The first clinically manifested case of angiostrongylosis in a dog in Slovakia

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Abstract
The first clinical case of canine angiostrongylosis from Slovakia, previously infection-free country, is described. 18-month old male Bernese mountain dog living in south-eastern part of Slovakia showed poor health condition characterized by weight loss, irritating cough, dispnoe, intense salivation, vomiting and bilateral scleral bleeding. Two times even the acute physical collapse occurred. Blood analysis was provided and revealed increase of total protein, eosinophilia, monocytosis, and mild thrombocytopenia. Anaemia characterized by reduced number of erythrocytes and reduced levels of haemoglobin, packed cell volume and iron was also diagnosed. Larvoscopic Baermann technique revealed the presence of Angiostrongylus vasorum species. The first clinical case of angiostrongylosis has evidenced that the new life-threatening parasitic disease of dogs has spread to the territory of Slovakia. A serious effort is therefore inevitable to increase the professional awareness and knowledge on diagnosis, treatment and prevention.

Keywords
Angiostrongylus vasorum, dog, clinical signs, Slovakia

Introduction
The present study describes the first clinical report of angiostrongylosis in a naturally infected dog from Slovakia. The agent of infection, Angiostrongylus vasorum, is the metastrongylid nematode with indirect life cycle. Definitive hosts are carnivores and several slug species and frogs play the role of the intermediate host (Koch and Willesen 2009). The parasite, known also as the French worm, causes severe, often fatal cardiopulmonary disease of dogs.

In recent years, the awareness of veterinarians and breeders on this parasitic disease has been growing due to reasonable spread of A. vasorum outside its endemic areas of southeastern France, England, Denmark and Canada (Bollet et al. 1994). The parasite has recently appeared in European countries where it previously did not exist – in central Europe A. vasorum was recorded in Hungary (Majoros et al. 2010) and a serological study confirmed its presence in Poland (Schnyder et al. 2013). In 2013 the infection was confirmed during routine faecal examination in a dog in the Czech Republic (Svobodová, personal communication) and in Slovakia (Hurníková et al. 2013).

Herein we present the first clinically manifested case of canine angiostrongylosis in Slovakia, diagnosed in an 18-month old male Bernese mountain dog. The dog was found strayed in the village Horovce (Michalovce district, Eastern Slovak Lowland) in June 2013. Estimated age of the individual at that time was approx. 9 months. The dog was rescued through the shelter and has been living with new owners in Kosice, eastern Slovakia.

At the time of adoption, the dog showed poor general condition with sporadic cough and was severely infested with ticks. Finding of routine faecal examination performed at veterinary clinic stated the presence of Trichuris spp. eggs, strongylid eggs (Ancylostoma/Uncinaria spp.), and "some un-
specified larvae”. The dog was given fenbendazole (Fenbion, 250 tbl. a.u.v., Mevak®) in two doses and subsequently his overall health improved. The owner observed persisting moderate intermittent cough, primarily after physical effort. Blood sample was therefore examined for dirofilariosis with negative result.

After four months the health condition of the dog deteriorated rapidly and significant lethargy, loss of appetite and weight loss of 14 kg appeared, associated with strenuous walking and hunched posture. Two times even the acute physical collapse occurred. Hemorrhagic diarrhoea, intense salivation, vomiting, difficulties with urination and haematuria were also reported. Scleral bleeding was evident bilaterally. The dog suffered from severe irritating cough and dyspnoea.

With regard to the anamnesis, the faecal sample from the dog was delivered to the Institute of Parasitology SAS, where the modified larvoscopic Baermann technique (Willesen et al. 2004) was performed and revealed the presence of morphologically distinctive Angiostrongylus vasorum first stage larvae (Fig. 1.). The length of the larvae ranged between 320 and 360 µm and their caudal end showed distinct indentation on the dorsal side and also small indentation on the ventral side. Larvae isolated from fresh faeces were all coiled. Infected dog excreted larvae in high numbers – in 10 g of the faecal material more than 800 larvae were counted. Considering the stray episode in the case-history, it is possible that the dog was compelled to feed on alternative food sources, including snails and thus we can assume that the infection pressure was eminent in this case.

DNA from larvae (10 specimens) was extracted using the DNeasy®Blood&Tissue Kit (QIAGEN Group, Germany). PCR reaction for the amplification of rDNA second internal transcribed spacer (ITS2) was performed in a final volume of 25 µl using specific A. vasorum pairs of primers AV5 and AV4 designed by Al-Sabi et al. (2010). Template DNA (5 µl) was amplified by PCR reaction started with a pre-heating step at 94°C for 2 min and 38 cycles consisted of denaturing at 94°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 30 s. The PCR was terminated with a final extension at 72°C for 7 min. Amplicons were visualized on a 1.5% agarose gel. DNA analysis confirmed the presence of A. vasorum in infected dog.

Fig 1. The first stage larvae (L1) of Angiostrongylus vasorum, immobilised
The blood sample of the dog was also subjected to laboratory tests and haematological and biochemical profile was provided. Blood analysis revealed several abnormal findings (Table I.).

The increase of total protein, eosinophilia, monocytosis, and mild thrombocytopenia were recorded. Anaemia characterized by reduced number of erythrocytes and reduced levels of haemoglobin, packed cell volume (PCV), and iron was also diagnosed (Table I.). Due to several specific clinical signs, the dog’s blood was also examined for the presence of *Babesia canis* with negative result.

