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 first stage larvae. Infected dog excreted larvae in high numbers – in 10 g of the faecal material more than 800 larvae were counted. DNA analysis using PCR confirmed 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 cardiopulmonal 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).

The first autochthonous case of canine angiostrongylosis in Slovakia was diagnosed in 7-month old Maltese pinch living in Kosice (eastern Slovakia). The dog showed no clinical signs of infection (Hurníková et al. 2013). After treatment, consecutive follow-up examinations were negative for the presence of A. vasorum larvae in faeces.

Case report

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 stray 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