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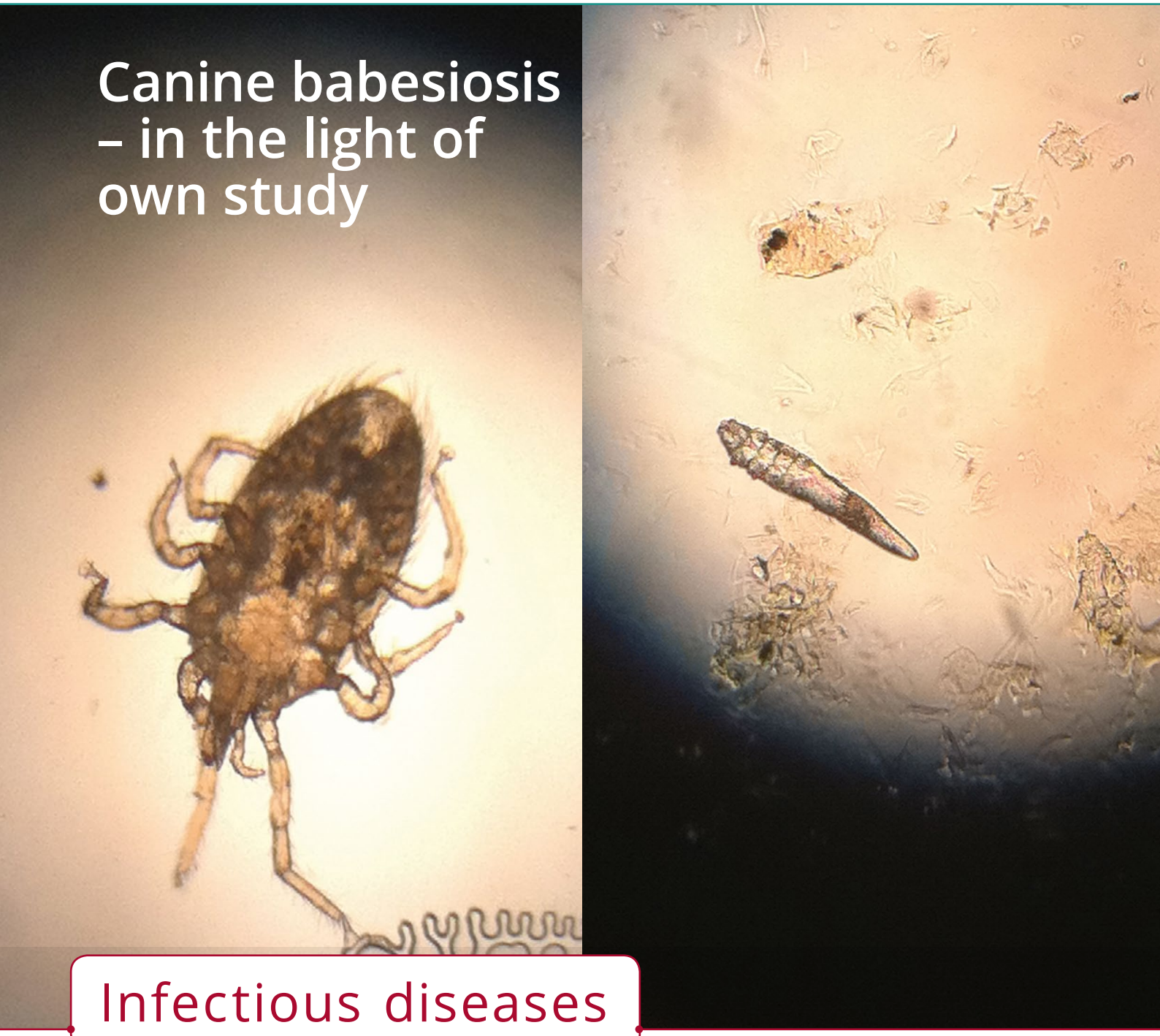
Veterinary LiFE

Magazine for companion-animal practitioners

No. 2. August 2017

Canine babesiosis – in the light of own study



Infectious diseases

Vector-borne Diseases

Feline Haemothropic mycoplasmosis

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Dear Readers,

the earliest archaeological discoveries suggest that humans and animals have come down with infectious diseases since time out of mind. It is believed that the main cause had been the development of agriculture and domestication of animals (followed by a transition to a settled way of life).

The first descriptions of infectious diseases and their treatment date back to ancient China. An important milestone came with the discoveries of Louis Pasteur, including the rabies vaccine.

Advances in knowledge have led to the development of more effective diagnostic methods, as well as better prevention and treatment strategies. On the other hand, the growth of civilization, the disappearance of borders, the free flow of humans and animals, and the migration of pathogen vectors continue to spread diseases throughout the world.

We have decided to bring together the expertise of specialists on the subject, and share their knowledge in this issue, hoping that you will find it useful and instructive.

Anna Rutkowska
Editor-in-chief

Veterinary LiFE

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Photo 1. Dermanyssus gallinae
Photo 2. Demodex canis

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Vector-borne Diseases

Janina Łukaszewska DVM, Center for Hematology and Veterinary Diagnostics. Wroclaw.

In recent years, the frequency of tick-borne diseases has significantly increased and numerous cases of these diseases occur even in places where previously they would occur only rarely (e.g. Silesia, Lower Silesia, Greater Poland, Lesser Poland). So far these diseases have been observed mostly in dogs, but now the frequency of feline infections is growing as well (1, 2). The diagnostics of the tick-borne diseases, with regards to their varied clinical symptoms, is extremely difficult as the symptoms may also suggest some other diseases. The mixed infections, such as *Babesia* and *Anaplasma*, *Borellia* and *Anaplasma*, *Anaplasma* platys and *Ehrlichia canis*, make the diagnostic process even more complicated or difficult. Such cases were also described in Poland – in dogs infected simultaneously with *Babesia sp.* and *Anaplasma sp.* or *Borrelia sp.* and *Anaplasma sp.* or with the two above together with *Dirofilaria repens* (Fig. 1) (6, 8, 12).

The author observed a similar mixed infection with varied symptoms in a cat with a non-exudative peritonitis: this was a simultaneous infection with *A.phagocytophla*, *Mycoplasama felis* and *Toxoplasma gondi*.

Abnormalities in biochemical blood parameters (3,4,5,7,8,13,15,16,18)

As these diseases are accompanied by a damage of parenchymal organs – some abnormalities in results of biochemistry tests are seen. They may also suggest other diseases with accompanying complications or a disease affecting solely one organ. Usually, the infection with the tick-borne germs is accompanied with an increase of the activity of liver enzymes (ALT, AST, ALP) and parenchymal liver disease resulting from the necrosis of hepatic cells. Either together with this infection or independently from it, an interstitial nephritis, pyelitis or glomelluritis and renal insufficiency might occur. Also cases of pancreatitis in dogs have been described. If haemolytic anaemia occurs, the inflammatory factors occurring together with haemolysis as well as hypoxia will be then the outcome of the haemoglobin level decrease, and they may cause or increase the damage of parenchymal organs.

The haemolysis may be accompanied by an increased level of iron and AST, frequently with correct ALT level and also by haemolytic jaundice.

The abnormalities in the activity of liver enzymes occur mostly in the infections with granulocytic anaplasmosis and ehrlichiosis, affecting monocytes; yet in these diseases a renal failure may also occur. The infection with Babesia canis is accompanied first of all with the lesions in kidneys, or the kidney damage accompanied with an increase of urea and creatine, caused by massive haemolysis; yet also the liver may be affected.

In turn, a frequent complication of the Borrelia infection is a glomellular nephritis of immunological origin, with protein losing nephropathy, significant proteinuria and decreased level of albumins in blood. Other complications comprise renal failure, azotaemia, increase of phosphorus level, hypercholesterolaemia, glycosuria and numerous vitreous rod cells in urine sediment (Fig. 2).

Bernese Mountain Dogs have a breed-related inclination to be infected with this germ and a frequent tendency to develop Glomerulonephritis together with it (7).

Boreliosis and anaplasmosis may be complicated by myositis, leading to an increase of creatine kinase and AST activity (17, 18).

The infection with granulocytic anaplasmosis and monocytic ehrlichiosis is usually accompanied by hyperproteinaemia caused by hyperglobulinaemia (4.8-7.2 mg/dl), whilst the level of albumins, as negative proteins of acute phase of inflammation, is decreasing. In such cases, their level drop even to 1.4 – 2.2 mg/dl, which might lead to accumulation of exudates in body cavities.

In such cases blood protein electrophoresis reveals polyclonal gammopathy, i.e. the increase of the level of β,γ globulins or monoclonal gammopathy – only the increase of γ globulins with Bence-Jones protein in urine (5, 9,10, 20).

Similar abnormalities in the concentration of blood proteins, yet perhaps less drastic, are observed also in the B. canis infection (our own observations).

One of the frequent clinical symptoms accompanying tick-borne diseases is lymphadenopathy. In these cases there is an enlargement of one, two or all lymph nodes available for evaluation sometimes together with the lymph nodes in the abdominal cavity or the chest. Such a node is often painful, but still normal lymphocytes are predominant (above 60%), yet also there are numerous plasma cells, neutrophils or, sometimes, macrophag-

Table 1. The abnormalities in blood parameters seen in biology test in tickborne diseases

No.	The abnormalities in blood parameters seen in biology test in tickborne diseases
1.	Increase of liver enzyme activities, parenchymal hepatitis, anaplasmosis in particular ehrlichiosis, less frequently, an increase of the urea and creatine level, and less frequently babesiosis. Hypoxia-induced liver damage and inflammatory factors in haemolysis (Babesia, AIHA)
2	Haemolytic liver disease – babesiosis, mycoplasmosis – of bilirubin concentration, unconjugated in particular, also AIHA
3	Nephritis and renal failure – parenchymal, mostly babesiosis or glomerular one with boreliosis: increase of the urea and creatine level, phosphorus, hypercholesterolaemia, albuminuria, glycosuria), vitreous rod cells and granulomatous cells in urine sediment
4	Increase of AST, Fe- haemolysis
5	Decrease of iron concentration, even a significant one, as a result of inflammation. Bleeding, petechiae at thrombocytopaenia
6	Increase of creatinine kinase level, AST – myositis (boreliosis, anaplasmosis, ehrlichiosis)
7	Hypoglycaemia – babesiosis
8	cPLi increase – pancreatitis – babesiosis, anaplasmosis

Table 2. Monoclonal gammapathy in the infection with *Anaplasma phagocytophila*. Decreased fibrinogen concentration in thrombocytopaenia suggests a development of DIC.

Fibrinogen	0.63 g/l (normal range: 1-4 g/l)	Decreased
Total serum protein	133 g/l	Extremely elevated
Albumins	14.47 g/l (normal range: 25-44 g/l)	Significantly decreased
α-1-globulins, α-2-globulins	8.82 g/l	Atypical distribution, very low
β-1-globulins, β-2-globulins		
γ-globulins	10.97 g/l	Very high

es (Fig.3). Reactive splenomegaly is likely to occur – with numerous plasma cells or, in the case of reactive anaemia, it might be accompanied with extramedullary erythropoiesis (Fig. 4 and 5), (5, 6, 16, 20).

CBC abnormalities

In transmissible diseases there are qualitative and quantitative abnormalities in blood, concerning red and white blood cells as well as blood platelets.

Blood abnormalities in infections with A*naplasma spp., Ehrlichia spp. and Borrelia spp.* (4, 5, 9, 12, 13, 15, 16, 18, 20):

- normocytic, normochromic, non-regenerative anaemia, in 95-100% dogs, accompanying the inflammation;
- non-regenerative anaemia with a decrease of the iron level with numerous microcytes in a chronic disease (Fig. 6);
- AIHA in 14% dogs (Fig. 7);
- Anaplastic or dysplastic anaemia (anaplasmosis, ehrlichiosis) (Fig. 8);
- Thrombocytopenia with macro-thrombocytes or without them (Fig. 9);
- Leukopenia with neutropenia;
- Lymphopenia and eosinopenia;
- Neutrophilia;
- Morules in neutrophils and eosinophils, sometimes in lymphocytes (Fig. 10, 11, 12);
- Leukocytes in adhesions, neutrophils agglutination (Fig. 13);
- Monocytopenia or monocytosis;
- A.platys – thrombocytopaenia and leucocytosis;
- The presence of reactive lymphocytes (Fig. 14, 15, 16);
- Rouleaux phenomenon (Fig. 17).

When the course of disease is long, and initially accompanied by normocytic, normochromic anaemia accompanying inflammations, the iron level might be significantly decreased and then followed by anisocytosis, with a significant number of small erythrocytes – microcytes. Then the mean erythrocyte volume (MCV) is reduced, reaching even the bottom level of the reference value, or – more rarely – hypochromia in extreme cases.

Figure 18 presents the CBC of a dog with a chronic granulocytic anaplasmosis and with thrombocytopenia – here the MVC value is correct, yet in the RBC histogram there is a clear peak reflecting small erythrocytes count and this is confirmed by an increased anisocytosis index – RDW. At the same time, there is a non-regenerative anaemia, neutropenia and thrombocytopenia – all the abnormalities are typical for bone marrow aplasia.

Figure 19 presents a similar case, yet without neutropenia or thrombocytopaenia but with a decreased MCV.

Another figure (Fig. 20) presents the blood count of a dog infected with granulocytic anaplasmosis and secondary autoimmune haemolytic anaemia –numerous spherocytes and agglutination can be noted in blood (high MCV).

Abnormalities in the blood accompanying the infections with parasites colonising erythrocytes: *Babesia canis, Mycoplasma canis, Mycoplasma felis*. (1, 3, 11, 14, 19):

- Haemolytic and regenerative anaemias, both intravascular and extravascular haemolysis – through the macrophages of the spleen and liver (Fig. 21, 22, 23);
- AIHA – a frequent complication accompanying the infections with B. canis and A. phagocytophila;
- Thrombocytopaenia, with the presence of macro-thrombocytes;
- Neutropoenia – frequently;
- Neutrophilia – less frequently;
- Leucocytes in agglutinations;
- Reactive lymphocytes;
- Erythrophagocytosis (Fig. 24);
- Post-bleeding anaemias with thrombocytopaenia (< 50000/µl);
- Intravascular coagulation syndrome;
- Rouleaux phenomenon as a result of high concentration of globulins.

With the infection of red blood cells with Babesia canis, in dogs and cats there are haemolytic anaemias, which also occur in the infections with *Mycoplasma felis* (Fig. 21, 24) and *Mycoplasma canis* (Fig. 25, 26).

In Poland, so far, there have been isolated cases (on the basis of spoken information – 4 cases) of canine infections caused by *Mycoplasma canis*. In cats mycoplasmas are present on the surface of erythrocytes in an isolated form or in aggregates of two particles or rods, sunken in the erythrocyte membrane; they rarely occur in chains, whereas in dogs, chains in very strange shapes are predominating. Every "different erythrocyte" is soon eliminated from infected organism either by intravascular haemolysis, so that the "shadows" of erythrocytes are made up, or by extravascular haemolysis (by spleen, liver or bone marrow macrophages, sometimes they might also be present in the peripheral blood) (11, 14).

Table 3. Causes of thrombocytopaenia.

Immunological destruction of thrombocytes with regeneration (with macro-thrombocytes)
Increased platelet consumption by macrophages of frequently enlarged spleen
A reduction of bone marrow production as a result of its aplasia or dysplasia (mainly anaplasmosis), without its regeneration
Adhesion to the damaged endothelium of blood vessels
DIC
Connection of all these mechanisms

Thrombocytopaenia is the most frequent characteristic symptom of all tick-borne diseases present in our country. In some cases, however, thrombocytopaenia may not be present or the platelets may occur in agglutinations on the margins of the sample – in such cases we deal with "pseudo-thrombocytopaenia" (Fig. 27). As a result of the reduction of the platelet count in the peripheral blood, caused by their destruction, new platelets – so called "macro-thrombocytes" appear: in dogs they are 1/3 of the erythrocyte size, and in cats – above the normal erythrocyte size. Most of them are located at the edges of the smear (Fig. 9).

Such forms will not be found with the bone marrow aplasia (anaplasmosis, ehrlichiosis).

A significant thrombocytopenia accompanies the infection of *Anaplasma platys*, these organisms may be found in the infected platelet cells and in the megakaryocytes of the bone marrow.

Neutropenia (leukopenia) is, together with thrombocytopenia, one of the main symptoms of babesiosis, anaplasmosis and ehrlichiosis in animals, yet also a correct or elevated leucocyte count may be observed in animals affected with these diseases. Leukopenia is usually accompanied with neutropenia and lymphopenia or, there might be cases when the lymphocyte count is correct or sometimes elevated. Neutropenia is most probably caused by immunological factors and bone marrow hypoplasia or dysplasia. Within the course of disease one might also observe neutrophilia with a left shift or a correct granulocyte count.

In neutrophils, eosinophils (A*naplasma spp.*) and monocytes (*Ehrlicha spp.*) within peripheral blood, bone marrow and articular fluid, there might also be inclusion bodies of rickettsia – morules. The rate of infected cells is 3-8%. The inclusions are composed of numerous, delicate (rod-like, round or oval) initial bodies, measuring 0.18-1.4 µm, of

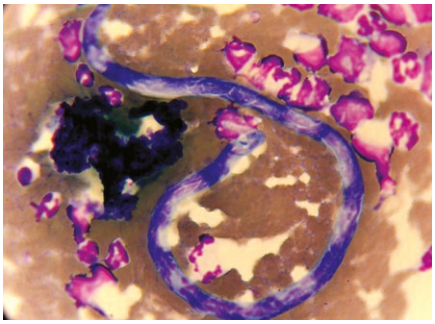

Figure 1. Dirofilaria repens larva in blood

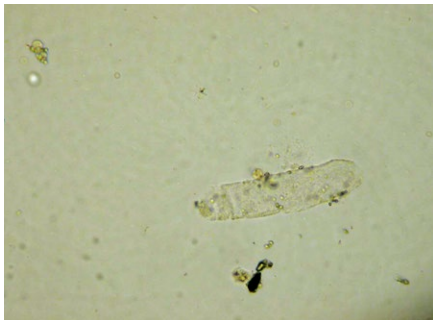

Figure 2. Vitreous rod cell in urine sediment

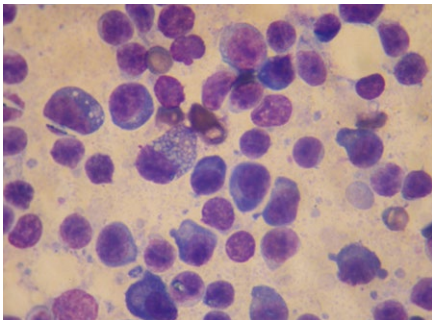

Figure 3. Reactive lymph nose with numerous plasmocytes (anaplasmosis)

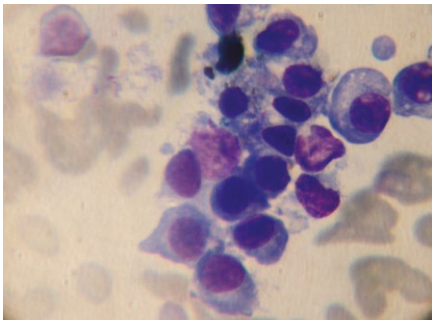

Figure 4. Plasmocytes in spleen (anaplasmosis)

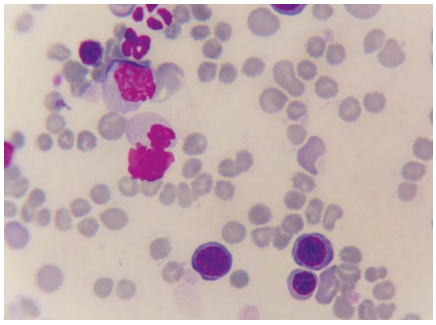

Figure 5. Extramedullary erythropoiesis in spleen (babesiosis)

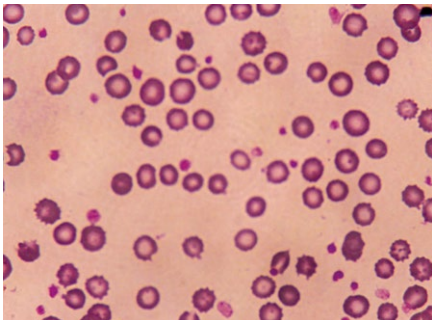

Figure 6. Anisocytosis with microcytosis – hypochromaticity

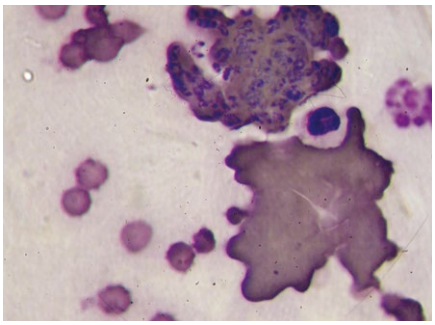

Figure 7. Agglutination (AIHA) – babesiosis

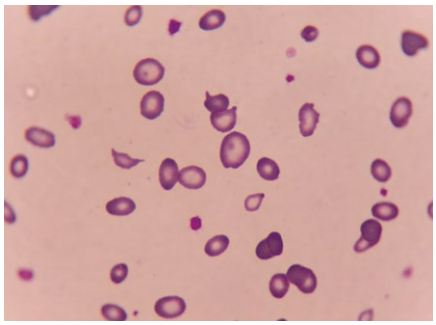

Figure 8. Peripheral blood smear in bone marrow dysplasia (anaplasmosis)

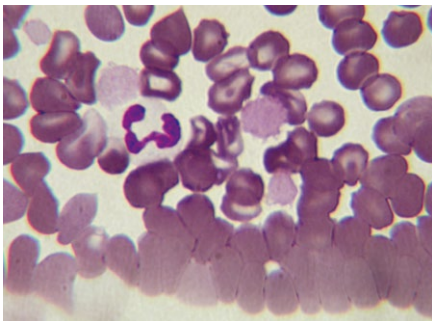

Figure 9. Thrombocytopaenia – macrothrombocytes in the smear periphery

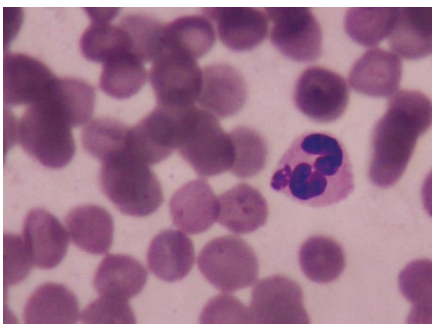

Figure 10. *A. phagocytophila* in dog neutrophil

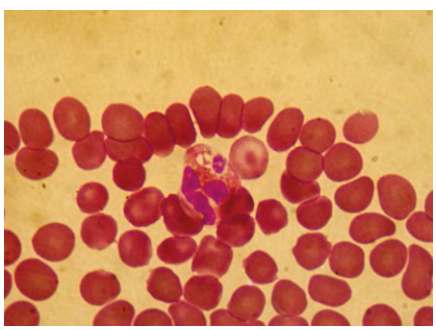

Figure 11. *Anaplasma phagocytophila* in dog eosinophil

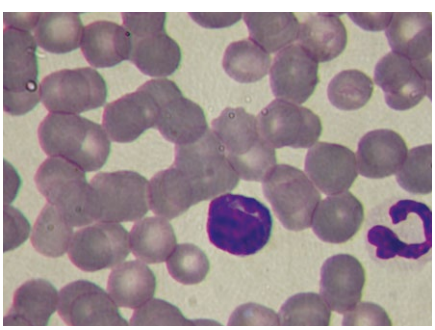

Figure 12. Anaplasma in a lymphocyte

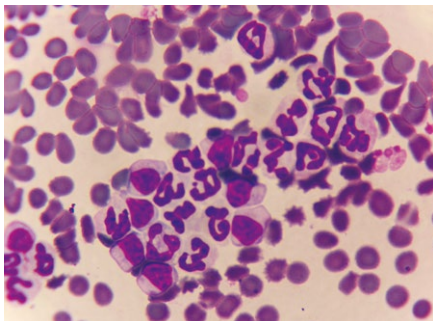

Figure 13. Leucocytes in agglutinations, numerous reactive and granulomatous lymphocytes

