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Review

Monocytic Ehrlichiosis in dogs

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Abstract

Ehrlichiosis is the multiorgan infectious disease caused by small, intracellular rickettsias from the genus *Ehrlichia*. These microorganisms are known as an etiologic factor of infections world wide in humans and in different species of animals. Dog ehrlichiosis can be caused by several species of *Ehrlichia* attacking different groups of blood cells, but most often an infection by *Ehrlichia canis* is diagnosed with special relation to monocytes. A vector for *E. canis* are *Rhipicephalus sanguineus* and *Ixodes ricinus,* commonly occurring in Poland. Disease caused by *E. canis* is known as Canine Monocytic Ehrlichiosis (CME). The disease most often has an asymptomatic course which can, in favourable circumstances, run into acute or chronic forms. The acute form of CME proceeds usually with fever, apathy, weakness and accompanying respiratory symptoms, lameness and disturbances in blood coagulation. In laboratory examinations thrombocytopenia, anemia and leucopenia are ascertained. The chronic form of CME proceeds among gentle, unspecific symptoms which may last even 5 years. The CME diagnosis is difficult and often demands parallel different diagnostic methods. A medicines of choice in the ehrlichiosis treatment are antibiotics from the group of tetracyclines, given at least for 28 days. They are largely efficient during treatment of the acute CME, causing the quick improvement. Instead, in the case of chronic form, answer for treatment can be weak, and cases of resistance to antibiotics ave known.

Key words: ehrlichiosis, rickettsias, dog

In recent years a growth in the incidence of transmissible diseases in Europe can be observed. A still worsening epidemiological situation in tick-borne diseases is closely connected to climatic and landscape changes that have been taking place over the last 10 years and which influence the development of tick populations and the circulation of the pathogens of tick-borne diseases (Randolph 2002). What is particularly important in the growth of the tick population and its activity is the rise of average temperatures in the spring months as well as the earlier start of plant vegetation period. In Poland the most common tick-borne disease at the moment is boreliosis (Lyme disease) – in 2009 as many as 10313 clinical cases in

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humans were reported. The problem of transmissible diseases also applies to veterinary medicine, particularly in accompanying animals – especially dogs. It has been proven that the percentage of health problems of veterinary patients connected to tick exposition is 15 – 21% of all cases. At the moment the biggest problem in canine medicine is babesiosis (red water fever), particularly in the northern and north-eastern regions of Poland – where the disease is considered endemic (Hułas and Dobrzyński 1995). However, ongoing climate changes and the increase of tourism cause a territorial expansion of ticks and their pathogens to areas which have not been their primary ecological niche. As a result, the appearance of diseases which had not been diagnosed in these newly affected areas, an example being ehrlichiosis, can now be seen. Ehrlichiosis is a multiorgan, transmissible, infectious disease of people and animals. It is caused by microorganisms belonging to the *Rickettsiales* order, showing tropism mainly towards leucocytes and being exclusive intracellular parasites. They have been known for years mainly as an ethiological factor of infections of different species of animals. Their presence has been proven in the blood of horses, cattle, sheep, goats, foxes and dogs (Platt-Samoraj et al. 1998). However, despite their common presence in animals, the beginning of intensive research on these microorganisms was caused by cases of ehrlichiosis being found in people. This has brought changes in the systematics of these bacteria and a better cognition of the details of their pathogenicitity and epidemiology of the infections they cause, as well as perfecting the diagnostic methods used for the recognition of ehrlichiosis in human and also in veterinary medicine.

The ethiological factor of ehrlichiosis is currently classified to the order of *Rickettsiales***,** which has been created on the basis of reorganization the families of *Rickettsiaceae* and *Anaplasmataceaea*, genus *Ehrlichia*. This genus presently consists of 11 species of *Ehrlichia* which have been separated because of their morphological, serological and genetic properties and divided into three gene groups (Dumler et al. 2001). They can be found both in people and animals and experimental research has proven the possibility of transmission of the pathogene between different species, e.g. the infection of dogs with *E. chaffensis* which is specific to humans. It has not been proven though, that in natural conditions such infections are possible (Egenvall et al. 1997).

