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Sub conjunctival *Dirofilaria repens* infection in a dog resident in the UK

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Dirofilaria repens infection was diagnosed in a 5 year old, female, German Shepherd Cross, originally from Romania but rescued and brought into the UK in February 2014. The dog presented with conjunctivitis in March 2015 and then again 2 months later with additional ocular and nasal mucopurulent discharge. Bacterial cultures from the nasolacrimal duct were negative for bacterial growth. The case was referred in August 2014 for ophthalmic examination, which revealed abnormalities in both eyes but more pronounced in the left. They included a mild palpebral conjunctival hyperemia and marked follicular conjunctivitis, as well as a dorso-nasal bulbar conjunctival mass. Serum biochemistry was unremarkable and a conjunctival biopsy taken from the dorso-nasal bulbar conjunctival mass revealed an eosinophilic/ lymphoplasmacytic conjunctivitis. At re-examination, nematodes were found in the area of the previous biopsy site and in the ventral palpebral conjunctival fornix. Polymerase chain reaction and sequencing confirmed these to be *D. repens*. Treatment with imidacloprid 10% and moxidectin 2.5%

(Advocate® Spot On) was successful, and clinical signs resolved over a 6 week period. This case report indicates that *D. repens* infection should be considered as a possible aetiological cause of ocular lesions in dogs in the UK, especially those with a history of foreign travel. Implications for establishment and spread of *D. repens* in the UK are discussed.

Key Words: Dirofilariosis, *Dirofilaria repens*, eosinophilic conjunctivitis, ophthalmology, nematode, dog, zoonosis

Introduction

Dirofilaria repens is a parasitic nematode of canids, although a wide range of mammals may be infected, including humans (Tarello, 2002). Transmission is vector borne with Culicine mosquitoes introducing infective L3 larvae into a host when feeding. These then develop into adult worms in the subcutaneous tissues. L1 stage larvae enter and circulate in the bloodstream, where they infect mosquitoes while feeding. Humans are not suitable hosts for *D. repens* infection and rarely develop patent infections; however, there is zoonotic potential, with worms forming subcutaneous nodules, lesions in the ocular region and less commonly migrating to other parts of the body (Pampiglione et al, 1995). In Europe, infection is more common in the south (Simón et al, 2012), and is rarely encountered in northern countries. However a number of factors are increasing likelihood of spread of the parasite to northern regions. These include climate change, and increased pet travel and relocation due to progressive relaxation of the Pet Travel Scheme (PETS) and increased human migration throughout Europe (Genchi et al, 2009). Recent autochthonous cases in Germany and climatic analysis highlight the potential for the parasite to spread into and

establish in northern European countries (Sassnau et al, 2014). As a result, veterinarians need to be aware of the increased risk of clinical cases of *D. repens* presenting in UK dogs that have travelled abroad or been imported. This report describes a case of ocular dirofilariosis in a dog imported into the UK from Romania. To the authors' knowledge, it is the first reported case of *D. repens* infection in a dog resident in the UK, highlighting the unusual clinical forms that infection with the parasite can take, and the long potential time lag between travel and onset of clinical signs.

Case history

A 5 year old, fully vaccinated, female neutered, German Shepard Cross weighing 39 Kg was referred to a diplomate veterinary ophthalmologist in Lancashire. The patient, originally from Romania, was rescued and brought into the UK in February 2014. She was presented to her referring veterinarian in March 2014 with a history of conjunctivitis and ocular discharge in both eyes (OU) but worse in the left eye (OS). In May, she again presented to her veterinarian, this time with ocular discharge OU and a mucopurulent nasal discharge from the left nostril. She was placed under general anesthesia and her nasal lacrimal apparatus was flushed OU. The veterinarian noted "white flecks" exiting from the left nares when the left nasolacrimal duct was flushed. Bacterial cultures from the duct were negative for growth and no yeast or fungi were seen. She was referred in August 2014 for ophthalmic examination.

