VET EXPERT
BASED ON EVIDENCE

VET EXPERT'S RAPID TESTS QUALITY EVALUATION

WE ARE BASING ON EVIDENCE

The study was performed in 2020 at the Department of Epizootiology and Clinic of Infectious Diseases of the Faculty of Veterinary Medicine, University of Life Sciences in Lublin, under the direction of Prof. Łukasz Adaszek, DVM PhD.

VET EXPERT

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Vet Expert has promoted animal health and wellbeing since 2008.

Our mission is to support global veterinary professionals and pet parents in their daily decisions concerning pet care and treatment so every animal can look, feel and perform at its best.

Our products are being choosen by vets, breeders, and pet owners in more than 20 countries. In Poland, Vet Expert ranks as the most trusted brand among Doctors of Veterinary Medicine. High product quality and efficacy have already provide us wide acclaim and many awards, and the concept behind our portfolio, "Vet Expert. Based on Evidence" guarantees tangible, science-based results.

No other brand offers such a broad range of specialist products:

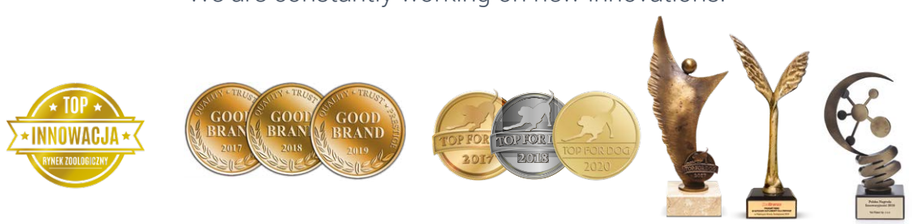
- dietary supplements for companion animals
- rapid diagnostic tests for dogs, cats, and farm animals
- an innovative line of cosmetics
- unique hygiene products
- veterinary diets and maintenance pet food
- compound feeds for farm animals

Facing your expectations, caring about mutual trust and the highest quality of our products, we performed comparison studies of two most popular rapid tests from the Vet Expert portfolio. We evaluated the CaniV-4 test, being useful tool in the diagnosis of vector-borne diseases in dogs, and the Giardia Ag test, for the diagnosis of one of the most common protozoan diseases of dogs and cats, giardiasis.

We hope that this study will serve as a reminder of these diseases and will be a useful tool in your daily clinical practice.

THANK YOU FOR TRUSTING US.

We are constantly working on new innovations.



GIARDIASIS IN DOGS AND CATS

Giardiasis is a disease caused by the protozoan *Giardia duodenalis (G. lamblia, G. intestinalis)*, that could be potentially pathogenic for both animals and humans.

Several genotypes of Giardia are currently distinguished, from A to H, which differ in host specificity. Interspecies infections are possible and furthermore, this protozoa has zoonotic potential.

	***Giardia duodenalis* genotypes**
A-1	humans, dogs, cats, cattle
A-2	humans, dogs
B	humans, dogs, guinea pigs, chinchillas
C	dogs
D	dogs
E	ruminants
F	cats
G	rats, cats
H	sea mammals

The occurence of giardiasis is variable and depends mainly on **environmental conditions**. The disease is often found in young animals under one year of age. This makes Giardia the most common endoparasite among this age group. Excretion of cysts is observed in both healthy and ill animals. The infection has been reported to provide temporary partial immunity. However, this is highly variable between individuals.

Giardia spp. are relatively easy to transmit from host to host as there are no intermediate hosts in their life cycle. Infection occurs through direct ingestion of invasive oocysts from faeces-contaminated water, food or the environment. Even small quantity of cysts (approx. 10) can cause a disease. Excretion of cysts occurs periodically, irregularly and they are fully invasive at the time of excretion. The prepatent period is 4-16 days.

Cysts can survive in the environment for many months but are quite sensitive to desiccation and extreme temperatures, so the intensity (extensiveness) of infestations decreases noticeably during winter. The most effective way of cyst inactivation is to use hot steam.

Giardia has a fairly simple development cycle involving the presence of two development forms in the small intestine of the host: a trophozoite and a cyst. The ingested cysts enter the stomach where they release excizoites due to the influence of hydrochloric acid. After cell division, they give rise to the progeny trophozoites, which are the active, mobile stage of the parasite that inhabit the duodenum and the early part of the jejunum. The trophozoites attach to the epithelial cells of the small intestine by using a clinging disc. Trophozoites divide into progeny trophozoites, which populate more and more of the intestinal mucosa. Periodically some of the progeny trophozoites form cysts which are excreted with feces to the external environment.

The consequence of parasites' localisation in the intestines is the destruction of the intestinal mucosa due to mechanical irritation of the mucosa and enzymes secreted by protozoa such as proteinase, peptidase and hyaluronidase. The consequences are digestive and absorption disorders. Giardia invasion is also accompanied by impaired absorption of fats, vitamins A and B12 and carbohydrates. Protozoa can also be located in the gallbladder, bile ducts and pancreatic duct.

Giardiasis is usually asymptomatic but may cause chronic, recurrent, pasty diarrhea, rich in mucus. The affected animals show anorexia, vomiting, weight loss and apathy. Symptoms are particularly severe and the course of the infection is more severe in young animals, immunocompromised animals, or animals co-infected with other gastrointestinal pathogens.

There are 3 main methods used to diagnose giardiasis: microscopic examination, immunological tests and molecular tests. The gold standard method is the immunofluorescence test, but according to also microscopic examination of stools using zink sulfate is possible. Performed by a skilled parasitologist and with sufficiently high intensity of infestation, gives reliable results. The sensitivity of these tests also depends on the number of samples tested. This is due to the fact that cysts (and sometimes trophozoites) of the parasite are excreted irregularly and in variable numbers. Because of the intermittent nature of cyst excretion, several (usually three) stool samples taken daily or every other day should be examined.

Microscopic methods include direct stool smear examination and flotation, and the sensitivity and specificity of these tests depend on the knowledge, experience, skill, and accuracy of the laboratory technician. Direct smears of feces stained with Lugol's solution can reveal the presence of cysts and occasionally trophozoites, but due to the widespread contamination of samples with environmental elements, great skill is required. The flotation test enables the detection of Giardia cysts. The flotation solution is critical to the reliability of the test. Zinc sulphate solution and sugar solution are considered as the best for this purpose. The commonly used saturated saline solution may cause damage to the cysts.

Immunological methods with the usage of specific antibodies, can detect, the antigens of cysts and trophozoites of the parasite. There are three groups of immunological methods used to diagnose giardiasis: immunofluorescence tests, enzyme-linked immunosorbent assays (ELISA) and immunochromatographic (IC) tests. The immunofluorescence test is considered as the gold standard by some sources, but due to its high cost, it is not routinely used in veterinary practice. Therefore, ELISA and immunochromatography (IC) are the most commonly used immunological methods. In the ELISA assay, the color reaction indicates the presence of G. intestinalis antigen to which enzyme-labeled antibodies have bound. Similarly, the rapid IC test is considered as a simple and reliable diagnostic method of Giardia infection and is far less demanding and less expensive.

