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Cytological diagnostics of subcutaneous dirofilariasis imitating proliferative lesions in dogs

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Abstract: Subcutaneous dirofilariasis is a disease in animals caused by invasions of nematodes, most often of the following species: *Dirofilaria repens, D. tenuis,* and *D. striata* transmitted by mosquitoes. Until recently, *D. repens* was endemic to the Mediterranean countries in Europe, but, in recent years, it has also been increasingly reported in Central and Eastern Europe. Cytological preparations collected by a fine-needle aspiration biopsy from nodular lesions located in the subcutis and skin of dogs were used to diagnose suspected proliferative lesions of a cancerous or inflammatory nature. The microscopic examination of the delivered cytological preparations revealed erythrocytes (very numerous), neutrophils and eosinophils (quite numerous), macrophages (single), and whole and/or damaged fragments of microfilariae of *Dirofilaria* sp. in various numbers. It should be noted that the described infection of *Dirofilaria repens* in Poland and other countries of this latitude will be an increasingly common pathology in dogs. Due to the mosquitoes transmitting the microfilariae, it is a zoonosis that is an increasingly frequent and a more serious threat to humans. In the differential diagnosis of various types of skin and subcutis lesions of unknown aetiology, dirofilariasis should be considered.

Keywords: canine dirofilariasis; cytology; dermal nodules; tumor-like lesions

Subcutaneous dirofilariasis is a disease of animals (domestic and wild canids) caused by infections of nematodes, most often of the following species: *Dirofilaria repens, D. tenuis,* and *D. striata* transmitted by mosquitoes (Orihel and Eberhard 1998; Taylor et al. 2015; Otranto and Deplazes 2019). The distribution of *D. repens* is restricted to the Old World, with no autochthonous cases reported in North America. The *D. striata* species are endemic to North and South America, while *D. repens* occurs in Southeast Asia, the Middle East, Africa, and Europe. Until recently, *D. repens* was endemic to the Mediterranean countries in Europe, but, in recent years, it has also been increasingly reported in Central and Eastern Europe (Ferasin and Knight 2005; Demiaszkiewicz et al. 2009; Demiaszkiewicz 2013; Ilyasov et al. 2015). *Dirofilaria repens* affecting domestic and wild canids is transmitted by several species of mosquitoes. It usually causes a non-pathogenic subcutaneous infection in dogs and is the principal agent of human dirofilariasis. Over the last several years, *D. repens* has increased in prevalence in areas where it has already been reported, and its distribution range has expanded into new areas of Europe (Capelli et al. 2018; Otranto and Deplazes 2019). According to these authors, this represents a paradigmatic example of an emergent pathogen. Subcutaneous dirofilariasis is a disease

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that is most often mild in animals (the course of the infection is not severe). The invasion may manifest itself in the form of small subcutaneous nodules, rarely painful or itchy, inside of which the adults and microfilariae (nematode larvae) are localized. In some cases, the parasite may also be subconjunctival (Ferasin and Knight 2005; Zarnowska-Prymek et al. 2008; Demiaszkiewicz et al. 2013; Taylor et al. 2015). In dogs, asymptomatic invasions are quite common, less frequent in cats. Cases of infections *D. repens* in porcupines, bobcats, red foxes, wolves, coyotes, and weasels, *D. tenuis* in raccoons, *D. striata* in wild cats (in North America) have also been reported (Otranto and Deplazes 2019).

Human beings can be an occasional host of parasites from the *Dirofilaria* genus, and the changes that these parasites cause are most often located in the conjunctiva, eyelids, scrotum, inguinal region, buttocks, legs, breasts, and arms. These changes are initially diagnosed as nodules or granulomas of unknown aetiology. Similar cases have been reported in dogs. Only additional research, including cytology or histopathology, will indicate their cause, which is often a surprise, especially in areas to which the disease is not endemic (Ng et al. 2002; Albanese et al. 2013; Pazdzior-Czapula et al. 2018).

