

Case Report

Rare case of *Angiostrongylus vasorum* intraocular infestation in an asymptomatic dog

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Summary

The presented clinical observation shows an atypical case of *Angiostrongylus vasorum* intraocular infection in an 18-month-old male beagle from north-eastern Slovakia. The dog presented with a motile worm in the anterior chamber of the right eye. No ocular signs or symptoms of a systemic disease were observed. The faecal examination using Baermann's technique and flotation was negative. Diagnosis was established following surgical removal of the worm. The specimen was determined as an *A. vasorum* female based on morphological features and confirmed by means of PCR technique and sequencing. To the best of our knowledge, the presented manifestation is the first ocular case of angiostrongylosis with absence of typical symptoms or signs of the disease.

Keywords: *Angiostrongylus vasorum*, French Heartworm, canine lungworm, ocular angiostrongylosis

Introduction

Angiostrongylus vasorum (Nematoda, Metastrongyloidea), commonly known as French heartworm, is a life-threatening nematode for dogs. General findings suggest that *A. vasorum* is occurred in isolated endemic areas. Since the first finding in Southern France in 1853 (Serres, 1854; Guilhon, 1963), the parasite was observed in Ireland (Roche & Kelliher, 1968), Switzerland (Wolff, 1969), Uganda (Bwangamoi, 1972), England (Simpson & Neal, 1982), Italy (Poli *et al.*, 1984) and Denmark (Bolt *et al.*, 1992). However, in recent years, autochthonous cases of infection are increasingly diagnosed in other European countries where the parasite previously did not occur, including Slovakia (Elsheikha *et al.*, 2014). The indirect life cycle of *A. vasorum* involves slugs and snails as intermediate hosts and frogs as paratenic hosts that harbour infective third stage larvae (L3). The definitive host becomes infected after ingestion of infected intermediate or paratenic host (Guilhon, 1963; Bolt *et al.*, 1993).

The disease is most frequently presented as cardiorespiratory distress with a history of gagging, coughing, exercise intolerance and dyspnoea. Bleeding abnormalities, coagulopathy, neurological symptoms, general malaise, uveitis, depression, and anorexia are also described. Occasionally, cases of aberrant migration of *A. vasorum* to liver, pancreas, kidney even to CNS have been observed (Koch & Willeesen 2009). The present study describes an atypical case of *A. vasorum* localisation in the anterior chamber of the right eye of an 18-month-old beagle from Slovakia.

Case Presentation

An 18-month-old beagle male from Bardejov, north-eastern Slovakia was referred to a private veterinary clinic in Prešov (Slovakia) for sudden appearance of a filiform foreign body in the right eye. The dog was kept indoors in the city, was usually walked in the vicinity of a river and never moved outside the region. Initial clinical ophthalmological examination revealed the presence of a

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Fig. 1. Adult *Angiostrongylus vasorum* worm in the anterior chamber of the right eye of an 18-month-old male beagle from Slovakia.

motile intraocular nematode in the anterior chamber of the right eye (Fig. 1). Apart from the infection, the eye appeared ophthalmologically normal. Clinical examination revealed no evidence of a systemic disease. The faeces were investigated by the Baermann technique and flotation method with zinc sulphate solution (s.g. 1.2) (Bowman, 2014) with negative results. Other examinations were declined by the owner.

Surgical removal of the parasite was performed under injection anaesthesia (Dexmedetomidine, Diazepam, and Ketamine). Removal of the parasite was performed by anterior chamber paracentesis and aspiration of the worm using 0.9 mm port. A 2 % methyl cellulose solution was injected into the anterior chamber and the inputs were sutured with 7/0 absorbable filament, then the eye was *pro tem* surgically covered with third eyelid.

Postoperative medication consisted of topical treatment with steroid solution of fluoremetolon twice per day (Efflumidex Liquifilm Int Opu, Allergan Pharmaceuticals, Ireland), antibiotic instillation of tobramycinum four times per day (Tobrex Int Opo, S.A. Alcon-Coureur, Belgium), and 1 % solution of atropine sulphate twice per day. The patient wore an Elizabethan collar until the stitches were removed day 4 post surgery. A follow up at day 4 revealed complete resolution without observable ophthalmologic after-effects seen in the right eye (Fig. 2). Specific anthelmintic treatment of angiostrongylosis consisted of single topical application of imidacloprid 250 mg/ moxidectin 62.5 mg (Advocate®, Bayer, Germany). A telephonic follow-up was performed six months post-surgery and the owner reported no evidence of systemic or ocular clinical signs.