The **PCR** test for the detection of Giardia DNA is currently one of the most expensive tests for the diagnosis of giardiasis, and is therefore particularly useful when the concentration of cysts in the sample is low, or when other techniques fail. PCR also allows the identification of the genotypes causing the infection, which is important for the determination of the zoonotic potential of parasites.

Monitoring the effectiveness of treatment is sometimes problematic because even after successful therapy combined with disinfection of the environment, diagnostic tests may provide false positive results. In case of antigen tests, there is a risk of false positive results for 2-4 weeks after treatment. The method recommended to control the treatment is a microscopic evaluation of the direct stool smear performed several times. Only by obtaining several negative results from subsequent examinations can answer the question if the therapy applied was appropriate and the patient has been cured of giardiasis. In case of persistent or recurrent diarrhoea despite antiparasitic treatment, the possibility of drug resistance or reinfection should be considered. Reinfection can be a consequence of contact of the treated animal with protozoan infected individuals (e.g. humans or asymptomatic carriers) or contact with parasites that are present in the environment. Therefore, proper hygiene, especially bathing of treated individuals, is crucial.

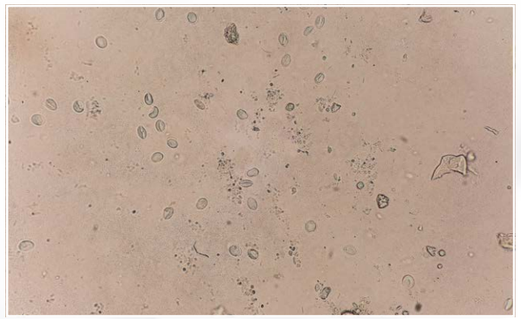


Photo 1. ***Giardia* cysts in faecal examination by flotation with zinc sulphate, magnification 400x.** Photo by Dawid Jańczak, DVM PhD.

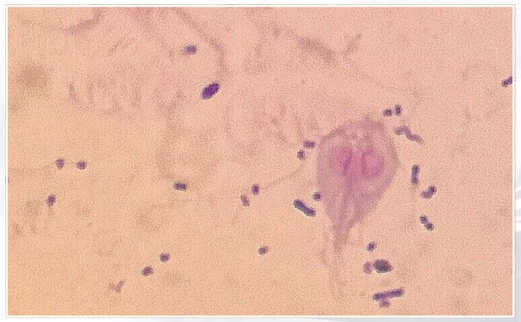


Photo 2. ***Giardia* trophozoite in smear from dog's feces, stained with DiffQuick, magnification x400.** Photo provided by Monika Wiśniewska (Przychodnia Weterynaryjna Jacek Bany, Legionowo).

QUALITY EVALUATION OF VET EXPERT Giardia Ag TEST

Objective: comparison of results obtained with classic PCR technique with results of microscopic analysis of fecal smears and results of **Vet Expert Giardia Ag** and rapid tests of different manufacturer that detects *Giardia spp.* in feces of dogs and cats.

Study material: fecal samples taken from 40 dogs and cats with clinical signs of diarrhea and and samples taken from 10 healthy animals.

	Vet Expert Giardia Ag	Rapid test of different producer	Direct fecal smear
Consistency with **PCR** method - positive results	97,5%	85%	85%
Consistency with **PCR** method - negative results	100%	100%	100%

The consistency of the Vet Expert Giardia Ag test with the PCR test is 97.5%.

The **Vet Expert Giardia Ag** test is a reliable and useful tool in clinical practice. It is a quick and easy way to confirm giardiasis in a patient by detecting *Giardia spp* antigen in the feces with an accuracy of 97.5%.

The **Vet Expert Giardia Ag** test shows 100% consistency for negative results, i.e. all cases of lack of cysts resulted negative.

Furthermore, in terms of positive results, it showed a significantly higher consistency (97.5%) than the smear test direct faecal smear test and different manufacturer's test (85%).

It is advised to perform the **Vet Expert Giardia Ag** test on any dog or cat showing signs of diarrhoea or other gastrointestinal symptoms, as a part of the differential diagnosis. It is also recommended for animals with recurrent gastrointestinal problems.

Regular testing of young animals is recommended, especially in spring and autumn when the infection rate is high. The test is helpful if co-infections with other intestinal parasites are suspected. Mixed infections complicate treatment and extend recovery time.

CANINE DIROFILARIASIS

Dirofilariasis is a parasitosis of dogs, cats and wild carnivores, that is common in southern Europe, especially in the Mediterranean basin. Once exotic, now it is one of the vector-borne diseases also present in Poland.

The disease is caused by nematodes of Dirofilaria species, mainly Dirofilaria immitis and Dirofilaria repens. Both pathogens differ in biology and virulence.

D. immitis localizes in the blood system, in the right ventricle and the pulmonary artery and it is the etiologic agent of **cardiopulmonary dirofilariasis**, whereas *D.repens* localizes mainly in subcutaneous and intramuscular connective tissue, giving the picture of **cutaneous dirofilariasis**.

***Culicidae* mosquitoes** act as intermediate hosts and vectors. The definitive hosts are various vertebrates including dogs, cats and other carnivores.

The life cycle of *Dirofilaria*, regardless of species, is similar and includes several development stages.

Mature female nematodes produce numerous larvae, called microfilariae, at the site of infestation in the body. Microfilariae circulate in the blood of hosts and are most numerous in skin capillaries late at night, at the same time as increased mosquito activity.

While feeding, mosquitoes take the blood of infected animals with the microfilariae in it. In order for the larvae to develop to the invasive stage in the mosquito's body, a temperature of at least 14°C is required throughout their development. In the mosquito's body, the microfilariae develop into the invasive stage, the L3 larva, within 2-3 weeks. During the mosquito's next blood meal, the invasive larvae penetrate under the skin of the definitive host and after 13-14 days and another sheding, they reach stage IV. The larvae then begin to migrate to their permanent location. During their migration they moult once more and reach sexual maturity within 6 to 9 months. Adult nematodes live from 2 to 4 years.

Depending on the *Dirofilaria species*, two forms of the disease can be distinguished: **cutaneous and cardiac**.

