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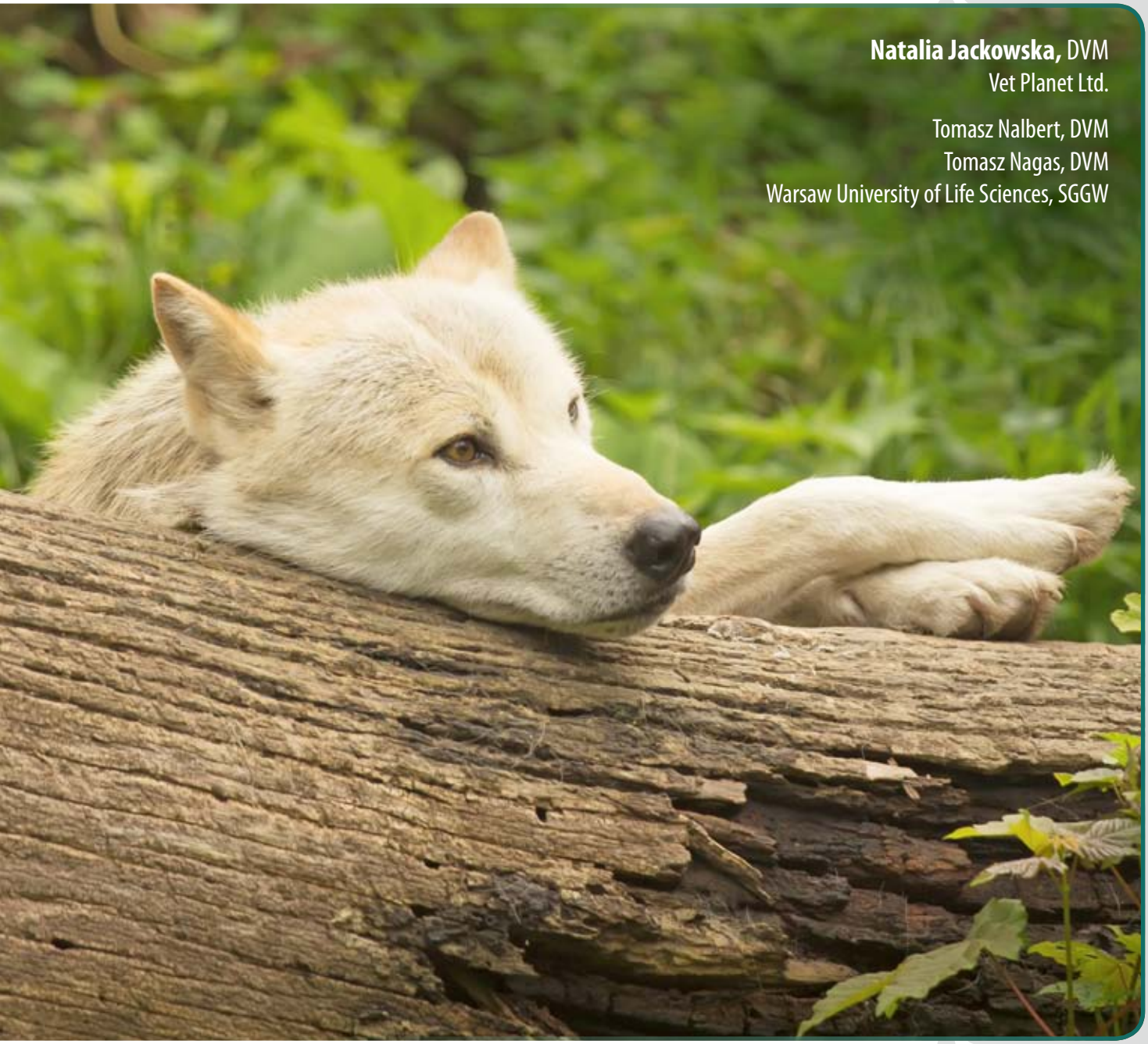
Selected vector-borne diseases – description and differentiation



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Selected vector-borne diseases – description and differentiation

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As a result of warming of the climate, Poland witnesses diseases that used to be associated with the warm Mediterranean climate only. The ever milder climate makes life of ticks and insects much easier. There are report from other countries (Germany, Austria) saying that dogs coming back from holidays were diagnosed with diseases not typical for their native country, namely ones brought from other countries (6). This means that when we take our four legged friends for holidays in warmer regions, we can bring back not only nice memories from there. Expansion of ticks and insects leads to the increase of cases of leishmaniosis, borreliosis (Lyme disease), ehrlichiosis, anaplasmosis - diseases whose vectors are different types of insects and ticks, some of which might cause cross infection. Knowledge about intermediate hosts, vectors and diseases transmitted by them is very useful in everyday practice.

