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Image diagnosis of zoonotic onchocercosis by *Onchocerca lupi*

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ABSTRACT

Onchocerca lupi, a zoonotic nematode infecting the eyes of carnivores, has been increasingly reported in dogs from Europe and the USA. In order to improve the current status of knowledge on this neglected filarioid, diagnostic imaging tools (i.e., ultrasound scan, computed tomography and magnetic resonance imaging) are herein used to diagnose canine onchocercosis in two dogs, which scored positive for *O. lupi* microfilariae at the skin snip test and to assess the anatomical location of the nematode within the ocular apparatus. Results indicate that ultrasound tools are useful to address the diagnosis of *O. lupi* in dogs and to evaluate the localization of nodules or cysts containing the adult nematode.

1. Introduction

Onchocerca lupi (Spirurida, Onchocercidae) is a parasite of carnivores that has been increasingly diagnosed in symptomatic (Széll et al., 2001; Komnenou et al., 2002; Hermosilla et al., 2005; Faísca et al., 2010) and asymptomatic (i.e., animals that did not display any overt sign upon clinical examination) (Otranto et al., 2013a) dogs from Europe (i.e., Hungary, Greece, Germany, and Portugal) and dogs and cats from the United States of America (Labelle et al., 2011, 2013). Interestingly, *O. lupi* has also been recognized as a zoonotic agent in different countries from both the Old and New Worlds, causing highly debilitating clinical conditions in humans (Otranto and Eberhard, 2011; Otranto et al., 2011, 2012; Eberhard et al., 2013; Mowlavi et al., 2013; Ilhan et al., 2013) and gaining the interest of parasitologists and physicians.

In the majority of cases, canine onchocercosis is associated with acute clinical conditions, featured by discomfort,

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lacrimation, photophobia, conjunctivitis, exophthalmos and periorbital swelling, with cyst-like structures containing adult worms mostly on the conjunctival surface (Eberhard et al., 2000; Egyed et al., 2001; Széll et al., 2001; Sréter et al., 2002). In addition, chronic cases have also been described, with varying degrees of inflammation around the ocular apparatus (e.g., conjunctivitis, ocular discharge, uveitis, blepharitis, and corneal ulcers) often associated with nodule-embedded parasites in the eyelids, orbital fascia or retro-bulbar space (Orihel et al., 1991; Gardiner et al., 1993; Eberhard et al., 2000; Egyed et al., 2001; Széll et al., 2001; Komnenou et al., 2002; Hermosilla et al., 2005; Zarfoss et al., 2005; Sréter and Széll, 2008). However, in the only epidemiological study conducted in canine populations from Greece and Portugal, a prevalence of up to 8% of positive dogs was diagnosed in asymptomatic animals by the detection of microfilariae in skin snip sediments (Otranto et al., 2013a). In spite of being the only diagnostic method, the detection of microfilariae from skin is time consuming, requires considerable technical skills for the isolation and identification of the parasites and may give false-negative results in cases of pre-patent infections (Otranto et al., 2013b). In addition, skin snipping is invasive and may be hindered by the unwillingness of pet owners in cases of asymptomatic infections. Conversely, ultrasound scan (US), computed tomography (CT) and magnetic resonance imaging (MRI) are non-invasive, highly sensitive, complementary diagnostic methods enabling the assessment of the location and extent of diseases affecting the ocular and orbital tissues (Penninck et al., 2001). However, in cases of filarioid infections, these tools have been seldom used during human ocular dirofilariasis (Smitha et al., 2008; Gopinath et al., 2013) and onchocercosis (Sallo et al., 2005; Faísca et al., 2010; Ilhan et al., 2013; Mowlavi et al., 2013), in the pre-operative phase to evaluate the extent of granulomatous nodules or cysts.

Therefore, this study reports the application of US, CT and MRI for diagnosing canine onchocercosis in dogs, which scored positive for *O. lupi* microfilariae at the skin snip. In addition, the anatomical localization of the parasite within the ocular apparatus has been assessed and potential applications of these data are also discussed.

2. Materials and methods

In June 2013, a 3-year old male mongrel (dog 1) and a 4-year old female mixed breed (dog 2), living in the municipality of Olhão, Portugal (latitude 37°01′42″ N, longitude 7°50′33″ W) were submitted to clinical investigation because they had been found positive for *O. lupi* microfilariae upon skin snip examination (Otranto et al., 2013a). Dogs were considered asymptomatic based on the lack of any apparent clinical alteration of the eye. On physical examination, they were in good clinical condition, although eosinophilia (1430 cells/μL) and a mild neutrophilia (9724 cells/μL) were detected in dog 2. Upon ophthalmological examination, two pigmented and rounded retinal scars were observed on the fundus of the right eye of dog 1. Contrarily, dog 2 did not present ocular alterations. The neuro-ophthalmic examination of both dogs was normal, including globe movement in all directions. The US, CT scan and a MRI of the head were performed, with the owners consent, to investigate any possible involvement of the eyeballs and retro-orbital space.