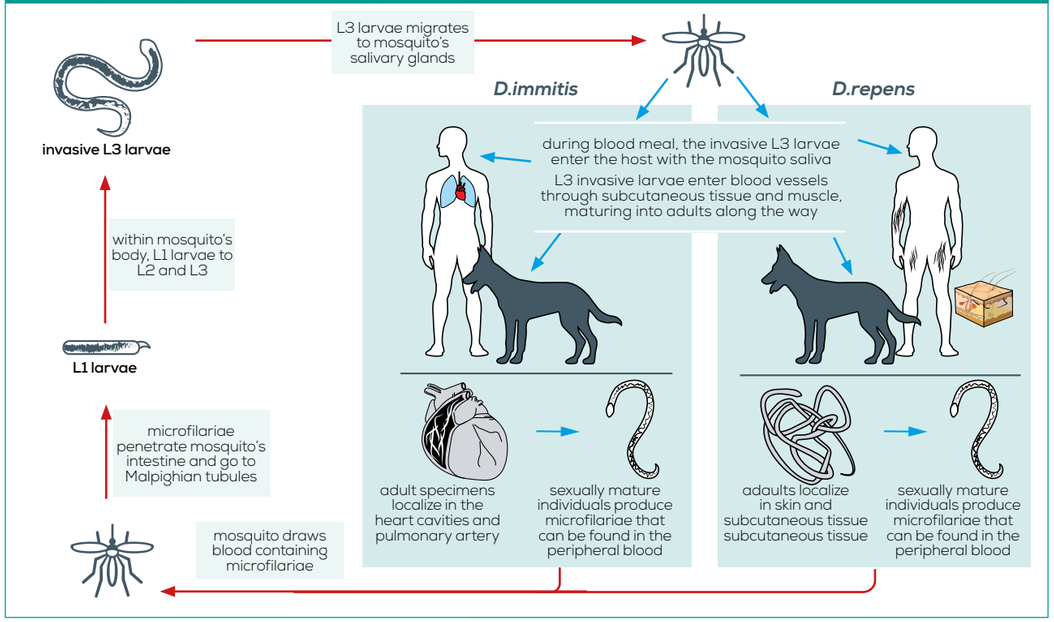
D. immitis causes cardiopulmonary dirofilariasis, dangerous and difficult to treat, often causing a direct life-threatening state. Parasites that die naturally or as a result of treatment cause obstructions in the blood vessels and trigger a severe inflammatory response. The body reacts to nematodes by producing antibodies, which form complexes with antigens (larvae, microfilariae, adults), which accumulate in the kidneys leading to glomerulonephritis. Multiple organs and systems can be affected by the invasion, and the clinical symptoms that develop are usually nonspecific. Regardless of the location of the nematode in the body, cardiopulmonary dirofilariosis is usually accompanied by cough, exercise intolerance, progressive respiratory failure, and sometimes splenic enlargement.

Infection with D. repens that is causing cutaneous dirofilariasis may be asymptomatic. However, nodular multifocal dermatitis or itchy papular eruptions, multifocal alopecia and erythema, hyperpigmentation and hyperkeratosis are frequently observed. Occasionally, purulent inflammatory lesions develop in the skin. Skin lesions are accompanied by the presence of mature nematodes under the skin or microfilariae in the skin. General symptoms such as lack of appetite, lethargy, weight loss and conjunctivitis may also occur.

Diagnosis of dirofilariasis should follow a two-step approach. The first step involves a clinical examination of the patient with additional tests, and the second step involves laboratory tests to confirm the presence of nematodes in animal's organism.

Diagnostic imaging techniques – X-ray, ECG, echocardiography – are used in the diagnosis of cardiopulmonary dirofilariosis. Based on their results, it is possible to assess the condition of the patient and the disease progression. Chest radiography in patients with advanced disease stage shows dilatation of the pulmonary artery, pulmonary infiltration, and enlargement of the right side of the heart. Echocardiography can demonstrate parasites in the pulmonary artery, right atrium, and right ventricle. There are multiple laboratory tests that are available for the detection of canine dirofilariasis.

Development cycle of *Dirofilaria spp.*



Source: Centers for Disease Control and Prevention (cdc.gov)

Microscopic examination of a blood drop or peripheral blood smear may reveal the presence of microfilariae. Vigorously moving microfilariae are most easily detected by drawing blood in the evening, during mosquito feeding, when mosquito activity is significantly increased. Adding a drop of isotonic saline solution is also helpful. Although there are significant differences in the morphology of microfilariae of the two nematode species that can be seen on stained blood smears, PCR is recommended for accurate species identification. However, this requires specialized equipment and is quite expensive. Rapid tests for the detection of *D.immitis* antigens in blood, based on immunochromatographic or ELISA methods, are extremely helpful in the diagnosis of cardiac dirofilariosis. In the past, such tests detected only adult female antigens, creating the risk of false-negative results in the case of infestations of only males or immature individuals. Current tests detect *D.immitis* regardless of the developmental form and are therefore useful tools in everyday clinical practice.

While *D.repens* infections appear to be common nowadays, *D.immitis* arises no significant risk to dogs in Poland. However climate change, as well as the increased frequency of travel with animals may cause the disease to appear in Poland and this makes it necessary to constantly monitor the disease.

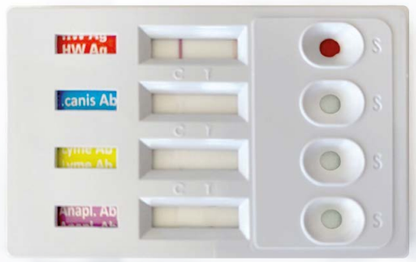


Photo 3. **Negative result of Vet Expert CaniV-4 test for *D.immitis*.** Photo provided by Klaudiusz Szczepaniak, DVM PhD and Prof. Łukasz Adaszek, DVM PhD.

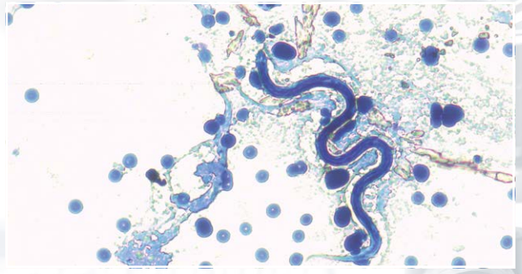


Photo 4. **Microfilaria in dog's peripheral blood smear, stained with MGG.** Photo provided by Klaudiusz Szczepaniak, DVM PhD and Prof. Łukasz Adaszek, DVM PhD.

QUALITY EVALUATION OF VET EXPERT CaniV-4 TEST

Dirofilaria immitis

Objective: comparison of results of classical PCR technique with results of microscopic blood smear analysis and results of **Vet Expert CaniV-4** and different manufacturer's rapid tests for Dirofilaria spp. antigen detection in dogs, combined with an evaluation of tests for possible cross-reactions between *Dirofilaria species*.

Study material: blood and serum samples taken from 20 dogs with signs of cutaneous dirofilariosis and 10 healthy dogs.

	Vet Expert CaniV-4 Test	Rapid test of different producer
Consistency with **PCR** method towards ***Dirofilaria immitis*** - negative results	100%	100%
Cross-reaction with ***Dirofilaria repens***	0	0

Due to lack of material positive for *Dirofilaria immitis*, the experiment was limited to comparing only negative samples.

The **Vet Expert CaniV-4** test for *Dirofilaria immitis* showed 100% consistency in terms of negative results, and is therefore a useful tool for ruling out adult nematode infestations.

