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CANINE EHRLICHIOSIS

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Abstract: *Ehrlichia* spp. are obligate intracellular bacteria with tropism for hematopoietic cells. Monocytic ehrlichioses in dogs and humans are transmitted by ticks and primarily caused by *E. canis* and *E. chaffeensis*, respectively. *E. canis* causes canine monocytic ehrlichioses (CME), a potentially fatal disease in dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. CME is characterized by three stages; 1) acute, 2) subclinical and 3) chronic. Dogs infected with *E. canis* remain infected for their entire lives, even after receiving antibiotic treatment with doxycycline. The prevalence of *E. canis* is dependent on the distribution of the vector, *Rhipicephalus sanguineus* tick, which occurs mainly in tropical and subtropical regions. The agent causing canine granulocytic ehrlichiosis (CGE) in Europe has been determined by nucleotide sequencing of the 16S rRNA gene to be similar to both *Ehrlichia equi* and *E. phagocytophila* (*Anaplasma phagocytophila*), and is identical to the agent of human granulocytic ehrlichiosis (HGE). The vector of this pathogen in Europe is the common European tick, *Ixodes ricinus* and its reservoir - wild and domestic animals. Two distinct clinical disease syndromes, including chronic, moderate to sever anemia and polyarthritis, are associated with CGE. In areas infested with vectors of tick-borne agents known to be endemic for Lyme disease, veterinarians may suspect ehrlichiosis in dogs.

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INTRODUCTION

Tick-borne diseases represent a problem of growing importance for public health. The multiple outbreaks of new tick-transmitted maladies and tracing their identity increased has public awareness about these zoonotic diseases [42]. The best known and the most frequently diagnosed are tick-borne encephalitis (TBE) and Lyme borreliosis. A less frequently detected tick-transmitted disease is ehrlichiosis. Its etiological factors are bacteria of the genera *Ehrlichia* and *Anaplasma*. It has become evident, from the international literature, that the infections with spirochetes of *Borrelia burgdorferi*, causing borreliosis, frequently coexist with co-infections inflicted, for example, by *Ehrlichia* spp.

The genus *Ehrlichia*, because of certain genetic affinities between its species, has been divided into three genogroups. Genogroup I incorporates three species i.e. *E. canis*, *E. chaffeensis*, and *E. ewingii*. Genogroup II includes *E. phagocytophila*, *E. equi*, and the human granulocytic ehrlichiosis (HGE) agent. Genogroup III covers two species: *E. sennetsu* and *E. risticii*. The name of each genogroup is consistent with the name of the first species described [4, 17, 49]. The above-mentioned classification was based on the gene encoding 16S rRNA. Detailed molecular analyses, carried out in recent years and based on the gene 16S rRNA and other genes (e.g. *gro ESL*, encoding heat shock protein), resulted in a systematic re-arrangement of the genus *Ehrlichia*. For example, *E. phagocytophila* was assigned to the genus *Anaplasma* and renamed *Anaplasma*

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phagocytophila [18]. Therefore, both taxonomic systems - old and new - can be encountered in the literature. *Ehrlichia* spp. are obligate intracellular bacteria with tropism for hematopoietic cells.