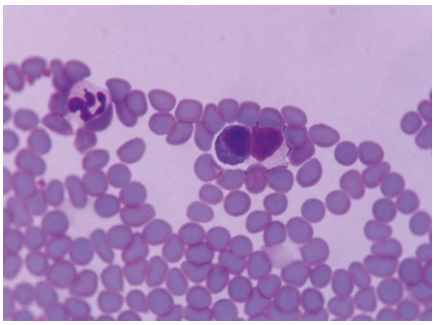

Figure 14. Reactive lymphocytes – boreliosis

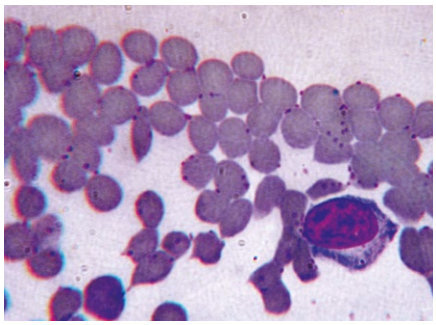

Figure 15. *M. felis* and a reactive lymphocyte (plasmocyte)

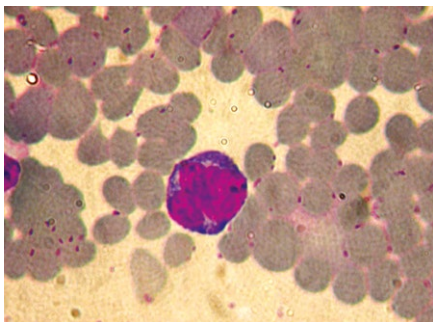

Figure 16. *Mycoplasma felis* – a reactive lymphocyte with an undulating nucleus

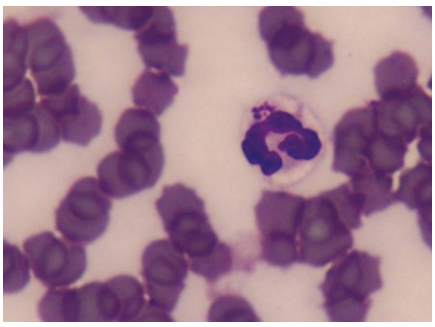

Figure 17. Anaplasmosis, Rouleax

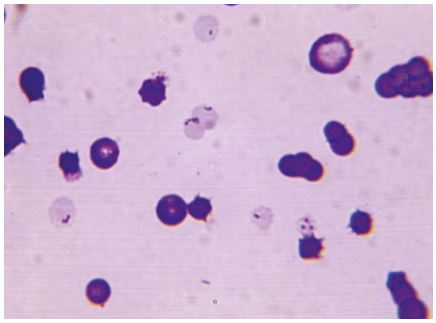

Figure 21 *Mycoplasma felis* – AIHA (intervascular haemolysis with erythrocyte shades and auto-agglutination)

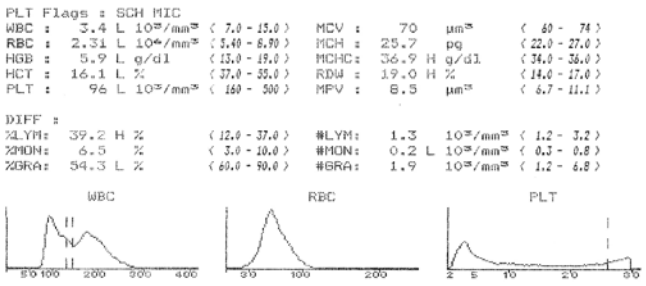

Figure 18.

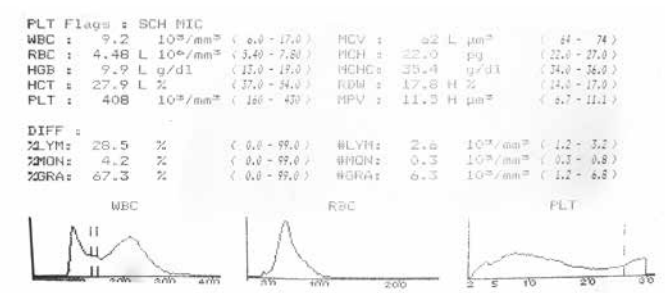

Figure 19.

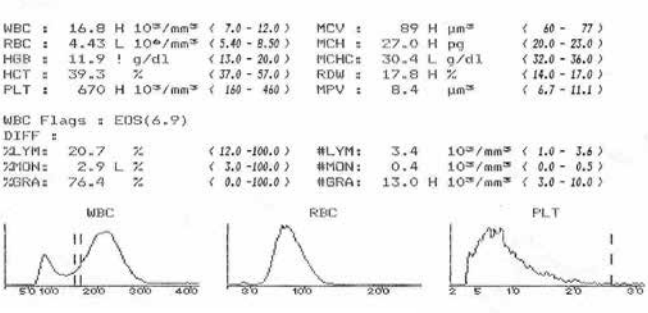

Figure. 20.

dark blue to scarlet colour, placed in vacuoles surrounded by a membrane. In some granulocytes they are disintegrated into basal bodies and their remains are in these vacuoles (Fig. 28), (4, 13).

Morule Ehrlichia canis is sometimes found in lymphocytes (Fig. 12). If neutropenia did not occur previously, haemolysis is accompanied by neutrophilia – as a reaction to the inflammation, caused by haemolysis, usually with a left shift. Sometimes it may be of significant degree – this is so-called leukaemic response.

Monocytosis is sometimes a characteristic symptom of infection with Anaplasma phagocytophila, yet cases with a decreased monocyte count in this disease have also been described. This disease was described together with neutrophilia in mycoplasma infections in cats.

Reactive lymphocytes
(4, 8, 11, 12, 13)

Although rickettsia or babesia infections may be accompanied both with lymphocytosis and lymphopenia; in most cases, in affected animals lymphopenia is diagnosed. Always, however, there are various types of reactive lymphocytes, characteristic of these diseases (about 50%) – these are plasmocytes, reactive lymphocytes with undulating nuclei and dark blue cytoplasm as well as granulomatous lymphocytes (Fig.13,14,15,16). This is indicative of the immunological stimulation.

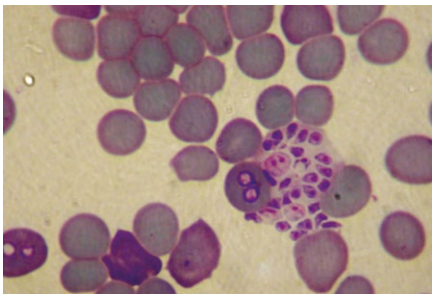

Figure 22. Babesia – intravascular haemolysis

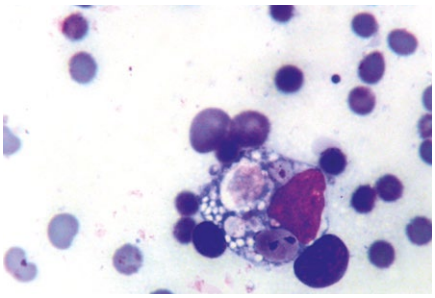

Figure 23. Macrophage – erythrocytes with babesias and neutrophilia (extravascular haemolysis)

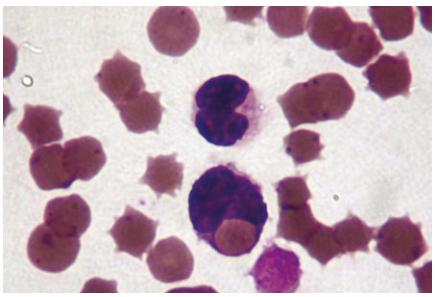

Figure 24. *Mycoplasma felis* – erythrocyte phagocytosis with feline mycoplasma

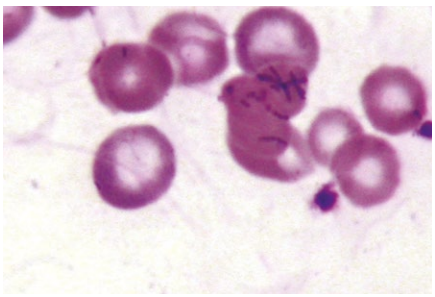

Figure 25. *Mycoplasma canis*.

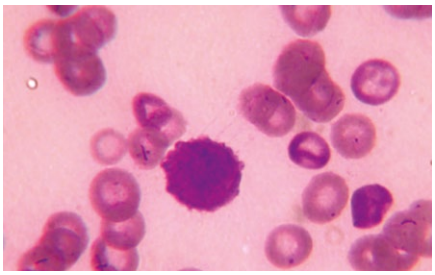

Figure 26. *Mycoplasma c*., thrombocytopaenia with mega-platelets, numerous reticulocytes

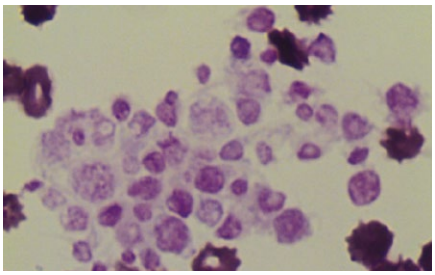

Figure 27. Platelets in agglutinations – pseudo-thrombocytopaenia in babesiosis

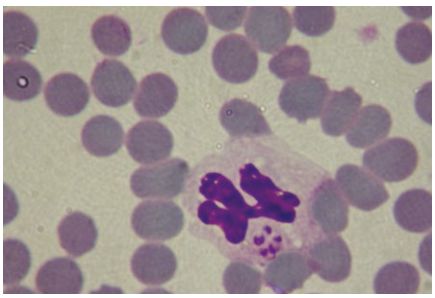

Figure 28. A vacuole with anaplasma in a horse with anaplasmosis

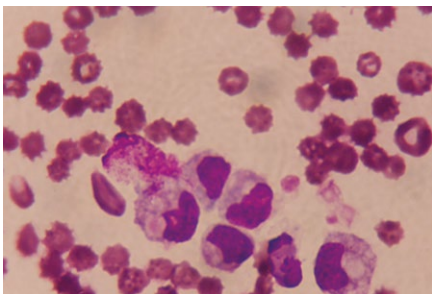

Figure 29. Erythro-phagocytosis – *B. canis*

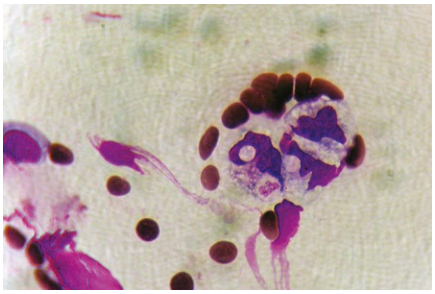

Figure 30. Neutrophil in macrophage (peripheral blood – babesiosis with neutropenia)

Phagocytosis in peripheral blood (author's own observations)

The destruction of red blood cells containing parasites, covered with antibodies, neutrophils with morules as well as blood platelets in the above diseases, takes place via their phagocytosis in macrophages. This occurs mostly in the spleen, yet in some isolated cases, this phenomenon may also be found in peripheral blood, in particular on the margins of the blood smear. This process is carried out by monocytes (macrophages) or neutrophils phagocytising the blood cells mentioned above. This is a rare phenomenon, yet it is very helpful in the diagnostics of these diseases (Fig. 24, 29, 30).

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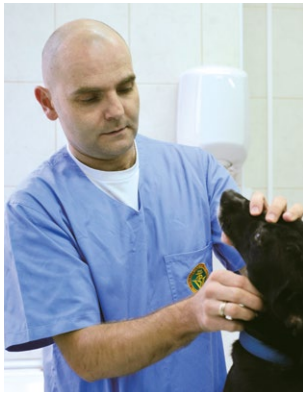
Canine babesiosis – in the light of own study



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Canine babesiosis is a tick-borne disease caused by protozoal parasites, which are most commonly transmitted by tick bite. Its etiological factor is an inter-erythrocyte protozoa of the Babesia genus, family: Babesidae, order: Piroplasmida, and type: Apicomplexa (1). On the basis of parasite cell morphology, two groups of these canine pathogenic parasites can be distinguished – larger ones, measuring 3–5 µm named *B. canis* and smaller ones, measuring 1–3 µm – *B. gibsoni* (2). The analysis of the 18S RNA, Bc28, 5,8S, hsp70 genes of cytochrome B showed that in fact the disease is caused by numerous Babesia species. Within small piroplasms, the following species were found: *Babesia conradae*, *Babesia microti*-like defined also as *Theileria annae*, or "Spanish isolate" and *Theileria spp* (3,4,5). Within large piroplasms three species are distinguished and they were initially regarded as subspecies of *B. canis* – *B. rossi*, *B. canis* and *B. vogeli* and relatively recently found in dogs in USA, large Babesia still unnamed (6,7,8,9). All of them are characterized by identical cell morphology, yet their geographic scope, genetic structure and virulence vary. These protozoa are transmitted by diverse species of ticks (9, 10, 11).

So far, only *Babesia canis* has been found in Poland (12, 13, 14, 15, 16). The disease caused by these protozoa may be uncomplicated, manifested by anaemia, or complicated, and then multiorgan failure and generalised inflammatory reaction develop. The complicated babesiosis is reported much more frequently in the registers of veterinary clinics than the uncomplicated one. This is probably caused by the fact that generally, the patients with the protozoic invasion are reported to veterinarians a few days after the occurrence of the disease, when the clinical symptoms are exacerbated (complications occur).

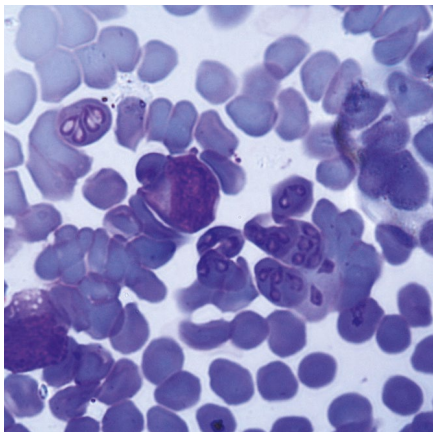

Figure 1. Blood smear stained with Diff-Quick methods. The *Babesia canis* merozoites are present in the dog's erythrocytes

The objective of this paper is to present the cases of babesiosis diagnosed within the last three years (2014-2017) in dogs in the Infectious Disease Clinic of the Faculty of Veterinarian Medicine of the University of Life Sciences in Lublin.

The site's own observations

The study included 253 dogs of varied breeds and sexes (148 males and 105 females), at various ages ranging from 5 months to 13 years; they were all the patients of Infectious Disease Clinic of University of Life Sciences in Lublin and in all of them babesiosis infection was confirmed by microscopic and/or molecular (PCR) evaluation (Fig. 1).

The animals were brought to the Clinic with various clinical symptoms and in diverse stages of the disease (Table 1). In 228 dogs, before the occurrence of the symptoms, the owners found ticks on the integuments of the animal bodies. In the case of 25 patients, no invasion of these arachnids was found. There were varied reasons for medical and veterinarian consultations. In 128 dogs, the owners observed apathy and bad mood within a short period (up to 4 days) from the moment of finding ticks on the pet's skin. In 53 other patients, initially dark colour of urine was observed, followed by anuria. Additionally, in 47 cases emesis occurred. In all 53 cases, blood serum biochemistry revealed an increased level of urea (68-181 mg/dl) and creatinine (1.9-3.6 mg/dl).

In 30 dogs difficulties in breathing occurred, with short and rapid breathing, intolerance of exercise, rapid heart rate and bruising of mucosal membranes. The ECG examination, performed in 22 patients, revealed the change of the picture and/or amplitude of the T wave and in 9 cases – accelerated sinus rhythm, in 6 cases – axis deviation and in 4 – widened QRS complex, in 5 cases – ventricular ectopics and in 3 cases – ventricular tachycardia. Also, in 12 patients, the ECG examination detected increased fractional shortening (FS%).

Another group of 28 patients were reported to the clinic with symptoms of jaundice, emesis and diarrhoea. Abdominal ultrasound allowed revealing increased liver and spleen (Fig. 2) and serum biochemistry showed elevated activity of asparagine aminotransferase (AST = 68-112 IU/l)) and alanine aminotransferase (ALT= 76-185 IU/l) as well as increased level of bilirubin (0.8-2.1 mg/dl). Additionally, in 19 cases, an elevated activity of alkaline phosphatase was observed (ALP>155 IU/l).

Consultations of another group of 11 dogs was done due to occurring difficulties in walking, caused in particular by problems with the pelvic limbs (muscle pain, difficulties in standing up), accompanied with a brown colour of urine.

Haematological assessment of all animals revealed thrombocytopenia (PLT 200x109). Haematocrit decrease below 37% (lower limit of normal range) was observed in 164. The drop of erythrocyte count below 5.5 x 1012 (lower limit of normal range) was found in 143 cases. Leukopenia (WBC < 6 x109) occurred in 126 cases and leucocytosis (WBC > 10 x109) in 45 cases.

The causative therapy in all animals included imidocarb dipropionate at the dose of 5 mg/kg of body mass, divided into two portions, every 24 hours, administered subcutaneously. Symptomatic treatment depended on the form of the disease.

As a result of the treatment, 244 dogs made complete recovery. Nine patients died in spite of the therapy. These were 4 dogs in whose case renal failure develop as well as all the three dogs with neurological symptoms and two patients who developed hepatic failure. It must be pointed out that all animals that died were of elderly age (11-13 years), which definitely affected the efficiency of treatment.

Discussion

The clinical course of babesiosis described above suggests that this disease may have many faces. On the basis of the clinical pic-

ture, dogs infected with this protozoa may be categorised into six groups: with the uncomplicated babesiosis (128 patients), babesiosis accompanied with renal complications (53 dogs), babesiosis accompanied with cardiological disorders (30 dogs), babesiosis accompanied with hepatic failure (28 dogs), babesiosis accompanied with walking difficulties (11 dogs), and babesiosis accompanied with neurological complications (3 dogs).

As it was mentioned in the introduction, on the basis of the clinical course of the disease two forms might be distinguished –complicated and uncomplicated one. The typical symptoms of uncomplicated piroplasmosis comprise anaemia, fever, lack of appetite, apathy, pale mucosa, splenomegaly and increased heart rate (17).

The patomechanism of anaemia is complex (18). In the course of canine babesiosis, both extravascular and intravascular haemolysis occur, manifested with degenerative anaemia, haemoglobulinaemia, haemoglobinuria and bilirubinuria. The development of haemolytic anaemia involves many processes. As Zygner and Gójska-Zygner (19) report, erythrocytes' disintegration may be caused by their mechanical damage by replicating parasites but also by the damage of erythrocytes with antibodies, complement system or oxygenating factors. Also the spleen is involved in the damage of the red cells attacked by protozoa. Babesiosis-induced anaemia may also be the outcome of oxidative stress and increased peroxidation of the lipids of the cellular membranes. The last of the above mechanisms contributes to the damage of the structure of the erythrocyte cellular membranes, the loss of their continuity and increased permeability for ions. An increased peroxidation contributes to the accumulation of oxidative ions in red blood cells and their lysis (19).

The complicated babesiosis has the symptoms not related to haemolysis. This form of disease is driven by increased inflammatory reactions of an organism, leading to Systemic Inflammatory Response Syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) (20).

Frequently the course of the disease is complicated, as it was shown above, with renal insufficiency, myocardial damage, neurological disorders, hepatic failure or shock. Sometimes the disorders of the alimentary tract, fatigue and myalgia have been observed (17). The most frequent complication observed in the patients of the centre was the renal failure, manifested with oliguria or anuria. The damage of the renal function occurs both in complicated and uncomplicated babesiosis, but the renal failure does not have to develop in every case. A diagnosis of renal insufficiency based only on the parameter of elevated serum urea is a mistake. Increased level of serum urea may be the outcome of the catabolism of damaged erythrocytes. Therefore, the following criteria must be met

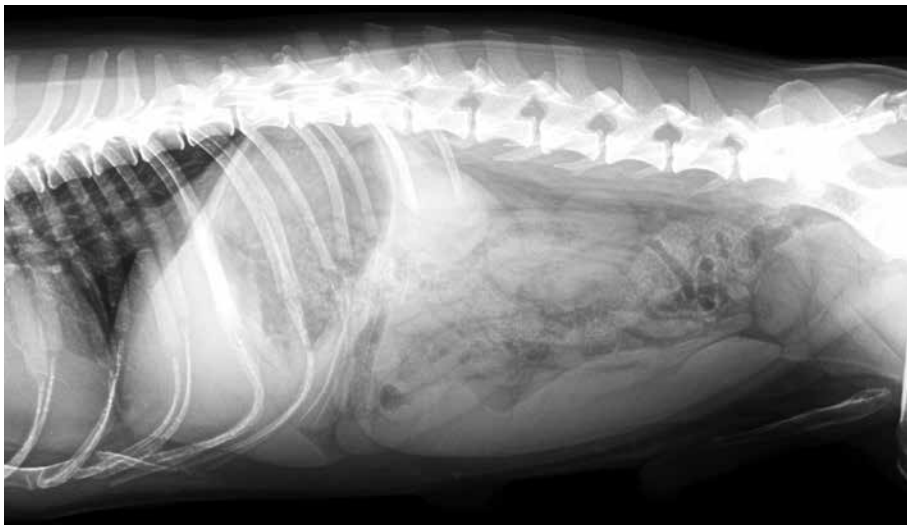

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Figure 2. Splenomegaly seen in the radiogram of a dog affected with babesiosis

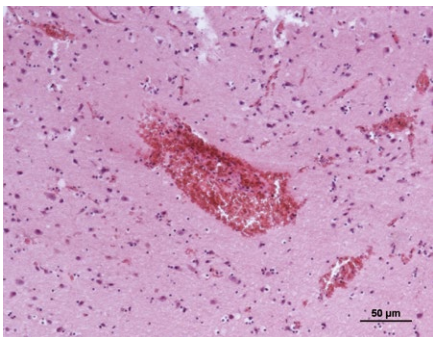

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Figure 3. Histopathological image of the brain of a dog which died because of babesiosis. The damage of the brain vasculature, resulting from the blood stasis and/or erythrocyte sequestration is seen,

before renal insufficiency is confirmed: decreased volume of the produced and excreted urine, typical results of urine analysis and the blood serum azotaemia confirmed by blood serum biochemistry. There are often theories explaining the development of acute renal failure in dog babesiosis with haemoglobinuria. Such view is only partly correct. Haemoglobin per se does not damage kidneys. After the release from red blood cells, it may be converted to methaemoglobin, which produces such an effect. The factors responsible for the development of this disorder are the inflammatory reaction mediators and hypotension. It is assumed that about 30% of the patients with babesiosis develop acute renal failure (21).