Dogs with confirmed cutaneous dirofilariosis were also tested. The test results were negative in all of them, which means that the **Vet Expert CaniV-4** test does not cross-react with *D. repens* infestations.

At the time the test was performed, cardiac dirofilariasis was not a significant problem in Poland, so the test was limited to comparing negative samples only. However, due to the warming climate and widespread migration of dogs, *D.immitis* infections require continuous monitoring. Patients with a positive rapid test and respiratory or circulatory signs should undergo additional investigations, including imaging.

CANINE MONOCYTIC EHRLICHOSIS

Monocytic ehrlichiosis is a multi-organ transmissible disease of animals and humans caused by the intracellular bacteria *Ehrlichia canis*. Its presence has been detected in the blood of horses, cattle, sheep, goats, cats, foxes, llamas, deer and also humans. Other *Ehrlichia* species pathogenic for animals are *E. ewingii*, etiological agent of granulocytic ehrlichiosis and *E. chaffeensis* causing monocytic ehrlichiosis in humans.

The vector responsible for disease transmission are **ticks** of the *Rhipicephalus sanguineus* species as well as *Ixodes spp*, and *Dermacentor*, which are also involved in babesiosis transmission. In the tick population, the infection spreads transstadially (from larva to nymph and then to adult).

The **endemic areas** of canine monocytic ehrlichiosis are mainly Mediterranean countries, but it is becoming more common in other regions, including Poland. The disease spreads to new areas due to a chronic, subclinical course of the infection in dogs, which often becomes fully developed only months or years after the initial contact with the pathogen.

Dog breeds that are particularly susceptible to ehrlichiosis are German Shepherds and Siberian Huskies, in which the disease lasts longer and its course is described as much more severe.

In the initial phase of infection, *E. canis* multiplies in mononuclear cells of the phagocytic system – monocytes and platelets – and spreads throughout the body. This leads to bone marrow damage, pancytopenia, especially thrombocytopenia, which in combination with inhibition of platelet aggregation may contribute to the development of life-threatening hemorrhagic lesions. Immune processes play a major role in the pathogenesis of the disease. The mechanism of thrombocytes destruction is not fully understood, but it is suspected to be a result of autoimmune reaction against own platelets glycoproteins.

The **incubation period** of the disease is 1-3 weeks, and the first clinical symptoms are non-specific. The course of ehrlichiosis depends on individual predisposition, the strain of bacteria and the possible presence of coexisting diseases. Initially, apathy, weakness, high temperature are being observed.

During **acute course**, there may be a body weight loss, bleeding from mucous membranes, enlarged spleen and generalized lymph node enlargement (the effect of microbial multiplication in the reticuloendothelial cells) and, less frequently, mucopurulent nasal discharge. Vomiting, diarrhea, arthritis, swelling of peripheral parts of the limbs and sometimes even neurological symptoms (seizures, ataxia, vestibular symptoms, hypersensitivity or impaired cranial nerve function) may also occur as a result of meningitis or extravasations in the central nervous system. Immunosuppression may be followed by secondary bacterial infections, e.g. limb skin ulcers. After 2-4 weeks of acute symptoms, dogs may spontaneously recover, or the infection may progress to a subclinical or chronic phase that can last for months or even years. Due to antigenic mimicry, *Ehrlichia spp.* may avoid contact with antibodies, resulting in long-term persistence.

Chronic monocytic ehrlichiosis is characterized by a very complex clinical picture. It is presumed that the development of chronic monocytic ehrlichiosis is due to the genetic predisposition of dogs to this type of infection. It is very often in chronically infected dogs that the bone marrow hypoplasia occurs resulting in pancytopenia. The most common chronic signs are apathy, fever, inappetence, polyuria, polidypsia, persistent weight loss, spontaneous bleeding with petechial haemorrhages on mucous membranes and skin, lymphadenopathy, splenomegaly, swelling of the pelvic limbs and scrotum in males, pale mucous membranes, and ocular inflammation. In addition, interstitial pneumonia with dyspnea, renal dysfunction, glomerulonephritis, and arthritis with lameness may develop. Less commonly, musculitis is observed, resulting in muscle weakness and sometimes even quadriplegia. Because strong immunosuppression develops in the course of the disease, monocytic ehrlichiosis is very often accompanied by secondary bacterial infections and parasitic infections (mainly protozoan).

Haematology shows anaemia, thrombocytopenia, decreased haematocrit and monocytosis, while biochemical examination may show hyperglobulinaemia, hypoalbuminaemia, hyperproteinaemia, increased alkaline phosphatase, lactate dehydrogenase and hepatic aminotransferases, increased urea and creatinine. Laboratory tests can show lymphocytosis and bone marrow plasmocytosis, sometimes accompanied by monoclonal gammopathy. These abnormalities may be the cause of misdiagnosis of E. canis infected dogs with diseases such as lymphocytic leukemia or multiple myeloma. Immune-mediated glomerulonephritis may be followed by the development of protein-losing nephropathy.

In the diagnosis of ehrlichiosis, the simplest method to detect the presence of rickettsiae is Giemsa or Diff-Quick staining of blood smears. With the microscopy method we can observe inside monocytes inclusions resembling the appearance of mulberry fruit – morulae. Unfortunately, they are only visible between 4 and 14 days after infection, so the sensitivity of this test in the case of chronic disease is low. Additionally, morulae can be present in monocytes from only 4-6% of infected individuals. The polymerase chain reaction (PCR) is a test for the detection of *E. canis* DNA. It has a higher sensitivity for early detection but it's usefulness is limited in chronic disease.

The diagnosis of ehrlichiosis is generally issued by basing on clinical symptoms and the results of serological tests (ELISA, IC, indirect immunofluorescence or Western-blott). The seroconversion occurs 1-4 weeks after exposure to the pathogen, so dogs in the acute phase of infection, may be serologically negative.

A positive rapid serological test combined with the presence of clinical signs raises the suspicion of chronic ehrlichiosis and requires therapeutic activities. In doubtful cases, it is recommended to confirm results by using additional diagnostic methods.

Photo 5. **Positive result of Vet Expert CaniV-4 test for *E. canis* antibodies.** Photo provided by Klaudiusz Szczepaniak, DVM PhD and Prof. Łukasz Adaszek, DVM PhD.

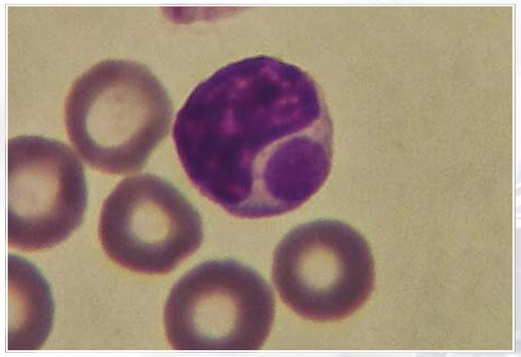


Photo 6. ***Ehrlichia canis* morula in dog's monocyte.** Photo provided by Klaudiusz Szczepaniak, DVM PhD and Prof. Łukasz Adaszek, DVM PhD.