Ehrlichia are small rickettsias, usually round in shape, however they can show a high degree of pleomorphism, especially in cell cultures. The structure of their cell wall is similar to those of Gram-negative bacteria however, it is best to stain them according to the Giemza method, which makes them turn dark blue or navy blue. They show an affinity to monocytes, granulocutes and thrombocytes (Harrus et al. 1997, Platt-Samoraj et al. 1998, Dumler et al. 2001,). In the infected host cells *Ehrlichias* are found in three forms, creating cytoplasmatic inclusion bodies – initial corpuscles, morulas formed by their aggregation and elementary corpuscles created after the morula;s disintegration (Greene and Harvey 1984, Platt-Samoraj 1998).

The vector and reservoir for most of the species of *Ehrlichias* are the *Arthropoda* among which the most important ones are ticks. Within their bodies the microorganisms multiply inside epithelial cell vacuoles as well as inside the salivary gland cells (Mathew et al. 1997). The infected tick becomes the carrier of the rickettsias for up to 155 days, and inside the tick population transstageal transmission of the germ often takes place (Mathew et al. 1997). The infection of the V*ertebratae* takes place after a tick bite. The microorganisms in the saliva of the *Arthropoda* enter the bloodstream of the host and multiply in the infected blood cells creating morulas. After their disintegration elementary corpuscles from a destroyed blood cell attack the next blood cells. Together with them *Ehrlichias* are carried all over the body, accessing the liver, spleen, bone marrow and lymphatic nodes, where again they multiply (Rikihisa 1991, Brouqui et al. 1994).

Ehrlichiosis in dogs can be caused by a few species of *Ehrlichia*, attacking different groups of blood cells. The most common diagnosed species are *E. canis*, showing an affinity to monocytes (Stockham et al. 1992, Harrus et al. 1997, Pusterla et al. 1998) and *E. phagocytophila* (act. *Anaplasma phagocitophilum*), together with *E. ewingii* which affects neutrophillic and acidophilic granulocytes (Inokuma et al. 2005, Poitout et al. 2005). Infections caused by *Ehrlichia* (act. *Anaplasma*) *platys* which has affinity to blood platelets are seen much more rarely (Harrus et al. 1997, Hua et al. 2000). These days canine ehrlichioses are diagnosed all over the world. They can take different clinical forms depending on the particular rickettsia that is causing the disease. The greatest threat to a dog is the infection with *E. canis*. The disease caused by this infection is called CME – Canine Monocytic Ehrlichiosis or TCP – Tropical Canine Pancytopenia (Frank and Breitschwerdt 1999, Stiles 2000). The disease can take a subclinical, acute or chronic course. It affects all dogs, regardless of their age and sex, however a clear breed predisposition in Alsatians can be observed (Nyindo et al. 1980, Kuehn and Gaunt 1985). The vectors for *E. canis* are mainly ticks *Rhipicephalus sanguineus* – a brown canine tick, attacking the carnivore (Reardon and Pierce 1981, Keefe et al. 1982). This species, originally met mainly

in the Mediterranean, has currently spread to a colder climate zones and it can be met in the middle and northern Europe, including Poland (Beugnet 2002). In our country *Ixodes ricinus* can also be a vector (Płoneczka 2004, Kalinova et al. 2009).

CME was first described in 1935 in Algeria (Pyle 1980) but interest in the disease increased towards the end of 1960 after an outbreak of the acute form of ehrlichiosis with a high mortality rate among dogs working for the US Army in Vietnam (Rikihisa 1991). Novadays the disease is common in the USA (Stockham et al. 1992, Pusterla et al. 2000, Poitout et al. 2005), northern and southern Africa (Inokuma et al. 2005), southern Asia and India. As for Europe, it has occurred in Switzerland (Pusterla et al. 1998), Great Britain (Shaw et al. 2003), Italy (Buonavoglia et al. 1995), Germany (Goethe 1998), Portugal (Bacellar et al. 1995) and Sweden (Egenvall et al. 1997). Data concerning canine ehrlichiosis is scarce (Płoneczka and Śmielewska-Łoś 2003, Płoneczka 2004, Adaszek 2006). The disease can take a subclinical, acute or chronic course (Kuehn and Gaunt 1985, Harrus et al. 1997, Frank and Breitschwerdt 1999, Stiles 2000). Natural *E. canis* infections can very often be symptomless. Despite the lack of clinical symptoms, the microorganisms can reside in the infected dog's organism for many months or even years, constantly stimulating the dog's immune system which shows through the high levels of antibodies. The subclinical form of the disease can be self-cured or turn into the chronic or acute form of the disease (Waner et al. 1998).