With the exception of the ophthalmic findings, a complete physical examination was within normal limits (WNL). Nasal discharge was WNL through both nares. Menace response, dazzle reflex and PLR (direct and indirect) were WNL OU. The auriculopalpebral reflex was normal with complete palpebral closure OU. Normal ocular discharge was noted in the right

72 eye (OD). Moderate mucopurulent ocular discharge was present in the nasal canthus OS.

73 Schirmer tear test® values (STT) were 19 mm/minute OU. Corneal fluorescein retention was

74 negative OU. A decreased tear film break time of 10 seconds OU was noted. Intra ocular

75 pressure (IOP) values measured by rebound tonometry were 16mmHg OD and 13mmHg OS.

76 There was a normal nasolacrimal flow of Fluorescein® stain from both nares. Both nares

77 appeared wet and smooth.

78 Slit lamp biomicroscopy revealed severe palpebral conjunctival hyperemia of the superior

79 eyelid and inferior eyelid OS as well as the conjunctival surface of the anterior third eyelid

80 OS. There were marked, large, multifocal follicles located in the palpebral conjunctiva, most

81 notably in the superior-nasal conjunctival fornix OS. A raised, firm, 0.5-1 cm, hyperemic

82 mass was noted in the dorsonasal bulbar conjunctival surface also covered in large follicles

83 OS. Following instillation of anesthetic (Proxymetacaine) OU, moderate to severe follicles

84 were also found on the conjunctiva of the posterior third eyelid OS. The corneal surface had

85 decreased luster OS. The anterior chamber and iris were WNL OS. Biomicroscopy of the

86 right eye (OD) noted mild palpebral conjunctival hyperemia of the superior and inferior

87 eyelid, the rest of the biomicroscopy exam was unremarkable. Binocular indirect

88 ophthalmoscopy was unremarkable OD. Two, linear, hyper-reflective lesions radiating

89 peripherally from an area adjacent to the optic nerve head were noted in the dorso-lateral

90 tapetum OS.

91 Possible etiology for a follicular conjunctivitis and a bulbar conjunctival mass in a 6yr old dog

92 include; hypersensitivity reaction, neoplasia, infectious disease (e.g. leishmaniosis) FB

93 reaction, abscess with further diagnostic tests required to differentiate between them.

94 Following topical anesthesia with proxymetacaine OS, a conjunctival biopsy was taken of the

95 dorso-nasal bulbar conjunctival mass OS. Due to her travel history, blood work was also

performed which included the following; CBC, serum biochemistry; Polymerase chain reaction (PCR) for *Babesia* spp., *Ehrlichia* spp. and *Leishmania* spp., and antigen testing for *Angiostrongylus vasorum* and *Dirofilaria immitis* (Nation Labs®). All the above results were negative.

Histopathology from the conjunctival biopsy (CytoPath Labs®) diagnosed a mild to moderate eosinophilic and lymphoplasmacytic conjunctivitis indicating chronic-active conjunctivitis with the eosinophils supportive of an allergic/hypersensitivity etiology. There was no evidence of neoplasia or infectious agents.

On follow-up ocular examination, numerous, thin, white nematodes were found exiting the bulbar conjunctival mass in the area of the previous biopsy site. In addition, there were nematodes in the ventral palpebral conjunctival fornix. The nematodes were submitted for identification and initially thought to be *Thelazia* spp. However, gross morphological results from a second nematode sample sent in for identification disagreed with the initial diagnosis, with worm length excessive for *Thelazia* spp. Also, the presence of a bulbar conjunctival mass containing some of the worms, and the patient's travel history, made *Dirofilaria repens* a likely differential. *Dirofilaria repens* is endemic in Romania, from where the patient had travelled.