QUALITY EVALUTATION OF VET EXPERT CaniV-4 TEST

Ehrlichia canis

Objective: comparison of **immunofluorescence (IF)** test results with **Vet Expert CaniV-4** rapid tests and rapid tests from different manufacturer in detecting antibodies to *Ehrlichia canis* in dogs.

Study material: blood and serum samples taken from 40 dogs with signs of monocytic ehrlichiosis and 10 healthy dogs.

	Vet Expert CaniV-4 Test	Rapid test of other producer
Compatibility with reference method **IF** – positive results	85%	68,75%
Compatibility with reference method **IF** – negative results	100%	100%

The **Vet Expert Cani-V 4** test for *Ehrlichia canis* showed 85% consistency in antibody detection with respect to the reference method. In clinically symptomatic dogs, a positive test result is helpful in issuing a diagnosis. It is important to remember that the time required to produce specific antibodies varies individually and ranges from 7 to 28 days.

Compared to different manufacturer's test, the **Vet Expert** test showed a higher sensitivity in detecting antibodies to *E. canis* in sick dogs.

In addition, no false positives were observed in the tested dogs in the absence of infection. The **Vet Expert** test showed 100% consistency for negative results.

BORRELIOSIS IN DOGS

Borreliosis, also known as **Lyme disease**, is a multi-organ tick-borne disease caused by bacteria belonging to the Spirochetaceae family – highly pathogenic Borrelia burgdorferi sensu lato. The most pathogenic for humans and animals are *B. afzelii*, *B. garinii, B. bavariensis* and *B. burgdorferi sensu stricto*.

Bacterias are **transmitted by** ticks of the Ixodes genus, of which *Ixodes ricinus* is the main representative in Europe, and the interaction between the bacteria, their vector and the organism of the infected host determines whether the disease will develop or not. It is believed that in order to transmit Lyme disease, the parasite has to stay on the host for more than 24 h. Therefore, the rapid removal of the parasite significantly reduces the risk of disease development.

In Europe, the main reservoir of *Borrelia spp*. are small rodents (mice, rats and rabbits) as well as some species of reptiles and birds.

Recent epidemiological data show that in Europe on average 13.7% (0-49.1%) of ticks are infected with *B. burgdorferi*, and in Poland up to **40%**.

The spirochetes of *Borrelia burgdorferi sensu lato* have several specific surface proteins (e.g. OspA, OspC, OspF, C6) allowing their classification into specific genogroups and also commonly used in diagnostics. After the blood meal of the tick, the change of temperature and pH within its digestive tract occurs. This causes a change in the conformation of surface proteins of bacteria (mainly OspA to OspC) and their migration from the intestine to the salivary glands of the tick. The change in the surface proteins of the bacteria is necessary to cause infection. Bacterial entry into the body causes activation of macrophages and neutrophils, release of large amounts of proinflammatory cytokines, and subsequent local inflammation. The spirochetes initially multiply in the skin, then enter the blood and from there into the tissues where they settle. Due to their predilection for collagen-rich tissues, these include the skin, joints, heart muscle and nervous system. After entering the body, B.brugdorferi persist permanently within the body. It has been shown experimentally that the spirochetes localize extracellularly (in the skin they multiply in the intercellular spaces) and inexplicably avoid the immune system for a long time.

The course of Lyme disease in dogs is highly variable and may be acute or chronic. Not every contact with the pathogen leads to the disease. Clinical signs are observed in only 5-10% of infected dogs and the time that elapses from contact to manifestation of the disease is several months and generally correlates with an increase in antibody titres.

In dogs, migratory erythema that is characteristic for humans is not observed. Very often, only high fever (> 40°C), apathy and lymphadenopathy are observed. In such animals it is difficult to diagnose Lyme disease, especially when the owners have not recorded the presence of a tick on the body of the sick animal.

The most common form of Lyme borreliosis found in dogs is **joint borreliosis**. Joint inflammation is caused by the proliferation of bacteria and increased expression of interleukin-8, which attracts neutrophils to inflamed areas of the synovial membrane and increases the severity of arthritis. The joint closest to the site of the tick bite is the first to become inflamed. The inflammation is accompanied by lameness, joint swelling, and fever. The lameness may be migratory, meaning that it initially affects one limb and then, after a few days, affects the other limbs. The pathological changes in the joints are progressive and may worsen even with antibiotic treatment.

Acute glomerulonephritis is much less common in dogs with Lyme disease. The prognosis for this form of disease is poor. It has been reported that golden retrievers, Labrador retrievers, Bernese Mountain Dogs and Shetland Sheepdogs are predisposed to this form of Lyme disease. Nephritis may be accompanied by polyarthritis and thrombocytopenia. Mechanism of the development of glomerulonephritis is not clear. It is suspected that the glomeruli may be blocked by immune complexes and improvement of patients have been observed after administration of immunosuppressive drugs. Glomerulonephritis is accompanied by loss of protein in the urine, which results in edema and fluid accumulation in body cavities. Animals lose weight, and when they develop uremia, vomiting may occur.

Occasionally Lyme disease in dogs results in myocarditis. Unlike in humans where Lyme borreliosis can be accompanied by meningitis and encephalitis, neuroborreliosis is not observed in dogs.

The diagnostics of Lyme disease is difficult. The clinical course of Lyme disease can vary greatly and a suspected case should always be confirmed by laboratory tests. The diagnosis is made based on a detailed medical history, clinical examination and laboratory tests.

Diagnostic methods for Lyme disease can be divided into **two groups**. The first group includes techniques allowing direct detection of bacteria in the examined material (microscopic examination, PCR and culture tests), the second group includes methods of indirect diagnosis (serological tests). Of the direct methods for detecting bacteria, PCR is the most effective. It allows detection of spirochete DNA in a variety of material. It is a sensitive test, although it can sometimes give false-negative results if the material for PCR was improperly collected. In the case of swollen joints, the best material for PCR is synovial fluid, in the cutaneous form – skin biopsies, and in case of kidney damage – urine. It is important to note that the spirochetes in the blood persist only during bacteremia, so the PCR test performed with the blood is not commonly used in the diagnosis of Lyme disease.

Also, the Borrelia culture test has limited usefulness for the diagnosis as well. Bacteria require special media and are best isolated from inflamed tissues (joint fluid, skin). The sensitivity of this method is relatively low.