Ticks belong to arachnids. In Poland there are about 20 species of ticks. The most common ones are Ixodes ricinus (the castor bean tick) and Dermacentor reticulatus, more rare ones being Rhipicephalus sanguineus, Ixodes hexagonus, Ixodes cerenulatus (14). In order to fully appreciate the threat posed by these arachnids it is good to review their physiology. One should remember that every tick goes through three development stages: an egg is transformed into a larva, a larva molts into a nymph, and a nymph molts into an adult form. The important thing is that already larvae feed on blood. In every life stage tick needs at least three hosts. This means that every adult tick must have had at least nine hosts. After mating, the female lays about 2000 eggs, from which after about 3 to 36 weeks larvae emerge. Depending on environmental conditions and access to intermediary hosts, a larva molts into a nymph within five weeks to 5 months. Molting of a nymph into an adult form takes 5 to 8 months. Ticks live as long as four years, feeding on a host short: from two hours to seven days. Ticks are vectors of several bacterial and viral diseases. The most frequent tick-borne disease affecting dogs in Poland is babesiosis, caused by protozoan Babesia canis. In our region it is transmitted by Dermacentor reticulatus, while in the US the main vector is Rhipicephalus sanguineus. There is common awareness of the existence of Babesia canis, and diagnosis and treatment in case of infection are known. Thus, the aim of this article is to discuss other selected vector borne diseases and to present the most frequently used diagnostic methods in recognising them. The authors focused on the rare vector-borne diseases, namely leishmaniosis, boreliosis (Lyme disease), anaplasmosis, ehrlichiosis and dirofilariosis. According to study on 107 randomly selected dogs, in Tri-City (northern Poland) the extensiveness of invasion of A.phagocytophilum is 10.3%, B.burgdorferi 7.4%, E. Canis 1.8% (19).

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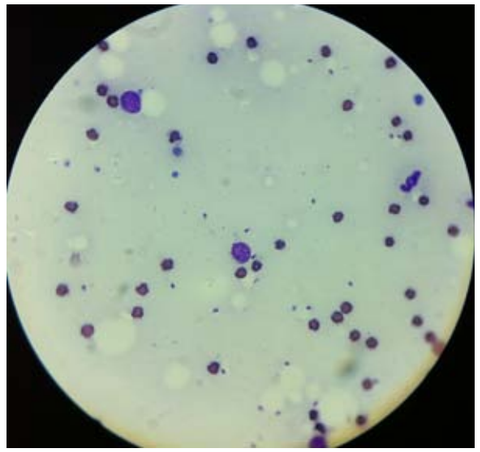
Leishmania infantum

Leishmania infantum was described for the first time by William Leishman in early 20th century in India (1). Leishmania is a protozoan belonging to Trypanosomatidae, transmitted by blood sucking Phlebotomus and Lutzomyia flies (3). The life-cycle of Leishmania spp. requires two hosts (13) – an insect and a mammal. It is very common in countries with temperatures over 16°C: the Mediterranean, Middle East, South America, some areas of the USA. It is claimed that in Greece or Italy almost 80% of dogs are seropositive and it is estimated that as much as 2.5 million dogs in Southern Europe