Parasitological Findings

The extracted nematode was identified based on morphometric and characteristic morphological features according to species description by Costa *et al.* (2003) under the Leica DM4000B light microscope, Leica DFC 290 HD camera and Leica Application Suite V 3.8.0 software (Leica Microsystems GmbH, Germany).

The worm was identified as an adult female of *A. vasorum*. The body measured 24.3 mm in length and maximum body width was 0.534 mm. The buccal capsule directly joined the rhabditoid oesophagus with length 257.81 µm. The nerve ring was situated approximately in the middle of oesophagus. The excretory pore was located near oesophago-intestinal junction, 478.89 µm from the anterior extremity. The caudal end was ventrally curved and rounded. The vulva was situated 306.57 µm from the anus. The female cuticle was transparent. The ovarians filled with oocytes was twisted along the reddish intestine and created a “barber pole” appearance (Fig. 2).

The nematode was homogenized with 5 mm stainless beads (Qiagen®, Hilden, Germany) and ATL buffer in Qiagen TissueLyser (Qiagen®, Germany) for 30 Hz/6 min. DNA extraction was provided by a commercial isolation kit DNeasy Blood & Tissue (Qiagen®, Hilden, Germany), following the steps in protocol. A fragment of ITS2 rDNA was amplified by conventional PCR assay using the *A. vasorum*-specific primer set AV4/AV5 designed by Al-Sabi *et al.* (2010). A DNA fragment of 250 bp was separated on a 1.5 % agarose gel. The positive template was purified using NucleoSpin®



Fig. 2. Female *Angiostrongylus vasorum* extracted from the eye of a beagle dog from Slovakia. A - anterior extremity, B - caudal end (v-vulva, a - anus), C - Ovaria twisted along reddish intestine - a "barber pole" appearance, D - uterus

Gel and a PCR Clean-up kit (Macherey-Nagel GmbH & Co., KG, Germany) and sequenced by Sanger sequencing in both directions. Sequences were compared by BLAST (Basic Local Alignment Search Tool) with sequences available in GenBank.

A 156 bp long overlapping fragment of *A. vasorum* revealed 99 % similarity with the isolate obtained from a dog from Italy (KF270683). The nucleotide sequence of *A. vasorum* ITS2 rDNA gene fragment obtained during the study was deposited in GenBank under accession number MH018578.

Discussion and Conclusion

The herein presented description of *A. vasorum* specimen found intraocularly in a dog from north-eastern Slovakia is an atypical but not sole case of ectopic location of *A. vasorum* in the eye. The ocular localisation of the parasite has to date been reported several times as summarized by Colella *et al.* (2016), however this patient is to the best of our knowledge the first reported case where any

ocular pathology and any other clinical signs associated with *A. vasorum* infection were absent. Dogs of all ages can be infected with *A. vasorum*, however, several studies showed that dogs younger than one year are more susceptible to clinical infection, presumably due to riskier behaviour when scavenging or playing with snails (Chapman *et al.* 2004; Koch & Willesen, 2009). This is true also for the herein reported case where the dog was in the age of 18 months and the owner confirmed its curiosity regarding snails and frogs.

The previously reported 8 cases of ocular *A. vasorum* migration originated from endemic countries such as France, Great Britain, Denmark, Canada and Italy (Colella *et al.*, 2016). In Slovakia, the first canine angiostrongylosis cases were reported in 2012 and 2013 from eastern Slovakia (Hurníková *et al.* 2013; Miterpáková *et al.* 2014). The following copro-epidemiological research in the territory of Slovakia revealed a relatively high prevalence of *A. vasorum* in dogs and red foxes (4.13 % and 5.43 %, respectively) (Miterpáková *et al.* 2015; Čabanová *et al.* 2018a, 2018b).

Despite the fact that angiostrongylosis is usually presented with cardio-respiratory clinical signs, the majority of infected dogs identified within the above mentioned study were asymptomatic. Also here presented ocular localisation of *A. vasorum* reveals that angiostrongylosis should be included into differential diagnosis of unexplained canine eye disorders.

Ethical Approval and Informed Consent

No animals were killed for the purpose of this study.

Conflict of interest

The authors declare that they have no conflict of interest.

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