The acute form of the disease is relatively rare and is generally connected to some propitious circumstances. The important factors that encourage its development are, among others, stress with accompanying immunosuppression and bad environmental conditions. The acute form of ehrlichiosis can also be connected to the presence of other infestation, especially with *Babesia canis* (Rikihisa 1991, Matthewman et al. 1993). The acute form of ehrlichiosis lasts from 2 to 4 weeks, it's symptoms are non-specific and can periodically disappear. The disease usually starts with a significant rise of the inner body temperature (40-41°C), general weakness and apathy. It can be accompanied by progressive weight loss, slight limping as well as an unwillingness to move due to arthritis, together with pains in musculo- skeletal system (Egenvall et al. 1997). In the acute phase of the disease symptoms in the respiratory system can be observed. They are: dyspnoea, seropurulent discharge from the nostrils and conjunctival sacs and even interstitial pneumonia (Reardon and Pierce 1981, Kuehn and Gaunt 1985). Some authors have also described neurological disturbances that can accompany the acute form of CME: ataxia, tremor of the head and epilepsy-like symptoms (Stockham et al. 1992, Goldman et al. 1998). However, the most characteristic disturbances for this type of the disease are the disturbances of the heamostasis, resulting in a tendency to bleed and extravasculations present in the mucous membranes. In dogs with acute ehrlichiosis uni-or bilateral epistaxis, extravasculations in injection sites, extravasculations into the auterior chamber of the eyes, the presence of blood in urine and faeces have also been described. In the skin of the abdomen as well as in the visible mucous membranes small, pinpoint extravasations can be observed (Kuehn and Gaunt 1985, Brouqui et al. 1991). A tendency to bleed in the course of the *E. canis* infection is the result of the affinity of the rickettsia to the cells of the endothelium of the blood vessels. The result of the damage of the blood vessels is bleeding and the activation of the platelets which will aggregate at the damaged spot and which leads to their excessive utilization. Thrombocytopenia is the dominating change, noticeable in the laboratory tests in the acute CME (Harrus et al. 1996, Cockwill et al. 2009). The mechanism leading to it is not quite clear, although the main role seems to be played by autoimmunological processes (Burghen et al. 1971). One of the hypothesis assumes that during the *E. canis* infection, in the organism of the infected dog, antibodies against glycoproteines of the dog's own platelets are produced, which leads to their malfunction (Burghen et al. 1971). An important role is also played by PMIF (*Platelet Migration Inhibition Factor*) produced by activated lymphocytes which inhibits the platelet migration (Rikihisa 1991). Besides thrombocytopenia, hemathological tests in the acute form of erlichiosis show normocytic anaemia, non-regenerative and slight leucopenia preceded by leuko- and monocytosis. Biochemical tests of the serum show hypoproteinemia, hypoalbuminemia, hyperglobulinemia, the rise of the activity of the alkaline phosphatase, alanine aminotransferase and the rise of the concentration of creatinine and urea (Harrus et al. 1997, Egenvall et al. 1998).

If not treated, the acute form of CME can lead to the death of the animal due to severe progressive coagulopathy or changes after 4 – 6 weeks into the subclinical or chronic form of the disease. In the course of the chronic form of canine ehrlichiosis usually mild, non- specific symptoms, which last up to 5 years, can be observed. A particular predisposition to the chronic course of the disease can be observed in Alsatians (Nyindo et al. 1980, Rikihisa 1991). Also, in the chronic type of ehrlichiosis, severe emaciation, tumors of the liver and spleen, autoimmunological disorders such as immune-mediated hemolytic anemia (IMHA), a bleeding disorder with symptoms such as nose-

bleeds, blood in stool, brusing of the skin, *endocarditis* and *poliarthritis,* can also be observed. These symptoms are accompanied by severe anaemia, thrombocytopenia and leucopenia, resulting from the hypoplasia of the bone marrow (Pyle 1980, Cockwill et al. 2009). In the blood smear examination, due to impaired bone marrow function, non-typical blood cell forms can be noticed which can lead to the misdiagnosis of leucaemia (Waner et al. 1998). Immunosuppression accompanying the chronic CME can lead to severe, secondary bacterial infections resulting e.g. in ulceration of the limbs (Pyle 1980).