leishmania in skin cytology

might be infected. The greatest number of imported cases of leishmaniosis were diagnosed in Germany, Holland and Belgium (6). Some breeds, such as German Shepherd, Boxer, Rottweiler, Doberman Pincher and Cocker Spaniel are particularly sensitive, while some other, like Podenco from Ibiza, are considered to be naturally resistant. The life-cycle of Leishmania spp. is not very complicated: the promastigote forms, after making their way from an insect's intestine to the host's skin, are phagocyted by macrophages, where they are transformed into amastigotes (6). Leishmania proliferates in the infected cells (macrophages) and goes to local lymph nodes, spleen, liver, blood, bone marrow. It infects the whole organism, the presence of the protozoan being confirmed in all excretions: saliva, urine, semen or synovial fluid. The incubation period of the disease is long and, depending on the immune status of the infected animal, can last up to several years. The invasion of the parasites in dogs does not always lead to the symptoms of the disease (6), and asymptomatic hosts are the main reservoir for infection. In dogs the disease usually takes a skin form, however cases of visceral form were described in the USA. Usually the first symptoms are observed in the areas most prone to bites (pinnae, nose, skin of the abdomen), there are single or multiple follicular lesions, ulcerations, alopecia, hyperkeratinisation, exfoliative dermatitis. The skin symptoms are accompanied by weakness, muscle dystrophy, splenomegaly, nose bleeding, glomerulonephritis, haematuria, arthritis, ocular lesions (blepharitis, conjunctivitis, keratitis). Laboratory tests usually show non-regenerative normocytic normochromic anaemia, rarely thrombocytopenia, leukopenia, as well as hyperglobulinaemia and hypoalbuminaemia; in case of kidney defect also azotemia, proteinuria. The enlargement of one or all peripheral lymph nodes might lead to an erroneous suspicions of lymphoma. In differential diagnosis one should take into account several diseases causing skin lesions, including bacterial, fungal and parasitic ones, and even neoplastic conditions. To confirm the diagnosis of leishmaniosis, dermatologists usually perform cytology. Finding developmental stages of Leishmania infantum in biopsy samples from skin lesions, lymph nodes or internal organs is easy (13). In case of symptomatic patients, or as an alternative to cytology, quick diagnostic tests for Leishmania spp. antibodies can be used. Antibodies against Leishmania develop within 2 to 3 months from the infection. It should be borne in mind that leishmaniosis is a disease that is dangerous for humans too, and dogs constitute its main reservoir. Treatment is difficult and expensive, therefore if we want to take a dog in a region with big leishmaniosis problem, we should protect the dog by using repellents against bloodsucking insects.

Anaplasma phagocytophilum and Anaplasma platys

First cases of anaplasmosis have been described in the beginning of 1980s (1). Anaplasma is a gram-negative intracellular bacteria from Anaplasmataceae family. Anaplasma phagocytophilum attacks neutrophils, and it is transmitted by Ixodes spp. ticks, while Anaplasma platys binds to platelets and most probably is transmitted by Rhiphicephalus sanguineus ticks, however it was also found in Dermacentor auratus and Ixodes persulcatus ticks (16).

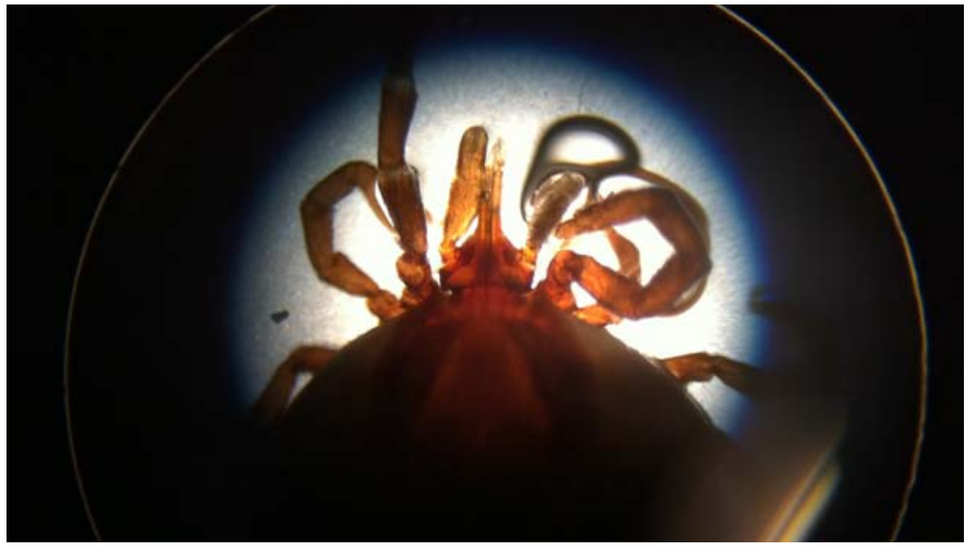
Anaplasma phagocytophilum is a pathogen for several species of animals (dogs, cats, ruminants, as well as for humans) and causes granulocytic anaplasmosis. In some regions of Europe almost 50% of dogs can be infected, with the disease usually manifested in early spring and in autumn. In case of granulocytic anaplasmosis there is a risk of coinfection with Lyme disease, as both bacteria are transmitted by the same tick species. Already the nymph form of a tick can be the vector for bacteria. Tick feeding time necessary to transmit the infection (transmission time) is 36 to 48 hours (1), which means that quick removal of a tick might prevent the infection.

