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Research paper

Clinical characteristics of *Spirocerca lupi* migration in the spinal cord

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ABSTRACT

Spirocerca lupi is a nematode infecting dogs mostly in tropical and subtropical areas. Although its typical target is the esophageal wall, aberrant migration is not uncommon, including migration of unknown incidence into the spinal cord. While successful treatment of intraspinal *S. lupi* (ISSL) infection depends on early diagnosis, tools for definitive ante-mortem diagnosis are unavailable. We therefore aimed at characterizing clinical signs and clinical pathology findings of ISSL in dogs.

For that, we analyzed medical records of dogs hospitalized in 2005–2016 presenting with neurological signs consistent with ISSL, which were diagnosed definitively post-mortem. Retrieved information included signalment, medical history, chief complaint, physical and neurological evaluation, neuroanatomical localization at presentation, clinical pathology, imaging findings, treatment, outcome and post-mortem findings. Ten midsize to large breed dogs were included, 7 of which had received prophylactic treatment. In all 10 dogs, onset was acute and neurological deterioration until presentation (2 h–6 d) was fast. Neurological examination localized the lesions within the spinal cord and paresis or paralysis was asymmetric in all dogs. Spinal pain was documented in 9/10 dogs. Cerebrospinal fluid (CSF) analysis was abnormal in all dogs and was characterized by pleocytosis in 8/10, whereas cytology revealed the presence of eosinophils in all dogs. Advanced imaging excluded spinal cord compression in all dogs tested. Post-mortem examination detected spinal cord migration tract in all cases. Nematodes were found in the spinal cord parenchyma (8/10) or adjacent to it (2/10) in all dogs. A larva was found in the subarachnoid space of one dog and an adult nematode in the thoracic intervertebral artery of another. Esophageal nodules were found in 5/10 dogs.

These findings suggest that the combination of sudden onset of acute asymmetric paresis accompanied by pain, presence of eosinophils in the CSF and lack of compressive lesion may serve as sufficient evidence for tentative diagnosis of ISSL in endemic areas.

1. Introduction

Spirocerca lupi is a nematode of worldwide distribution, but is most commonly found in tropical and subtropical areas. Dogs are the definitive hosts and become infected by ingesting the intermediate host coprophagous beetle or via a paratenic host (Bailey, 1972). After ingestion, the 3-mm long, third larval stage (L3) larvae are liberated in the gastric lumen, migrate through the gastric mucosa and arteries and through the thoracic aortic wall to the caudal esophagus. Typically, the worms settle within the esophageal wall, mature to adults of up to 70-mm in length and promote formation of a granulomatous nodule (Bailey, 1972). Aberrant migration is not uncommon and has been reported in various sites including every thoracic organ (Babero et al., 1965; Harrus et al., 1996), the gastrointestinal and urinary tracts (Wandera, 1976; Georgi et al., 1980) and the subcutaneous tissues (Turk, 1960; Harrus et al., 1996). Known involvement of the nervous system has so far been confined to the spine, with one extradural (Du Plessis et al., 2007) and 7 intradural reported cases of nematode migration, respectively termed extra- or intra-spinal *S. lupi* (E/ISSL) (Smith and Knottenbelt, 1987; Tudury et al., 1995; Dvir et al., 2001; Chai et al., 2008). In two reports, the intraspinal nematode was an incidental finding on post-mortem examination; hence, clinical description is lacking (Smith and Knottenbelt, 1987; Tudury et al., 1995).

The nematode *S. lupi* is endemic in Israel (Mazaki-Tovi et al., 2002; Aroch et al., 2015). The average number of dogs admitted to the Koret School of Veterinary Medicine Veterinary Teaching Hospital (KSVM-VTH) with new cases of esophageal spirocercosis (ES) is 22.5 per year (Aroch et al., 2015). Spirocercosis remains prevalent despite

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prophylactic treatment with ivermectin given every 3 months to most dogs in Israel. The incidence of dogs with aberrant migration of *S. lupi* into the spinal cord has not been determined due to failure to achieve a definitive ante-mortem diagnosis.

Treatment of intraspinal nematode migration in humans and animals includes anthelmintic agents, which may be combined with corticosteroids to reduce the inflammatory response (Vidal et al., 2003; Lee et al., 2010; Jabbour et al., 2011; Mitchell et al., 2011; Agbedanu et al., 2015). Rate and quality of recovery depend on the ability to stop the nematode's migration through the spinal cord, thereby preventing additional nervous tissue damage, as well as on the initial degree of neuronal loss. Early diagnosis and treatment are therefore crucial for recovery (Vidal et al., 2003).

In the absence of available tools for definitive ante-mortem diagnosis, tentative diagnosis is made based on characteristic clinical and clinical pathology findings. It is therefore important to provide clinicians with the most accurate and typical clinical characteristics to allow early diagnosis and treatment of the condition. The aim of this study was to characterize clinical signs, clinical pathology findings and risk factors of ISSL in dogs.

2. Materials and methods

Medical records of dogs admitted to the KSVM-VTH between 2005 and 2016 and presenting with suspected ISSL were reviewed. Only dogs that were definitively diagnosed in post-mortem examination were included. Definitive diagnosis of ISSL was made only in cases where *S. lupi* was found in the spinal cord or in close proximity to it (i.e., in the spinal column), together with the presence of a migratory tract in the spinal cord.