Some other complications observed among the dogs in our stud, were cardiological disorders. A probable cause of their occurrence was the generalised inflammatory reaction and hypoxia. Abnormal readings of the ECG, found in affected animals are the outcome of myocardial ischemia and myocarditis. The first of them causes abnormalities in the T wave, whilst the damage of the myocardium leads to its elevation, or, in rare cases, its depression. Myocardial necrosis, in turn, leads to widening of the QRS complex and the development of the atrioventricular bloc. In myocarditis, there are also premature ventricular contractions and ventricular tachycardia (23). The studies by Dvir et al. (27) suggest that the frequency of abnormalities on the ECG reading in dogs with babesiosis is as follows: elevated T wave (42%), heart axis deviation (40%), widened QRS complex (32%), depressed R wave amplitude (23%), atrioventricular bloc (7%), premature ventricular contractions (7%). Both myocarditis and myocardial ischemia may develop as a result of the thrombus in small vessels and the anaemia of the immunological origin. Yet it should be noted that in the course of canine babesiosis, there are many factors which might affect the ECG reading, such as: anaemia, hypoxia, hypokalaemia, metabolic acidosis, or uraemia. Therefore, on the basis of the ECG reading only, it is impossible to determine whether myocardium damage occurred or not. What might be helpful here is the determination of the blood serum level of cardiac troponin I (cTnI) and T troponin (cTnT). These are myocardial proteins which participate in the regulation of heart contractility. As it is suggested in the studies by Lobetti (17) the level of cardiac troponins is significantly elevated in the blood serum of dogs with babesiosis complicated by the myocardial damage, and this elevation corresponds to the severity degree of the disorder.

Hepatic failure is a relatively frequent complication in the course of babesiosis. In this study, hepatic insufficiency was found in 28 patients, which made up 11% of all animals in the study. Its permanent symptom is jaundice, developing as a result of haemolysis or bile ducts obstruction. Hepatic jaundice may be a consequence of the damage of liver parenchyma by cytokines or hypoxia (17). Extrahepatic jaundice, in turn, may develop as an effect of an inflammation of the bile ducts or of gallbladder (22).

Other complications – gait disorders and neurological symptoms – observed in the studies of the dogs with babesiosis, are less

Table 1 Abnormalities observed in dogs with confirmed babesiosis

Type of complication	The number of dogs with confirmed babesiosis
General symptoms	128
Renal symptoms	53
Cardiological symptoms	30
Gastrointestinal symptoms	28
Gait disorders	11
Neurological disorders	3

frequent. The first of the listed symptoms might be connected with rhabdomyolysis. Rhabdomyolysis is a complex of symptoms caused by the striated muscular tissue damage, which leads to the presence of myocardial unconjugated myoglobin in the blood and then this myoglobin is filtered by renal glomeruli, which might lead to their damage and acute renal failure. Muscle damage may not be accompanied by any symptoms. Sometimes muscle swelling, generalised muscle pain or haematuria are observed. Laboratory tests may reveal the increase of CPK and LDH activity, hypocalcaemia during oliguria and hypercalcemia in the recovery period, and also the presence of myoglobin in urine. Neurological disorders, in turn, are the effect of the accumulation of erythrocytes affected by parasites in the small vessels of the brain, which leads to inflammation and prefusion disorders. Some other mechanisms leading to the development of neurological symptoms are metabolic disorders induced by hypoxia and hypoglycaemia, as well as neurotransmission disorders induced by nitrogen oxide (25).

Neurological form of babesiosis manifests itself with the motor disorders, palsies and convulsions. The abnormalities observed in the dogs with this form of the disease comprise abnormal pupil dilation, strabismus, transient loss of consciousness, excessive aggression or vocalisation. In many cases, the outcome of this form of the disease is death of the affected animal (24,26), whereas the brain histopathology may reveal the damage of cephalic vessels and the presence of parasites in the CNS (Fig. 3).

The final diagnosis of the encephalic form of babesiosis is possible when the following criteria are met: identification of the parasites which are the underlying cause of the disease, diagnosis of impaired brain function and exclusion of co-existing infections. If falls occur, the diagnosis may be confirmed with the histopathological or molecular analysis revealing the presence of parasites within the brain (24).

In the study group of patients no other complications described in publications, such as acute respiratory distress syndrome, acute pancreatitis or shock were found. Nevertheless, babesiosis is still a disease with a diversified course, posing in many cases a great diagnostic or therapeutic challenge for veterinarians. Continual monitoring of the invasion and search for the markers of many forms of this disease, allow for its better understanding, prediction of its course and elaboration of new methods of treatment.

Canine babesiosis – in the light of own study

Canine babesiosis is a common and clinically significant tick-borne disease caused by hematozoan parasites of the genus Babesia. The pathophysiology of canine babesiosis has been extensively studied but many questions remain unanswered, especially regarding the diversity of disease manifestations.

This paper presents the possible forms of the canine babesiosis reported in dogs during the last three years.

Keywords: Babesia canis, dogs, clinical manifestation

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Feline Haemothropic mycoplasmosis – diagnostics and treatment

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Introduction

Feline haemothropic mycoplasmosis (FHM) is caused by gram-negative non-acid resistant bacteria, which attach to the external surface of erythrocytes [1,2]. Although the incidence of this disease is currently increasing in Poland, it is still quite rarely diagnosed in everyday practice. The clinical signs are most frequently non-specific. In some cases complications, such as haemolytic anaemia occur. The treatment is based on fighting the etiological factors, and also on treating the disease outcomes [3].

Aetiology

Mycoplasmas are staining, gram-negative pleomorphic cell-wall-deficient bacteria [3]. For many years they have been classified as rickettsia belonging to the genus of Haemobartonella or Eperythrozoon.

The organisms which have been strongly attached to the erythrocyte surface occurring as forms of granulomas or rods were classified as belonging to the genus of Haemobartonella. The organisms present between the erythrocytes, yet also attached to the red cells with a clear ring-shape form were classified as belonging to Eperythrozoon. These criteria seemed, however, inadequate to establish two separate genii [2].

The development of molecular diagnostics and sequencing method allowed for classifying all these parasites as mycoplasmas. As a consequence, the genii of Haemobartonella and Eperythrozoon were transferred to the genus of Mycoplasma. In cats, three different mycoplasmas have been identified: *Mycoplasma haemofelis* (previously defined as large forms of Haemobartonella felis), *Candidatus Mycoplasma haemominutum* (previously specified as small forms of *Haemobartonella felis*) and *Candidatus Mycoplasma turicensis* (2,5).

Diagnostics

The microscopic evaluation of the smears stained with the May Grunwald-Giemsa or Diff-Quick methods used in animals infected with mycoplasma, may reveal the presence of small, basophilic-stained, round, irregular structures on the surface of erythrocytes [3,4]. Within the course of infection, there are cyclic bacteraemias, that is why, even in acute infections, the result of the smear might be negative with regards to the presence of the above microorganisms [3]. Under EDTS, the mycoplasmas tear off the cell membrane of red blood cells and fall between the cells, giving an impression of a dirty smear. That is why it is recommended to make smears directly from a needle so that the blood should have no contact with an anti-coagulant [4]. It is also recommended to perform a few smears within a few days. Each positive result of a microscopic evaluation of the smear as for the presence of Mycoplasma species, should be confirmed with a PCR test, as these microorganisms may be easily confused with Howell-Jolly bodies, basophilic spots-on or other artefacts occurring in the sample as a result of an incorrect staining procedure. In the patients in whose case the result of the microscopic evaluation is negative, but clinical symptoms and disease history suggest the presence of an infection, an analysis of a blood sample with molecular diagnosis is recommended, as this procedure will detect bacterial DNA. It should be remembered that PCR diagnostics is sensitive enough to detect subclinical infections [3].

Pathogenesis

It is suspected that the infection vectors are the fleas (Ctenocephalides felis). In laboratory conditions, an infection might be induced by means of intravenous or intraperitoneal administration of a germ. It was also found that kittens may be infected by mothers-carriers. However, it was not established whether an infection occurs in an intrauterine manner, during the labour or during the care of the litter. In cats the course of the disease is usually asymptomatic and mild, or solely accompanied with a mild anaemia [7]. A direct parasitic damage of the blood cells is limited and a key role in the development of anaemia is played by immunological mechanisms.

Clinical symptoms thus depend on the stage of infection development, anaemia intensity and the immunological status of an infected pet. The condition of a patient reveals the signs of developing anaemia, such as pale mucosa, depression, anorexia, and sometimes jaundice or splenomegaly. In acute infections, fever occurs. In chronic infections, the temperature increase may fluctuate. The weight loss is observed in chronic patients and also, in these patients, clinical symptoms of infection may occur periodically, in particular during the periods of immunity decrease caused by stress [8]. The most frequent type pf anaemia observed in the course of mycoplasmosis is macrocytic normochromic anaemia or macrocytic, hypochromic anaemia when the infection is accompanied by other conditions leading to a chronic condition. In some infected cats also neutrophilia and monocytosis were observed [8]. In acute cases sever autoimmune haemolytic anaemia is developed.

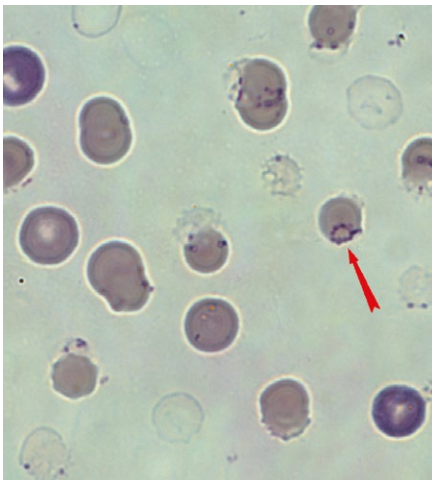

Figure.1 Mycoplasma haemofelis in MGG staining

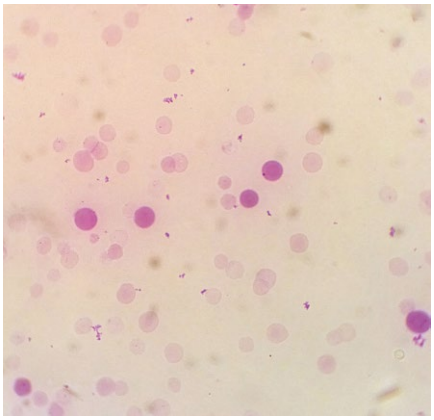

Figure. 2 The effect of a dirty smear. Mycoplasma haemofelis on the surface of erythrocytes and between them

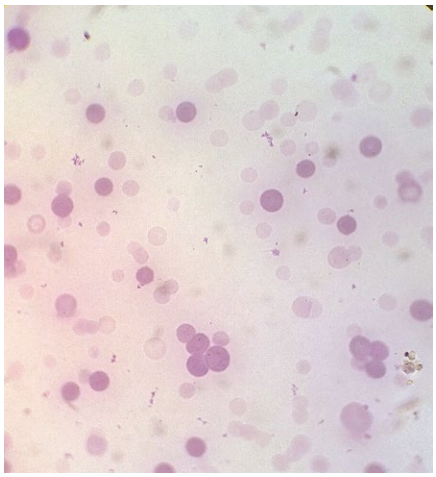

Figure. 3 The effect of a dirty smear. Mycoplasma haemofelis on the surface of erythrocytes and between them

Treatment

The treatment of choice consists in the administration of doxycycline at the dose of 10 mg/kg, PO, co 24 h, for 28 days.

If the infection has led to the development of autoimmune haemolytic anaemia it is necessary to implement immunosuppressive therapy with prednisolone at the dose of 3-4 mg/kg, PO, every 12 hours. Immunosuppressive therapy usually lasts several months and, in some cases, it is a lifelong therapy. It seems that tetracyclines used in the treatment of mycoplasmosis lead to the disappearance of clinical symptoms and reduction of parasitemia, yet they do not eliminate the infection. The recurrence of symptoms must be taken into consideration, even in a correctly conducted therapy. In the animals that do not tolerate doxycycline, enrofloxacin, at the dose of 10 mg/kg, PO, every 24 hours for 14 days must be administered. Depending on the clinical condition of a patient, sometimes fluid therapy and, in severe cases, blood transfusion is recommended [1,5,8].

The studies carried out recently prove that the risk factors in the infections with haemothropic mycoplasmas comprise the infections with immune deficiency viruses and feline leukaemia. The cats infected with feline leukaemia viruses or the viruses of immunological deficiency are much more exposed at the development of infection. It has been known for many years that in cats infected with FeLV, an acute form of mycoplasmosis with an accompanying anaemia develops much more often. Recent studies prove also that a co-infection of mycoplasma/FeLV increases the probability of microproliferative diseases in infected animals.

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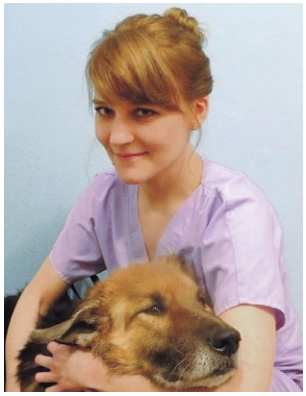
Leishmania Infantum in a Dog – a Clinical Case



Karolina Fidura DVM

Leishmaniasis is a serious disease affecting both humans and animals, caused by the *Leishmania spp.* parasite, belonging to protozoa. Many species of Leishmania have been identified (infantum, braziliensis, mexicana, donovani, tropica) and the majority of them has the zoonotic potential. This disease most frequently occurs among humans and dogs, yet also other mammals may become its reservoir.

Leishmaniasis generally occurs in various geographic latitudes. In Europe these are Mediterranean countries and Portugal, yet there are also reports about the incidence in France, Germany, Switzerland or the Netherlands. Apart from Europe, the endemic areas comprise South and Central America, Eastern, Western and Northern Africa, Eastern and Central India and China. The area where Leishmania can be found corresponds with the habitats of the Phlebotomus and Lutzomyia insects, which are the vectors of this disease.

The range of some endemic areas as well as the total number of infected animals has

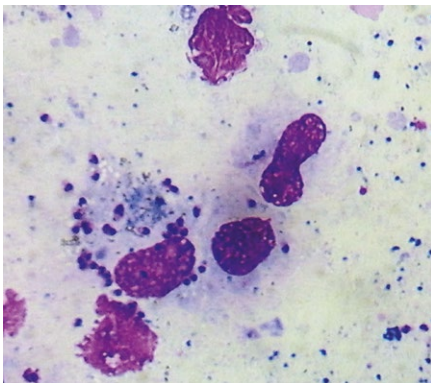


Figure.1

increased in the recent years. Most probably this is the consequence of larger mobility of the dog owners and the animals themselves, and also the climatic changes which enable the Tunga penetrans (chigoe flea) to colonize new areas. The cases of leishmaniasis, diagnosed in non-endemic areas occur most frequently in the animals imported from endemic areas or staying there even for short periods of time. However, there are reports about the episodes of this disease in the places where Phlebotomus does not occur. This is the reason for a suggestion of the existence of alternative vectors of the disease and the disease transmission by arthropods other than chigoe fleas. The largest risk of

morbidity concerns young adult dogs and older animals, yet there is no clear sex-related predisposition. An increased risk also concerns large dog breeds, such as boxers, German shepherd dogs or Rottweilers, whilst the lower infection risk is linked to the toy dog breeds. Most probably this is connected with their larger frequency of staying indoors. There are also some breeds which are resistant to infections, such as Podenco from Ibiza, which is resistant to Leischmania spp. infection. The frequency of infection increases in summer months when the number of vectors is increased.

PATOMECHANISM

Blood sucking insects which are the vectors, suck parasites in a form of amastigote (not flagellated tissue form), together with blood. Then Leischmania multiplies in the intestinal tissue of chigoe fleas and then transforms into promastigote body form – a flagellated one. Promastigotes, with their flagella, migrate to the sucking apparatus and then, during an episode of consumption, they are deposited in the skin of a mammal. Here, promastigotes are absorbed by macrophages and multiply inside them leading to the cell destruction and further spread of the parasite. Some other cells, apart from macrophages, such as Langerhans's cells or dendritic cells, may also be infected. These cells, presenting the parasite antigens on their surfaces, are able to induce the Th-lymphocyte response against the parasites. There are three types of responses which might occur after a contact with flagellates. The first type is based on the strong Th1-lymphocyte response, consisting in the production of cytokines, gamma interferon, TNF, interleukins IL-2 and IL-12. These cytokines stimulate the cellular response of an organism which is able to eliminate the infection. The animals which develop this model of response may have a positive titre of antibodies and keep the parasite in their organism before eliminating it. The other response model is based on Th2-lymphocyte response and increased production of interleukins IL-4,IL 5,IL- 6,IL-10, which stimulate B lymphocytes to proliferate and produce antibodies. Unfortunately, the antibodies do not perform a protective function, and may even have a harmful effect by producing and storing immunological complexes in the basal membrane. About 10-50% seropositive dogs do not present clinical symptoms, whilst in 80% of animals the symptoms of the disease may develop. The incubation period is very long and may last from 1 month to 7 years. During this period, the parasites are disseminated all over the organism with special predilection for haematopoietic organs, such as bone marrow, lymph nodes, spleen and liver. The damage caused by these parasites results from its direct effect on the tissue, stimulating the occurrence of such conditions as granulomatous infections, among other within the skin, liver, kidneys, intestines, eyes or bones as well as indirect effect consisting in immunological complexes storage in the basal membrane of the vessels, eyes, kidneys and joints, causing vasculitis, glomerulonephritis or uveitis.

Clinical symptoms

The clinical symptoms, which may be observed within the course of this disease, are generalised fatigue, decreased physical activity, loss of body weight with usually retained appetite, local or generalised enlargement of the lymph nodes and skin lesions. The dominating dermatological signs are symmetrical hair loss and skin dry seborrhoea – exfoliating dermatitis. Ulceration may also be present, mainly on limbs, bony eminences or dermal-mucosal junction, as well as nodules or pustules, mainly on the skin of the back. Less frequent signs comprise hyperkeratosis of the nasal planum, finger pads, onychogryposis (ram's horn nails), paronychia or depigmentation of the nasal planum. When it comes to the visual organ, among ophthalmologic disorders, the dominating conditions comprise uveitis, dry eye syndrome and blepharitis. Leishmania spp., when multiplying in the liver, may lead to its hepatitis, hepatomegaly, and also to emesis, polyuria and polydipsia and weight loss. Also chronic ulcerating colitis, gastrointestinal bleeding, acute haemorrhagic colitis and acute haemorrhagic pancreatitis have been described as the conditions occurring in the course of this disease. In the urinary system, the conditions which might occur comprise moderate to acute renal failure, which is related to the sedimentation of immunological complexes and development of glomerulonephritis. The accompanying proteinuria might lead to nephrotic syndrome and chronic renal failure. Heart failure and thrombosis have also been occasionally described, whilst nasal bleeding, unilateral in particular, is a frequent outcome of ulcerating lesions within the nasal mucosa.

Case description

On 26th August 2014, the clinic admitted a dog brought in by a foundation looking after stray dogs. The dog was found during an intervention around Warsaw. The case history was thus unknown. The animal was extremely emaciated with extensive skin lesions and strong pruritis. On the first day, the physical examination revealed mucosal paleness and slight viscosity, capillary refill of about 2 seconds, decreased heart rate and body temperature of 38 degrees Celsius. Popliteal lymph nodes were slightly enlarged. The abdominal palpation revealed no pain and chest auscultation was unremarkable. The rectal examination revealed light orange-coloured stool poorly filling the rectum with no anatomic abnormalities found in there. A strong pruritis was observed in the patient. The entire head and back were covered with scabs. The whole body was affected by the hair loss, excessive flaking, erythema and abrasions. Some slight erythema was also present in interdigital space and in perianal area, but there were no signs of erythema on internal side of the auricles and acoustic meatuses. Numerous fleas and ticks were found on the skin of the dog.

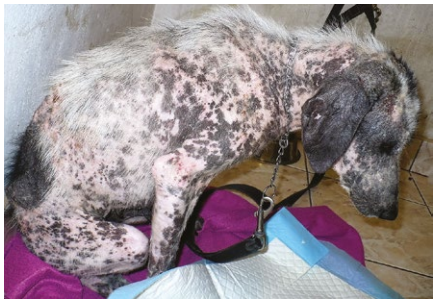


photo: Karolina Fidura

Blood biochemistry and lab tests were performed. CBC revealed moderate anaemia and blood smear – anisocytosis, some slight number of polychromatophilic cells, some isolated ovalocytes and target cells (codocytes), reactive lymphocytes and eosinophils. No microfilariae or developmental forms of Babesia spp were found. In blood biochemistry, some slight elevations of the liver parameters and urea were found together with the hyperalbuminemia and hyperglobulinaemia. On account of hyperalbuminemia, urine was tested together with the protein/creatinine ratio and also abdominal ultrasound was performed. The ultrasound did not reveal any perceptible lesions.

On account of the extensive skin lesions and large pruritis, deep surface epithelium scraping was performed, and hair was collected for microscopic evaluation, as well as skin lesion samples were collected for cytology and culture for dermatophytes was performed.