The course of the infection is initiated by an invasive L3 larva transmitted by blood-sucking mosquitoes. In the final host organism, which, in the case of *D. repens*, is most often dogs, the larva reaches an L5 stadium. Settles in the subcutis, and after maturing, the adults are capable of copulating to produce microfilariae. The diagnosis of an infection is based on the presence of adult nematodes in the material from the subcutaneous nodules or the presence of microfilariae in the peripheral blood. A modified Knott test can be used to detect the microfilariae in the peripheral blood. When suspecting subcutaneous dirofilariasis, it is also worth remembering that the length of the prepatent period ranges from 27 to 34 weeks. So, before the expiration of that time, it is not possible to determine the microfilariae in the blood (Ferasin and Knight 2005; Taylor et al. 2015).

The study aimed at describing cases of subcutaneous dirofilariasis in dogs that were diagnosed based on a cytological examination of nodular changes in the skin and hypodermis. These changes were clinically differentiated as proliferative (including neoplastic) and/or inflammatory lesions, without suspecting a parasitic aetiology.

MATERIAL AND METHODS

Cytological preparations collected by a fine-needle aspiration biopsy (FNAB) from nodular lesions located in the subcutis and skin of dogs were used in the diagnosis.

These changes occurred in the form of painless, single or multiple nodules with a diameter of 1 cm to 3 cm, protruding above the surface of the skin, some of them hairless. The single nodules occurred in different parts of the torso and/or on the head; the multiple nodules were most often located in the lumbosacral and perianal regions. The material for the cytological tests was collected to diagnose suspected proliferative lesions of a cancerous or inflammatory nature. The material (only cytological smears from the lesions) that was sent to the Department of Pathology in the years 2014–2018 came from 25 dogs of different breeds and mixed-breed dogs, both sexes, aged from 11 months to 12 years. The material came from patients from south-western and western Poland, i.e., from the Lower Silesia and Lubuskie voivodeships. About 8 000 (from 7 170 dogs) nodule biopsies were examined for the specified time frame. The received preparations were stained with the routine haematoxylin and eosin (H&E) method and viewed using an Olympus BX 53 light microscope coupled with a camera model UP-90. To take measurements, the cellSens Standard V1 software was used (Olympus, Japan).

RESULTS AND DISCUSSION

A microscopic examination of the delivered cytological preparations revealed erythrocytes (very numerous), neutrophils and eosinophils (quite numerous), macrophages (single), and whole and/or damaged fragments of microfilariae of *Dirofilaria* sp. in various numbers. In some cases, apart from the inflammatory cells, whole and damaged sebaceous gland cells and connective tissue cells lacking atypical features were visible. The predominant number of inflammatory cells, such as neutrophils and eosinophils, and a few macrophages indicate an acute inflammatory reaction of varying intensity. In none of the tested preparations, none features indicated a neoplastic process.

The observed microfilariae were characterized by a length of about 330–380 µm, a width of about 7–8 µm, a bluntly ended front part, and a thin,

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threadlike, pointed, and sometimes characteristically bent back end of the body (Figures 1 and 2).

All the doctors sending the material for the tests assumed that the lesions examined by them might be cancerous or inflammatory without showing any carcinogenic features. The results of the research were surprising for them, the more so that none of the dogs examined had been with its owner to the south of Europe, i.e., to areas considered endemic to the occurrence of parasites from the *Dirofilaria* genus. In many of the cited publications, the authors describe obtaining similar results as a kind of surprise, both in human medicine and in veterinary medicine (Ng et al. 2002; Manuali et al. 2005; Fleck et al. 2009). Pilsczek (2010) analyzed the cases described in the literature of parasitic invasions imitating proliferative lesions, among which were lesions also caused by nematodes, including *Dirofilaria repens*. In all of the cases analyzed by Pilsczek, parasitic invasions were not suspected, and the examined lesions were treated as potential proliferative changes. In this research, the diagnosis of dirofilariasis was performed based on the cytological image and information from the doctor, and by the data from the literature. According to Tzanetou et al. (2009), the diagnosis of dirofilariasis is based on the following clinical and histological features: a single small subcutaneous nodule, which does not usually exceed 2 cm, inflammatory cells, and the presence of a characteristic nematode or microfilaria in the center of the nodule.

The symptoms manifested by the examined dogs, i.e., mainly painless single or multiple and sometimes itchy skin nodules, including the subcutaneous tissue, resulted both from the mechanical irritation by the adults and microfilariae, as well as from their toxic effects on the surrounding tissues. The mechanical damage led to skin blood vessel blockages; toxins and metabolites led to the triggering of an immune response cascade. However, it should be remembered that a significant part of the infections proceeds in an asymptomatic manner, which, together with the lack of specific diagnostic tests, is the reason for the failure to combat the invasions and the possibility of the microfilariae spreading among the dog population (Ferasin and Knight 2005; Taylor et al. 2015).