Because of the low reliability of direct methods, the diagnosis of Lyme disease is now based on **serological tests**. IgM antibodies are appearing in the dog's blood within a week post infection, when the IgG antibodies appearing after 4-6 weeks (more than 40 days). The most commonly used serological tests are the enzyme linked immunosorbent assay (ELISA) and the indirect immunofluorescence (IF). It is important to remember that the antigens for both tests are prepared in different ways and there is no standardized procedure for obtaining them and the results vary between laboratories. If the ELISA test uses whole bacterial cells as antigen, it is possible to obtain false-positive results in the situation when a given individual had a history of contact with antigenically similar bacteria to Borrelia, such as Leptospira. It is therefore important to exclude possible cross-reactions. Commercially available **rapid tests** for the diagnosis of Lyme disease detect antibodies to specific proteins of the C6 and OspF class, which are only formed during natural infection. Therefore the danger of false positive results, even in animals vaccinated against Lyme disease, is very limited. Rapid diagnostic kits show high sensitivity and specificity, so they are successfully used in the daily veterinary practice. In case of doubtful results, immunoblotting, which allows detection of proteins of a specific mass, can be conclusive. Antibodies from naturally infected individuals react in the immunoblotting test with different Borrelia proteins than the immunoglobulins produced by vaccination (vaccines use OspA proteins or whole bacterial cells).

The diagnostics of Lyme disease is problematic and often inconclusive. To give the definite diagnosis of Lyme disease, the coexistence of 4 elements is required:

1. clinical signs typical of Lyme disease (lameness)
2. a positive serum Borrelia antibody titre
3. confirmed contact of the patient with ticks
4. positive response to treatment

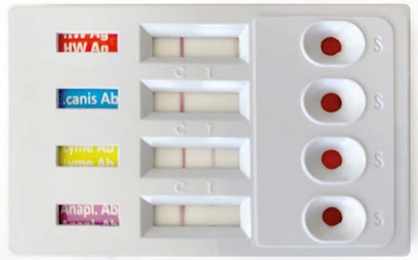


Photo 7. **Positive result of Vet Expert CaniV-4 test for antibodies to *B. burgdorferii*.** Photo provided by Klaudiusz Szczepaniak, DVM PhD and Prof. Łukasz Adaszek, DVM PhD.

Photo 8. **Positive result of ELISA test for antibodies against *B. burgdorferii*.** Photo provided by Klaudiusz Szczepaniak, DVM PhD and Prof. Łukasz Adaszek, DVM PhD.

QUALITY EVALUATION OF VET EXPERT CaniV-4 TEST

Borrelia burgdorferi

Objective: comparison of **ELISA** results with results of **Vet Expert CaniV-4** rapid tests and rapid tests of different manufacturer in detecting antibodies of *Borrelia burgdorferi* in dogs.

Study material: blood and serum samples taken from 40 dogs with signs of Lyme disease and 10 healthy dogs.

	Vet Expert CaniV-4 test	Rapid test from different manufacturer
Concordance with the **ELISA** - positive results	92,5%	87,5%
Concordance with the **ELISA** - negative results	100%	100%

The **Vet Expert CaniV-4** test for *Borrelia burgdorferii* showed over 92% consistency with the accepted reference method in dogs with Lyme disease symptoms. It is therefore a helpful tool in the diagnosis of Lyme disease in this species.

It is notable that a minimum of 40 days is required to produce specific antibodies detected by this test. In relation to a test from another manufacturer, the **Vet Expert** test showed a higher sensitivity in detecting antibodies against *B. burgdorferii* in sick dogs.

The **Vet Expert** test showed 100% consistency in terms of negative results. No false positives were observed in the tested dogs in the absence of infection.

GRANULOCYTIC ANAPLASMOSIS IN DOGS

Granulocytic anaplasmosis is a multi-organ disease of humans and animals with thrombocytopenia caused by *Anaplasma phagocytophilum*, which belongs to the *Rickettsiales* order in the *Anaplasmataceae* family. Until recently it was included in the *Rickettsiaceae* family, *Ehrlichia genus*. Currently it is classified within the *Anaplasma genus*.

Anaplasma platys, which attacks platelets and causes thrombocytic anaplasmosis, also belongs to the same genus. In contrast to granulocytic anaplasmosis granulocytic anaplasmosis, the disease has not clinical significance at this time.

The reservoir of *Anaplasma phagocytophilum* is a variety of wild mammals (mice, rats, deer) and birds, whereas the vector responsible for the transmission of Anaplasmosis are ticks of the Ixodes spp. genus. For transmission, arachnids must reside on the host for at least 48 hours.

The **incubation period** for granulocytic anaplasmosis is 1-2 weeks. Clinical signs in dogs are quite non-specific and include apathy, weakness, high fever. Coughing may also occur.

During **acute phase** the liver, spleen and lymph nodes are enlarged, mucosal bleeding (e.g. nosebleeds) and weight loss may also occur. In addition, vomiting, diarrhea, often containing blood in the stools, and arthritis or muscle soreness with lameness may occur. Nervous symptoms also occur in humans, so the possibility of their occurrence in dogs should not be excluded.

One of the characteristic symptoms of granulocytic anaplasmosis is thrombocytopenia which is a consequence of platelet destruction by immune cells, increased phagocytosis by macrophages and increased breakdown in the spleen. An additional cause of thrombocytopenia may also be the development of bone marrow hypoplasia.

Hematologic studies show mild to severe thrombocytopenia, lymphopenia, and non-regenerative anemia as a result of bone marrow hypoplasia or a chronic form of the disease. Hypoalbuminemia and increased ALP activity are observed in biochemical tests.

Rickettsiae of *Anaplasma phagocytophilum* persist in the infected organism inside neutrophils and eosinophils. They multiply inside the granulocytes and form structures called morulae. In the infected host organism, this microorganism inhibits neutrophil apoptosis, which results in prolonged morulae persistence.

The diagnosis of granulocytic anaplasmosis, could sometimes be difficult, is sometimes difficult. Diagnosis should be based on a patient history, a detailed clinical examination, and the results of additional examinations (microscopic evaluation of blood smears, molecular and serological tests).

The **history** should provide information on whether the dog has been in an area endemic for anaplasmosis (in Poland most cases are reported in the Lublin, Mazovia, Warmia-Masuria and Subcarpathia Provinces), whether ticks were found on the body, and whether the dog shows clinical signs of the disease.

A **clinical examination** will evaluate the current status of the patient.

Microscopic examination of blood smears stained with Diff- Quick, Wright or Giemsa stain can demonstrate the presence of *A. phagocytophilum morulae* in the cytoplasm of granulocytes. In the acute phase of infection, they may be found in 7-32% of neutrophils. The morulae appear 4-12 days after infection and persist only 4-8 days. For this reason, microscopic examination has a very low sensitivity. In addition, the result depends on the intensity of the bacteremia and the experience of the person evaluating the slide.

Available serological tests detect antibodies of Anaplasma phagocytophilum, which can persist in the blood for up to several months after contact with the pathogen. However, they do not differentiate between antibodies arising after infection with Anaplasma platys. Serological methods, including rapid tests, are based on the detection of antibodies directed against a surface protein that is identical in Anaplasma phagocytophilum and Anaplasma platys.