Diagnostics of ehrlichiosis in dogs can be difficult, especially if it appears in the area where it had not been diagnosed before. The clinical picture is usually non-specific and it is not always accompanied by the most characteristic symptoms such as a tendency to bleed or limp. An additional difficulty is the fact that the course of ehrlichiosis is changeable depending on the geographic region. That is why the diagnosis of the suspected ehrlichiosis, based on the symptoms, must be preceded by epizootic anamnesis together with complex microscopic, hemathological, biochemical, serological and molecular examinations.

The simplest method used in the diagnosis of ehrlichiosis is the microscopic smear staining test using the Giemsa, Wright-Leishmann or Diff-Quick methods (Harrus et al. 1997, Inokuma et al. 2005). This test allows for the discovery of morula made of inclusion bodies, which look like a mulberry fruit inside the leukocyte cytoplasm. This method is most useful in the diagnosis of the acute form of the disease, as in the chronic form *Ehrlichias* are difficult to find (Frank and Breitschwerdt 1999). The lack of morula in the examined samples does not exclude the suspected ehrlichiosis thus the final diagnosis should be supported by serological and molecular tests (Nyindo et al. 1980, Harrus et al. 1997, Pusterla et al. 2000). The most commonly used serological tests to diagnose ehrlichiosis are ELISA test and IFAT (the test of indirect immunofluorescence) allowing to find IgM antibodies from the 7th day after infection and IgG from 14 – 15 days after the infection (Waner et al. 2000, Okewole and Adejinmi 2009). Recently, commercial tests based on immunochromatographic methods have become available. They identify antibodies specific for *E. canis* which are bound to specific, purified, recombined antigen of *E. canis*. The most sensitive diagnostic method of erlichiosis is, however, the identification of the microorganisms in cell cultures of histiocytes or monocytes, together with the isolation of the *Ehrlichia's* DNA with PCR technique (Brouqui et al. 1994, McBride et al. 1996, Pusterla et al. 2000, Peleg et al. 2010).

The best drugs to treat ehrlichiosis, regardless of the species of *Ehrlichia* or the form of the disease, are tetracyclines (Sainz et al. 2000). The most effective antibiotic to fight *E. canis* is doxycycline administered *per os* in the dose of 11 mg/kg b.m. (Sainz et al. 2000, McClure et al. 2010) once a day, for 28 days. The doxycycline therapy can be supported by the therapy using imidocarb in the dose of 3-6 mg/kg b.m. in injections given every 14 days (Rikihisa 1991, Matthewman et al. 1993, Harrus et al. 1997). Study by Price (1980) showed that the action of imidocarb is three times stronger than that of tetracyclines and it's additional advantage is the effectiveness against *Babesia canis* which can accompany ehrlihiosis. Instead of doxycycline, other tetracyclines can be used – tetracycline in the dose of 22 mg/kg b.m. every 8 hours or oxytetracycline in the dose of 25 mg/kg b.m. every 12 hours. As adjunctive therapy liquids, corticosteroids and vitamins are used. In severe cases blood transfusions can be used (Mylonakis et al. 2004).

Symptoms of acute ehrlichiosis usually subside within 48-72 hours from the administration of the antibiotic. Hemathological parameters also improve (Kuehn and Gaunt 1985, Sainz et al. 2000). However, the treatment of chronic ehrlichiosis can be more difficult, as dogs do not respond well to tetracycline treatment. Cases of drug resistance have been reported as well (Igbal and Rikihisa 1994).

Canine ehrlichiosis is a disease which is rarely diagnosed in Poland. Multiform course of the disease and non-defined clinical symptoms make the diagnosis significantly more difficult, especially in its chronic form. However, taking into account the presence of the etiological factors in Poland and the abundance of vectors, which are ticks *Rhipicephalus sanguineus* and *Ixodes ricini*, the disease should be taken into account in differential diagnostics of diseases with symptoms such as thrombocytopenia, limping of unknown origin or a tendency to bleed.

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