Anaplasma phagocythophilum deteriorates the function of neutrophils, limits the adherence of cells to vascular endothelium and their migration to tissues, and prolongs the lifetime of neutrophils, preventing apoptosis. As a result, the life-cycle of neutrophils and the time of their migration in the bloodstream is much longer, with the functions of neutrophils severely inhibited. A.phagocytophilum may invade other cells as well, for instance eosinophils (14), bone marrow cells, megakaryocytes, endothelium, however the consequences of this invasion are not well known yet. Some animals do not have clinical symptoms of the disease. In other cases, within one to two weeks from the infection, the animal presents fever and weakness, in rare cases diarrhoea, vomiting, cough, joint pain. The response of the lymphatic system causes the enlargement of lymph nodes, splenomegaly, and – as a result of an autoregressive reaction – an autoimmune haemolytic anaemia might develop, while the presence of antibodies against platelets might be manifested by moderate thrombocytopenia. In differential diagnosis other vector-borne diseases should be taken into account.

Anaplasma platys is pathogenic for dogs and it's very common worldwide. The incubation period for the disease usually lasts from 1 to 2 weeks (14). A.platys attacks platelets causing cyclic thrombocytopenia. The platelet level cyclically drops down to under 20 000/µl, to come back to normal levels within a few days, and after 7 to 14 days dramatically drops again. The mechanism of the thrombocytopenia has not been recognised yet. The disease is accompanied by fever (15). The Anaplasma platys infection can be accompanied by Ehrlichia canis, transmitted by the same tick species. The symptoms of the disease in case of cross infection are more pronounced. Cytology is useful in diagnosing Anaplasma infections. The greatest probability of finding morulae inside neutrophils or thrombocytes is within first 24 to 72 hours of infection (16). This method is not very sensitive because of the extremely limited time in which bacteria can be found. Alternatively, to diagnose anaplasmosis quick diagnostic tests for Anaplasma phagocytophilus/Anaplasma platys antibodies can be used. Antibodies can be found already 2 weeks after infection (4). However this method does not differentiate Anaplasma platys from Anaplasma phagocytophilum. PCR is the most sensitive test, yet its use is limited by its very high cost. Therapy is based on a long-term administration of doxycycline.

