

FIRST REPORT OF AUTOCHTHONOUS CANINE ANGIOSTRONGYLOSIS IN BOSNIA AND HERZEGOVINA

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Abstract

This report describes a case of autochthonous canine angiostrongylosis in a mixed-breed dog in Bosnia and Herzegovina. The owner noted the dog had lost appetite and weight, accompanied by a non-productive and intermittent cough. Physical examination revealed increased bronchovesicular pulmonary sounds and a caudally expanded lung field. Haematological parameters suggested mild dehydration in the dog. Radiological examination of the dog's thorax showed a mild bronchointerstitial pattern with alveolar patches in the cardiac lobes. Coprological examination revealed the first stage (L1) *Angiostrongylus vasorum* larvae (Baillet, 1866). The dog was treated with two doses of 0.4 mg/kg of ivermectin administered subcutaneously with a time interval of 21 days between the two applications. Five weeks after the initial diagnosis, a repeated coprological examination revealed no evidence of L1 *A. vasorum* larvae in the dog's faeces. This case report confirms the presence of *A. vasorum* in a dog from Bosnia and Herzegovina.

Key words: *Angiostrongylus vasorum*, Bosnia and Herzegovina, dog

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CASE PRESENTATION

This paper reports the first case of autochthonous and chronic angiostrongylosis in a mixed-breed dog from Bosnia and Herzegovina, along with clinical, haematological and radiological findings and therapy control.

In early December 2018, a two-year-old, non-castrated male mixed-breed dog from the suburban area of Banja Luka was brought to a local veterinary clinic for a physical examination. The household the dog came from was located in Vrbanja, a suburban area in Banja Luka (44°45' 54.306"N; 17°14' 33.936"E) surrounded by low-growing green vegetation and water reservoirs. The dog was a family pet and was adopted as a puppy. According to basic instruction from veterinarians, the owner regularly collected fresh faeces for coprology examination. Regular annual vaccinations had been performed along with anthelmintic treatments using a commercially available combination of praziquantel, pyrantel and fenbendazole. The last coprological examination before the patient presented at the clinic had been performed in May 2018, and it was negative. In the weeks prior to presentation at the clinic, the owner noted the dog had lost both appetite and weight. The animal also suffered from a non-productive and intermittent cough, mainly after physical exercise. Haematoma of the glans penis was diagnosed once in the dog before any respiratory symptoms were apparent.

On presentation at the clinic, physical examination of the dog showed good body condition. The dog was responsive and alert without any visible signs of general weakness or fever. The mucous membranes were pale with normal capillary refill time. Heart sounds were rhythmic and no signs of pathological murmurs were noticed. The clinical examination only revealed increased bronchovesicular pulmonary sounds and a caudally expanded lung field. A blood sample was collected for haematological and potential serological testing. Testing for the presence of heartworm (*Dirofilaria immitis*) was not performed. The observed haematological parameters suggested mild dehydration (increased haematocrit level). However, in general, the cell counts were within normal limits: white blood cells (WBC) $10.6 \times 10^3 \text{ mm}^{-3}$; red blood cells (RBC) $8.46 \times 10^6 \text{ mm}^{-3}$; haematocrit (HCT) 57.9 %; haemoglobin (HGB) 20.1 g dl⁻¹; platelets (PLT) $232 \times 10^3 \text{ mm}^{-3}$; mean cellular volume (MCV) 68 μm^3 ; mean cellular haemoglobin (MCH) 23.8 pg; mean cellular haemoglobin concentration (MCHC) 34.8 g dl⁻¹; lymphocytes $3.4 \times 10^3 \text{ mm}^{-3}$; monocytes $0.6 \times 10^3 \text{ mm}^{-3}$; granulocytes $6.6 \times 10^3 \text{ mm}^{-3}$. Radiological examination of the thorax showed a mild bronchointerstitial pattern with alveolar patches in the cardiac lobes.