In addition to their usefulness in diagnosing granulocytic anaplasmosis, **rapid tests** enable the simultaneous diagnosis of other tick-borne diseases. They detect antibodies IgG class antibodies, which appear in the blood approximately 14 days after infection. If the acute phase of the disease is suspected, as a confirmation, the PCR testing should be considered. Antibody detection with the **indirect immunofluorescence test (IFAT)** requires sending samples to external, specialised laboratories specialised laboratories.

Molecular PCR testing allows detecting of genetic material of rickettsiae and identify the strain, but does not differentiate them, but does not differentiate between live and dead microorganisms. The test is expensive and requires the involvement of a specialised laboratory. It has the highest sensitivity in diagnosing infections up to 14 days after infection. Persistence of DNA fragments of killed bacterials in tissues after the antibiotic treatment, may cause false-positive results of the PCR test.

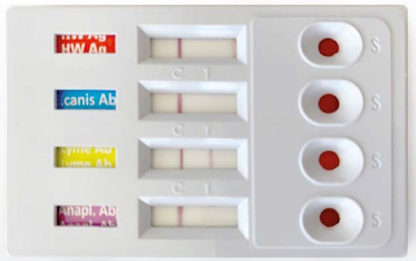


Photo 9. **Positive result of Vet Expert CaniV-4 test for antibodies to *Anaplasma spp.*** Photo provided by Klaudiusz Szczepaniak, DVM PhD and Prof. Łukasz Adaszek, DVM PhD.

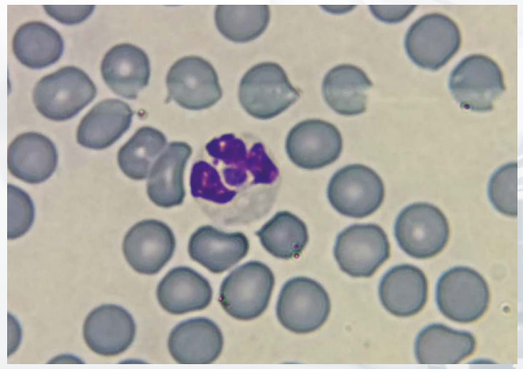


Photo 10. ***A. phagocytophilum* morula in canine neutrophil, peripheral blood smear stained with DiffQuick.** Photo provided by "Pani Krwinka".

QUALITY EVALUATION OF VET EXPERT CaniV-4 TEST

Anaplasma phagocytophilum

Objective: comparison of the **immunofluorescence (IF)** test results with **Vet Expert CaniV-4** rapid tests and a rapid test from different manufacturer in detecting antibodies of *Anaplasma phagocytophilum* in dogs.

Study material: blood and serum samples taken 40 dogs with signs of granulocytic anaplasmosis and 10 healthy dogs.

	Vet Expert CaniV-4 Test	Rapid test of other producer
Concordance with **IF** method – positive results	100%	100%
Concordance with **IF** method – negative results	100%	100%

The **Vet Expert Cani-V 4** test for *Anaplasma phagocytophilum* showed 100% consistency in terms of positive and negative results. It is therefore a useful tool in the diagnosis of canine anaplasmosis. The test detects specific antibodies that appear 14 days after infection.

SUMMARY

In comparative studies, the **Vet Expert CaniV-4** and **Giardia Ag** tests showed a high level of results consistency. They are therefore a useful tool for the diagnosis of canine vector-borne diseases and canine and feline giardiasis.

We recommend performing the **Vet Expert CaniV-4** test as soon as clinical signs suggestive of a tick-borne disease are observed and repeat the test after a minimum of 40 days. This will enable detection of antibodies of *Anaplasma* and *Ehrlichia*, as well as *Borrelia*, for which seroconversion takes the longest time. Coming to the topic of *Dirofilaria*, we recommend performing the Vet Expert Giardia Ag test on every patient living in areas of endemic cardiac dirofilariasis and in case of presence of microfilariae in the peripheral blood.

The **Vet Expert Giardia Ag** test is worth performing on any patient with diarrhoea as a part the differential diagnosis.

When assessing the usefulness of rapid tests in clinical practice, it is important to have in mind the **sensitivity and specificity** of the diagnostic test. These are based on a comparison of test results of the same sample, obtained by two different methods or kits. These parameters can vary considerably depending on the methodology used.

Sensitivity (consistency of positive results) tells us about the ability of the test to detect the pathogen or disease among animals that are actually sick. The higher the sensitivity is, the fewer false-negative results are obtained. On the other hand, a high **specificity** (consistency of negative results) ensures no false positives and no detection of the disease or pathogen in healthy animals. Tests with high specificity can therefore be helpful to exclude the presence of a given infectious agent in a test sample.

In the case of **antibody detection** tests, it is crucial for correct interpretation not only to relate the test result to the clinical picture and the patient history, but also to take into account the time of antibody production (seroconversion), which is different for each disease entity. Each test should therefore be performed and evaluated at a specific time point, when the organism has already produced antibodies and their level is detectable.

The **Vet Expert Giardia Ag** test, detecting ***Giardia intestinalis*** cyst antigen in canine and feline faeces showed a 100% consistency in terms of negative results and 97.5% consistency in terms of positive results, compared to the PCR method.

The **Vet Expert CaniV-4** test for ***Dirofilaria immitis*** has only been compared within the range of negative results and gave a consistency of 100%, and it is therefore a very helpful tool to exclude infection with the nematode ***Dirofilaria immitis***, the etiological agent of serious cardiac dirofilariasis. When microfilariae are found in peripheral blood smears and the test is negative, the presence of ***Dirofilaria repens*** infection, responsible for cutaneous dirofilariasis, can be assumed with high probability. No cross-reactivity between D. repens and D. immitis was observed in this study.

The **Vet Expert CaniV-4** test for ***Ehrlichia canis*** showed a 100% consistency for negative results and 85% consistency for positive results, and is therefore a useful tool in ruling out disease in a suspected patient. In doubtful cases, it is worth to repeat the test after 3 weeks or considering an additional diagnostic method.

The **Vet Expert CaniV-4** test for ***Borrelia burgdorferi*** showed a consistency of 100% for negative results and 92.5% for positive results, and is therefore an important and low-cost tool for Lyme disease diagnosis.

The **Vet Expert CaniV-4** test for ***Anaplasma phagocytophilum*** showed 100% consistency for positive and negative results and can therefore be successfully used for the diagnosis of chronic anaplasmosis. If an acute phase of the disease is suspected and the test is negative, additional PCR testing should be considered.