Borrelia burgdorferi

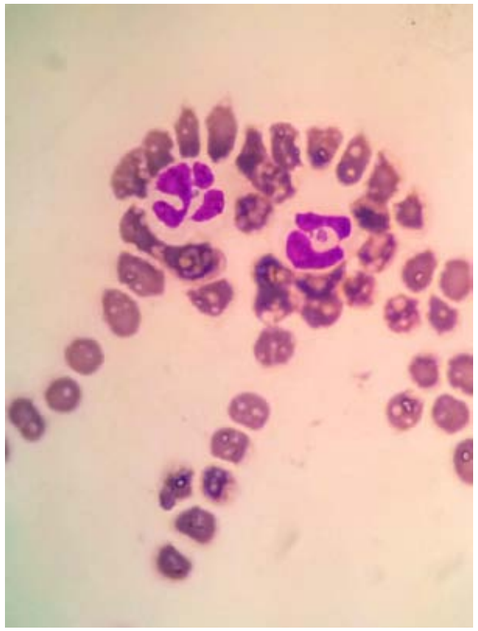
Borrelia burgdorferi sensu lato causes Lyme disease. The name Lyme disease was taken from a small town, Old Lyme in Connecticut (USA), where for the first time in 1975 several cases of disease in children were recorded (17). After this epidemic the disease was described by Alan Steere from Yale University (18). Seven years later, in the beginning of 1980s, Burgdorfer (1) identified Borrelia burgdorferi spirochete as the cause of the epidemic (19). The first case of the disease in dogs was described in 1984 (19). Borrelia burgdorferi sensu lato is a gram-negative intracellular bacteria in the shape of a spirochete, belonging to the Spirochaetaceae family, transmitted by the common Ixodes spp ticks. Despite numerous screening and prevention programs, Lyme disease remains the most frequently diagnosed tick-borne disease in humans and animals on the northern hemisphere (10). The disease is global and concerns majority of animal species and humans. In Europe, it constitutes the greatest problem in Poland, Slovakia, Slovenia (1). The B.burgdorferii sensu lato group consists presently of 18 genotypes of spirochetes (9). Pathogenicity for humans and animals is related predominantly to B.afzelii, B.garinii, B.burgdorferi sensu stricto, B. bavariensis and B. spielmanii. Pathogenicity of the remaining spirochetes (including B. lusitaniae, B. bissetti) is not known yet (11). Probably tick larvae and nymphs, too small to be noticed, are the main infection vector in humans, while adult forms are as pathogenic in dogs as larvae and nymphs. It is believed that the transmission takes place after 24 hours from the tick bite, though there are suspicions suggesting that it happens earlier (1). Antibodies against B.burgdorferii are seen after 2 to 4 weeks (17). Simultaneously, a coinfection with Anaplasma phagocytophilum in can be present, complicating the clinical image. Dogs, as opposed to humans, do not present with skin symptoms (erythema migrans) as a first sign of invasion. It is suspected that in case of dogs the disease usually takes the latent form, and the symptoms become visible only after 2 to 5 months. The symptoms are not very specific: fever, decreased appetite, joint pain, thrombocytopenia, in some cases vomiting, loss of body weight, polyuria, polydipsia and proteinuria being the effect of damage of renal glomeruli, and enlargement of lymph nodes. Blood count and biochemistry do not show any characteristic changes (9). The most sensitive breeds are Labradors, Golden Retrievers, Rottweilers (1). The differential diagnostics should include immune-based arthritis, joint injuries, other vector-borne diseases; kidney related symptoms could suggest leptospirosis or amyloidosis. Depending on the host or infection stage, B.burgorferi is characterised with big antigenic variation of surface lipoproteins, which results in quickly developing drug resistance, thus decreasing the efficiency of treatment and making diagnostics difficult. Lime disease definitely constitutes a huge problem in humans, while in dogs probably 90% of infections are asymptomatic (1). Majority of the currently known immunological methods can be used to diagnose borreliosis, however one should remember that diagnostics methods remains far from perfect (14). The main reason for diagnostic problems is expression of surface proteins (4). The recommended and the most sensitive method is determining the IgM and IgG antibodies level with Western blot method. The drawback of this method is long waiting time for the result, lower availability and high price. Quick diagnostic tests are limited in their efficiency. Choosing a test, one should pay particular attention to its specificity. If it's high, the test can be used to exclude the disease. The sensitivity of the test is limited because of the high variability of proteins, therefore the positive result should be strictly correlated with clinical symptoms and the history of the disease, and in case of doubts it should be confirmed with Western blot method.



mounthpart of the tick

Ehrlichia canis

First reports about Ehrlichia canis date back to 1930s and come from Algeria (1). Ehrlichia canis is a gram-negative intracellular bacteria from Ricketssiaceae family, closely related to Anaplasmataceae family. Its vecctors are ticks, predominantly Rhiphicephalus sanguineus (2), however there are a lso reports that Ixodes ricinus and Dermacentor spp. can transmit E.canis. Already larvae transmit bacteria. The time of transmission of the disease is very short, it can take place already after three hours of tick feeding on a host (5), so even fast removal of the arachnid does not give full chance to avoid the disease. E.canis is present worldwide, causing monocytic ehrlichiosis in dogs. There is a suspicion of possible infection of humans as well, because DNA of E. canis was isolated in a few cases of monocytic ehrlichiosis in humans. The extensiveness of invasion in some countries is high. In Portugal antibodies against E. canis in dogs were found in as much as 50% of the tested serum samples (2). The most sensitive breed is German Shepherd, in their case prognosis in case of the infection is guarded (1). The disease might have three phases: acute, subclinical and chronic, however the precise recognition of subsequent phases tends to be problematic. The incubation period varies and may last from 8 to 20 days. The antibodies are found after seven days from the beginning of the invasion. The bacteria proliferates in vacuoles of mononuclear phagocytes. When phagocytes rupture, the bacteria spread in the organism. The most frequent symptoms of the disease are unspecific: fever, decrease of appetite, lethargy, loss of body

