

УДК 619

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SPONTANEOUS BLEEDING IN A DOG WITH SUBCUTANEOUS DIROFILARIASIS – CASE REPORT

This report describes a case of spontaneous bleeding in a dog suffering from *D. repens* invasion. Authors suggest incidence of monoclonal gammopathy in the course of disease.

Key words: *dog, subcutaneous dirofilariasis, bleeding disorders, monoclonal gammopathy, thrombocytopenia*

Introduction. Filariasis is a disease group caused by roundworms belonging to the superfamily Filarioidea. Two main filarial parasites affect domestic carnivorous in Europe: *Dirofilaria immitis*, and *Dirofilaria repens* [12, 13]. The parasite has a complex life cycle. These are transmitted from host to host by mosquitoes (order *Diptera*, suborder *Nematocera*, family *Culicidae*). Domestic carnivorous are a dead-end host for *Dirofilaria* [12].

Subcutaneous dirofilariasis due to *Dirofilaria repens* is endemic in some country of Southern and Eastern Europe [2, 4, 5, 12, 13]. Dirofilariasis is an underestimated problem in Poland. Invasion with this nematode is generally asymptomatic [5] or clinical symptoms are usually restricted to a subcutaneous nodule containing a single infertile parasite [9]. So far hemorrhagic sequelae of *Dirofilaria repens* invasion have not been reported.

History, clinical examination and laboratory data. A 12-year-old, 28kg, female, mix breed dog was admitted to the Clinic of Infectious Diseases with 3-month history of anorexia, lethargy and weight loss. The dog has lived entirely outdoors at the rural area near Lublin for several last years. On the day before admission, the patient had episode of temporary bleeding from the oral cavity. On clinical examination the animal was moderately dehydrated with sunken eyeball, conjunctival mucus membrane of the eye and the skin of the ventral abdomen appears pale. The lymph nodes were not enlarged. The rectal temperature was 40,5°C. Based on such clinical symptoms and history, the animal was suspected for babesiosis. A diurnal peripheral venous blood sample was drawn from the dog within 2 hours of admission. Blood analysis revealed severe anemia with a hematocrit value of 16.6 %, leukopenia and thrombocytopenia $35 \times 10^9/l$. The total plasma protein level was also observed to have increased to 10.12 g/dl, urea to 100,8 mg/dl, ALT and AST to 310 U/l and 152 U/l respectively. The patient's blood was also tested for coagulation abnormalities including prothrombin time (PT), activated partial thromboplastin time

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(APTT), plasma fibrinogen, the euglobulin lysis test (ELT) and fibrin monomer paracoagulation test. The coagulation profile showed mildly prolonged PT and APTT and low level of fibrinogen. The values obtained were as follow: PT 14,8s (INR 1,19), APTT 24,2 s, fibrinogen 89 mg/dl and ELT 54 min. A fibrin monomer paracoagulation test was negative. It is interesting also to note that the plasma euglobulin fraction was very reach (Sia test).

Babesia parasites were not identified on review of the peripheral blood smear but many microfilariae were found in the slide. Microscopic characteristics indicated that the worm was *Dirofilaria* spp. Subsequent PCR analysis of the DNA identified the parasite as *Dirofilaria repens*, which confirmed morphological means in accurate identification. Therefore, the patient was diagnosed as having subcutaneous dirofilariasis and it has been treated with imidaclopride and moxydectin and ivermectine. The specific source of haemorrhage could not be found and it was presumed to have originated from blood vessels subjacent to the walls of nasopharynx mucosa. Bleeding episodes were managed with etamsylate and vitamin K. Clinical improvement was not achieved. The dog died on the 30th day from admission.

Discussion. In the last decade many important aspects of the diseases have been studied and elucidated, including pathogenesis and parasite transmission, however, there are still many unanswered questions. A review of the literature indicates, that the range of occurrence of *D. repens* will spread significantly towards central and north Europe. Native dirofilariasis of dogs caused by *D. repens* may be common in Poland [4,10]. Mean prevalence of invasion among dogs from some examined region is 37.5% [4]. Most affected dogs have patent infections with circulating microfilariae in peripheral blood, although infected dogs sometimes develop occult infections characterized by the absence of microfilariae [10, 13]. Clinical manifestations of invasion include dermatological problems such as nodular multifocal dermatitis, presence of several pruriginous papule, erythema and alopecia [5, 13]. In cases with high microfilaremia, gross and histopathological changes in many organs, like spleen, liver, kidneys, lungs, heart and brain were reported [5]. Symptoms and signs other than dermatological include: conjunctivitis (46%), anorexia (35%), vomiting (26%), fever (25%), lethargy (20%), and lymphadenomegaly (10%) [13]. Serious hemorrhagic sequelae of invasion have been reported in dogs with hookworms [10] but not in subcutaneous dirofilariasis. Some authors believe that most common cause of bleeding in the course of *Dirofilaria immitis* invasion is disseminated intravascular coagulation (DIC) [10]. This is not supported by any clinical and experimental research. However finding described in the above case were similar to those observed in overt DIC, the soluble fibrin monomer complex was negative. This allowed us to exclude the presence of DIC as the cause of hemorrhage. In experimental research performed by Kitoh [8], thrombi were not found in dog's blood vessels of any organ at necropsy after shock induced by injection of heartworm extract, although subclinical coagulopathy was present. Blood dwelling parasites and worms which lives in the bloodstream avoid stimulating the coagulation system and posses anticoagulant properties. This could be achieved by inhibition of coagulation proteins, inhibition of platelet function or promotion of

fibrynolysis [3]. For instance in hookworms, the smapins are responsible for the anticoagulant properties of these blood-feeding parasites [6, 7].