The coprological examination was performed using the passive flotation tube test with saturated 1.350 s.g. zinc sulphate solution. The microscopic examination revealed *Metastrongyloidea* larvae and *Toxocara canis* eggs. However, species determination of *Metastrongyloidea* larvae was not possible because the larvae were deformed due to the flotation fluid's osmotic pressure. After the zinc sulphate flotation, a Conboy's modification of the Baermann technique was used to isolate metastrongyloid larvae from the faecal sample (Conboy et al., 2017). After 24 hours, metastrongyloid larvae

with dorsal notched tail were recovered. The dorsal and ventral indentation could be seen in the examined larvae with a typical cephalic button located at the head of larvae (Fig 1). The morphological analysis was performed according to descriptions given by McGarry and Morgan (2009). According to this morphological key, it was concluded that the recovered larvae belonged to *A. vasorum*. The dog was treated with two doses of 0.4 mg kg⁻¹ of ivermectin administered subcutaneously, with a time interval of 21 days between the two doses. Clinical control was conducted after the first three weeks and, according to the owner, the previously observed cough had disappeared. A control coprological examination was performed five weeks after the diagnosis and the first administration of ivermectin. No *A. vasorum* larvae were found during this coprological examination using the Baermann technique. In the meantime, a moxidectin- and imidacloprid-based preparation (Advocate®, Bayer) was registered in the country and was preventively applied to the dog in early spring 2019, but it was also recommended for continuous use throughout the year. The last coprological examination of the dog was performed in December 2019, in which no parasitological elements were identified.



Figure 1. First stage larvae of *Angiostrongylus vasorum* with cephalic button (Cb) and dorsal notched tail with dorsal (black arrow) and smaller ventral (white arrow) indentation (scale bar: 50 µm)

DISCUSSION

A. vasorum (Baillet, 1866) or French heartworm is a well-known cardiorespiratory metastrongyloid nematode species in dogs and wild canids – foxes, wolves, and golden jackals (Spratt, 2015). *A. vasorum* adults are located in the pulmonary arteries and right heart of the definitive host. The first stage (L1) *A. vasorum* larvae can be found in lung tissues, especially in alveoli and arterioles. The larvae are swallowed and finally excreted *via* faeces into the environment by the definitive hosts. Like

most Metastrongyloidea, *A. vasorum* has an indirect life cycle with terrestrial gastropods – snails and slugs serve as the intermediate hosts (Bolt et al., 1994). The clinical presentation of angiostrongylosis can be very variable in dogs, ranging from asymptomatic cases to severe clinical signs and even sudden death (Helm et al., 2010). Diseased animals generally show cardiorespiratory signs including cough, dyspnoea, and tachypnoea due to bronchopneumonia (Traversa et al., 2013). In addition to the cardiorespiratory symptoms, signs of neurological and gastrointestinal dysfunction, as well as coagulopathies have been described (Helm et al., 2010; Traversa et al., 2013). *A. vasorum* has been recorded in most western and central European countries, as well as in Scandinavia (Helm et al., 2010; Di Cesare and Traversa, 2014; Taylor et al., 2017; Grandi et al., 2017). Data on current *A. vasorum* distribution remain poorly researched in eastern regions of Europe, and studies published on this topic are limited (Simin et al., 2014). In the Balkans, *A. vasorum* was reported in Greece (Papazahariadou et al., 2007), Serbia (Simin et al., 2014; Gavrilović et al., 2017), Albania (Shukullari et al., 2015) and Croatia (Rajković-Janje et al., 2002).

An epidemiological study conducted by Hodžić et al. (2016) revealed that *A. vasorum* was not present in red foxes (*Vulpes vulpes*), but the foxes sampled in that survey did not cover the complete geographical region of Bosnia and Herzegovina. The lack of extensive surveys and misdiagnoses and/or underdiagnoses in clinical practice are possible reasons why natural *A. vasorum* infections are rarely reported in the Balkan region. To the authors' knowledge, this is the first published report of *A. vasorum* presence in Bosnia and Herzegovina. However, a limitation of this case report is that our coprological finding of *A. vasorum* was not confirmed by molecular analysis – PCR.