Molecular methods were used to establish the identity of the nematode. Briefly, the submitted adult nematode was crushed using tweezers before DNA extraction. DNA was extracted using DNEasy Blood and Tissue Kit (Qiagen, Germany) according to the animal tissue extraction protocol, with final elution volume of 100µl. The DNA repeat region (IpS) of 246bp was isolated and amplified using *D. repens* specific primer, DIR 3: 5'-CCGGTAGACCATGGCATTAT-3' (forward) and DIR 4: 5'-CGGTCTTGGACGTTTGGTTA-3' (reverse) as used established by Rivasi et al (2006). Polymerase chain reaction (PCR) assays

were performed in a final reaction volume of 25µl that consisted of 5µl of 5x polymerase buffer (Promega), 0.5µl of dNTPs (10mM each), 3µl of MgCl₂ of 25 mM (Promega), 1µl of each primer (12.5µM), 0.2 µl a Taq DNA polymerase (5 U/µl) (Promega), 4.3 µl dH₂O and 10µl DNA. PCR was carried out with cycling conditions of initial denaturation at 94°C for 5 min, 48 cycles at 94°C for 30 seconds, 51°C for 30 seconds and 72°C for 25 seconds, followed by a final extension at 72°C for 5 min. PCR products were separated on a 2% (w/v) agarose gel and visualised using 10mg/ml ethidium bromide and UV illumination to confirm product size. Amplified PCR products were sequenced for further phylogenetic analysis as follows. PCR products were purified using QIA quick purification kit (QIAGEN, Germany) and sequenced using the cycle sequencing technology (dideoxy chain termination / cycle sequencing) on ABI 3730XL sequencing machines (Eurofins MWG Operon, Germany). Sequences obtained were assembled, corrected by visual analysis using DAMBE 5 (Xia, 2013) and subjected to BLAST Identity Search (NCBI) to study their identity with the reported sequences for *D. repens* found in GenBank. PCR amplification resulted in a 246 base pair product, which is specific for *D. repens*. Sequencing demonstrated 100 % identity with *D. repens* obtained from naturally infected dogs in Sri Lanka (accession no. L15324.1) and in South east Serbia (accession no. GU971359.1).

Based on initial clinical examination, histopathology and the presence of nematodes in the palpebral conjunctiva OS, a diagnosis of parasitic conjunctivitis was made. Given the absence of *D. immitis* antigen (in which case macrocyclic lactones would be contraindicated), treatment with imidacloprid 10% and moxidectin 2.5% (Advocate® Spot On) was commenced.

Following initial treatment, the conjunctivitis and ocular discharge significantly worsened OU, with bilateral mucopurulent ocular discharge developing. Follow-up fundiscopic

examination revealed an intraconal lesion causing tenting of the dorso-lateral globe OD. An ocular ultrasound confirmed the presence of a retrobulbar, hyperechoic mass measuring approximately 1cm diameter. Over the following six weeks, this intraconal mass resolved and was no longer found on subsequent ocular ultrasound examination OD.

Discussion

Autochthonous and imported cases of *Dirofilaria repens* infection in dogs have been reported as far north as Germany (Hermosilla et al, 2006; Sassnau et al 2014), Poland (Masny et al, 2011) and Norway (Sævik et al, 2014), but this represents the first case reported in the UK. This case is also an unusual presentation of clinical *D. repens* infection. Although nodules are a well-recognised sign of *D. repens* infection in dogs, pruritus, erythema and papules are more common outcomes, occurring in 100, 79, and 62 % of cases respectively. In comparison, only 12 % of cases have subcutaneous nodules and 46 % conjunctivitis (Simón et al, 2012). A time lag of 2 months between arrival in the country and onset of clinical signs, followed by a failure in first opinion practice to recognise this case as possible *D. repens* infection, led to a delay of 6 months from arrival in the UK to diagnosis. Culicine mosquitoes capable of transmitting *D. repens* are present in the UK, presenting a risk of endemic foci developing in the country if cases are not diagnosed quickly. This case demonstrates the need to consider *D. repens* in dogs initially presenting with conjunctivitis that have a foreign travel history, especially if present in combination with nodules or dermatological signs. This case responded well to treatment with a licensed moxidectin/imidacloprid spot on and canine cases of *D. repens* usually carry a favourable prognosis. However, *D. repens* also has zoonotic potential and therefore avoiding the establishment of the parasite in the UK is highly desirable. This will only be achieved if *D.*

repens is considered as an early possible aetiological cause of ocular and dermatological lesions in dogs, especially those with a history of foreign travel.

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