Table 1. The results of CBC on admission:

MCV	65.2 ft	60 – 72
MCH	24.5 pg	19 – 25.5
MCHC	37.5 g/dl	32 – 38.5
Erythrocytes	3.35 million /µl	5.5 – 8.5
Haematocrit	21.80 %	37 – 55
Haemoglobin	8.2 g/dl	12 – 18
Leukocytes	8.4 thousand /µl	6 – 16.5
Thrombocytes	248 thousand /µl	200 – 500
Lymphocytes	1.3 thousand /µl	1.2 – 5
Lymphocytes %	16.4	
Monocytes	0.8 thousand /µl	0.3 – 1.5
Monocytes %	9	
Granulocytes	6.3 thousand /µl	3.5 – 12
Granulocytes %	74.6	

Table 2. The results of blood biochemistry on admission:

Alt	165.8 U/l	< 60
Aspat	47.5 U/l	< 45
AP	258 U/l	< 155
Creatine	0.5 mg/dl	0.5 – 1.7
Urea	66.0 mg/dl	20 – 50
Glucose	70 mg/dl	70 – 120
Total protein	6.5 g/dl	5.5 – 7.5
Albumins	1.9 g/dll	3.3 – 5.6
Globulins	4.6 g/dl	2.1 – 4.5
Sodium	320 mg/dl	320 – 360
Potassium	22.6 mg/dl	16 – 21
Chlorides	393 mg/dl	350 – 410
Iron	137 µg/dl	94-195
Thyroxine	0.95 µg/dl	1.5-4

Table 3. Urine analysis on admission

Urine protein	580 mg/dl
Creatine level	69 mg/dl
UPC	8.52
Colour	yellow
Clarity	Slightly turbid
Specific weight	1.025 (normal range: 1.016 – 1.045
pH	Present (+++)
Glucose	negative
Ketones	Negative
Bilirubin	Negative
Urobilinogen	Negative
Leukocytes	1-3 within the range of sight
Erythrocytes	10-15 within the range of sight
Mucosa	absent
Bacterial flora	poor
Squamous cells	Isolated round within the range of sight
Crystals	Absent

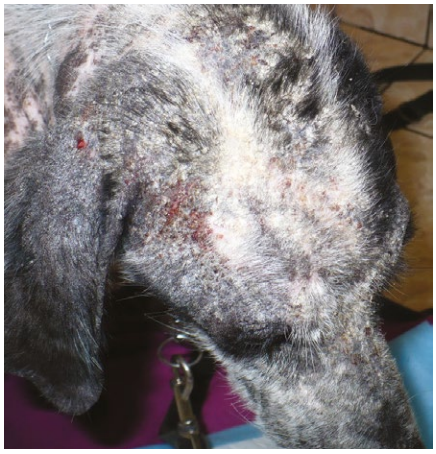


photo: Karolina Fidura

The evaluation of the scrapings revealed numerous adult forms of scabies eggs (Sarcoptes scabiei) as well as numerous flea faeces. The trichogram revealed hair in the telogen and anagen phases with broken hair shafts, yet no dermatophyte spores were found. The cytology findings comprised numerous degraded neutrophils phagocyting granulomas, isolated eosinophils, numerous corneocytes and nucleated epidermal cells. On the basis of the available examination results, fluid therapy was prescribed with NaCl applying the Duphylate preparation (the dog did not have appetite and was slightly dehydrated), as well as skin scabies therapy (selamectin in 3-week intervals), anti-flea and anti-tick prophylaxis with a preparation containing permethrin and imidacloprid every 3 weeks, baths in a shampoo with chlorhexidine every 4 days and deworming with Aniproazol for 5 consecutive days, antibiotic therapy with cefalexin at the dose of 250 mg every 12 hours and a diet based on fish as the sole source of proteins. The dog stayed in the hospital to be monitored with regards to the dynamics of blood lab parameters (CBS was recommended every 2 days). On the following day, the dog was in a much better condition. She was joyful and interested in the surroundings with good appetite, increased thirst and polyuria. For the next days, the dog's urine was tested to verify the protein level with the use of strip tests. Each time the results were positive, whilst the protein content was assessed to be 3+. On the fourth day, the patient's condition deteriorated; and loss of appetite and apathy were observed. Blood CBC and biochemistry tests were performed. Blood microscopic examination revealed merozoites of *Babesia spp.* On account of a persistent hyperglobulinaemia, serum protein electrophoresis was recommended, whereas with regards to a significant proteinuria – urine protein. When the electrophoresis was still pending, a non-aspiration fine needle biopsy of the enlarged popliteal lymph nodes was performed. The cytology of the lymph nodes specimen revealed amastigotes of *Leischmania spp.* located inside and outside macrophages. The finding was really surprising, as we had been informed that the dog was found in the Warsaw area; and Poland does not belong to Leischmania endemic areas. The person who found the dog was then questioned about their previous place of residence. It turned out that the animal was a stray dog brought from Monte Negro, so the diagnosis of Leishmania was very likely. With regards to the new developments, apart from the test of the *Leischmania spp.* antibody titre, the analysis of other pathogens which might occur in the above area was performed, such as *Hepatozoon canis, Hemotropic Mycoplasmas, Borellia burgdorferii, Anaplasma spp. Ehrlichia canis and Dirofilaria immitis.*

The treatment against Babesiosis was applied with Imidocarb (twice with 14-day intervals), atropine and intravenous fluid therapy. On the following day a significant improvement of the patient's condition was seen: the appetite returned and the overall condition was better. However, polyuria, polydipsia and proteinuria remained.

Table 4. Blood biochemistry 4 days after admission

Alt	98.7 U/l	< 60
Aspat	48.7 U/l	< 45
AP	173.3 U/l	< 155
Creatinine	0.8 mg/dl	0.5 – 1.7
Urea	75.8 mg/dl	20 – 50
Glucose	108 mg/dl	70 – 120
Total protein	6.5 g/dl	5.5 – 7.5
Albumins	2.0 g/dl	3.3 – 5.6
Globulins	4.6 g/dl	2.1 – 4.5
Sodium	327 mg/dl	320 – 360
Potassium	16.3 mg/dl	16 – 21
Chlorides	386 mg/dl	350 – 410
Total bilirubin	0.11 mg/dl	< 0.20
Calcium	8.9 mg/dl	8.4 – 11.5
Phosphorus	4.5 mg/dl	2.5 – 6.3
Iron	223 µg/dl	94 – 195

Table 5. CBC 4 days after admission

MCV	67.8 fl	60 – 72
MCH	25.1 pg	19 – 25.5
MCHC	37.1 g/dl	32 – 38.5
Erythrocytes	3.54 million /µl	5.5 – 8.5
Haematocrit	24.00 %	37 – 55
Haemoglobin	8.9 g/dl	12 – 18
Leukocytes	8.5 thousand /µl	6 – 16.5
Thrombocytes	140 thousand /µl	200 – 500
Lymphocytes	2.1 thousand /µl	1.2 – 5
Lymphocytes %	25.5	
Monocytes	1 thousand /µl	0.3 – 1.5
Monocytes %	10.3	
Granulocytes	5. 4 thousand	3.5 – 12
Granulocytes %	64.2	

The electrophoresis of the serum proteins showed a decrease of the albumin/globulin ratio, a significant hypo-albuminemia and polyclonal hyperglobulinaemia. In dogs infected with Leishmania, hypo-albuminemia may be caused by protein losing nephropathy, liver disease or malabsorption. Hyper-

Table 6. the results of tests for other pathogens

Hepatozoon canis (DNA) (real-time PCR)	negative	
Canine Haemotropic Mycoplasma		
Mycoplasma haemocanis DNA (real-time PCR)	negative	negativ
Cand. M. haematoparvum DNA (real-time PCR)	negative	negative
Filaria species-DNA (PCR)		
Dirofilaria repens	negative	negative
Dirofilaria immitis	negative	negative
Panfilarial-(6-species-PCR)	positive	+ negativ

Uzyskano również ujemny wynik dla Borellia burgdorferi, Ehrlichia canis, Anaplasma spp. oraz dodatni dla Dirofilaria immits.

Table 7. The results of tests for other pathogens

Serum electrophoresis (agarose gel)				
Total protein	62		54 - 76	g/l
A/G	0.3	- -	> 0.8	
Albumin (%)	22.9	- -	44.5 - 62.2	%
alpha-1 globulin (%)	3.2		2.3 - 4.2	%
alpha-2 globulin (%)	13.5		11.4 - 19.0	%
beta-1 globulin (%)	6.9		3.2 - 8.9	%
beta-2 globulin (%)	32.7	+ +	9.8 - 18.7	%
gamma globulin (%)	20.8	+ +	5.7 - 17.0	%
Albumin (abs.)	14.1	- -	24.0 - 47.0	g/l
alpha-1 globulin (abs.)	2.0		1.3 - 2.8	g/l
alpha-2 globulin (abs.)	8.3		6.0 - 13.0	g/l
beta-1 globulin (abs.)	4.2		1.8 - 6.6	g/l
beta-2 globulin (abs.)	20.1	+ +	5.1 - 13.0	g/l
gamma globulin (abs.)	12.8	+ +	3.5 - 9.4	g/l
Leishmania antibodies (ELISA)	70.4	+	< 7.0	TU

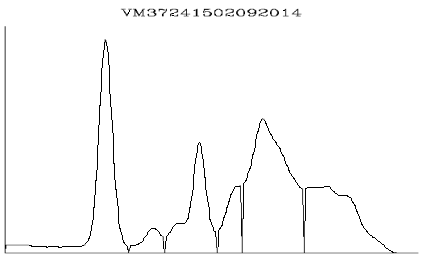
globulinaemia, in turn, with an increase in beta and gamma-globulins levels is the result of a polyclonal activation of B lymphocytes and antibodies production. This patient presented the protein distribution in electrophoresis which was typical for Leishmaniasis, and a significantly elevated level of antibodies against *Leishmania spp.*

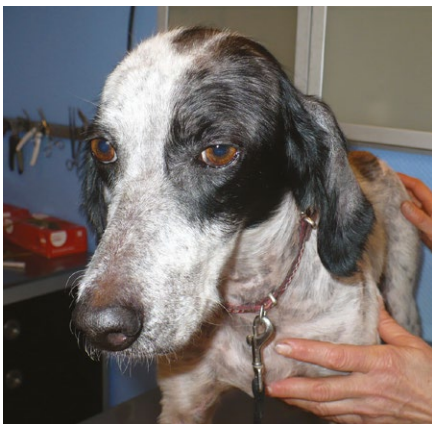
The electrophoresis of urine proteins revealed some glomerulotubular injury, which might accompany renal failure as secondary to the glomerular nephritis or diabetes, hypertension or pyelonephritis-induced nephropathy. The presence of proteins with large and smaller particle weight, such as

photo: Karolina Fidura

IgG, albumins, micro-globulins was seen. Electrophoresis performed in polyacrylamide gel revealed 2 groups of proteins with very low particle weight, suspected of belonging to monomers and dimers of Bence-Jones proteins.

On the basis of the tests performed so far, an additional diagnosis of Leishmania infection was made. Together with the dog caregivers, a decision was made to start a combined therapy with allopurinol at the dose of 10 mg/kg every 12 hours and meglumine antimoniate at the dose of 100 mg/kg 1 daily





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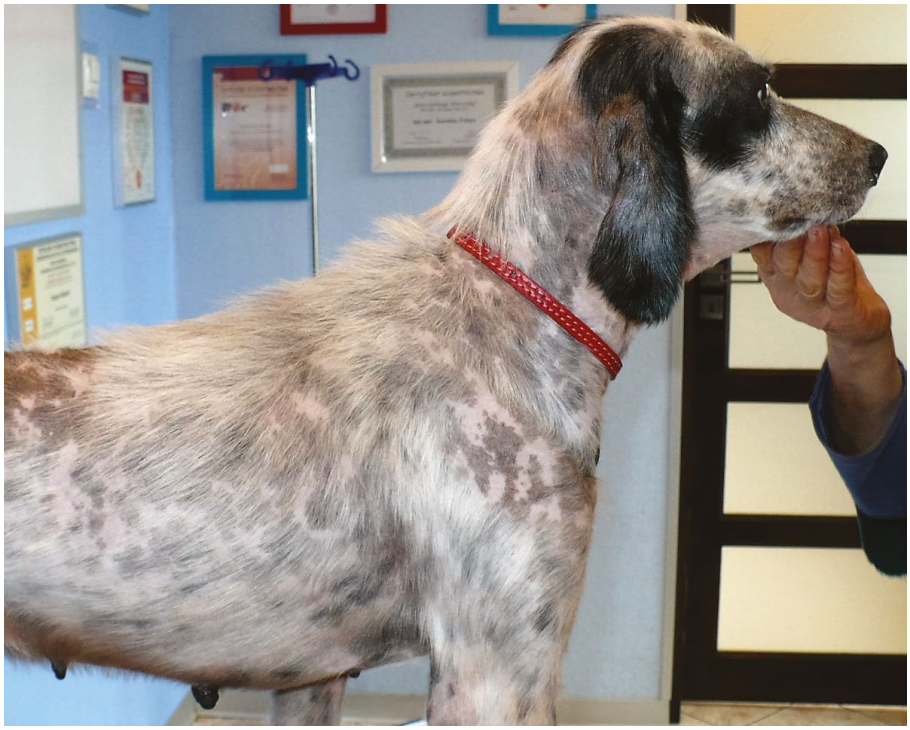

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(Glucantime preparation). While awaiting the delivery of Glucantime (the medication was not available in Poland and had to be imported from Spain) the therapy with allopurinol alone began.

Allopurinol is a hypoxanthine analogue, which is first converted and then built into the parasite RNA, where it inhibits its multiplication. It is very little toxic for dogs, hence it is appropriate for a prolonged use. Unfortunately, it does not eliminate the parasite from the host organism. That is why it is used in combination with other agents acting against Leischamnia spp., such as, for instance, meglumine antimoniate, which interferes with the process of parasite glycolysis. The use of meglumine antimoniate does not prevent the recurrences, which are present in about 75% cases after 6-8 months. The combination of this agent with Allopurinol increases the therapy efficacy and decreases the recurrence rate. Unfortunately, none of the drugs is able to eliminate the parasite completely from the host organism. The available treatment protocols suggest the use of Glucantime at the dose of 100mg/kg per day for 20-30 together with Allopurinol, administered for 9-12 months or even for the animal's lifetime. After the completion of the Glucantime therapy, the dogs should be monitored with serum protein electrophoresis, as they remain potential carriers of the parasite. If a recurrence is expected (the return of the changes in the serum protein electrophoresis), the re-administration of meglumine antimoniate is recommended. The patient should be monitored every 3-6 months till the end of the life. The test for the Leishmania antibodies level is not used for monitoring the disease.

Within 2 weeks of the administration of Allopruinol alone, proteinuria and anaemia were reduced in our patient whilst the globulins level decreased and albumin level was elevated. The dog felt very well with a good apatite and thirst, decreased pruritus and improvement of the skin condition. In the meantime, the result of the dermatophyte culture for the presence of dermatophytes was negative.

After 2 weeks of Allopurinol administration, Glucantime was added – it was administered once per day in a subcutaneous injection, and subsequently when it was seen that the dog tolerated the treatment well, the patient was discharged home. The anti-flea and anti-tick prophylaxis was recommended to be used every three weeks with a preparation which was also a repellent. The caregivers were informed about possible side-effects of the drug, such as pain in the injection site and its potential nephrotoxic effect; the prompt visit at the clinic was recommended if there were signs suggesting dehydration related to diarrhoea, emesis or adipsia. The follow-up visit took place in the 18th day of the combined therapy. The dog looked very well, having gained weight of about 5 kg; the hair grew back and the patient had a very good appetite and thirst. In the opinion of the caregivers, the patient tolerated the medication very well. The appearance of oestrus was observed. CBC, blood biochemistry and urine analysis were performed and the examination for the presence of *Dirofilaria immitis* was repeated.

The test for the presence of *Dirofilarii*

Table 8. CBC 18 days of the introduction of treatment

MCV	67.4 fl	60 – 72
MCH	24.9 pg	19 – 25.5
MCHC	37 g/dl	32 – 38.5
Erythrocytes	4.2 million/µl	5.5 – 8.5
Haematocrit	28.30%	37 – 55
Haemoglobin	10.4 g/d	12 – 18
Leukocytes	13.9 thousand/µl	6 – 16.5
Thrombocytes	288 thousand//µl	200 – 500
Lymphocytes	3 thousand//µl	1.2 – 5
Lymphocytes %	21.6	
Monocytes	0.9 thousand//µl	0.3 – 1.5
Monocytes %	6.6	
Granulocytes	10 thousand/µl	3.5 – 12
Granulocytes %	71.8	

Table 9. Blood biochemistry 18 days of the introduction of treatment

Alt	36.2 U/l	< 60
Aspat	23.4 U/l	< 45
AP	37.2 U/ll	< 155
Creatinine	1.1 mg/dl	0.5 – 1.7
Urea	79.2 mg/dl	20 – 50
Glucose	83 mg/dl	70 – 120
Total protein	7.1 g/dl	5.5 – 7.5
Albumins	2.7 g/dl	3.3 – 5.6
Globulins	4.4 g/dl	2.1 – 4.5
Sodium	339 mg/dl	320 – 360
Potassium	20.2 mg/dl	16 – 21
Chlorides	415 mg/d	350 – 410
Iron	112 µ/dl	94 – 195
Thyroxine	1.19 µ/dl	1.5 – 4

Table 10. Urine analysis

Urine protein	69 mg/dl
Creatinine level	30 mg/dl
UPC	2.3
Colour	Light yellow
Clarity	Clear
Specific weight	1.011 (normal range: 1.016 – 1.045)
pH	6 (normal range: < 6.5)
Protein	Present (++)
Glucose	Negative
Ketones	Negative
Bilirubin	Negative
Urobilinogen	Negative
Leucocytes	1-3 within the range of sight
Erythrocytes	3-5 within the range of sight
Mucosa	Absent
Bacterial flora	Poor
Squamous cells	Quite numerous polygonal, isolated round within the range of sight
Crystals	Absent
Haemoglobin traces	Negative

immitis gave a positive result again, whilst Knott's test for the presence of microfilaria was negative. Hypoalbuminaemia and hyperglobulinaemia decreased and the serum albumin/globulin was increased. The urine analysis revealed a reduction of protein loss and a decrease of the protein/ creatinine ratio from 8.52 to 2.3.

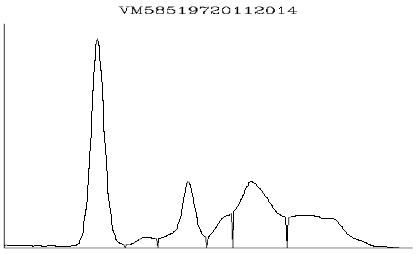
The continuation of the treatment was recommended with a follow-up blood and urine tests after one week if the dog felt well. In case of worrying symptoms, some earlier check-up was recommended. Additionally, a spot-on preparation, containing moxidectin and Imidacloprid was recommended to be administered every 4 weeks to prevent the prospective development of microfilariae.

After one week, the caregivers brought the dog to the clinic because of her poor condition. Vomiting persisted for two days and the dog had no appetite or thirst. One day before that the Glucantime therapy was discontinued. The physical examination revealed mucosal viscosity, slightly tense abdominal wall and slight pain in the abdominal cavity, the body temperature was 38.6 Celsius.

CBC revealed leucocytosis: 28 thousand/µl; anaemia was maintained at a constant level, whilst biochemistry showed a significant elevation of renal parameters (creatinine – 4.7 mg/dl, urea – 206 mg/dl). The test for pancreatitis, measuring the level of canine pancreatic lipase was positive. The abdomi-

Tabela 11. The results of tests for other pathogens

Serum electrophoresis (agarose gel)				
Total protein	72		54 - 76	g/l
A/G	0.5	- -	> 0.8	
Albumin (%)	34.1	- -	44.5 - 62.2	%
alpha-1 globulin (%)	2.6		2.3 - 4.2	%
alpha-2 globulin (%)	12.9		11.4 - 19.0	%
beta-1 globulin (%)	6.3		3.2 - 8.9	%
beta-2 globulin (%)	25.1	+ +	9.8 - 18.7	%
gamma globulin (%)	19.0	+ +	5.7 - 17.0	%
Albumin (abs.)	24.5	- -	24.0 - 47.0	g/l
alpha-1 globulin (abs.)	1.9		1.3 - 2.8	g/l
alpha-2 globulin (abs.)	9.3		6.0 - 13.0	g/l
beta-1 globulin (abs.)	4.5		1.8 - 6.6	g/l
beta-2 globulin (abs.)	18.1	+ +	5.1 - 13.0	g/l
gamma globulin (abs.)	13.7	+ +	3.5 - 9.4	g/l
Leishmania antibodies (ELISA)	68.0	+	< 7.0	TU



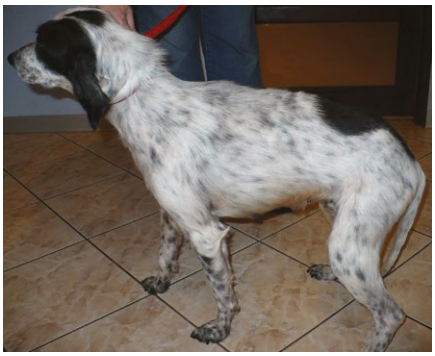


photo: Karolina Fidura

nal ultrasound revealed a "non-homogenic renal echogenicity and incorrect corticomedullary ratio with significantly enlarged renal pelvis". In order to exclude other diseases which might lead to renal failure and which do not develop as the complication of Leishmaniasis, a test for leptospirosis was performed (the dog's history and risk of exposure were unknown). The result was negative. Intravenous fluids were administered and antiemetic (maropitant) and analgesic therapy (buprenorphine) were given; also the drugs protecting the gastric mucosa (ranitidine), oral Alugastrin and enrofloxacin were administered. The dog was hospitalised. The patient kept urinating, yet her condition was continually deteriorating. Haematochezia appeared. In spite of the use of a combination of analgesic agents, the pain in the abdominal cavity increased. After 2 days the blood biochemistry was repeated and it revealed a further increase of the renal parameters (creatinine: 6.1 mg/dl, urea 235.3 mg/dl, phosphorus: 10.5 mg/dl, potassium: 21.7 mg/dl, chlorides: 420 mg/dl) and an elevation of leucocytosis to 40 thousand /µl. Within the next two days of fluid therapy and the above-listed mediation, the creatinine level still kept increasing, reaching the level of 8.6 mg/dl, urea – to 318 mg/dl and leucocytosis to 50 thousand /µl. On account of the deteriorating condition (also neurologic symptoms and dyspnoea appeared with a lack of possibility of maintaining body temperature) and a clear suffering of the animal and her poor prognosis, a decision was made, after consulting the caregivers, to euthanize the dog for humanitarian reasons.

photo: Karolina Fidura

Leishmaniasis is a disease which might render diverse clinical symptoms. Frequently the symptoms of the disease are masked by the comorbidities (e.g. babesiosis, skin scabies, tickborne diseases, such as ehrlichiosis or boreliosis) or may imitate other diseases, such as systemic lupus or multiple myeloma. The prognosis varies from good to poor, depending on the stage of the disease in which an animal is seen by a doctor. Hence, an early diagnosis, therapy and disease monitoring are of great significance for the further prognosis.

"Exotic diseases" which are not endemic in Poland should be taken into consideration in differential diagnosis with an increasing frequency; as our patients, when travelling with their owners are exposed to contacts with the vectors unusual for our geographic region. Therefore, a detailed history taking is often the key to a diagnostic success.