2.1. Ultrasound scan

The US examination was performed with a portable ultrasound MyLab 30 Gold Vet® (Esaote, Italy). A few drops of benoxidate hydrochloride, as local anaesthetic, were placed into the eye, with animals restrained in sternal recumbency. Eyelids were manually retracted and the transducer was placed gently on the cornea surface, following the application of sterile gel film (Cogel Ultrasuoni®, Comedical, Italy). The eye and the retro bulbar space were scanned through trans-corneal, trans-scleral and trans-palpebral approaches, along horizontal, vertical and oblique planes. In particular, a linear, high frequency, transducer 10–18 MHz was used by the corneal contact method, while a convex transducer 5–8 MHz, with supraorbital access, was preferred for the investigation of the retro bulbar space.

2.2. Computed tomography and magnetic resonance imaging

Native and contrast-enhanced CT scans and unenhanced MRI were performed with dogs under general anaesthesia. A four slice-MDCT scanner (Philips MX-8000®, Philips Medical Systems, USA) was used with animals, in sternal recumbency, on the CT table. Images were acquired before and after the manual intravenous injection of iodinate contrast medium (Iopamigita®, Insight Agents GmbH, Germany) using the following technical parameters: standard acquisition algorithm, 120 kVp, 250 mAs, 0.6-mm slice thickness, pitch of 0.8, and 0.75 s/rotation. Three-dimensional (3D) multiplanar reformatted and volume-rendered images were obtained using a dedicated 3D workstation (Pixmeo OsiriX DICOM-viewer®, Pixmeo, Switzerland).

Unenhanced MRI was performed using a 0.25 T permanent magnet (Esaote Vet-Mr Grande, Esaote, Italy). The protocol included Spin Echo T1-weighted images acquired along the sagittal, transverse and dorsal planes, a fast Short-Tau Inversion Recovery (STIR) in the transverse and dorsal planes and RF-Spoiled 3D Gradient Echo T1-weighted images along the dorsal plane.

3. Results

In dog 1, two hyper-echogenic structures (about 1.6 mm wide) were observed in the right eye during the US, the first located dorsal to the sclera-corneal junction, and the second dorso-medially (Fig. 1A). A similar, hyper-echoic round lesion (2.7 mm in diameter) was detected within the insertion of the medial rectus muscle of the left eye (Fig. 1B). In dog 2, a 1.7 mm-wide lesion was found in the retro-bulbar space of the right eye (Fig. 1C). In addition, a small and irregular granular lesion hyperattenuating to soft tissue (3.3 mm × 2.5 mm × 5.8 mm) was detected during CT on the left globe of dog 1, close to the insertion of

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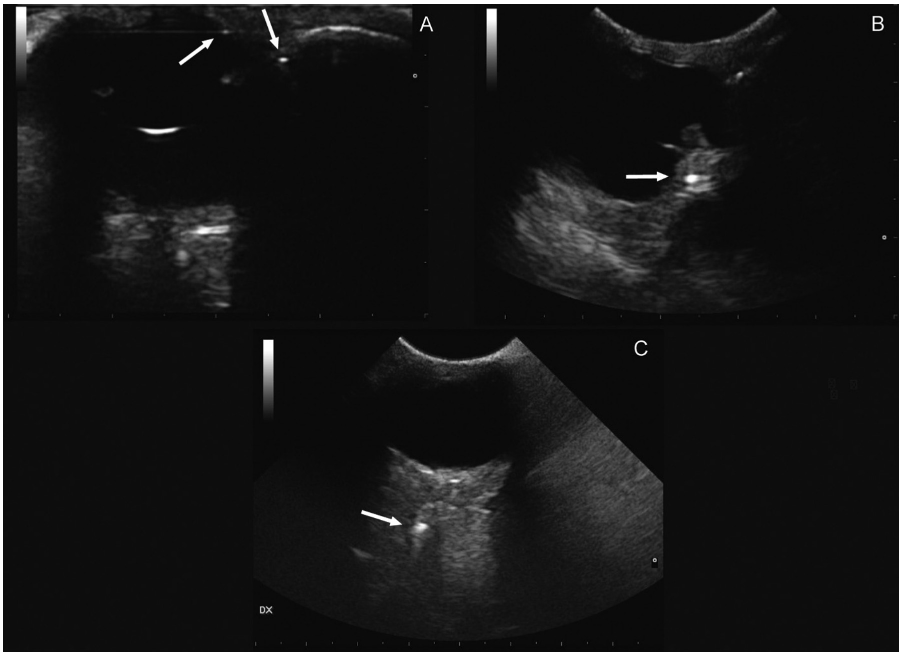

Fig. 1. (A) Dog 1, US: two hyper-echoic episcleral structures (arrows) on the medial sclerocorneal junction of the right eye (trans-corneal approach, horizontal dorsal scan). (B) Dog 1, US: hyper-echoic round lesion (arrow) within the insertion of the medial rectus muscle of the left eye (trans-scleral oblique scan). (C) Dog 2, US: hyper-echoic round lesion (arrow) in the retro-bulbar space of the right eye (trans-scleral dorsal scan).