MONOCYTIC EHRLICHIOSIS

A number of *Ehrlichia* species can infect dogs and their affinity for hematopoietic cells may result in leukopenia and thrombocytopenia. Monocytic ehrlichioses in dogs and humans are transmitted by ticks and caused primarily by *E. canis* and *E. chaffeensis*, respectively. *E. canis* is the most important species of *Ehrlichia* in dogs. Canine monocytic ehrlichiosis (CME), sometimes referred to as the tropical canine pancytopenia, was described for the first time in Algeria in 1935 by Donatien and Lestoquard [16]. In the United States it was reported for the first time in 1963, and subsequently has been found in widely separated regions of the USA [9, 10, 11, 12, 32, 34, 54, 60]. At present, it is widely distributed around the world, particularly frequently in tropical and subtropical areas [3, 6, 27, 30, 37, 40, 41, 45, 55, 58, 61, 62]. The first human ehrlichiosis ever described was sennetsu fever, a mononucleosis-like illness, caused by *E. sennetsu*, since renamed *Neorickettsia sennetsu*, in patients from Japan and Malaysia in the 1950s [39]. Human monocytic ehrlichiosis (HME), caused by *E. chaffeensis*, was detected for the first time in the United States in 1987. At that time the disease was blamed on *E. canis* because the sera of the patients reacted positively only with the antigen of the above species [33]. In 1990, based on the differences (1.8%) in the sequence of the gene encoding 16S rRNA between *E. canis* and the bacteria isolated from a patient with HME, the pathogen was considered to represent a new species *E. chaffeensis* [1]. The number of the positively identified cases in the USA has been growing ever since and the serological studies suggest that this disease is also present in Europe, Africa, Mexico, and Australia [48]. The two species of *Ehrlichia* mentioned earlier are transmitted primarily by three tick species: *E. canis* - by brown dog tick, *Rhipicephalus sanguineus* and by American dog tick, *Dermacentor variabilis*, while *E. chaffeensis* - by *Ambyloma americanum* [21]. The natural reservoir hosts are also different: representatives of the family Canidae - for *E. canis*, and white-tailed deer, *Odocoileus virginianus* - for *E. chaffeensis* [24]. *E. canis* causes a febrile systemic diseases that is often severe and can be fatal in dogs, like *E. chaffeensis* in humans [41]. However, in experimentally infected dogs, *E. chaffeensis* causes only a mild febrile response with no hematological disorders [15], and vice versa - *E. canis*-like agent (a new strain of *E. canis*) inflicts a chronic asymptomatic infection in humans [43].

Magnarelli and Anderson [35], in a retrospective study by IFA methods, detected antibodies against *E. risticii* (causative agent of equine monocytic ehrlichiosis) in sera of dogs and they concluded that canine- and equine ehrlichiosis coexist with Lyme borreliosis in Connecticut and the lower River Valley of New York.

In Europe, the principal vector of tick-borne pathogens is the common European tick, *Ixodes ricinus*, which does not transmit *E. canis*. In some western and southern regions of Europe, however, another species occurs - *R. sanguineus*, known as a potential vector for *E. canis* and, cases of CME are described. The presence of anti-*E. canis* antibodies in dogs with clinical signs suggesting a rickettsial infection were recorded in Italy [8] and Spain [59]. In Portugal, 50% of clinically healthy pet dogs were seropositive for *E. canis* [2]. CME was recognised in Greece [40], and a single case of this disease in England was associated with a dog imported from Sardinia [23]. In Switzerland, despite the presence of *R. sanguineus*, the antibodies against *E. canis* have rarely been detected, and this species is not indigenous to Switzerland as suggested by the studies of Pusterla *et al.* [47]. Canine monocytic ehrlichiosis does not occur in Sweden mainly due to the lack of ticks *R. sanguineus*, although this condition has been diagnosed in imported dogs [24].

CME - STAGES OF DISEASE

E. canis causes a potentially fatal disease in dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favourable prognosis [38]. CME, the disease caused by *E. canis*, is characterized by three stages. The first, acute stage, beginning after 8–20 days following transmission by infected tick, lasts 2–4 weeks. The acute phase may be manifested by fever, depression, dyspnoea, anorexia, and slight weight loss. In laboratory findings: thrombocytopenia, leucopoenia, mild anaemia, and hypergammaglobulinemia. The second phase is subclinical and follows the acute phase and may last 40–120 days or even years, in which dogs can remain persistently infected for years without clinical signs but with mild thrombocytopenia. The ultimately stage is chronic, characterized by haemorrhages, epitasis and edema, with clinical signs; the results of laboratory study resemble the first phase of the disease. The course of this phase may often be complicated by superinfections by other micro organisms [7, 10, 28, 48, 50] which, for many dogs, become progressively become worse, due to bone marrow hypoplasia which results in a bad prognosis [56]. Dogs infected with *E. canis* remain infected for their entire lives, even if they received antibiotic treatment with doxycycline [63].

Waner *et al.* [61] reported in experimentally infected dogs the subclinical phase of CME with mild haematological abnormalities (thrombocytopenia and significant decrease in leucocytes). In order to determine whether dogs in the subclinical phase of CME are carriers of *E. canis*, Harrus *et al.* [26] infected dogs experimentally through inoculation. After 34 months post infection the samples of the spleen, bone marrow, and the blood were studied and four out of six dogs were PCR positive. The samples of the spleens of all four of these dogs were PCR positive. The data obtained in this study demonstrates that clinically healthy dogs in the subclinical phase of CME are carriers of the ehrlichiae for years. The authors suggest that the spleen is

the organ most likely to harbour *E. canis* parasites during the subclinical phase and the last organ to accommodate the bacteria before elimination. Treatment of those animals should be taken into consideration before the chronic phase of this disease develops [13].