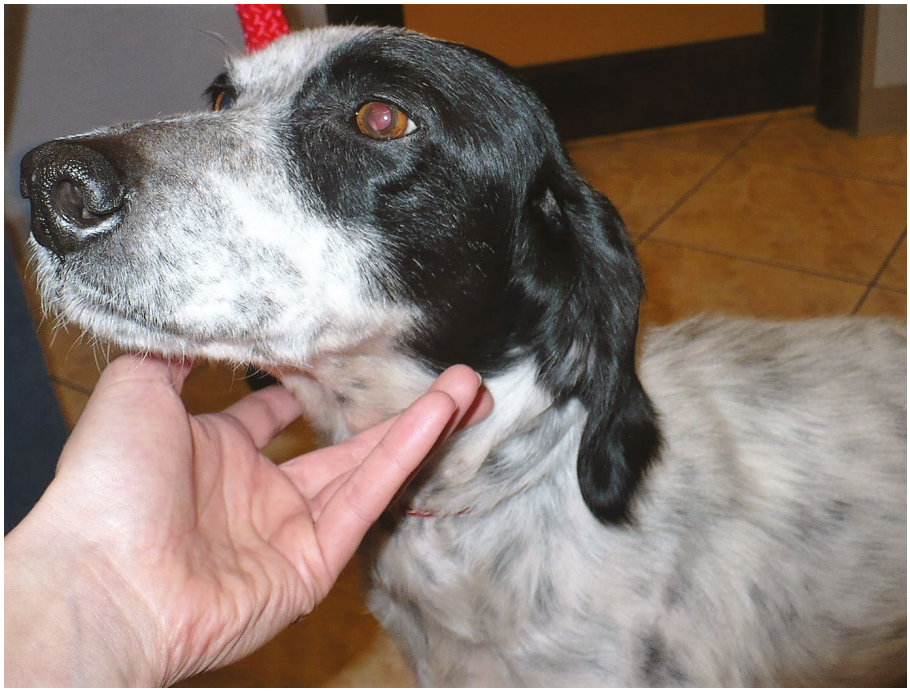


photo: Karolina Fidura

CRYPTOSPORIDIOSIS IN HEDGEHOGS

Katarzyna Ptak, DVM, "Animal Care" Veterinary Clinic, Kłodzko
Jerzy Gara, "Jerzy dla Jeży" Hedgehog Rehabilitation Center, Kłodzko

Hedgehogs are a very important element of our ecosystem, our silent daily companions. These spiky animals feed on insects and thus help reduce the population of plant pests. They are able to eat as many as 200 grams of insects in a single night; those killed with pesticides, however, often turn out poisonous for them. In temperate climates, hedgehogs go into hibernation in winter. In Poland, two main species of European hedgehogs can be observed: the northern white-breasted hedgehog (Erinaceus roumanicus) and the West European hedgehog or common hedgehog (Erinaceus europaeus). Both species have been covered by partial protection since 2014. Just like most insect-eating species, hedgehogs prey at night; whenever they are seen by day, this should be a cause for alarm.

A large proportion of hedgehogs in Poland build habitats in urban centers and struggle with related problems; many fall victim to lawn mower accidents, suffer when leaves are burnt in gardens, and are often bitten by dogs.

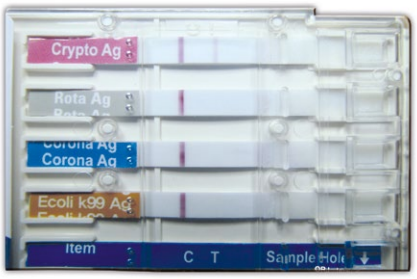


photo: Jerzy dla Jeży

A universal problem that affects all hedgehogs regardless of habitat has to do with internal and external parasites. The most vexatious among the latter include ticks and house fly larvae, which often breed in wounds.

Internal parasites most commonly found in hedgehogs include nematodes from the Capillaria and the Crenosoma genus, tapeworms, flatworms, and coccidia. For this reason, every sick hedgehog should have its feces tested for the presence of dispersing parasites (oocysts, eggs, or larvae).

An interesting, albeit rarely diagnosed, parasitic disease is cryptosporidiosis, which occurs mainly in young hedgehogs living in the countryside.

The Cryptosporidium genus comprises parasitic protozoans that attack the epithelium of intestinal villi and cause diarrhea in animals with impaired immunity. The infection may prove persistent and life-threatening. The incubation period takes 1-14 days (7 days on average) and the disease usually subsides around day 8 or 9, even though there are reports of infections that last as long as 100-120 days.

Relapse is frequent and occurs in up to

photo: Jerzy dla Jeży

photo: Jerzy dla Jeży

photo: Jerzy dla Jeży

photo: Jerzy dla Jeży

40-70% of patients. The first clinical symptoms include watery diarrhea, often quite profuse and with traces of blood or mucus.

Oocysts of the parasite are ubiquitous in the environment. Transmission may be direct or indirect, either through food or water.

In order to differentiate between species within the Cryptosporidium genus, it is not enough to rely on the morphological features of the developmental cycle of the parasite and the nature of its host. Nor can differentiation be based on oocysts, the smallest external forms of the protozoan.

Instead, the features to take into consideration include:

1. the morphometric properties of oocysts
2. the features of the genetic code saved and deposited in the GenBank
3. the natural and experimentally possible hosts
4. conformity with the International Code of Zoological Nomenclature.

Twenty five species of the Cryptosporidium genus have been officially identified in hedgehogs to date.

Cryptosporidium may cause clinical symptoms that sometimes prove fatal. One such case has been reported in a Baltimore zoo; developmental stages of the parasite were discovered in the ileum, jejunum, and colon of the animal. A report also exists of a West European hedgehog with hemorrhagic diarrhea that showed a moderate to severe degree of atrophy of intestinal villi in the jejunum and the ileum. Oocysts of Cryptosporidium were also detected (Sturdee 1999) in the feces of free-living hedgehogs in Great Britain, in the amount of3000 oocysts/g of feces. In Germany (Dyachenko 2010), 30% of 188 studied hedgehogs tested positive for Cryptosporidium parvum of the gp60 subtype of the IIa and IIc family, as well as the gp60 subtype of the XIIa (formerly VIIa) family. Even if tested hedgehogs manifested diarrhea, its connection to Cryptosporidum spp. was not demonstrated.

Several cases of cryptosporidiosis among young West European hedgehogs admitted to rehabilitation centers around Europe were identified as caused by Cryptosporidium spp from the VIIa family, which is closely related to C. parvum but genetically distinct and probably specific for hedgehogs (a genotype has a yet unknown zoonotic potential)(Clinical veterinary advisor– Jorg Mayer, Thomas Donnelly).

Even though the incidence of cryptosporidiosis in Polish hedgehogs is still being investigated at the moment, the disease should be kept in mind when an untypical patient shows up at our clinic.

CASE STUDY

On 12 August 2016, a 255g common hedgehog was admitted to the Hedghehog Rehabilitiation Center. The animal was in bad overall condition, emaciated, apathetic, and without appetite. After it was rehydrated and began to eat on its own, a parasitological test based on the modified McMaster method was conducted (Roepstorff and Nansen 1998); the result pointed to Capillaria spp 220 epg. Effective treatment eliminated the parasite and the hedgehog returned to good overall condition; however, diarrheas with pieces of undigested food persisted. A test was then conducted to rule out Cryptosporidium; the hedgehog tested positive. The animal was put on treatment with Gabbrocol administered every 24 hours for 5 days. It recovered and is currently being prepared for hibernation at the center, to be released into the wild next year.

In the treatment of cryptosporidiosis, especially in crowded centers, it is necessary to maintain adequate hygiene and isolate sick animals.

This year, the center used the VetExpert Rapid Cryptosporidium Ag test to screen 30 hedgehogs for cryptosporidiosis; of those, eight tested positive, equal to the prevalence rate of 27%.

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photo: Jerzy dla Jeży

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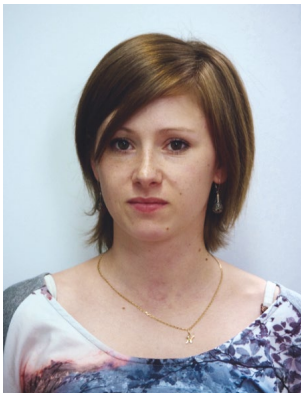
Giardiasis - are cross-species infections possible?



Natalia Jackowska, DVM

Everybody heard about the infections with Giardia duodenalis and everybody knows that Giardia belongs to "not very picky" parasites and interspecific infections are possible. In this paper I will try to describe the capabilities of this protozoan in detail, trying to find an answer to the basic question: is Giardia dangerous?

Giardia duodenalis belongs to flagellates, parasitizing on the cell surface of the epithelium of the small intestine in many animal species (from rodents through dogs, cats, pigs, ruminants to primates, including humans). Giardiasis is the main cause of parasitic diarrhoeas in humans and animals. The number of cases of Giardia infections in Poland in 2008-2011 was from 1736 to 3182 per year (according to the data of the NIP-PZH Institute: The reports of infectious disease cases, infections and poisonings in Poland), in animals the invasion prevalence varies from 5% to even 80%. In the studies carried out in Great Britain, the Netherlands, Belgium and Italy, the *Giardia* invasions were found in about 25% dogs and 20% cats with gastrointestinal disorders. The study, carried out in Germany, also showed a high rate of invasions: in 16% dogs and in 12% cats. According to the studies carried out in Poland in 2001-2006 in animals below 1 year of age, the prevalence in dogs was about 10% in Poznań, in Warsaw – from 9% to even 50%, in Lublin – about 53%, in Puławy – 10% and in Gdańsk – more than 16%. Some significant differences in the rate of infected animals resulted from the differences in the numbers of specific age groups of the studied animals and their diverse clinical conditions. The study carried out in 2007-2010 in Wrocław and the Lower Silesia region found giardiosis in 27.2% dogs and in 26.6% cats. What is interesting, *Giardia* invasion was more frequently found in pedigree animals, and first of all in young ones <6 months of life.

Giardia duodenalis was for the first time observed under a microscope in 1681 by Leeuwenhoek, a Dutch natural scientists, called the father of microbiology. Giardia though, owes its name to two other scientists, as almost 200 years later, an Austrian physician Wilhelm Dusan Lambl and a French zoologist, Alfred Mathieu Giard, independently from each other, discovered it again and described it. The correct nomenclature of this protozoan raises a lot of dispute, as the World Federation of Parasitologists recommends the use of the name of Giardia duodenalis, yet the name which is seen with an equal frequency in European publications is Giardia intestinalis, whilst in USA the most popular name is Giardia lamblia. The terms lambliosis or Lamblia intestinalis are regarded as incorrect.

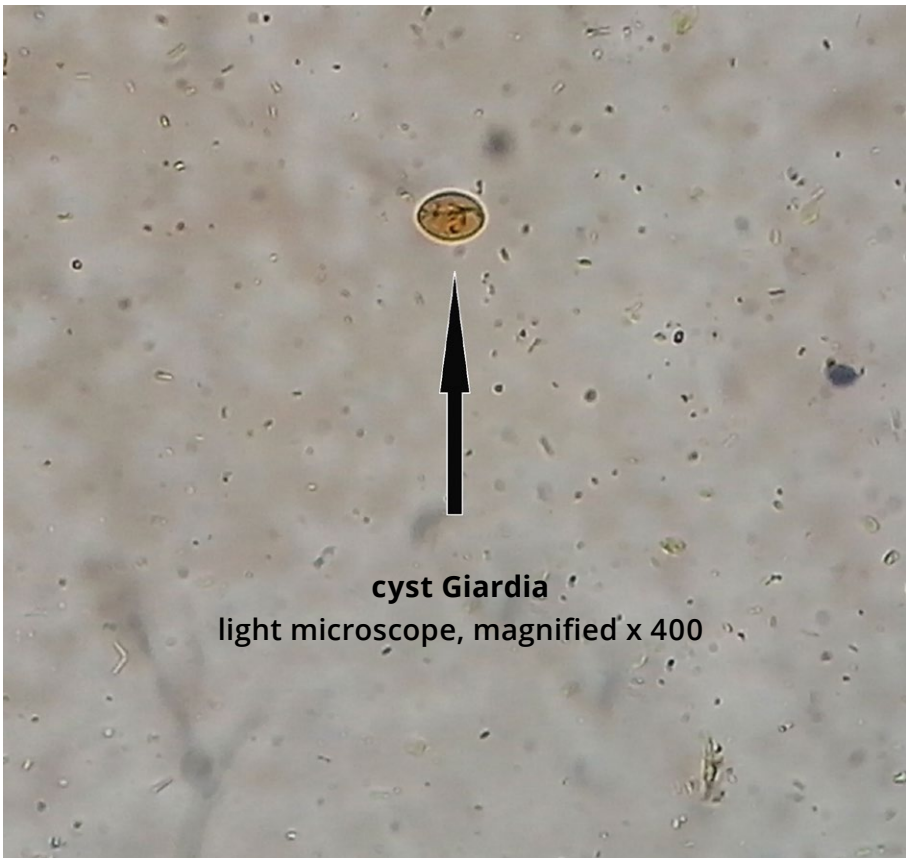


Figure 1. A Giardia cyst, light microscope, magnified x 400

Giardia duodenalis lagellate occurs in a form of trophozoites and cysts. The infection occurs via an oral route by consumption of infected water or food. *Giardia* does not have intermediate hosts; it has a cosmopolitan distribution with the frequency of occurrence depending on the region. Trophozoites are released from swallowed cysts in a duodenum. A trophozoite is bilaterally symmetrical, piriform and rounded on the front pole, sharpened in its posterior part; convex on the dorsal part and flattened ventrally. It may be 9-21 µm long and 5-15 µm wide. Below its front pole, two oval nuclei, resembling a pair of eyes may be observed. A trophozoite has four pairs of flagella which function as its motor organella. On its ventral side there is a large tendril disc which allows it to be attached to the erythrocyte surface. *Giardia* multiplies by means of longitudinal fission. A cyst is oval, measuring

8-18 x 7-10 µm, with four visible nuclei and primordial flagellae. This protozoan colonises first of all, a small intestine, yet it may also spread to bile ducts, gallbladder and pancreatic ducts. A complete formulation of the cyst wall takes about 16 hours. The prepatent period of invasion takes 5-16 days, whilst the period of cyst excretion in faeces varies from 4 to 5 weeks, yet it may also last whole months or years. The cysts are excreted with the faeces of the host in an irregular way and varied quantities.

Trophozoites destroy the surface of the intestinal epithelium, disrupting the excretory and absorptive function of the intestine. A intense invasion leads to mucosal inflammation of the small intestine, where the trophozoites attach with their tendrils. Usually the cysts are passed in faeces, but trophozoites may be found also in diarrhoeic faeces. Once trophozoites are spread into outside environment, they are unable to cause the disease, as they are very sensitive to environmental factors and they soon die, but cysts are very resistant to the environmental conditions.

The most important symptom of giardiasis is diarrhoea, which may occur already 5 days after the infection. The disease leads to a decrease of the appetite, acute or chronic colitis, diarrhoea (faeces may contain mucus, or, more rarely, blood) or fatigue. In young animals (<12 months) the course of the disease is severe and more acute. In adult animals, giardiasis may be asymptomatic, chronic with periodic shedding of cysts. Asymptomatic shredders pose a real threat to human health!

There are a few diagnostic methods in the diagnostics of giardiasis. The most popular method in human medicine, is to look for trophozoites and cysts in the direct smear of the faeces. In veterinary medicine, the classical method consists in looking for trophozoites and cysts in the samples stained with Lugol's iodine. An alternative for this method is provided by commercial tests, which are based, among others, on immunochromatographic method detecting the Giardia antigen in the examined faeces. A few facts must be taken into consideration while selecting the diagnostic method, namely: the classical microscopic method is more time consuming and requires a lot of experience so that Giardia cyst could not be overlooked and not mistaken with, for example, yeasts. It is easy to make a mistake in a dense sample in which all the constituents are stained yellow! The advantage of the classical method consists in a satisfaction in finding a trophozoite or cysts and, first and foremost, a lower price! The diagnosis based on immunological techniques is by all means faster and does not require any experience and allows us to diagnose the disease even in a situation when only few cysts are in the faeces. Fast diagnostic tests may also be used for monitoring the course of treatment. In the

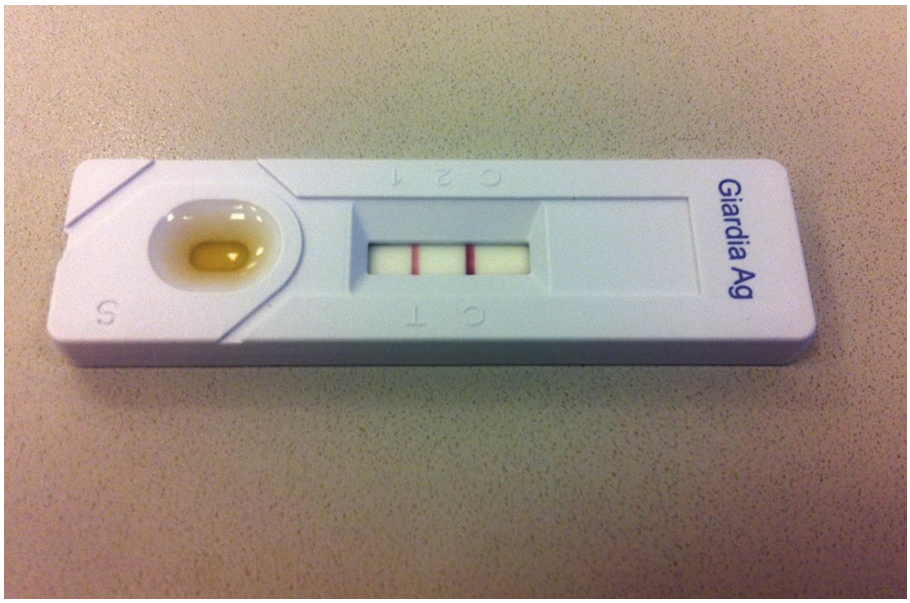


Figure. 2. A positive result in the VetExpert Giardia Ag quick test

correct diagnostics of giardiasis, whether according to the classical or immunological method, one must remember that cysts are excreted irregularly so their number drops below the level of detectability, which might render false negative results! When preparing a sample for analysis, it is recommended to collect a few samples from an entire week e.g. 3 samples every second day. The faeces should be stored in a fridge. A sample may be mixed and then a pooled sample analysed. There are 8 genotypes within the species of Giardia duodenalis. In order to classify the samples to specific genotypes, the molecular biology techniques are necessary. In the majority of cases the genotypes of group A or B are identified (among others in humans); genotype C and D are frequently isolated from dogs, genotype E is most frequently found in ungulates, genotype F is isolated from dogs and genotype G from rats. It must be remembered that genotypes A and B have a vast group of hosts, including humans and animals, which is illustrated in the table below: Giardiasis is a zoonosis and perhaps even a "reversed zoonosis"? If a human is able to be infected from a dog with genotype A or B, a dog may also be infected from a human being … Children are the most prone to infections as their immunological system is less efficient. The largest number of infections is observed in children below 1 year of age. A study was carried out in 2007 in southern Italy: the incidence and genotype of G. duodenalis was determined in 14 children and 14 dogs from a closed population living at the social margin. It turned out that about 50% children and more than 50% dogs were infected with genotype A1 of G. duodenalis! The invasion in children is accompanied with diarrhoea, lack of appetite, abdominal pain, nausea and vomiting. The symptoms may resolve spontaneously after a few days, yet the disease may also be chronic and wasting. Taking into consideration the possibilities of interspecific transmission and asymptomatic course of the disease, when Giardia infection is diagnosed in one family member, all other members of this family must be carefully examined to check whether they have been infected!

Giardia duodenalis	
A-1	Humans, dogs, cats, cattle
A-2	Humans, dogs
B	Humans, dogs, guinea pigs,
C	Dogs
D	Dogs
E	Ruminants
F	Cats
G	Rats

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Nutrition in the treatment of viral diseases that affect the digestive system of cats and dogs

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Diet therapy is not the basic form of treatment for infectious disease, but may play an important role in certain conditions, especially in viral and bacterial infections that affect the digestive system. The present article aims to discuss the fundamental principles of nutrition for patients with infectious diseases.

In the veterinary medicine of companion animals, infectious diseases play a vital role for a number of reasons. Some may be zoonoses, which usually require intensive therapy; in addition, no causal treatment is available for most infections, especially those transmitted by viruses, which makes prevention, including vaccines, especially important. From the dietary perspective, the greatest challenge is posed by diseases in which the pathogen causes damage to the digestive tract, which, on the one hand, affects the animal's ability to digest and absorb nutrients, often leading to malnutrition, and on the other, causes chronic clinical symptoms such as diarrhea and vomiting. These are particularly frequent in the course of infections caused by parvoviruses, coronaviruses, and rotaviruses. In all these cases, the chief clinical symptom involves acute gastroenteritis.

Is fasting necessary?

Fasting is one of the chief principles of viral gastroenteritis treatment in dogs; it is recommended that the affected animal should be taken off food for 24-48 hours to give irritated intestines the chance to "rest", decrease the risk of vomiting, boost the proliferation of bacteria in the digestive tract, and reduce the severity of osmotic diarrhea. In recent years, however, it has been shown that the animal may in fact benefit more from being "fed despite diarrhea", primarily because the practice helps maintain the integrity of the intestinal barrier, which is of key importance for preventing microorganisms and their toxins from entering the body through damaged intestinal walls. Studies have demonstrated beyond any doubt that even a single day of fasting significantly reduces the length of intestinal villi and decreases the depth of intestinal crypts; it also decreases the activity of important enzymes, especially disaccharidases (lactase, maltase). As a consequence, the digestive function is severely impaired, which will become apparent once food is reintroduced. This is true for the rotavirus infection, for instance, which often causes a temporary lactase deficiency. Studies with dogs suffering from parvovirosis shave have shown that the early introduction of enteral nutrition considerably shortens the time required to normalize behavior, appetite, vomiting and diarrhea, and improves the regulation of intestinal permeability, as compared with dogs that are kept off food until vomiting has subsided (Mohr and Leisewitz, 2003). Accordingly, it would seem that nutrition at the early stages of the disease should not do any harm to the animal; moreover, complex diets have better effects than highly purified variants (based on a single ingredient). However, a lot depends on the state of the individual patient; feeding animals in a state of incomplete consciousness may result in aspiration pneumonia. For this reason, it is now recommended that in severe cases of gastroenteritis, fluids should be administered for the first 3-4 hours, before any food is introduced. It stands to reason that the animal will not be able to ingest its normal food intake, which is why it should be limited to 25% of the daily energy requirement in the form of highly digestible, low-fat diet, in the spirit of "minimal luminal nutrition", i.e. providing the animal with the minimum amount of food to ensure the normal functioning of cells in the digestive system.

A diet for patients with viral or bacterial gastroenteritis

A diet for patients with viral or bacterial gastroenteritis should be characterized by:

1. High digestibility. Digestibility is not declared on commercial product labels and it is difficult to estimate based on the appearance and composition of the food in question. However, the market currently offers a variety of prepackaged commercial products advertised as highly digestible, such as intestinal or convalescence diets, and some ingredients of home-cooked meals, such as fresh chicken, fish, cottage cheese, and eggs, also fall into that category. Boiled potatoes and rice, and especially the latter, are an excellent source of carbohy-

Table 1. Home-cooked meals for cats and dogs with acute gastroenteritis (based on Cave, 2012):

Recipe	Energy value	% of calories from protein	% of calories from fat	% of calories from carbohydrates
Cottage cheese (1% fat) and boiled white rice; weight ratio: 1:1	1 kcal/g	33%	6%	61%
Cooked chicken breast (peeled) and boiled white rice; weight ratio: 1:4	1,3 kcal/g	26%	6%	68%

drates. Importantly, boiled rice contains a small-molecule, non-protein lipophilic compound capable of blocking chloride channels in the intestinal epithelium, whose permeability typically increases during secretory diarrhea. Its beneficial effects were also reported in humans (Mathews et al., 1999; Gore et al., 1992).