Immediately after diagnosis the dog was treated with two administrations of spot-on imidacloprid/moxidectin solution (Advocate®, Bayer Animal Health). One month later, the fae-

<table>
<thead>
<tr>
<th>Parameter (reference range)</th>
<th>Haematological findings</th>
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<tbody>
<tr>
<td>Total Protein (55–75 g/l)</td>
<td>↑95 g/l</td>
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<tr>
<td>Thrombocytes (200–500 × 10⁹/l)</td>
<td>↓145×10⁹/l</td>
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<tr>
<td>PCV (0.37–0.55 l/l)</td>
<td>↓0.29 l/l</td>
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<td>Haemoglobin (120–180 g/l)</td>
<td>↓101 g/l</td>
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<td>Erythrocytes (5.5–8.5×10¹²/l)</td>
<td>↓4.1×10¹²/l</td>
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<tr>
<td>Iron (16–21 µmol/l)</td>
<td>↓13.7 µmol/l</td>
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<tr>
<td>Eosinophils (0–5%)</td>
<td>↑12%</td>
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<tr>
<td>Monocytes (2–10%)</td>
<td>↑23%</td>
</tr>
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**Table I.** Clinical and abnormal laboratory findings registered at diagnosis in *Angiostrongylus vasorum* in naturally infected dog

**Clinical findings**
- poor general conditions, lethargy, appetite and weight loss,
- cough, dispnoe, fever, acute collapse,
- hemorrhagic diarrhoea, vomitus, intense salivation, haematuria,
- bilateral scleral bleeding

**Abnormal laboratory findings**

- **Parameter (reference range)**
  - Total Protein (55–75 g/l)
  - Thrombocytes (200–500 × 10⁹/l)
  - PCV (0.37–0.55 l/l)
  - Haemoglobin (120–180 g/l)
  - Erythrocytes (5.5–8.5×10¹²/l)
  - Iron (16–21 µmol/l)
  - Eosinophils (0–5%)
  - Monocytes (2–10%)

**Fig. 2.** Location of two cases of canine *Angiostrongylus vasorum* infection in eastern Slovakia (AUT – Austria, CZE – Czech Republic, HUN – Hungary, POL – Poland, UKR – Ukraine)
 registered leading

Consequently, insufficient dose of fenbendazole was admin-

istered during treatment. In the aforementioned case, the diagnosis was not determined correctly. August 2013, the diagnosis was not determined correctly. Although the dog was al-

ready infected at the time of the first faecal examination in

parallel with difficulties in determining the correct diagnosis. Herein described second case, manifested by significant clinical symptoms and almost fatal course, has clearly confirmed this assumption.

Hitherto, both reported angiostrongylosis cases in Slovaki

dog have been diagnosed in dogs from the region of south-eastern Slovakia (Fig. 2.). Here presented second case was ascertained in an individual coming from the area of the Eastern Slovak Lowland characterized by specific ecological conditions. Major part of the territory is covered by woodland meadows, flood plain forests and swamps, the winters are very mild, often without snow cover, and the average temperature is among the highest in Slovakia. This geographic and climate terms provide suitable conditions for both, survival and re-

production of many parasite vectors – from mosquitoes and ticks to snails. Recent parasitological and epidemiological studies indicate that the area is endemic for dirofilariosis (Iglódyová et al. 2012) and babesiosis caused by Babesia canis (Majláthová et al. 2011) with both agents occurring as co-infections (personal observation). The significance of this region in terms of circulation of the vector-borne parasites is evidenced by the fact that the Eastern Slovak Lowland was up until the 60’s of the 20th century considered a malaria endemic area (Dziuban 1962).

While practicing veterinarians have already apparently good knowledge on dirofilariosis and babesiosis, the angiostrongylosis emerging nowadays in Slovakia might cause major difficulties in determining the accurate diagnosis. Furthermore, this may be complicated by the occurrence of mixed infections – as for example in the case of A. vasorum and Diro-

filaria immitis mixed infection in a dog from Lombardy (D. immitis hyperendemic area) reported recently by Traversa et al. (2013). In addition, clinical signs in course of angiostrongylosis are very diverse and might evoke a whole range of other diseases, including pulmonary form of dirofi-

lariosis or babesiosis, in particular in patients from known endemic areas, as was apparent in here presented case.

Another problem that may occur in newly infected areas in parallel with difficulties in determining the correct diagnosis is the embarrassment to provide adequate treatment, as confirmed by hereby presented case. Although the dog was already infected at the time of the first faecal examination in August 2013, the diagnosis was not determined correctly. Consequently, insufficient dose of fenbendazole was admin-

istered during treatment, within a few months, to emergence of malign-

ant course of infection. Existing experience with therapy of angiostrongylosis confirms the fenbendazole administration being highly effective, when using proved therapeutic dose 20–50 mg/kg for 5–21 days (Chapman et al. 2004). In some cases even the prolonged use of the preparation for a period of 35 days is necessary (Gallagher et al. 2012). Therapeutically effective is also the administration of imidacloprid/moxidectin spot-on solution in single topical dose (Willesen et al. 2007), which was approved in the treatment of our patient.

The first clinical case of angiostrongylosis has evidenced that the new life-threatening parasitic disease of dogs has spread to the territory of Slovakia. A serious effort is therefore inevitable to increase the professional awareness and knowl-

dge on diagnosis, treatment and prevention. Our experience (particularly with dirofilariosis prevention and control) indicates that the monitoring and research related to emerging dis-

eases is very challenging (Miterpáková et al. 2012), and longer time is required for establishing effective and continuous co-

operation with veterinary practitioners.

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