Perform the Vet Expert Giardia Ag test in dog or cat:

- √ in any case of diarrhoea, especially in young animals
- √ with gastrointestinal signs other than diarrhoea
- √ adopted or purchased from breeders
- √ in case of no recovery and clinical improvement after treatment

Perform the Vet Expert CaniV-4 test in a dog:

- √ in any case of suspected vector-borne disease
- √ after contact with a tick (seroconversion period is important), it is recommended to peform the retest after 40 days
- √ that are travelling, especially to disease endemic areas
- √ at least once per year as a screening test

Pathogen	Antibodies detectable	Recommended retest time
Anaplasma spp.	after 14 days	antibodies can be present for many months
Borrelia burgdorferi	after day 40	around 3-4 months
Ehrlichia canis	after 7 days (some dogs after 28 days)	3-9 months (individual)

Table 1. Seroconversion in chosen tick-borne diseases in dogs

Pathogen	Vector	Transmission time	Clinical symptoms	Laboratory examination results
Anaplasma phagocytophilum	*Ixodes spp.*	36-48 hours	fever, apathy, diarrhoea, vomiting, arthritis, spontaneous bleeding	severe thrombocytopenia, IMHA
Borrelia burgdorferi sensu lato	*Ixodes spp.*	~24 hours	often subclinical, fever, loss of appetite, arthritis, weight loss	thrombocytopenia, hyperglobulinemia
Ehrlichia canis	*Rhipicephalus sanguineus, Ixodes ricinus, Dermacentor spp.*	< 3 hours	often subclinical, fever, weight loss, lymphadenopathy, splenomegaly	thrombocytopenia, non-regenerative anemia, hyperglobulinemia, azotemia, APTT elongation, bone narrow hypoplasia or aplasia
Dirofilaria immitis	Mosquitoes of the genus *Aedes, Anopheles, Culex*	Invasive larvae are transmitted during blood drawing	often subclinical, cough, tachypnoe, pulmonary embolism, ascites	eosinophilia, thrombocytopenia, leucocytisis, hyperglubulinemia, changes in radiogram

Table 2. Selected informations on the most common vector-borne diseases in dogs in Poland.

References:

1. C.E. Green, Choroby zakaźne psów i kotów, Wyd. Galaktyka 2011
2. Ł. Adaszek, S. Winiarczyk: Rozpoznawanie granulocytozy monocytarnej, Magazyn Weterynaryjny 07/2018
3. Ł. Adaszek: Czas potrzebny na przeniesienie chorób odkleszczowych, Magazyn Weterynaryjny 09/2019
4. Przewodnik ESCCAP 05 Zwalczanie chorób przenoszonych przez wektory u psów i kotów, Wydanie trzecie - marzec 2019
5. L. Kidd, S. Ghaffari: Rozpoznawanie chorób przenoszonych przez wektory u psów. Jak najlepiej wykorzystać dostępne panele diagnostyczne, Magazyn Weterynaryjny 03/2014
6. Ł. Adaszek, M. Staniec, M.Kalinowski, J. Zietek, S. Winiarczyk: Choroby zakaźne psów, Wyd. Elamed 2019
7. Ł. Mazurek, R. Janecki, Ł. Adaszek, O. Teodorowski: Wykorzystanie szybkich testów diagnostycznych w wykrywaniu przeciwciał dla Borrelia burgdorferi i Anaplasma phagocytophilum u psów, Weterynaria w Partyce 11-12/2020
8. S. Winiarczyk, Ł. Adaszek, P. Łyp: Najczęstsze choroby odkleszczowe psów w Polsce, Weterynaria w Praktyce 03/2015
9. W. Zygner, O. Gójska-Zygner, P. Górski, J. Bartosik: Pasożytnicze choroby przenoszone przez stawonogi u psów w Polsce – kompendium lekarza praktyka, Magazyn Weterynaryjny 07/2018
10. Sarah J. Wooten Alarm boreliozowy u labradora! Ale czy na pewno? Weterynaria po Dyplomie 06/2018
11. Ł. Adaszek, P. Łyp, P. Dębiak, A. Śmiech, S. Winiarczyk, Borelioza psów – choroba przenoszona przez kleszcze Magazyn Weterynaryjny 12/2016
12. Ł. Adaszek, B. Dzięgiel, Ł. Mazurek Erlichioza monocytarna małych zwierząt, Weterynaria w Praktyce 4/2019
13. Ł. Mazurek, R. Janecki, Ł. Adaszek, K. Witt-Jurkowska, N. Jackowska-Pejko, Badanie seroepidemiologiczne w kierunku erlichiozy monocytarnej psów, Weterynaria w Praktyce 3/2021
14. A. W. Demiaszkiewicz, M. Radulska, A. M. Pyziel, Przypadek dirofilariozy powiekowej u psa w Polsce Magazyn weterynaryjny 04/2012
15. Ł. Adaszek, R. Janecki, K. Lachowicz, O. Teodorowski, S. Winiarczyk, Czy zarażenia Dirofilaria immitis stanowią zagrożenie dla psów w Polsce? Magazyn Weterynaryjny 04/2021
16. M. Klockiewicz, A. Jasic, K. Karolak, K. Humięcki M. Wysmołek, K. Woźniak, S. Mišków, Dirofilarioza – sercowa czy skórna? Niespodzianki diagnostyczne, Magazyn Weterynaryjny 02/2020
17. Ł. Adaszek, P. Łyp, K. Szczepaniak, T. Skrzypek, S. Winiarczyk, Komary jako wektory chorób ze szczególnym uwzględnieniem dirofilarioz psów, Magazyn Weterynaryjny 06/2017
18. W. Zygner, A. Bajer, Trudności w diagnostyce inwazji Giardia intestinalis u psów i kotów – wyniki fałszywie dodatnie, Magazyn Weterynaryjny 10/2011
19. W. Zygner, Giardioza u psów i kotów: patogeneza - powikłania - frustracje. Cz.I, Weterynaria w Praktyce 10/2019
20. M.Klockiewicz Giardioza kością niezgody? Nowe perspektywy leczenia, Magazyn Weterynaryjny 06/2012
21. Przewodnik ESCCAP 06 wydanie drugie – luty 2018 Zwalczanie pierwotniaków jelitowych u psów i kotów
22. Giardioza. Wytyczne Europejskiej Rady Ekspertów ds. Chorób Kotów (Advisory Board on Cat Diseases – ABCD), Weterynaria po Dyplomie 04/2021
23. Luciana Laranjo Amorim Ventura1, Dirce Ribeiro de Oliveira2, Maria Aparecida Gomes1, Marcia Regina Fantoni Torres: Effect of probiotics on giardiasis. Where are we? Braz. J. Pharm. Sci. 2018
24. Matheus Diniz Gonçalves Coelho, Francine Alves da Silva Coelho, Ismael Maciel de Mancilha; Probiotic Therapy: A Promising Strategy for the Control of Canine Hookworm, Journal of Parasitology Research, vol. 2013
25. Epe C., Rehkter G., Schnieder T., Lorentzen L., Kreienbrock L.: Giardia in symptomatic dogs and cats in Europe -Results of a European study. Vet. Parasitol. 2010
26. Saleh M.N., Heptinstall J.R., Johnson E.M., Ballweber L.R., Lindsay D.S., Werre S., Herbein J.F., Zajac A.M.: Comparison of diagnostic techniques for detection of Giardia duodenalis in dogs and cats. J. Vet. Intern. Med. 2019

Detailed study description and literature available on request from Vet Expert.

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