In some cases, dogs were subclinically infected (Conboy 2011; Hurníková et al., 2013; Simin et al., 2014). The affected dog from this report displayed mild, chronic and unspecific symptoms: recent loss of weight and appetite, intermittent cough, increased bronchovesicular lung sounds and a patchy alveolar pattern, which was observed in the radiological examination. There are no specific and pathognomonic haematological and biochemical findings to support a definitive diagnosis of angiostrongylosis (Di Cesare and Traversa 2014). The most notable radiological feature is a diffuse interstitial pulmonary infiltrate with a transient alveolar pattern (Di Cesare and Traversa 2014). Other reports describe diffuse, multifocal or peripheral alveolar infiltrate depending on the duration of the disease and lung inflammation (Di Cesare and Traversa 2014). Similar to the haematology and clinical biochemistry, the radiological signs are not specific in the diagnosis of canine angiostrongylosis. The glans penis haematoma in the dog from this report could be associated with *A. vasorum* infection.

For many years, benzimidazoles (e.g. fenbendazole) and macrocyclic lactones have been used to cure angiostrongylosis in dogs worldwide (Conboy 2009; Di Cesare and Traversa 2014). In most cases, veterinarians advised the administration of 50 mg kg⁻¹ fenbendazole for 14 days to treat affected dogs (Simin et al., 2014). Also, ivermectin (0.2 to 0.4 mg kg⁻¹ subcutaneously 2-4 times with a three week interval) has been used with success and documented by several authors (Martin et al., 1993; Bourque

et al., 2002). This therapy protocol was included as a treatment option in the general review by Helm et al. (2010). In the last decade, effective chemotherapy with the “spot on” formulation of imidacloprid 10% and moxidectin 2.5% was reported in a recent survey by Schnyder et al. (2014). In our case, we used ivermectin for therapy as an alternative, since new “spot on” formulations were not licensed and available in Banja Luka at the beginning of 2019.

This case report of *A. vasorum* infection in a dog from the Banja Luka region confirms the presence of this cardiorespiratory nematode in Bosnia and Herzegovina. The case of *A. vasorum* infection described here is the reason why veterinarians should conduct coprological examinations and serological testing in order to diagnose angiostrongylosis. Use of new licensed products with scientifically confirmed effectiveness in prevention of *A. vasorum* infection is recommended.

Authors' contributions

OS diagnosed angiostrongylosis and drafted the first version of this manuscript; MD, DV, DN, IT, TI drafted the final version of this manuscript.

Competing interests

The authors declare that they have no competing interests.

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PRVI IZVEŠTAJ O AUTOHTONOJ ANGIOSTRONGILOZI PSA U BOSNI I HERCEGOVINI

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Kratak sadržaj

Ovaj prikaz slučaja opisuje prvi nalaz autohtone angiostrongiloze kod psa mešanca u Bosni i Hercegovini. Vlasnik je primetio da je pas izgubio apetit i da je smršao, što je bilo praćeno neproduktivnim i intermitentnim kašljem. Fizički pregled je ukazao na pooštreno vezikalno disanje sa kaudalno proširenim plućnim poljem. Nije bilo promena u hematološkim promjenama. Radiološki pregled toraksa psa je ukazao na blag mešani bronho-intersticijalni crtež sa alvolarnim mrljama u kardijačnim lobusima. Koprološkim pregledom su ustanovljene L1 larve *Angiostrongylus vasorum*. Pas je terapijan sa ivermektinom u dozi od 0.4 mg/kg datim subkutano sa intervalom od 21 dan između dvije aplikacije. Pet sedmica nakon inicijalne dijagnoze, koprološkim pregledom nisu ustanovljene L1 larve od *Angiostrongylus vasorum* u fekalnom uzorku psa. Ovaj prikaz slučaja potvrđuje prisustvo *Angiostrongylus vasorum* u Bosni i Hercegovini.

Ključne reči: *Angiostrongylus vasorum*, Bosna i Hercegovina, pas