The most predominant changes in biochemical profiles were: high total serum protein level and very reach euglobulin fraction. Such changes could occur in paraproteinemic patients with immunoproliferative disorders and also in malaria or filariasis [1, 2]. Since paraproteinemia is asymptomatic, it is usually detected incidentally during routine laboratory evaluation, but may be the cause of hemorrhagic diathesis. In patients with paraproteinemia, there is a positive correlation between an increased viscosity and a prolonged thrombin time. Serum viscosity is negatively correlated with platelet aggregation with collagen [11].

In our case, the patient presented with a severe thrombocytopenia of $35 \times 10^9/l$. Both non-immunological as well as immunological destruction of platelets have been implicated in causing thrombocytopenia in such cases, but the mechanisms involved are unknown. The thrombocytopenia and a possible additional defect of platelet aggregation may have contributed to the severity of the bleeding.

Conclusion

1. Spontaneous bleeding associated with massive *D. repens* invasion can occur in dogs, but the mechanism of hemorrhage is unclear.
2. *Dirofilaria repens* invasion may cause monoclonal gammopathy

References

1. Caprariis, de D., Sasanelli M., Paradies P., Otranto D., Lia R.: Monoclonal gammopathy associated with heartworm disease in a dog. J Am Anim Hosp Assoc. 1988; 45:296-300
2. Chopra, R. N., Mukherjee, S. N., Rao, S.S.: Studies on protein fractions of blood sera; normal and filarial blood sera. Indian J. M. Research 1934; 22: 171.
3. Craford G.P.M., Howse D.J., Grove D.I.: Inhibition of human blood clotting by extract of ascaris suum. J. Parasitol. 1982; 68: 1044-1047
4. Demiaszkiewicz A.W., Polańczyk G., Pyziel A.M., Kuligowska I., Lachowicz J.: Pierwsze ogniska dirofilariozy psów wywołanej przez *Dirofilaria repens* Railliet et Henry, 1911 w centralnej Polsce. Wiadomooci Parazytologiczne 2009; 55: 367–370
5. Džaja P., Beck A., Kiš G., Kurilj A G., Živičnjak T., Artuković B., Beck R., Hohšteter M., Zuckermann Šoštarić I.C., Grabarević Ž.: *Dirofilaria repens* infection in a dog in Croatia - a case report. Veterinarski Arhiv 2008; 78: 521-527
6. Dzik J.M.: Molecules released by helminth parasites involved in host colonization. Acta Biochemica Polonica, 2006; 53: 33–64
7. Haapasalo K., Meri T., Sakari Jokiranta T.: *Loa loa* Microfilariae Evade Complement Attack In Vivo by Acquiring Regulatory Proteins from Host Plasma. Infection and Immunity, 2009; 77: 3886–3893

8. Kitoh K, Watoh K, Kitagawa H, Sasaki Y.: Blood coagulopathy in dogs with shock induced by injection of heartworm extract. *Am J Vet Res.* 1994; 55:1542-1547
9. Oleaga A., Pérez-Sánchez R., Pagés E., Marcos-Atxutegi C., Simón F.: Identification of immunoreactive proteins from the dog heartworm (*Dirofilaria immitis*) differentially recognized by the sera from dogs with patent or occult infections. *Molecular and biochemical parasitology.* 2009; 166: 134-41
10. Rafał Niziołek, Katarzyna Rutkowska: *Dirofilarioza u psow i kotow.* *Życie Weterynaryjne* 2009; 84: 798-805
11. Robert F., Mignucci M., McCurdy S.A., Maldonado N., Lee J.Y.: Hemostatic abnormalities associated with monoclonal gammopathies. *Am J Med Sci.* 1993; 306 :359-366
12. Toparlak M., Gargili A., Ulutas Esatgil M., Cetinkaya H.: Canine Filariosis Around Istanbul, Turkey Employing Naphtol AS-TR Phosphatase. *Acta Vet. Brno* 2005; 74: 233–236
13. Walter Tarello: Clinical Aspects of Dermatitis Associated with *Dirofilaria repens* in Pets: A Review of 100 Canine and 31 Feline Cases (1990–2010) and a Report of a New Clinic Case Imported from Italy to Dubai. *J. Parasitol. Research* 2011; 2011: 578385

Summary

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*Subcutaneous dirofilariasis due to *Dirofilaria repens* is endemic in some country of Southern Europe. It is an underestimated problem in Poland. Clinical manifestations of the invasion include persistent high fever, cachexia and bleeding from the nose. Moreover the dog developed paraproteinemia, anemia and thrombocytopenia. To our knowledge, this is the first report of spontaneous bleeding associated with *D. repens* invasion in the dog.*