Retrieved information included signalment, medical history, chief complaint, physical and neurological evaluation (performed by a board-certified neurologist), neuroanatomical localization of infection at presentation, clinical pathology results, imaging findings, treatment, outcome and detailed description of post-mortem findings. Imaging methods used were radiography, computed tomography (Elscint Twin, Elscint, Haifa, Israel), myelography followed by CT scan and magnetic resonance imaging (GE 1.5 T, Milwaukee, WI or Intera 1.5 T, Philips Healthcare, Best, the Netherlands).

DNA was extracted from a formalin-fixed, paraffin-embedded spinal cord tissue containing the nematode from case #6, using QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Then, a high-resolution melt (HRM) real-time PCR that detects a 135 bp fragment of the ITS1 gene of *S. lupi* was performed (Rojas et al., 2017) using DNA of a complete *S. lupi* worm and dog tissue as positive and negative controls, respectively. In addition, a non-template control with PCR grade water (Biological Industries, Kibbutz Beit-Haemek, Israel) was added to the reaction. The obtained amplicon was cleaned and sequenced using the BigDye Terminator cycle sequencing chemistry from Applied Biosystems ABI3700 DNA Analyzer (ABI Carlsbad, USA). The sample was considered positive for *S. lupi* when < 97% of sequence identity to available *S. lupi* sequences (GenBank) was obtained using BLAST 2.2.28 program.

3. Results

3.1. Signalment and general information

Ten dogs met the inclusion criteria, with male (n = 5) and female (n = 5) equally represented. Mean and median age was 5 and 5.5 years, respectively (range, 1–9.5). All dogs were of midsize to large breed dogs, including 5 Labrador retrievers, 3 German shepherds, one Saluki and one Doberman. Mean and median weight was 31.5 and 32.5 kg (range, 20–40 kg). Retrieved information for all cases is shown in Table 1.

3.2. History and clinical signs

Prophylactic treatment with doramectin (200 µg/kg SQ) had been given to 7 dogs, of which two received the last treatment 21 and 30 days before the onset of clinical signs.In the other five dogs, doramectin treatment was given 1–3 months prior to the onset of clinical signs. Onset was acute in all 10 dogs and included paralysis of one hind limb in four dogs (4/10), non-symmetrical involvement of both hind limbs in 4 (4/10) and back pain in two dogs (2/10). Neurological deterioration rate from onset to presentation, which ranged from 2 h to 6 days, was fast in all dogs and

neuroanatomical localization of lesions was within the spinal cord in all cases. Spinal cord segments involved were T3-L3 (5/10), L4-S1 (3/10), L6-S1 (1/10) and T3-S1 (1/10). In the latter case, localization could not be narrowed further due to weak withdrawal reflexes in hind limbs and prominent mid-thoracic spinal pain. The lesion was asymmetric in all dogs and pain along the spine was documented in 9/10 dogs. Physical examination was normal in all 10 dogs.

3.3. Clinical pathology

Complete blood count (10/10) and chemistry profile (9/10) were normal in all dogs tested, except for relative eosinophilia in two dogs (2/10). Cerebrospinal fluid (CSF) analysis was abnormal in all dogs (10/10), ranging between 2–1920 cells/µl (mean, 419; median, 180) in cisternal samples (9/10) and 240–6400 cells/µl (mean, 2367; median, 460) in caudal lumbar samples (3/10; reference range, 0–5). The one dog with normal cisternal cell count of 2 had 33% of eosinophils (Table 1, case #7). CSF cytology (Fig. 1) revealed the presence of eosinophils in all dogs, representing 1–80% of nucleated cells in the slide. Neutrophils were evident in all slides, accounting for 12–80% of the cells. Macrophages were seen in the CSF slides of five dogs (5/10). Blood contamination was evident in two (2/10) of the cisternal and in two (2/4) of the lumbar CSF samples. Protein levels were elevated (> 25 mg/dl) in 5 out of the 7 dogs tested and ranged between 33.4–488 mg/dl.

Spirocerca lupi eggs were found by fecal flotation in one out of the three dogs tested (Table 1, case #6). Serology for *Toxoplasma gondii* and *Neospora caninum* was negative in all dogs tested (8/8).

3.4. Imaging findings

Survey radiographs of the spine were normal in 7/9 dogs examined. Mild spondylosis at the T8–T12 level was recorded in one dog and marked lamellar response at the T9–T12 level in another.

Advanced imaging of the spine was performed in 5 dogs, of which one underwent CT scan, 2 underwent myelography followed by CT scan and 2 were examined by MRI. The CT scan revealed spondylitis, aortic calcification and calcified mass between the aorta and the thoracic vertebrae at the level of T9–T10 in one dog (Table 1, case # 4). Post-myelography CT scan revealed non-specific abnormalities in two cases, including attenuation of the contrast material at the T3–T5 level in one dog (Table 1, case #9), and leakage of iohexol into the central canal at the T4–T8 level in another dog (Table 1, case #4). Spinal MRI revealed hyperintensity at the level of T2-L2 spinal cord segments in T2-weighted fluid attenuated inversion recovery (FLAIR) images in 1 dog (Table 1, case #7) and at the T12-L2 level in another (Table 1, case #8). Focal intra-medullary T1-weighted post-contrast enhancement was detected in both.

Typical *S. lupi* esophageal nodules were detected by endoscopy in 2/4 dogs (Table 1, cases #4 and #6). Turbulent flow in abdominal aorta was seen in ultrasound in one dog.

3.5. Outcome and post-mortem findings

As mentioned, intraspinal spirocercosis was definitively diagnosed