Fig. 2. Dog 1, CT: dorsal (A) and transverse (B) multiplanar reformatted images scan of the left globe and retro-orbital space. Irregular, granular lesion close to the insertion of the medial rectus muscle (mr) on left the globe (arrows). Hounsfield mean attenuation value of 224 ± 79 HU. Dog 1, CT: oblique volume-rendered (C) and sagittal (D) multiplanar CT reformatted image of the right globe (RE). Two episcleral micro-nodules hyperattenuating to soft tissue (arrows) are evident on the dorsal surface of the eyeball. Hounsfield mean attenuation value of 256 ± 64 HU.

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the medial rectus muscle (Fig. 2A and B). Two hyperattenuating episcleral structures (less than 1 mm in diameter) were observed on the dorsal surface of the right eye, contiguously to soft tissues (Fig. 2C and D). The mineral Hounsfield Unit mean attenuation values were detected (mean 224 ± 79 HU left eye lesion; mean 256 ± 64 HU right eye lesions). None of the lesions enhanced after IV administration of contrast material and no abnormalities of the eyeballs, optic nerves, and retro-orbital spaces were observed at the CT of dog 2 and at MRI of both dogs.

The owner of the dog 1 consented to an explorative surgery to confirm the diagnosis. After general anaesthesia a lateral canthotomy on the right globe was performed. The eye was prolapsed but since the nodules were not palpable, the incision was made on the conjunctiva under ultrasound guidance, in correspondence with the two hyper-echoic episcleral micro-nodules. Dissection of the overlying conjunctiva near the lesions revealed firm, immobile nodules attached to the sclera (about 2 mm in diameter). After excision of the lesions the dog recovered without any complications. Nodules were removed from the sclera by the dissection of the overlying conjunctiva, stored in saline solution and examined under a light stereomicroscope (Leica®, DL MB2). Following the removal of external tissues, fragments of a nematode were morphologically identified as an *O. lupi* female based on key characters (e.g., the body cuticle, transverse ridges and striae, shape of ridges) (Mutafchiev et al., 2013). The morphological diagnosis was confirmed molecularly by amplification and sequencing of a partial *cytochrome oxidase* subunit 1 gene fragment (Otranto et al., 2013a). A 100% nucleotide sequence identity was detected with sequences (EF521410, KC686701) of *O. lupi* in the Genbank database.

4. Discussion

Results indicate that imaging tools are useful in addressing the diagnosis of *O. lupi*, and in localizing the site of the infection in dogs, which are positive for microfilariae at the skin skip examination. Indeed, until now, all cases of *O. lupi* in dogs were clinically investigated because of overt clinical signs (e.g., retro-bulbar abscesses, tumours or prolapse of nictitate gland), which oriented the veterinarian towards the surgical removal of the globe (Sréter and Széll, 2008) and therefore to a parasitological diagnosis. However, dogs infected by this spirurid might be asymptomatic, based on the lack of any apparent clinical alterations of the eye (Otranto et al., 2013a,b), as inferred by the nodular lesions that cannot be detected during routine ophthalmologic examination (i.e., sclera-corneal junction or medial rectus muscle). Therefore, diagnostic imaging tools proved useful in improving the diagnosis of *O. lupi*, with US resulting in more compliance by pet owners than skin snips, as US is easy to perform, requires a relative low budget and is minimally invasive. Small-mineralized ocular and retro-orbital lesions were clearly evident on US and CT, but were not on MRI images. However, although CT provided superior bone detail and better detection of calcification, and MRI offered soft tissue discrimination, US was a more valid, less invasive, safe and fast procedure to evaluate ocular onchocercosis, also supporting the pre-operative phase, revealing the localization of *O. lupi* nodules before undertaking surgery.

In the absence of a sensitive and specific serological tool (Giannelli et al., 2014), image techniques should be considered as complementary diagnostic tests, associated with the detection of *O. lupi* microfilariae and/or to an accurate morphological and molecular identification of the parasite. Indeed, when infections of the eye apparatus is suspected, US should be routinely performed, especially in regions endemic for *O. lupi*. Additional cases would confirm the utility of this diagnostic technique in a larger canine population.

Accordingly, these procedures might be also useful in human medicine, with US routinely used when patients complain of ocular signs (Richter et al., 2003; Otranto et al., 2011). Indeed, the importance of diagnostic imaging tools for the diagnosis of *O. lupi* infection has recently been demonstrated in the central cervical channel of a human patient suffering from neck pain (Eberhard et al., 2013). Finally, veterinarians living in areas where *O. lupi* is endemic, should be aware of the wide range of non-specific lesions caused by this little known spirurid and include canine onchocercosis in the differential diagnosis of subconjunctival or periocular granulomatous nodules, fat pockets, neoplasms, cystic masses, and small mineralized ocular and retro-orbital lesions in dogs.

Supplement data

Movie documenting the detection of O. lupi nodules during the computed tomography.

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