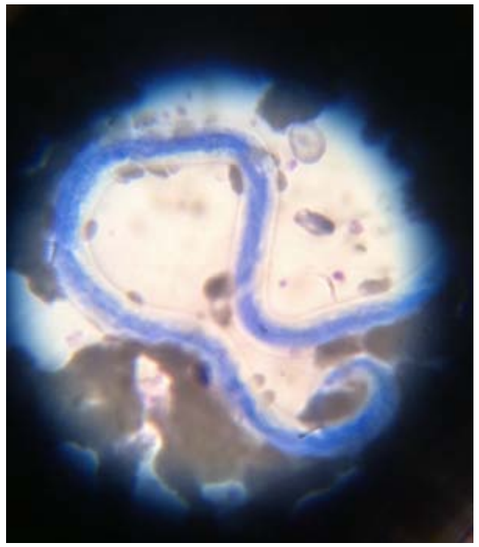


E.canis in blood smear

weight, enlargement of peripheral lymph nodes, splenomegaly, sometimes lacrimation, nasal discharge, oedemas (4). Frequently an immunological reaction is observed, leading to thrombocytopenia and haemorrhages. In the acute phase, laboratory tests show thrombocytopenia and non-regenerative anaemia. The chronic form of ehrlichiosis is accompanied by non-regenerative anaemia, thrombocytopenia, and rarely by leukopenia, giving the symptoms of pancytopenia. Other deviations in lab test results are hypoalbuminaemia and hyperglobulinaemia, prolonged aPTT, and in case of defect of renal glomeruli, azotaemia and proteinuria may develop. Chronic ehrlichiosis may lead to hypoplasia or aplasia of bone marrow. Acute symptoms might relapse continuously, and the disease itself might transform into subclinical form, lasting from a few months to a few years. Long-lasting subclinical form of the disease increases the risk of dissemination of the pathogen. Diagnosing ehrlichiosis might include cytology, however finding E. canis morulae in monocytes is not always possible, as morulae can be observed only within three days of the acute phase of the disease (14). Quick diagnostic tests based on detection of antibodies against E. canis are available. In experimentally infected dogs, antibodies were found already after seven days from the induced infection (4). The most sensitive diagnostic method in case of this disease is PCR (14). Treatment is based on long term administration of doxycycline, however starting the treatment in the chronic phase might not bring about the expected effect.

Dirofilaria immitis

Dirofilaria immitis is a parasite belonging to nematodes family, pathogenic mostly for canines and cats, but also for humans. The vector and the intermediate host for Dirofilaria (6) are mosquito species: Aedes, Anopheles and Culex. It is most common in tropical climate. The adult nematodes place themselves in the right atrium of the heart, right ventricle, pulmonary arteries and caudal vena cava. Adult males are 12 to 16 cm long, while females might reach up to 25-30 cm of length. In case of a multiple invasion, parasites are found post-mortem, cumulated in one mass. Dirofilarias are viviparous, their larvae (microfilariae) live in the bloodstream. Their life cycle is not complicated: microfilariae are ingested by a female mosquito during blood meal, in the mosquito's body microfilariae mature until they reach the invasive form (L3), and the process lasts 10 to 16 days (6). After it reaches the subcutaneous tissue of the host, the L3 form gets transformed twice more within a few months. Finally the young adult form of Dirofilaria immitis finds its way to the heart. The prepatent period lasts about six months (6), while the adults live even up to a few years. Dogs with few parasites might have very mild clinical symptoms. The first symptom observed by the owner is exercise intolerance. The clinical image of dirofilariasis is related to hypertrophy of vascular internal membranes and destruction of blood vessels, which leads to increased permeability of blood vessels, and as a result to pulmonary oedema, as cellular infiltration causes pulmonary fibrosis. In case of a massive invasion, the multiple parasites might lead to the development of endocarditis, pulmonary hypertension, pulmonary embolism. Other possible consequences are hypertrophy of right atrium, ascites or glomerulonephritis as a result of immune response. The clinical image of dirofilariasis depends on the number of parasites in the host's body. Exercise intolerance is usually accompanied by chronic coughing and weakness. Later the symptoms of heart insufficiency are present, with the possibility of shock developing, especially after exercise (7). There are cases of sudden death of dogs, for instance hunting dogs during the hunt. The laboratory tests show eosinophilia, thrombocytopenia, leucocytosis, moderate anaemia, hyperglobulinaemia, hypoalbuminaemia; radiological lesions are also found. In case of a massive invasion, the vena cava syndrome might develop, with dyspnea, jaundice, and haemolysis haemoglobinuria, bilirubinaemia