2. Low fat content. Preference should be given to foods with the lowest possible concentration of fat.
3. Source of proteins. Animals should not be given any new source of protein, even though some hydrolyzed diets may prove very beneficial.
4. Dietary fiber. While it is true that dietary fiber proves helpful in many digestive tract disorders, in acute enteritis its quantities should not exceed 5% of the total intake of raw fiber. In most convalescence diets, the concentration is limited to less than 1%. The digestibility level of fiber is zero, so if the objective is to supply highly digestible ingredients, the substance should be avoided.
5. Food intake. Food intake during the first several days of infection should not exceed 25% of the daily resting calorie requirement divided into three meals (Cave, 2014).

Nutrients essential in the diet of patients with viral and bacterial enteritis

Glutamine

Glutamine is an amino acid that serves as the principal source of energy for leukocytes and the epithelial cells of the small intestine. It also delivers the nitrogen needed to synthesize purines, i.e. special bases necessary for normal cell division in fast-dividing cells, such as enterocytes. Oral administration of glutamine boosts the growth and repair of the mucous membrane in the digestive tract, limits the migration of bacteria, and reduces inflammation. In order to achieve the beneficial biological effect, however, it is not necessary to supplement isolated glutamine; foods rich in the nutrient, such as casein, whey, or soy will be enough. Large quantities of glutamine are also found in many fluids designed for use in patients who are fed artificially. Glutamine supplements, however, are relatively expensive and unstable, which means they cannot be stored for extended periods of time (Decker, 2002). Literature recommends various doses: e.g. 0.5 g/kg month/day or 15 g/day divided into three doses.

Zinc

Oral administration of zinc helps restore the damaged epithelium, normalizes the processes of absorption, and decreases the permeability of the mucous membrane. It is also helpful in normalizing the absorption of water and electrolytes and stabilizing the enzymes of the brush border; for this reason, it is particularly beneficial in the treatment of damaged digestive tract. Zinc can be found in a variety of food supplements, but also in the liver. Dosage differs depending on whether the product is organic or non-organic, a salt or zinc chelate. The exact form needs to be determined before a specific dose is prescribed to the patient.

Conclusions

Acute viral or bacterial gastroenteritis should not be a reason to prescribe fasting until the disappearance of symptoms or for at least 24-48 hours. Instead, during the first days of the disease, the patient should receive around 25% of the normal calorie intake in the form of a highly digestible, low-fat and low-fiber diet. An excellent solution is boiled rice, which can be combined with cottage cheese or cooked chicken breast.

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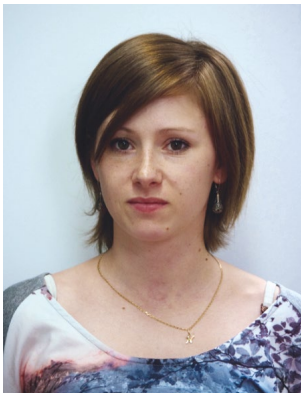
Quick diagnostic tests in daily practice



Natalia Jackowska, DVM

The history of quick bedside tests goes back to the 1960s, when the first pregnancy tests were introduced. Based on the immunochromatographic (IC) method, quick tests are now widely used to diagnose conditions such as flu, tonsillitis, and H. pylori infection. Despite their innocent appearance, they hide carefully selected gold-conjugated monoclonal antibodies under their plastic cover. The test strip is lined with antibodies specific for the diagnosed disease and the control strip with those for gold-conjugated monoclonal antibodies. For the result to be considered valid, the control line needs to show up. If the tested sample contains the antibodies or antigens in question, both the test line and the control line will appear. Most quick tests are qualitative assays: the result is positive no matter whether the line is thick or thin. The IC method does not entail any special requirements; tests can be stored at room temperature and allow to arrive at an accurate diagnosis in a matter of minutes. Words of praise are definitely in order, but one should also bear in mind certain limitations.

Quick tests for cats – FeLV/FIV

Feline medicine frequently looks for the antigen of the feline leukemia virus (FeLV) and the antibodies against the feline immunodeficiency virus (FIV). What should we keep in mind when diagnosing these conditions? Tests for the feline leukemia virus are designed to detect the antigen of the viral capsid – protein 27 (p27), and a positive test result attests to the presence of the virus in the bloodstream (viremia). The course of the disease may vary; permanent viremia may develop, the disease may progress into the latent phase, or the virus may be eliminated from the organism. In the second scenario the virus is incorporated into the host cell (proviral DNA is integrated with the genome of the host), and quick tests that detect the protein of the viral capsid will fail to diagnose the disease; in such cases, it is essential to conduct a PCR assay (to detect the proviral DNA). If the virus is eliminated, the test result will come out negative and the cat will remain immune to future infections. Importantly, the antigen used in vaccines is different from that in tests, so that vaccination should not affect test results.

Quick tests for FIV (feline immunodeficiency) are designed to detect antibodies. In this case, however, time is of the essence. FIV antibodies can only be detected after six months have elapsed from the moment of infection. In practice, this means that quick tests are not appropriate for kittens; in addition, whenever test results come out negative, they should be repeated six months later or confirmed with another diagnostic method.

Quick tests for dogs – vector-borne diseases

In recent years, there has been a growing interest in diagnostic tests for vector-borne diseases. Non-specific clinical symptoms (fever, general malaise, anemia, thrombocytopenia) mean that it is difficult to differentiate between dirofilariasis, ehrlichiosis, anaplasmosis, and borreliosis. A quick test makes differentiation possible, but each disease has its own requirements. Tests for D. immitis are now able to detect the relevant antigen in the reproductive tracts of mature females, but the maturation time of the heartworm (at least 6 months), as well as the fact that only males and immature females are invaded, means that the test can come out negative. The Polish market offers tests designed to detect the antigen of microfilaria, both for males and females, and the risk of a false negative is negligible. The test for E. canis is able to detect antibodies as early as on day 7 after the infection; in some dogs, however, antibodies do not show up before day 28. Anaplasmosis antibodies can be detected since day 14, but the tests are unable to differentiate between A.phagocytophilum and A.platys. A positive result should be interpreted in the light of other clinical symptoms. If these suggest disease, doctors usually decide to introduce targeted therapy. However, antibody tests are severely limited, because antibodies can persist in the body throughout the lifetime and even healthy animal may test positive, for instance after treatment. The final diagnosis should thus rely on the PCR assay and the detection of bacteria in the tested sample. Borreliosis (Lyme disease) is one of the most complicated to diagnose; difficulties are due to the large variability (expression) of bacterial surface proteins. During the first days of the disease in dogs, protein variability is huge; it is only after 35-40 days that two proteins, OspF (VetExpert CaniV-4) and C6 (Snap 4Dx), stabilize enough to serve as a basis for currently available quick diagnostic tests. The latter appears around 30 days after infection and the former is first detected around day 42 – this is important to keep in mind when deciding when to use the test. Moreover, borreliosis can be monitored, since the antibody titer drops below the threshold of detectability after 3-4 months of effective treatment. Because of the nature of the four discussed diseases, their often asymptomatic course, and great zoonotic importance, diagnostic tests should be used for routine check-ups at least once a year in every dog.

QUICK DIAGNOSTIC TESTS IN THE EYES OF VETEXPERT

FeLV – feline leukemia virus

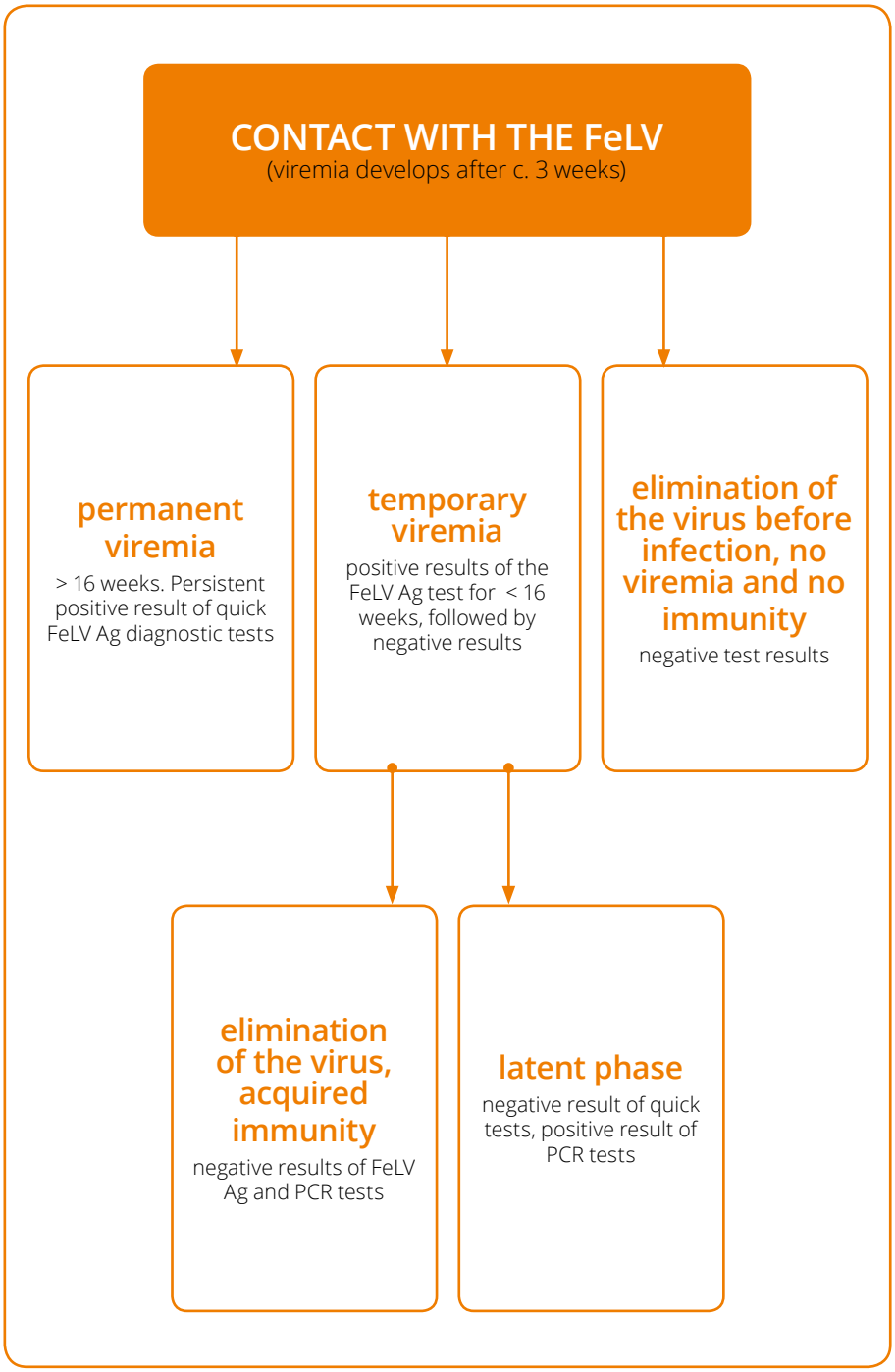
Feline leukemia is one of the most frequent viral diseases in cats; more than 10% of Polish cats are thought to be carriers of FeLV. Infection occurs primarily through saliva, feces, urine, milk, and other bodily secretions. Leukemia usually affects young animals, but that does not mean that adult cats cannot contract the pathogen. FeLV is a single-strand RNA virus that consists of just three genes, none of which, interestingly, is an oncogene, i.e. none is able to cause cancer on its own. Whether a FeLV infection will result in cancer depends on individual factors and the presence of oncogenes.

The course of the disease may vary, which is why FeLV represents a considerable diagnostic challenge.

Quick tests detect the antigen – the p27 protein in the viral capsid, which means that they can only diagnose viremia! Importantly, vaccinations do not affect test results.

Once ingested, the virus begins to replicate itself and viremia develops after 3 weeks from the moment of transmission (the quick test comes out positive). The course of viremia may vary and largely depends on the individual patient. For instance, the infection may be defeated by the immune system; in this case, the cat acquires lifelong immunity to the virus. In nearly half of the patients, however, viremia goes into what is referred to as a latent phase. Latent infection

means that the genetic material, or the viral RNA, is transcribed onto the DNA of the host cell, creating a provirus; cats remain carriers and the infection may be reactivated at any time. If viremia continues beyond 16 weeks, the animal is considered permanently infected and continues to secrete the virus with, for instance, saliva. Patients with permanent viremia usually live a short life of no more than 2-4 years. Some cats, however, destroy the virus even before infection occurs – in this case, neither viremia nor immunity develop.



From the perspective of available diagnostic methods: during viremia, quick results will come out positive, while latent infection can be diagnosed with a PCR assay conducted in a veterinary clinic (when requesting a test, it is worth making sure it is labeled as FeLV DNA provirus or FeLV latent phase). The complex course of infection may pose diagnostic difficulties.

FIV – feline immunodeficiency virus

FIV is mostly found in older cats, predominantly males who go outside and get into fights. Infection usually occurs through biting (contact with blood); detectable levels of the virus are also present in sperm, urine, and milk, but these are too low to cause infection.

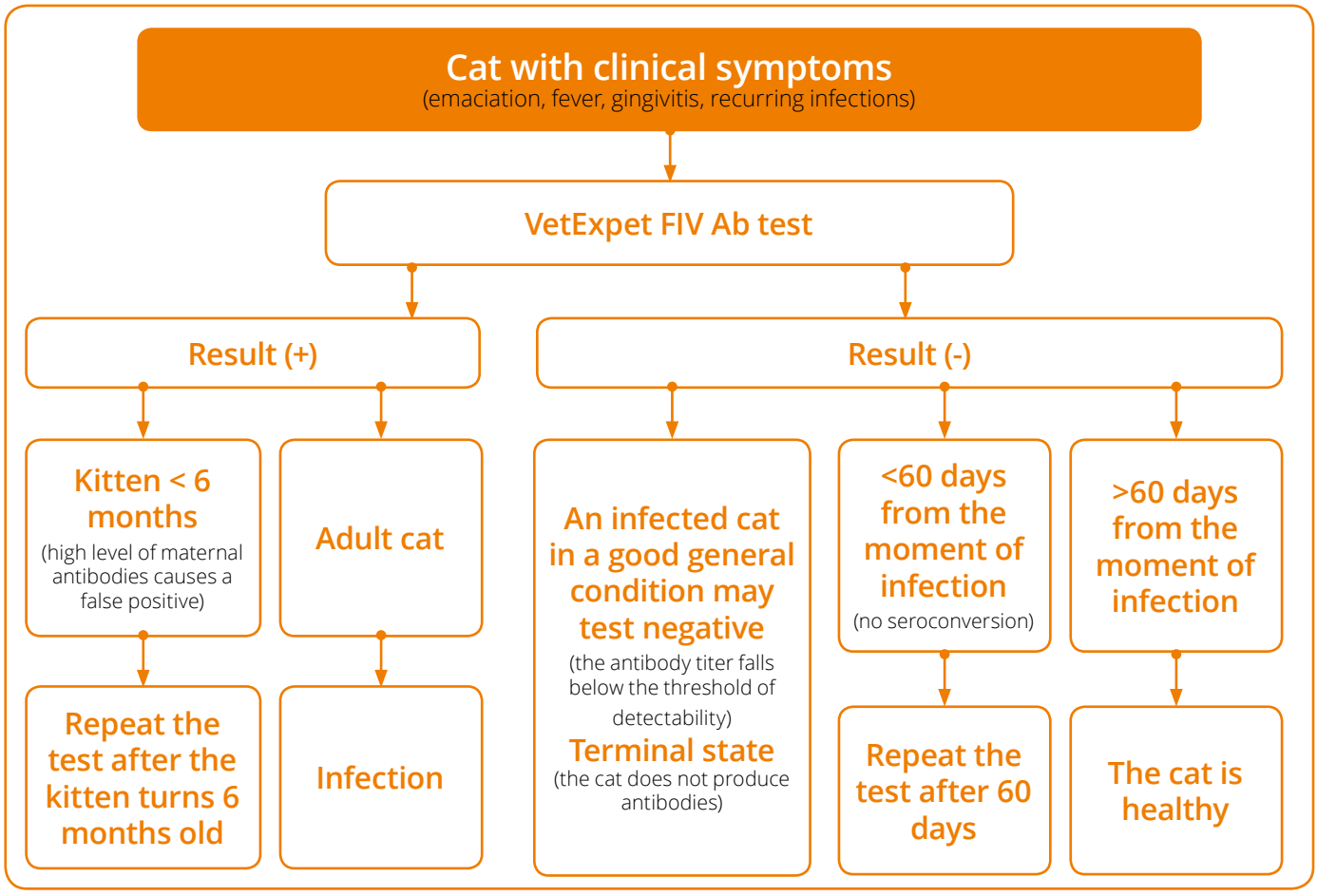
The feline immunodeficiency virus is an RNA virus belonging to the Lentivirus species; after the infection, its RNA is integrated into the host cell as a provirus. FIV infections only occur in the latent phase; the provirus slowly multiplies in the host cells of the immune system, such as macrophages and lymphocytes. First clinical symptoms often occur months or even years after infection. Carrier status is similar to that observed in HIV – long duration: impaired immunity; cats easily come down with other diseases. Infection does not directly lead to death; it is not a death sentence. But a FIV-positive cat should be kept away from other animals.

The typical symptoms of the disease are difficult to describe, since they are mostly the result of impaired immunity or co-morbid conditions. In general, FIV can be suspected in patients manifesting:

- Unexplained recurring fevers,
- Recurring infections and invasions, especially with a severe clinical manifestation, e.g. ear scabies is normal in a young, but not in an older cat, especially if it fails to respond to treatment,
- Mycosis – another disease typical of younger animals; if it affects the entire body of an adult cat, this should give us pause,
- Mange – a disease caused by Demodex parasites, much more commonly found in dogs; it practically never affects cats, it is always a secondary condition.

FIV diagnostics are not very complicated. A quick test detects FIV antibodies; however, these only appear two months after infection, which is why the test should not be used in kittens. If a kitten of several months is tested, we might detect antibodies that belong to its mother. Let us remember that FIV is incurable and a diagnosed cat should be considered sick. It may enjoy many years of healthy life, but since it can pass on the infection to other animals, it should be kept at home and never left to wander outside. Importantly, when the patient is in good clinical condition, the antibody titer often drops under detectable levels and, paradoxically, a FIV-positive cat may get a negative test result! A golden standard used to confirm a FIV diagnosis is the Western Blot method designed to detect antibodies; however, those detected by the Western-Blot method appear later than those used in quick tests. If the result of the latter is positive and that of the former negative, both tests should be repeated after around two months. If a PCR assay is chosen instead, it is essential to make sure that it covers all 5 subtypes of the FIV virus (from A to E), in other words, that the PCR method has 5 primers; otherwise there is an increased risk of a false negative result.

In countries where the FIV is a serious issue, FIV vaccines are sometimes available. Interestingly, the VetExpert FIV Ab quick test does not detect post-vaccination antibodies, which means that the vaccine will not affect its results.



FELINE CORONAVIRUS – TWO FACETS OF DIAGNOSTIC TESTS

Coronavirus infections are very common in cats, especially those kept in larger groups, e.g. in shelters or breeding centers. The coronavirus causes a temporary episode of diarrhea and kittens often contract the virus from adult cats. In terms of pathogenicity, two biotypes can be distinguished:

- intestinal (feline enteric coronavirus FECV)
- FIP-causing (feline infectious peritonitis virus – FIPV)

The latter is a mutation of the relatively harmless but very widespread intestinal biotype. Despite their radically different degrees of pathogenicity, the two biotypes cannot be differentiated by available diagnostic methods.

FIP (feline infectious peritonitis) is probably one of the least frequent viral diseases but tends to cause the most anxiety among doctors, breeders, and owners alike.

Unfortunately, the facts are harsh:

- FIP is incurable
- FIP is "undiagnosable"
- FIP may have a rapid progression
- FIP most frequently affects young kittens, only recently adopted from a breeding center or shelter

There is no cure for FIP but kittens can be protected from the mutation of the virus! In order to prevent the disease, coronavirus infections should be avoided or at least delayed as much as possible (the later the kitten contracts the virus, the lower the risk of FIP).

How?

- Early weaning (up to 5-6 weeks)
- Complete isolation of the mother and the litter from other cats (preferably until the kittens are sold)
- Limiting the number of cats in the group
- Good litter box hygiene: one box per 1-2 cats, cleaned daily and disinfected once a week, situated away from eating bowls.

In terms of diagnosis, it is necessary to distinguish between FIP and diarrheas caused by the intestinal biotype. The portfolio of VetExpert includes two diagnostic tests:

- VetExpert FCoV Ag – detects the coronavirus antigen in the feces of cats and ferrets
- VetExpert FCoV Ab – detects coronavirus antibodies in the serum, plasma, and full blood of cats.

Let us begin by discussing the VetExpert FCoV Ag test that detects the coronavirus antigen in feline feces.

When should it be used?

1) **The test should be used to diagnose the cause of diarrhea in a kitten as well as to differentiate against panleukopenia with the VetExpert FPV Ag/FCoV Ag/ Giardia Ag. It should be kept in mind that mixed infections with the panleukopenia and the coronavirus have a more severe manifestation and a worse prognosis. Whenever mixed infection occurs, all the big guns should be brought out to save the kitten.**

2) **The test can help eliminate coronaviruses from your breeding center. It's difficult but doable! How?**

- Cats should be divided into infected and healthy groups and the two groups should be kept apart:
  a) preferably in permanent subgroups of 3-4 animals (a group of up to 10 cats will be free of the virus after several months, as shown by a negative result of the VetExpert FCoV Ab test). If diarrhea occurs, repeat the VetExpert FCoV Ag test.
- Good litter box hygiene (one box for 1-2 cats)
- Successive reintroduction of cats into the healthy group.

The challenge is definitely worth taking,

VetExpert FCoV Ag		Negative	Positive	Positive	Negative
VetExpert FCoV Ab		**Negative**	**Negative**	**Positive**	**Positive**
Interpretation	FCoV	• No infection • No viral shedding • Possible incubation period	• Viral shedding • Antibodies cannot be detected since they show up 2 weeks from the moment of infection (seroconversion after 2 weeks)	• Viral shedding • Carrier status	• Nosicielstwo koronawirusa • Brak siewstwa
	FIP		• Low probability of FIP • A negative antibody titer may suggest that antibodies are bound up in an antibody-antigen complex	• the FIP antibody titer tends to be high • Reconsider the adoption of another cat or adding this cat to the group	• the FIP antibody titer tends to be high
After elimination of the virus, the antibody titer decreases within 3-12 months; cats who continue to test positive should be considered carriers. Carriers should not be introduced into breeding centers.					

since cats that never had any contact with coronavirus will not come down with FIP!

