *Ehrlichia canis* и *Borrelia burgdorferi* у 5-летней Немецкой овчарки. Совершены клинические, серологические и гистологические испытания, словно и некропсия, которые подтверждают диагноз.

Ключевые слова: *Ehrlichia canis, Borrelia burgdorferi*, собака, результаты некропсии, гистопатологические результаты