microfilaria in blood smear

found in lab tests (2). In cats the disease does not give typical symptoms, sometimes cough and increased respiratory rate is observed. Despite the seemingly mild course of the disease, it is frequently lethal. Cases of infection with Dirofilaria immitis are much more frequently diagnosed in southern Europe, compared to cases of skin dirofilariasis in dogs caused by Dirofilaria repens (6). In Poland, D.repens is much more frequent, with the first case of D. immitis found post-mortem in a dog in Wrocław in 2014 (20). In order to diagnose the disease, microscopic method can be used: evaluation of peripheral blood smears, Knott's test, as well ELISA texts detecting antigens or antibodies against D.immitis. Under the microscope, microfilariae of Dirofilaria immitis are rounded on one end, and have a sharp tip of the other end (2). Evaluation of microfilariae should not be the only method in diagnosing the disease. Quick tests to detect Dirofilaria immitis in drop of blood are commercially available, in dogs tests are based on detecting antigens secreted from the genital tract of adult D.immitis females (6), and their efficiency is high (4). False negative results of the test can be the result of invasion with male forms of D.immitis only, or immature females. Antigens can be detected from six months from the infection. In dogs antibodies against Dirofilaria immitis are not very specific, so detection of antibodies is not efficient. In cats the situation is different, the recommendations being to perform combined tests detecting both antibodies and antigens (4). If invasion is suspected, x-ray of chest should be performed, the diagnosis can be based on ECG (8).

Every year the climate in Poland becomes less and less harsh, with a longer periods of high temperatures being favourable for the feeding of ticks and insects. More and more frequently in our everyday practice we encounter diseases that were inexistent in Poland just a few years ago. Clinical symptoms are similar, frequently not very expressed, this is why the correct diagnosis seems to be extremely important, as it allows to start the targeted therapy. Majority of diseases described above are a threat for humans as well. The animals can constitute a reservoir for the disease and the source of its transmission. The most effective method of avoiding the disease in our pets is using products protecting them against the invasion of ticks and insects. Selecting the products, one should pay particular attention when the products starts to work, so that the time is shorter than the time of disease transmission, and whether the product guarantees the repellent efficiency, meaning if it also repels insects. Repellent properties are extremely important in case of diseases transmitted by mosquitoes, in which the transmission time is extremely short.

Pathogen	Vector	Transsmition time	Clinical symptoms	Laboratory symptoms
Leishmania infantum	„sand flies" *Phlebotomus* and *Lutzomyia*	At the moment bites	Often subclinical, various skin lesions, lymphadenopathy, joint pain	nonregenerative anemia, thrombocytopenia, hiperglobulinemia
Anaplasma phagocytophilus	*Ixodes spp.*	36-48 hours	Fever, weakness, diarrhea, vomiting, joint pain	Moderate thrombocytopenia, IMHA
Anaplasma platys	*Rhipicephalus sanguineus*	36-48 hours	Fever	Cyclic thrombocytopenia
Borrelia burgdorferi sensu lato	*Ixodes spp.*	~24 hours	Often subclinical, fever, loss of appetite, joint pain, weight loss	Thrombocytopenia, hiperglobulinemia
Ehrlichia canis	*Rhipicephalus sanguineus, Ixodes ricinus, Dermacentor spp.*	< 3 hours	Often subclinical, fever, weight loss, lymphadenopathy, splenomegaly	Thrombocytopenia, nonregenerative anemia, hiperglobulinemia, azotemia, prolonged aPTT, hypoplasia or aplasia of the bone marrow
Dirofilaria immitis	Mosquitoes: *Aedes, Anopheles, Culex*	At the moment bites	Often subclinical, cough, tachypnea, pulmonary embolism, ascites	osinophilia, thrombocytopenia, leukocytosis, hiperglobulinemia, changes in the chest radiograph

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