In Poland, no cases of subcutaneous dirofilariasis in humans and animals were found until 2007. The first case of the *D. repens* infection in our country was described in a man in Warsaw in 2007 (Zarnowska-Prymek et al. 2008). Since then, other cases have been found in other people coming from Warsaw or its vicinity, including those who did not leave Poland (Cielecka et al. 2012). In turn, the first cases of infections in animals were found in dogs from the Mazowieckie voivodeship in the summer of 2009 (Demiaszkiewicz et al. 2009). In the following years, further cases of subcutaneous dirofilariasis in dogs have been described (Sapierzynski et al. 2010). In all the cases mentioned, it was confirmed that the infections were caused by the *D. repens* species. Based on the above data, it can be concluded that currently, the infection of *Dirofilaria repens* occurs endemically in the Mazowieckie voivodeship, as confirmed by Osinska et al. (2014). They also showed numerous cases in dogs from eastern Poland, i.e., the Lublin and Podlasie voivodeships. This indicates the increasing

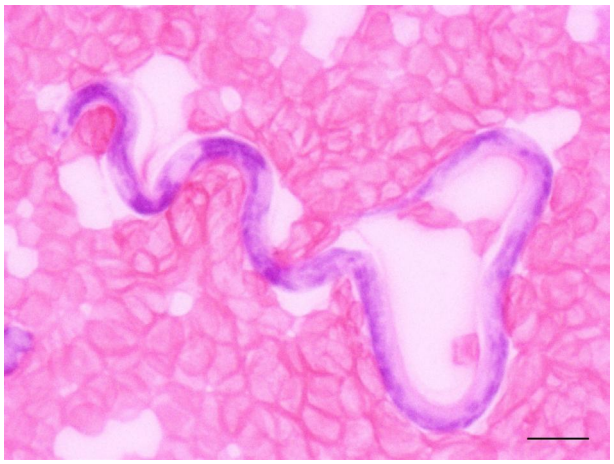

Figure 1. Microfilaria *Dirofilaria repens*, the front end of the body bluntly ended, the rear pointed. Length approx. 330 µm, H&E staining, × 400 magnification

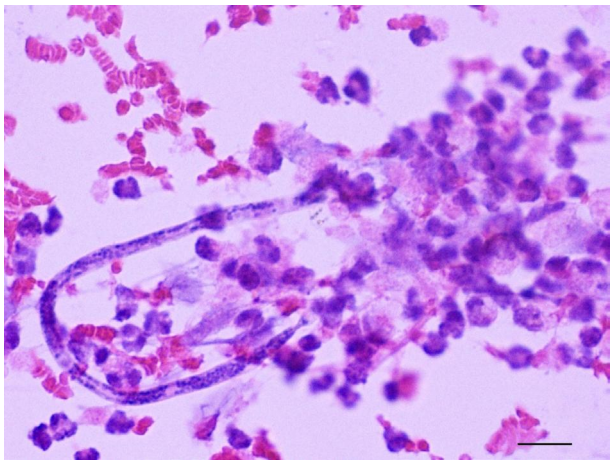

Figure 2. A fragment of microfilariae among the erythrocytes and numerous neutrophils and eosinophils. H&E staining, × 200 magnification

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range of parasites in this part of Europe (Svobodova et al. 2005; Demiaszkiewicz et al. 2009). This is confirmed by the fact that the *D. repens* infection was diagnosed in dogs in Slovakia for the first time in 2005, while, in the subsequent years, it has also been diagnosed in the Czech Republic, Germany, Greece, Norway, and the Netherlands (Svobodova et al. 2005; Miterpakova et al. 2008; Fleck et al. 2009; Tzanetou et al. 2009; Saevik et al. 2014; Traversa et al. 2010, respectively). The described cases are, in turn, related to dogs from the south and western Poland, i.e., the Lower Silesia and Lubuskie voivodeships. The territorial range of the parasites from the genus *Dirofilaria* is, in some way, limited by the presence of mosquitoes from the *Anopheles, Aedes*, and *Culex* genera that are the intermediate hosts and vectors. While, in the above-mentioned countries of Central and Western Europe, endemic infections are increasingly frequent, in Scandinavia, two cases of dirofilariasis have been described: one in a dog that was imported from southern Africa and the other from Hungary (Bredal et al. 1998; Saevik et al. 2014).