After the elimination of the virus, the antibody titer decreases within 3-12 months; cats who continue to test positive should be considered carriers. Carriers should not be introduced into breeding centers.

The other test, VetExpert FCoV Ab detects coronavirus antibodies. It should be kept in mind, however, that it does not differentiate between the two biotypes.

The presence of antibodies does not mean that the cat suffers from FIP! Their absence, on the other hand, does not mean that the cat is not infected!

If this is the case, how should the test be used in practice?

1) **Antibodies can show up in healthy cats (it is worth performing the test before or right after adoption):**

Negative result = the cat hasn't had any contact with coronaviruses, the risk of FIP equals zero

Positive result = 33% means carrier status (the higher the titer, the greater the risk of disease, which should be kept in mind when a FIP-positive cat is introduced into a FIP--negative breeding center)

2) **And what are the figures for affected cats?**

- >70% of cats suffering from FIP have a high antibody count
- 11-30% of cats suffering from FIP have a low (undetectable) antibody count… why? The antibodies may be bound up in an Ab-Ag complex. In other words, they are linked to antigens and are no longer detected by the test….

If a cat suffers from FIP, a quick test may be the only way to persuade the owner to put it out of its misery…

FIP diagnostics. What can help?

1. **Interview:**
- Young age (up to 18 months)
- Sudden onset of symptoms; at first the kitten feels good
- Recent stress – change of owner, stay at a different location, castration, accident

2. **Lab symptoms:**
- Examination of body cavity fluids: fluid in the pertitoneal/pleural cavity, usually transparent/ slightly cloudy, yellowish, gooey and foamy – high protein content with many cells (neutrophils and i macrophages), bacteriologically sterile
- Increased globulin level, reduced albumin level
- a positive result on the Rivalta test.

3. **Ruling out other diseases with similar clinical symptoms, for instance:**
- Bacterial infections e.g. as a result of biting or scratching
- lymphocytic inflammation of the liver (more common in older cats)

GIARDIA AG

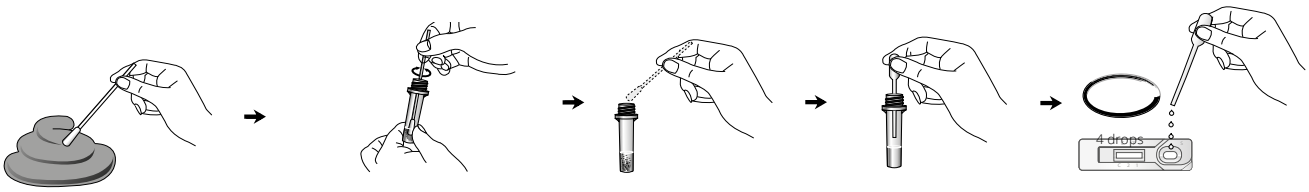
Giardia, also known as Lamblia, is a protozoan parasite of the small intestine that feeds on numerous animal species (ranging from rodents, cats, dogs, pigs, and ruminants, and all the way to primates, including humans). Giardiasis is the leading cause of parasitic diarrhea in humans and animals. Invasion occurs when cysts, found in water and other moist environments, are ingested. In the digestive system, they release a trophozoite and within 4-5 days new cysts are formed. To sum up, Giardia can be found in two forms, as trophozoites and cysts. Feces most frequently contain the latter, but the former can also be observed, especially in heavy diarrhea.

For diagnostic purposes, several things should be kept in mind:

Firstly, cysts are excreted in feces at irregular intervals; they might show up one day only to become undetectable the next day. Clinical symptoms observed in the patient are not correlated to the number of excreted cysts. It may well be the case (and my own experience tells me this is particularly likely in cats) that a beautifully formed stool will contain countless cysts and the test line will be very thick. An ideal test material, therefore, will be a pooled sample from the entire week, e.g. three samples taken every second day. The number of cysts in a single sample of feces also tends to vary; for this reason, it is extremely important to dip the swab stick in the sample several times and at different places. A pooled sample, however, has certain shortcomings...it is usually rather large and when the diluent is added, the mixture becomes rather dense, so that the test may get clogged. Among the VetExpert fecal tests, this applies to the VetExpert Giardia test in particular, as the lattice of its test window is denser in order to effectively filter the antigen out of the sample. In order to check if the sample was too dense, I recommend opening a new test and dripping the diluent alone; if the test works fine, this means that this was the case.

Once a sample of feces has been taken, excess material should be wiped off against the wall of the test tube, the sample should be dipped and thoroughly stirred in the di-

luent. The tube should then be sealed and put aside for 1-2 minutes for the feces to settle at the bottom. To perform the test, it is essential to use the "clean" supernatant from the top, where no fecal lumps are found. This is to ensure that the test does not get clogged!



dip the swab stick in feces several times

stir in the diluent

put aside for 1-2 minutes for fecal lumps to settle

take a sample of the supernatant from the top of the tube

drip 4 drops of the supernatant onto the test strip

The VetExpert Giardia Ag test detects fully formed cysts. This means that if we test a single sample taken during the first days of the disease, when no cysts have yet had time to form and fecal matter contains only trophozoites, our test will come out negative! The same will happen if we store the sample outside the fridge and the cysts are damaged by environmental conditions. It is essential to keep this in mind...Similar principles apply to the flotation method; if we collect our test samples correctly, performing the Giardia test should not pose any additional problems. A great asset of the VetExpert test is its low detectability threshold; as few as 125 cysts/100 µl are enough to give a positive result! The test line in this case will be very thin, but since quick tests are qualitative in nature, even a thin line should be interpreted as a positive result.

From the therapeutic point of view, I would like to touch on two more issues: firstly, cross-species infections, and, secondly, treatment time. Are cross-species infections with Giardia possible? Unfortunately, they are... As many as three genotypes show affinity for several animal species and humans. Genotype A1, genotype A2, and genotype B may invade all members of the household. Therefore, if a dog is diagnosed with giardiasis, everyone else should also be tested; not all patients will manifest severe clinical symptoms and asymptomatic carriers are the most dangerous. Different Giardia genotypes are shown in the table below. And what about treatment? There are several effective treatment methods. What seems of particular importance is their time; considering the time it takes for cysts to form and their sensitivity to medication, treatment should not be shorter than 5 days.

A quick test may be used to control the outcome of treatment; the result should come out negative already 2 days after the end of treatment.

Giardia duodenalis	
A-1	humans, dogs, cats, cattle
A-2	humans, dogs
B	humans, dogs, guinea pigs
C	dogs
D	d ogs
E	ruminants
F	cats
G	rats

COMBINED TEST CPV Ag/CCV Ag i CPV Ag/CCV Ag/Giardia

Differentiating the causes of diarrhea in cats and dogs

VetExpert offers special tests designed to differentiate the causes of diarrhea in dogs (VetExpert CPV Ag/CCV Ag and VetExpert CPV Ag/CCV Ag/Giardia Ag) and cats (FPV Ag/FCoV Ag/Giardia Ag). Why is it worth performing a double or triple test? For two reasons:

1) a combined test makes it possible to determine the precise cause of diarrhea, as it covers all the diseases that we normally take into account in differential diagnosis.

2) it allows us to establish a clear prognosis and indicates the correct treatment approach in the event of a mixed infection.

I have discussed the issue of feline parvovirosis, coronavirosis, and giardiasis in previous articles. Presently, let us focus on canine coronavirosis and the reasons why we should make sure that parvovirosis (panleukopenia) is not accompanied by a co-morbid coronaviral infection. Both viruses can cause similar clinical symptoms, especially in young unvaccinated animals.

The course of the disease is different, but symptoms may be alike.

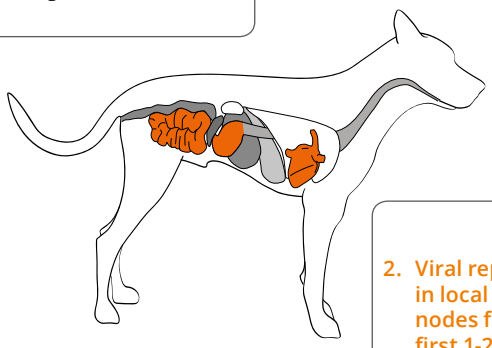
The essential difference is that:

- the parvovirus damages intestinal crypts – it penetrates deep into the intestine and causes significant damage to its structure
- the coronavirus only destroys enterocytes and villi – the damage is more superficial

The CPV infection cycle:

4. **Fecal excretion after c. 3-4 days from the moment of infection**
   - » Necrosis of intestinal crypt cells
   - » Absorption disorders, more severe symptoms, profuse bleeding from the digestive tract

3. **Viremia after 3-5 days from the moment of infection**
   - » Viral replication in the stomach, lymphatic system, and bone marrow



2. **Viral replication in local lymph nodes for the first 1-2 daysi**

1. **Infection**

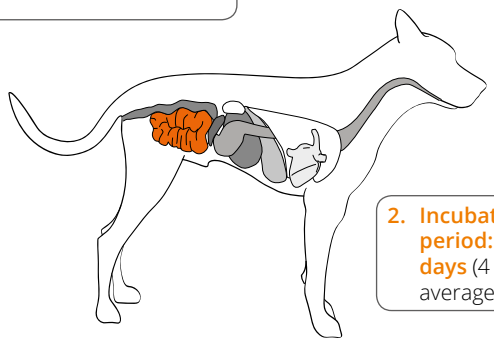
5. **Endotoxemia**
   - » Total intestinal permeability
   - » Penetration of the physiological intestinal microflora into the organism

The CCV infection cycle

CCV is the second most frequent cause of diarrhea in puppies

3. **After 2-3 days the virus already occupies 2/3 of the length of the small intestine**

4. **Diarrhea sets in 1 to 7 days after infection**



2. **Incubation period: 3-6 days** (4 days on average)

1. **Infection**

5. **The virus is excreted with feces for 6-9 days from the moment of infection**

	PARVOVIRUS	Coronavirus
Age	3-12 months	Up to 6 months
Breeds	All	All
Infectiousness	Yes	Yes
Mortality	High (puppies and young dogs)	Very low (only puppies)
Duration	6-10 days	6-10 days (or fewer)
Body temperature decrease	2-3 days, significant	No decrease in body temperature
Leukopenia	Frequent, but short-lasting	No leukopenia
Feces	Watery, greyish-white, often with traces of blood and occasional strands of mucus, soapy smell	Watery, yellowish-orange or greenish, foul smell
Response to intravenous rehydration	Gradual improvement	Fast improvement
Complications	Hypoglycemia, acidosis, hypopotassemia, hypoproteinemia, immunosupression, sepsis, endotoxemia, shock	No complications
Relapses	No relapses	No relapses (immunity is short-lived but subsequent infections are asymptomatic)

The course of a CCV infection is milder and recovery faster; the virus does not burden the organism to quite the same extent as the CPV. The prognosis is better. However, a mixed infection with coronaviruses and parvoviruses is also possible (in cats as well); in this case, radical measures are required and the prognosis is considerably less optimistic.

Virus	Severity of symptoms	Mortality	Remission
CCV	+	0%	100%
CPV	+++	50%	50%
CCV + CPV	+++++	89%	11%

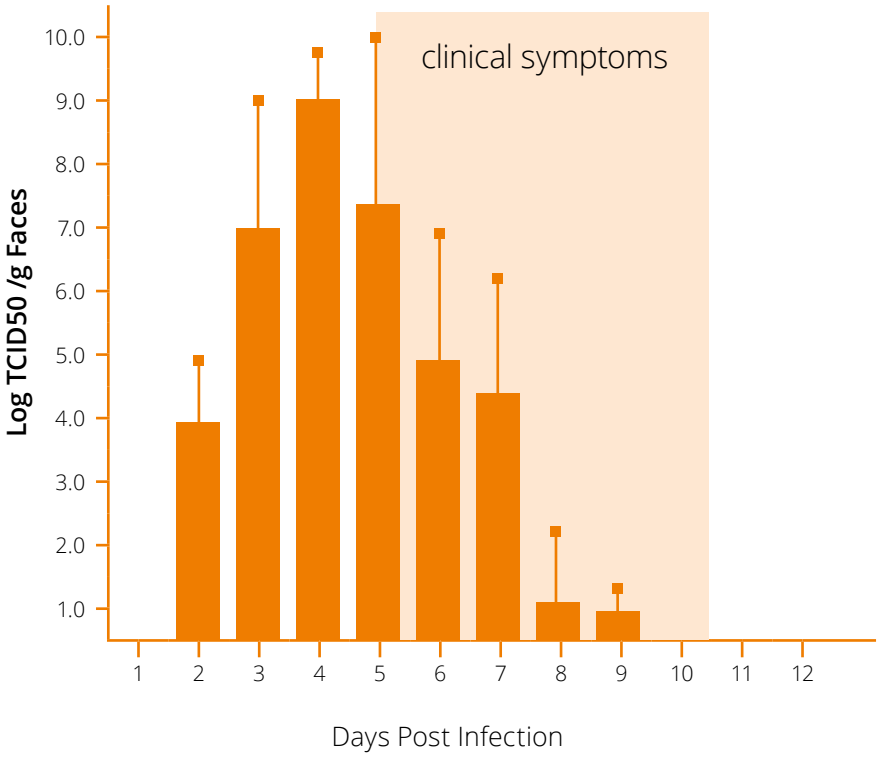
PARVOVIRUSES

Canine parvovirosis and feline panleukopenia are highly infectious viral diseases, whose chief symptoms include diarrhea, vomiting, heavy immunosuppresion, and decreased white blood cell count (more marked in cats). The feline parvovirus – FPV shows a strong antigen affinity to the canine parvovirus CPV-2, and, according to some theories, the CPV is derived from the FPV. The CPV-2 itself does not cause disease in cats, but its newer variants – CPV--2a, CPV-2b, and probably the most common variety, CPV-2c, do. "Canine" variants are often said to be crowding out the FPV. Interestingly, the FPV does not cause disease in dogs. In practice, therefore, it means that a cat can contract the virus from the dog, but the reverse situation is impossible; however, how can one know that the cat is infected with the FPV, rather than one of the canine variants, such as CPV-2a, CPV--2b, or CPV-2c? Rottweilers are particularly susceptible to CPV infections and should be vaccinated at least one extra time after the 12th week of life.

The course of panleukopenia and the CPV infection in dogs is similar, but several essential differences can also be observed.

The pathogen is ingested with food, multiplies in the nasal cavity and the throat, and quickly penetrates into the bloodstream, which takes it to its final destination. The incubation period takes from 2 to 10 days (usually 3-5 days). Initially, the disease is accompanied by a fever of 40-41.5°C and symptoms such as vomiting, diarrhea, and enteritis that lead to dehydration. The main lab symptom is leukopenia, shorter in dogs, longer and more severe in cats (even <1 thousand/µl). A decrease in the number of white blood cells leads to reduced immunity and results in frequent bacterial complications, including sepsis. As a result, hypoglycemia and hypothermia often set in. Mortality among young, unvaccinated animals can exceed 50%, especially if the owner takes the first symptoms too lightly and the animal arrives at the clinic too late in the day.

viral excretion after infection



Pollock RVH, *Comell Vet 72*: 103,1982

What clearly sets apart the course of the disease in cats and dogs is the fact that only very young puppies are affected by myocarditis, while reproductive issues, such as miscarriages, infertility, and cerebellar hypoplasia are only observed in cats.

In the diagnosis of these diseases, time is of the essence. What should be kept in mind is that the virus begins to show up in feces even before the first clinical symptoms have set in; importantly, its quantity increases up until the second day after the appearance of symptoms and then drops dramatically. The decrease is so huge that quick diagnostic tests may come out negative already on day 5-6 of the disease! This must be always kept in mind and parvoviral infections should not be ruled out in patients with clear clinical symptoms and negative results. If the animal arrives at the vet's office too late or the interview is ambiguous, the test should be taken as soon as possible, and even if the patient tests negative, a CPV diagnosis should often be made, based on clinical symptoms and experience. The relationship between the quantity of excreted virus and the appearance of clinical symptoms is shown in the graph above.

Diagnosis of vector diseases

It is also important to consider the possible impact of vaccination on quick diagnostic tests. The procedure does not affect the results of VetExpert CPV Ag. Cats, however, can test positive as long as 3 weeks after vaccination without manifesting any symptoms of disease. A positive test result in a kitten with heavy diarrhea, however, should not be taken lightly even after vaccination, especially that, as shown in the above diagram, the clinical symptoms of panleukopenia only set in after several days. If a kitten is tested before vaccination, sometimes no symptoms are observed; two days later, however, the pet may be admitted to the clinic with a diagnosis of panleukopenia.

The diagnostic portfolio of VetExpert includes a quick test designed to detect vector-borne diseases. VetExpert Cani V-4 allows us to detect the antigens of Dirofilaria immitis, as well as antibodies against Borrelia burgdorferii, Anaplasma platys/phagocytophilum, and Ehrlichia canis. The main question to ask in this context is: why are four different diseases caused by distinct pathogens tested together? Firstly, their clinical symptoms, such as fever, apathy, and joint pain, are very similar and non-specific. Their lab symptoms may also be alike and include thrombocytopenia and reduced count of red blood cells.

As a result of global warming, diseases that once seemed confined to the hot Mediterranean climate now begin to appear in Poland. Increasingly mild weather is propitious to the proliferation of ticks and insects. A vacation in a warmer country with our four-legged friends may also leave us with more than just nice memories. The expansion of ticks and insects contributes to the increased incidence of borreliosis, ehrlichiosis, and anaplasmosis, diseases transmitted by their different species, some of which can cause a mixed infection. These three diseases are transmitted by ticks; dirofilariasis, on the other hand, is passed on by mosquitoes. In our climate zone, infections with spirochaetes Borrelia and Anaplasma phagocytophilum are particularly common. Microfilaria are also often found in the blood smear; luckily, these are usually the larvae of the skin worm and heartworms are practically absent from our climate zone. In Poland, three types of ticks are particularly common: *Ixodes spp.*, *Dermacentor spp.*, and *Rhipicephalus spp.* Regardless of the species, each tick goes through three morphological stages: the egg gives way to the larva and the larva transforms into a nymph before the parasite reaches its adult stage. Importantly, larvae can already drink blood! Each stage needs at least three hosts to undergo metamorphosis. A fertilized female lays around 2000 eggs, which turn into larvae within 3-36 weeks. Once it has drunk blood, the larva begins to shed its skin and takes from 5 weeks to 5 months to change into a nymph. Nymphs then look for hosts and mature into adult specimens capable of reproduction. Ticks stay on a single host for a very short time, between two hours and seven days; their life, however, can last up to 4 years, while they change hosts and drink their blood. An adult tick extracted from a patient can be assumed to have traveled across half of Poland and fed on at least 6 hosts.

The table below puts together all the diseases mentioned above, along with a short list of the most frequent clinical and lab symptoms, as well as the vectors that transmit them. What is worth noting is that Ixodes shows up in as many as three rows. Another thing to keep in mind is the extremely short transmission time for E. canis and the fact that it is transmitted by the same tick as that responsible for carrying Babesia canis.

From the diagnostic point of view, a few nuances should be kept in mind when using the VetExpert CaniV-4 test.

Pathogen	Vector	Transmission time	Clinical symptoms	Lab symptoms
Anaplasma phagocytophilum	*Ixodes spp.*	36-48 hours	Fever, weakness, diarrhea, vomiting, joint pain	Moderate thrombocytopenia, autoimmune hemolytic anemia
Anaplasma platys	*Rhipicephalus sanguineus*	36-48 hours	Fever	Cyclical thrombocytopenia
Borrelia burgdorferi sensu lato	*Ixodes spp.*	~24 hours	Often subclinical, fever, appetite loss, joint pain, weight loss	Thrombocytopenia, hyperglobulinemia
Ehrlichia canis	*Rhipicephalus sanguineus, Ixodes ricinus, Dermacentor spp. (Babesia canis)*	< 3 hours	Often subclinical, fever, weight loss, enlarged lymph nodes, splenomegaly	Thrombocytopenia, non-regenerative anemia, hyperglobulinemia, azotemia, increased APTT, bone marrow hypoplasia or aplasia
Dirofilaria immitis	komary z rodzaju *Aedes, Anopheles, Culex*	Invasive larvae transmitted when the skin is pricked	Often subclinical, coughing, quickened breath, pulmonary embolism, ascites	eosinophilia, thrombocytopenia, leukocytosis, hyperglobulinemia, radiogram abnormalities

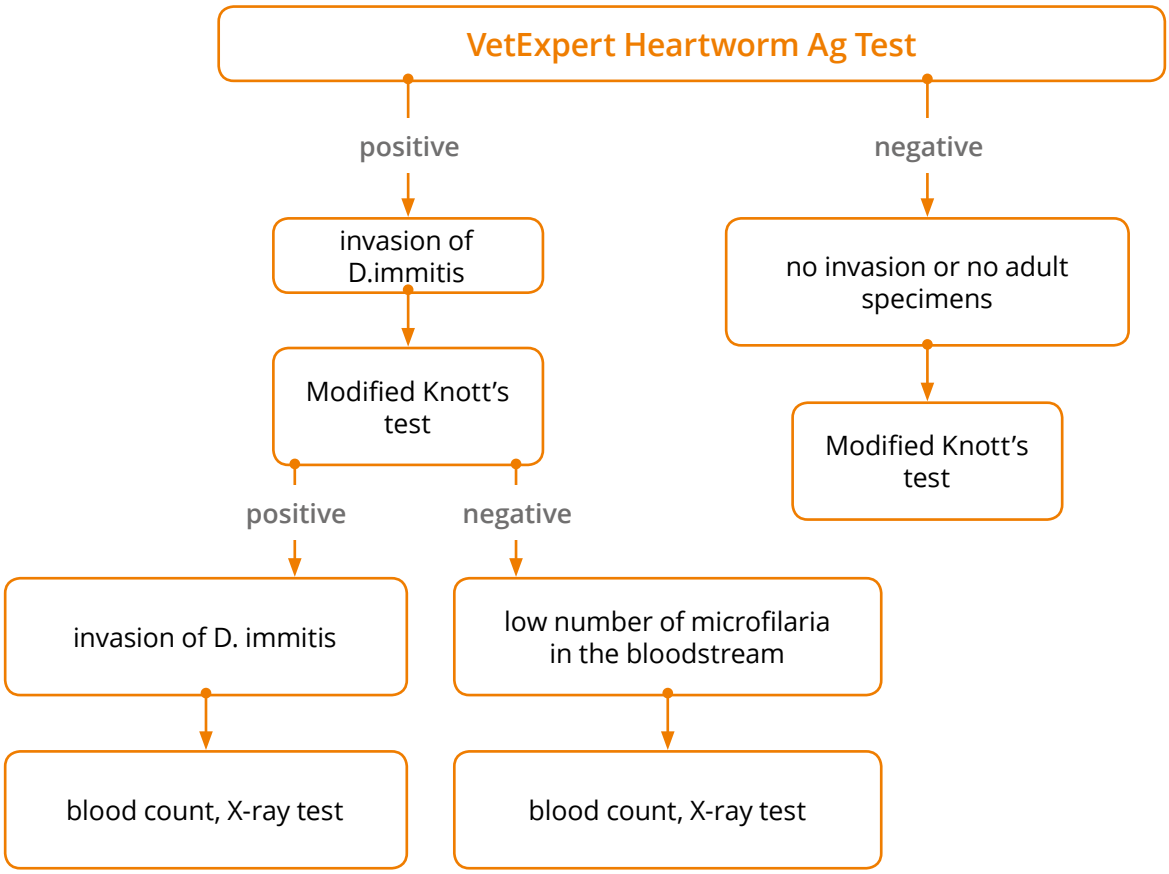
Heartworm (*Dirofilaria immitis*) cardiac necrosis

Antibodies used in the VetExpert Heartworm test make it possible to detect the somatic antigen and the secretory antigen of D. immitis of a specific size. The test detects both males and females. Its sensitivity is so high that the presence of one adult specimen is enough to give a positive test result. However, it is essential to remember two things: firstly, microfilaria are absent from the bloodstream in as many as 20% of cases (e.g. in invasions by males only). The Knott's test might thus give a negative result, while VetExpert Heartworm Ag comes out positive. The most reliable results are obtained several (even as many as 6) months from the beginning of invasion.