GRANULOCYTIC EHRLICHIOSIS

Reports by many authors suggest that along with *E. chaffeensis*, two additional species - *E. ewingii* and human granulocytic ehrlichiosis (HGE) agent - are zoonotic, and also occur in dogs and deer [5, 14, 17, 19, 21, 22]. Moreover, canine ehrlichiosis in dogs can be caused by other ehrlichiae with similar and unique hemopoietic cell tropism, including *E. phagocytophila*, *E. equi* and *E. platys*, and co-infections with multiple ehrlichial species have been reported in dogs [5, 31]. *E. platys*, which is also believed to be transmitted by *R. sanguineus*, infects platelets. This species has been reported in the USA and in southern Europe.

E. ewingii and *E. equi* both occur in the USA and infect predominantly neutrophils [22, 36, 64]. In the northeastern United States, *E. canis* coexists with *E. equi*, by *I. scapularis* tick-transmitted pathogen of horses and humans. Magnarelli *et al.* [36] demonstrated that dogs exposed to *I. scapularis* can develop a benign or subclinical forms of ehrlichiosis caused by *E. equi*, and distinct leucopoenia and thrombocytopenia or anaemia should be treated as evidence of suspected ehrlichiosis. Moreover, dogs living in the endemic areas of this tick species are exposed to many other tick-borne pathogens, such as *Borrelia burgdorferi*.

In Europe, the most frequently occurring *Ehrlichia* species, has been so-called human granulocytic ehrlichiosis (HGE) factor infecting people, but detected also in the blood of dogs. The vector of this pathogen in Europe is the common European tick, *I. ricinus*, and its reservoir - wild and domestic animals. Molecular comparison of isolates from dogs in Switzerland and Sweden, revealed that the factor causing canine granulocytic ehrlichiosis (CGE) is an *Ehrlichia* species, closely associated with the factor causing human granulocytic ehrlichiosis. The nucleotide sequences of their genes 16S rRNA are 100% homologous [29, 46]. The factor causing CGE cannot be serologically differentiated from *E. phagocytophila* and *E. equi*. Because strong cross-reactivity has been detected between the members of this gene group, the antigens of *E. phagocytophila* or *E. equi* can be used for serological diagnostics of CGE [18].

In Europe, cases of human granular ehrlichiosis have been described in many countries [44]. In Poland, two cases of ehrlichiosis have also been identified [57], and the screening study of people within the area of endemic borreliosis proved the existence of a population exposed to the HGE agent [25]. Also, ours studies carried on the population of *I. ricinus* and aimed at the presence of DNA of the HGE agent, indicated a potential threat of infection of people and animals with this pathogen [52] and a possibility of double and triple co-infections [51, 53].

CGE - CLINICAL SIGNS

Clinical diagnosis of CGE can be difficult. Two clinical distinct disease syndromes, including chronic, moderate to sever anaemia and polyarthritis, are associated with canine granulocytic ehrlichiosis CGE [22]. Clinical signs are nonspecific and include fever, lethargy, anorexia, vomiting, and diarrhoea. The most frequent laboratory abnormalities are normocytic, normochromic nonregenerative anaemia, moderate thrombocytopenia with large platelets, lymphopenia, and eosinopenia. In beagles, inoculated experimentally with granulocytic *Ehrlichia* organism, after an incubation period of 4–11 days, the most prominent clinical signs were high fever for 2–5 days, and depression [20]. All these dogs developed profound thrombocytopenia, moderate leucopoenia and strong serological antibody response. In blood smears, ehrlichial inclusions were detected in neutrophils from 4–14 days after inoculation for 4–8 days. DNA of ehrlichia could be detected (by PCR) during the parasitaemic period and were visible a few days before and after microscopic inclusion.

In areas infested with vectors of tick-borne agents known to be endemic for Lyme disease, veterinarians may suspect ehrlichiosis in dogs.

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