The cases examined in this research were based on the morphology of the identified parasites, which is also described by Liotta et al. (2013), who showed that advanced molecular techniques are not required to differentiate the microfilaria *D. repens* and *D. immitis*, but only the knowledge of the morphological differences and dimensions. However, more and more often in the diagnosis of parasitic infections, molecular techniques are used to accurately determine the aetiological factor despite the various and often early stages of the development of the parasites (Marusic et al. 2008; Sapierzynski et al. 2010). The research results of Sapierzynski et al. (2010) indicate that dogs who did not show skin lesions characteristic of dirofilariasis, but only a poor general condition, may also be infected with *D. repens.* Traversa et al. (2010) believe that due to the asymptomatic course of many infections, the actual state of the illness is underestimated, especially where the occurrences of parasites are endemic, e.g., in northern Italy. This opinion is confirmed by Pampiglione et al. (2001), describing numerous new cases in this region. According to Pampiglione et al. (2009), taking the increasing number of cases described in humans into account, each newly diagnosed case should be confirmed in detail due to possible false diagnoses of *D. immitis* and *D. repens*. In the case of geographical areas where dirofilariasis is endemic, other (only imported) parasitoses with similar symptoms (lesions and cutaneous nodules), i.e., infections caused by *Wuchereria bancrofti, Onchocerca volvulus, Loa loa,* or *Mansonella perstans*, should be included in the differential diagnosis (Ng et al. 2002; Simon et al. 2009). The more and more frequent occurrence of many new cases of *D. repens* infections in the countries of Central and Eastern Europe is related to the fact that dogs traveling with owners to the south of Europe frequently transfer it further. However, an equally large percentage of infected dogs are dogs that do not leave the country, and the infection is caused by mosquitoes living in their climate.

Nematodes of the genus *Dirofilaria* are currently considered one of the most expansive parasites in humans and animals. The subcutaneous dirofilariasis caused by them is considered one of the fastest spreading transmitted disease in Europe. This creates a high risk of invasion in both animals and humans. Until recently, the geographical area of *D. repens* in Europe was limited to the endemic Mediterranean regions. However, climate change, frequent flooding, and an increase in the number of mosquitoes have led to the extension of the invasion borders (Pampiglione et al. 2001; Pantchev et al. 2011; Saevik et al. 2014). The analysis of European meteorological data has shown that in the summer, thermal conditions enable the development of *Dirofilaria* in areas covering the territory of Poland. It seems, therefore, that the detection of vectors among the local mosquito population in Poland is only a matter of time (Cielecka et al. 2012).

To sum up, it should be noted that the described invasion of *Dirofilaria repens* in Poland and other countries of this latitude will be an increasingly common pathology in dogs. Due to the mosquitoes transmitting the microfilariae, it will be a zoonosis that is an increasingly frequent and more serious threat to humans. Therefore, in the differential diagnosis of various types of skin and subcutis lesions of unknown aetiology, dirofilariasis should be considered, and cytological tests and blood tests should be performed with the Knott test in such patients. However, it should be remembered that the result of this last study is influenced by periodic (in our climate, the peak is in August and September) and daily (the peak is in the evening) fluctuations in the intensity of the microfilaremia, which, in many cases of subcutaneous dirofilariasis, may give a negative result (Pantchev et al. 2011).

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Veterinary doctors should be aware of the danger and remember that this disease does not have to be an "imported" disease. It can occur in dogs that have never left the territory. In the cases of focal nodular and/or inflammatory lesions of an aetiology that is difficult to determine, the possibility of infections with *Dirofilaria* parasites should be considered. Our study shows that the relative frequency of subcutaneous dirofilariasis among other similar lesions is very rare (25 cases in circa 8 000 biopsies). Nevertheless demonstrating the microfilariae, as a cause of skin lesions, in a cytological examination is possible.

Conflict of interest

The authors declare no conflict of interest.

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