If microfilaria are found in the blood smear in our climate zone, the VetExpert Heartworm Ag test should be used to rule out the presence of heartworms and confirm a skin worm invasion. Importantly, the test sample should preferably be taken in the evening, as the number of microfilaria in the bloodstream increases at night in the anticipation of a potential mosquito that could transfer them onto another host.

The algorithm used in heartworm diagnostics, including other diagnostic methods is shown below:

VetExpert Heartworm Ag Test Diagnostic Algorithm



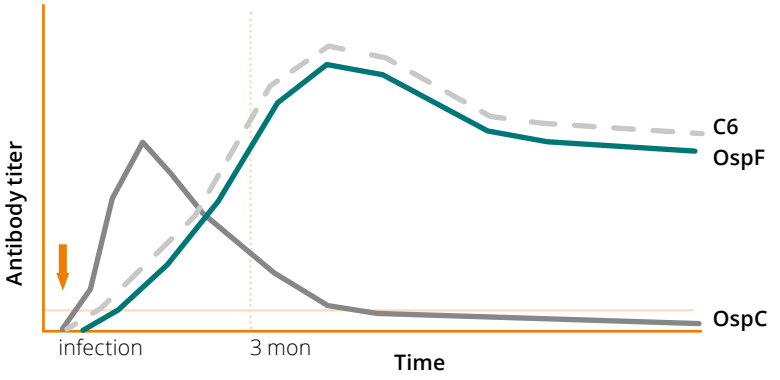
Lyme Ab (*Borrelia burgdorferi*) Lyme disease

The VetExpert Lyme Ab test looks for antibodies against the OspF surface protein, which in most dogs shows up around day 42 from the moment of infection. Unfortunately, surface proteins in borreliosis are very unstable, distinct for different species, and different depending on the duration of the disease.

Interestingly, erythema migrans, typically found in people, is not observed in dogs. The only other species in which erythema is known to occur is the cow. The most susceptible breeds include Labradors, Golden Retrievers, and Rottweilers.

- **OspC protein** – acute-phase protein, disappears after 4-5 months even though the infection continues
- **C6 protein** – appears around day 30 and persists until recovery (around 2-3 months from the beginning of effective treatment, antibodies are no longer detectable)
- **OspF protein** – appears around day 42 and persists until recovery (around 3-4 months from the beginning of effective treatment, antibodies are no longer detectable)

Surface proteins in canine borreliosis



Anaplasma Ab (*Anaplasma phagocytophilum/Anaplasma platys*)

The VetExpert CaniV-4 or Anaplasma Ab quick test cannot differentiate between infections with

A. phagocytophilum and A. platys. The test strip is lined with a surface protein associated with relevant antibodies produced by the dog. The antibodies can already be detected around day 14 from the moment of infection (and in some patients even earlier). Importantly, during the first several days, the blood smear contains morulas, which can be observed in the cytoplasm of granulocytes (A. phagocytophilum) and platelets (A. platys).

Unfortunately, the antibodies can persist throughout the lifetime, which means that a positive test result is always ambiguous: it is impossible to tell whether the dog is sick or had been sick and recovered in the past. If the test comes out positive, it is essential to analyze the clinical symptoms and consider confirming the result with the PCR method, which allows to detect an ongoing infection. The test cannot be used to monitor treatment.

VetExpert Anaplasma Ab positive + clinical symptoms

- confirm with the PCR method
- introduce treatment

VetExpert Anaplasma Ab positive + no clinical symptoms

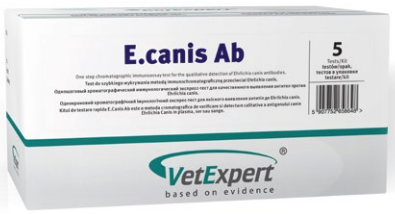
- confirm with the PCR method
- the dog is healthy; antibodies persist after previous infection

Ehrlichia canis

The VetExpert E.canis quick test detects antibodies against one of the surface proteins of Ehrlichia canis. In most dogs, these appear 7 days after the bacteria have entered the organism. It should be kept in mind, however, that in some dogs, seroconversion takes much longer, up until day 28; unfortunately, the exact time varies from one patient to another. Most infections are asymptomatic and clear symptoms, by then often irreversible (such as bone marrow hypoplasia/aplasia), only appear after many months. During the first days, the blood smear can be shown to contain morulas in the cytoplasm of monocytes. The VetExpert CaniV-4 and VetExpert E.canis can be used to monitor treatment, since the antibody titer decreases within 3-9 months of effective therapy (depending on the individual). What is particularly important to remember in our climate zone is that E.canis may be transmitted by the same tick as Babesia canis – Dermacentor reticulatus, and that its transmission time is extremely short (it may take less than 3 hours!).

With this in mind, the VetExpert CaniV-4 test should be administered when clinical symptoms first set in, and repeated after around 40 days. A repeat test increases the likelihood of correct diagnosis. Since vector-borne diseases may be asymptomatic, it is worthwhile to monitor and test every dog regardless of symptoms at least once a year.

CaniV-4 in the monitoring of disease:



Pathogen	Detectable from	Repeat test
Anaplasma spp.	Day 14	Antibodies may persist throughout the lifetime
Borrelia burgdorferi	> day 40	After 3-4 months
Ehrlichia canis	day 7 (in some dogs, day 28)	After 3 – 9 months (depending on the individual case)

Clinical studies of the VetExpert Total IgE test at the Dermavet dermatological clinic for cats and dogs

Joanna Karaś-Tęcza, DVM

Canine skin allergies are currently the most frequent cause of admission to veterinary clinics. Particularly common skin complaints include flea-allergy dermatitis (FAD), atopic dermatitis, and adverse food reactions. The latter two conditions are typically affected by fluctuations in the IgE (and/or IgG) titer. Accordingly, its level should be determined in order to establish a correct diagnosis. Serological tests are often used in the process; however, these techniques are not designed to diagnose atopy or food allergy, but rather to serve as a source of additional information that, if possible, allows to eliminate potential allergens or introduce allergen-specific immunotherapy. In order for atopic dermatitis to be diagnosed, all other skin conditions in which itching appears as a symptom should be ruled out.

Atopy is a genetic disease characterized by a propensity to develop IgE-dependent allergic reactions and produce IgE antibodies in response to low concentrations of environmental allergens, which normally cause no response in healthy dogs. Atopic dermatitis (AD) is a chronic, recurrent, inflammatory skin condition, with itching as the chief symptom. The disease presents a peculiar clinical image and the location of skin lesions is very characteristic; itching is seasonal and gradually intensifies. Atopic dermatitis is incurable; its treatment thus consists in trying to shorten the acute and prevent the chronic phase of the disease. Specific immunotherapy is designed to eliminate the acute symptoms.

The golden standard in veterinary allergology is to conduct an intradermal test and, based on its results, introduce allergen-specific immunotherapy. Intradermal tests are considered more reliable than blood tests, but the latter are becoming increasingly common in clinical practice. Blood assays serve to determine the titer of allergen-specific antibodies IgE and IgG.

The clinical image of food allergy in dogs is very similar to that of atopy in terms of the type and location of skin lesions. However, essential differences between the two conditions can also be observed: in food allergies itching is not seasonal, usually intensifies up to two hours after the meal, and is often accompanied by additional symptoms from the digestive tract, such as irregular stools and/or stools of abnormal consistency and/or variable volume, as well as flatulence.

Differentiating between atopy and food allergy requires not only close observation but also an analysis of initial symptoms; the problem is that during the medical interview, very few owners are able to remember and reliably report the initial phase of their dog's allergic reaction. What they do remember is that the itching set in, but when? They rarely can tell. This is why it is next to impossible to determine whether the dog suffers from a food allergy or atopic dermatitis based on the medical interview and clinical examination alone. The situation is further complicated by the fact that two types of food allergy can co-occur in one patient. In this case, the best thing to do would be to try out an elimination diet, preferably hydrolyzed, or a monodiet for at least 6 months. For many owners, such an experiment is a challenge since it requires that they remain consistent and avoid adding any snacks or delicacies throughout its duration. Whenever atopy and food allergy co-occur, a diet has a positive impact on the intensity of itching, reducing its severity and improving the overall clinical image. The most important method of diagnosing food allergies involves putting the dog on an elimination diet, followed by a provocation diet, i.e. a return to the previous eating habits.

Research aimed at developing tools to identify food ingredients responsible for food allergies has long been underway, but no ideal blood test has been created thus far; practically all serological assays currently available in the market may result in false positives and false negatives. It has been shown that healthy dogs may also occasionally have an elevated IgE antibody titer, which is why a decision to use these tests should be based on the clinical examination and medical history of each patient.

A veterinarian is thus faced with a double challenge. On one hand, he needs to educate dog owners and inform them that atopy is an incurable condition; on the other, he needs to tell them of the high cost involved in diagnosis and subsequent treatment. For this reason, veterinarians should keep track of the latest diagnostic developments and gradually introduce them into their daily practice. Recently, a new quick test designed to detect the whole pool of IgE antibodies in canine blood serum, VetExpert Total IgE, has appeared in the market.

The Dermavet veterinary clinic assessed its usefulness in the differential diagnosis of canine atopy and food allergy, trying to determine whether it may be helpful in the initial diagnostics of patients with an allergic reaction to unknown food and/or environmental allergens.

Dogs with atopic dermatitis, food allergy, or both conditions at the same time, represent more than 60% of all dermatological patients treated at the Dermawet clinic. Accordingly, detailed procedures have been developed to handle such patients. After a close analysis of the medical interview and clinical examination, all dogs with suspected atopic dermatitis or adverse food reactions were tested with the VetExpert Total IgE kit, based on the immunochromatographic method designed to qualitatively detect immunoglobin E in the serum. Subsequently,

all patients were put on a hydrolyzed diet for a minimum of four weeks, and finally, the test was repeated to determine the titer of IgE antibodies. Flea allergy dermatitis was ruled out in all patients beforehand through the prior introduction of anti-flea protection. The initial diagnostic process using the VetExpert Total IgE kit in the clinic proved very useful and well-accepted by the owners.

Detailed procedure

60 dogs whose medical records and clinical symptoms indicated atopic dermatitis and/or food allergy were included in the study. The following criteria were adopted:

1. non-seasonal generalized or topical itching in various forms – licking limbs, scratching, rubbing the mouth, sides, or back, or scooting;
2. skin lesions, such as recurring otitis externa;
3. skin lesions such as erythema or other primary exanthemas or secondary exanthemas in the area of the face, armpits, groins, abdominal part of the neck, and spaces between fingers and/or around finger pads and/or soles of the feet;
4. flatulence at least several times per week, irregular stools of varying consistency and mass, proneness to diarrhea and constipation;
5. commercial diet, traditional home-cooked diet, or mixed diet including leftovers;
6. flea-allergy dermatitis was ruled out in all the 60 dogs through long-term anti-flea protection.

Patients who met all the above criteria were tested with the VetExpert Total IgE kit. There were 60 such patients. 46 patients tested positive on the first attempt; 14 tested negative despite the presence of clinical symptoms typical of atopy and/or food allergy. Patients with a positive result were put on a hydrolyzed diet for 4 weeks and retested after its end.

After a minimum of 4 weeks on the diet, the VetExpert Total IgE test was employed once again. If the second result was negative, the diet had led to complete clinical recovery, and none of the previously mentioned symptoms were observed in the patient, the final diagnosis was established as food allergy. Ten patients fell into this category.

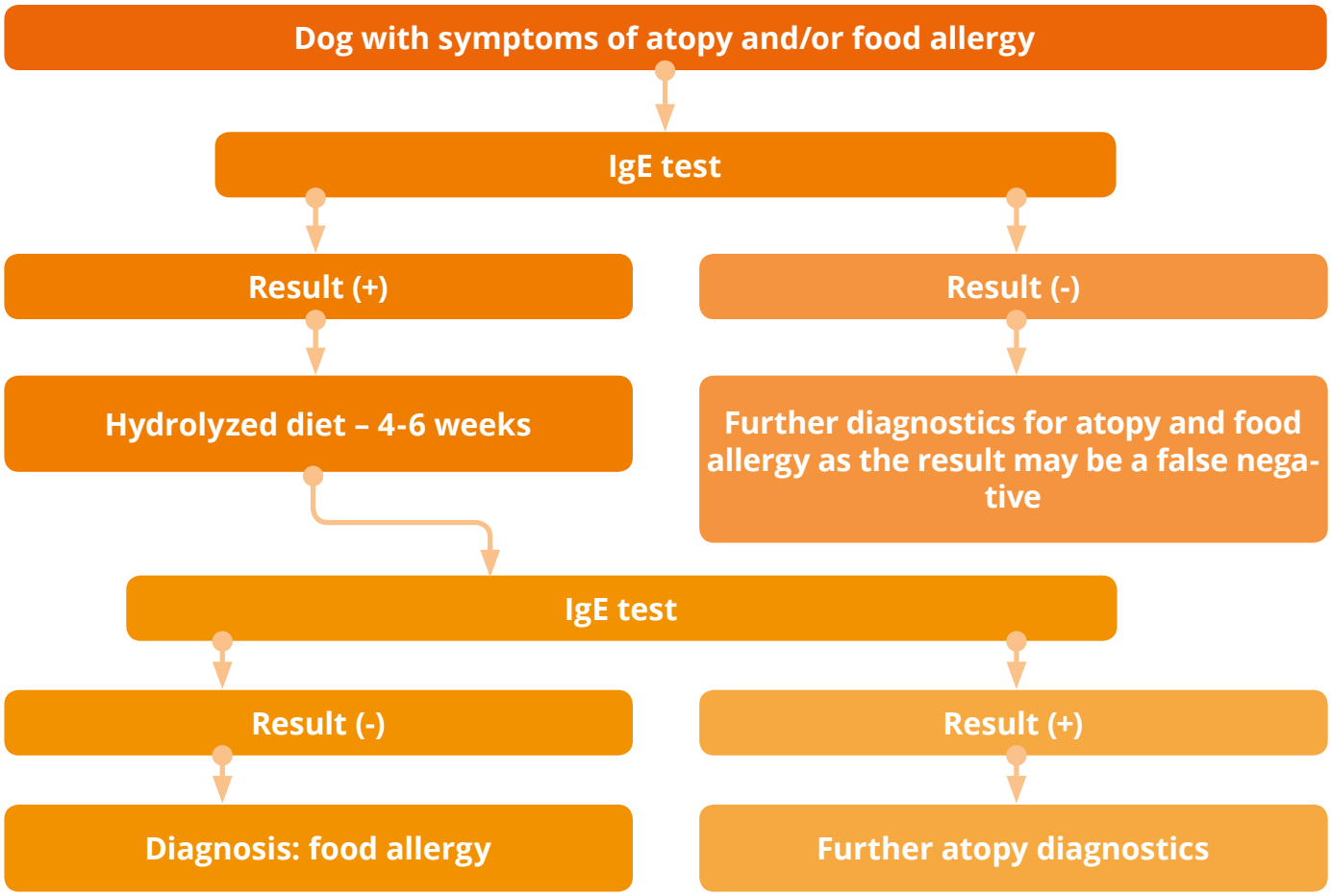
If the result came out positive and some clinical symptoms, especially those from the digestive tract, had subsided, the dog was diagnosed with co-morbid atopic dermatitis and food allergy and retested to identify environmental allergens. Additional procedures, normally used in atopic dermatitis, were prescribed: oral supplementation of omega-3 unsaturated fatty acids, topical use of omega-6 acids, shampoo therapy, and short-term treatment with corticosteroids, administered orally in patients with acute itching. Fourteen patients fell into this category.

These 24 patients were involved in further food allergy diagnostics designed to identify food allergens responsible for the reaction, through the detection of allergen-specific IgE and IgG antibodies and/or experiments with diets based on a single source of animal protein, exclusively vegetal protein, gluten-free diets, and traditional home-cooked meals using new ingredients, with which the patient had not had any prior contact.

If dietary experiments failed to bring about clinical improvement in the patient and the test result came out positive, diagnostics continued in the direction of atopic dermatitis. 22 patients fell into this category..

Comments:

The advantage of the test is that it is very affordable and enables fast initial diagnostics of atopic dermatitis and/or food allergy. From the perspective of the physician, it is also important that a positive test result is likely to make the owner more willing to cooperate and experiment with elimination diets. Based on my own observation, a positive result might be difficult to interpret, since any reading in which a second line, no matter how thin or shadowy, shows up should be read as such. At first, sometimes I found it difficult to tell whether the result was positive or negative. However, after a while, my eye became well-trained in distinguishing an empty field from one with even the thinnest trace of a line.



VetExpert Total IgE

One-step Canine Total IgE Antibody Test

VetExpert Rapid Total IgE Ab Test Kit

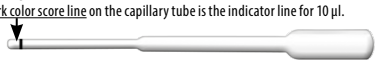
Principles

The VetExpert Rapid Total IgE Ab Test Kit is a chromatographic immunoassay for the qualitative detection of canine total IgE antibody in canine serum.
The Rapid Total IgE Ab Test Kit has the letter "T" and "C" as test line and control line on the surface of the device. Both the test line and control line in the result window are not visible before applying any samples. The control line is used for procedural control, and should always appear if the test procedure is performed properly and the test reagents of the control line are working. A purple test line will be visible in the result window if there are enough canine IgE antibodies in the specimen.
The specially selected anti-canine IgE antibody is used in the test band as both capture and detector materials. These enable the VetExpert Rapid Total IgE Ab Test Kit to identify canine IgE antibody in canine serum with a high degree of accuracy.

Materials provided (5 tests/kit)

1) 5 VetExpert Rapid Total IgE Ab Test Devices.
2) 5 Assay diluents (0,5 ml)
3) 5 Disposable capillary tubes
4) 5 Disposable droppers
5) 1 Package insert

◆ A dark color score line on the capillary tube is the indicator line for 10 µl.



Precautions

1) For veterinary diagnostic use only.
2) The Rapid Test Kit is only dog for use. Do not apply to other animals.
3) For the best results, strict adherence to the instructions is required.
4) Do not open or remove test kit from their individually sealed pouches until immediately before use.
5) Do not use the test kit if the pouch is damaged or the seal is broken.
6) Do not reuse test kit.
7) All reagents must be at room temperature before running assay.
8) Do not use reagents beyond the stated expiration date marked on the label.
9) The components in this kit have been quality control tested as standard batch unit. Do not mix components from different lot numbers.

Storage and Stability

1) The kit can be stored at room temperature or refrigerated (2~30°C). **DO NOT FREEZE**.
2) Do not store the test kit in direct sunlight.
3) The test kit is stable through the expiration date marked on the package label.

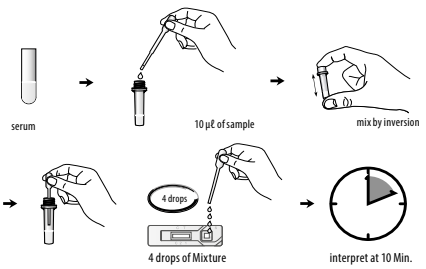
Specimen Collection and Preparation

1) The test should be used to canine serum.
   (1) Collected blood should be left at room temperature for 30minutes for coagulation and then separate serum by centrifugation.
   (2) Serum may be stored at 2~8°C for up to 1 weeks, for longer storage (1 year), freeze at or below -20°C.
2) Specimen should be brought to room temperature(18~25°C) prior to use.

Procedure of the test

1) Using a disposable capillary tube provided, add 10 µl of canine serum into assay diluents bottle.
2) Cap the assay diluents bottle and mix thoroughly by inverting bottle for a few seconds.
3) Remove the test device from the foil pouch, and place it on a flat and dry surface.
4) Using a disposable dropper provided, add 4 drops of mixed sample into sample well.
5) As the test begins to work, you will see purple color move across the result window in the center of the test device. If the migration has not appeared after 1minute, add one more drop of the mixed sample to the sample well.
6) Interpret test results at 10 minutes. Do not interpret after 10 minutes.

[Figure for Test Procedure]



Interpretation of the test

A color band will appear in the left section of the result window to show that the test is working properly, this is the Control band. The right section of the result window indicates the Test band, and it indicates a positive test result.

1) Negative result

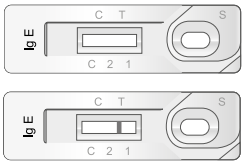
The only one band("C") in the result window indicates a negative result against canine total IgE antibody.

2) Positive result

The two color bands ("T" and "C") within the result window indicate the presence of total IgE antibody. The minimum indicative level and warning level of total IgE for allergic disease is generally agreed to be 10 ug/ml.

3) Invalid Result

If the control line ("C") fails to appear, the result is considered invalid. The test has been defective or was not used correctly. The sample should be retested.



Limitations of the test

1) A negative result means that the total concentration of IgE antibodies in the serum is lower than 10 µg/ml, but does not need to mean that the dog is not allergic. When in doubt, other clinical tests are required. The final clinical diagnosis should not rely solely on the results of a single test but should be established by a qualified veterinarian based on the analysis of all clinical and lab findings.
2) The VetExpert Rapid Total IgE test is a qualitative assay. The thickness of the test line needn't be proportional to the concentration of IgE in the serum.
3) Corticosteroids and anti-histamine drugs may affect test results. Two weeks should elapse before the test can be administered.
4) Parasitic infections should be eliminated prior to the test.

sensitiveness – 97,1%
specificity – 96,4%

UroTest - 10

VetExpert Reagent Strips for Urinalysis

Packagning: 100 strips

- Determination of 10 parameters:
  1. Leukocytes
  2. Nitrite
  3. Urobilinogen
  4. Protein
  5. pH
  6. Blood
  7. Specific gravity
  8. Ketones
  9. Bilirubin
  10. Glucose
- Fast and reliable analysis in 60 seconds
- For the following animal species:
  cattle, pigs, horses, dogs, cats, sheep, rabbits, guinea pigs

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Success is the result of correct decisions

SUPPLEMENTS DIETS COSMETICS DIAGNOSTIC HYGIENE CATTLE

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