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The first report of serratospiculiasis in Great Tit (*Parus major*) in Slovakia

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Summary

Serratospiculiasis is a parasitic disease caused by filariid nematodes of the genus *Serratospiculum*, the subfamily *Dicheilonematinae*. *Serratospiculum* spp. parasitizes the air sacs and members of this genus have been found in various species of Falcons, Bald Eagles and Cooper's Hawk from all around the world. In the present study, infection with *Serratospiculum* was confirmed for the first time in the Great Tit in Slovakia. Nematode infestation was identified post mortem. Lesions in the respiratory system (airsacculitis, pneumonia) were associated with the presence of this nematode. Smears of the necrotic lesions in the crop and oesophagus contained the large numbers of embryonated eggs. Faecal samples were examined by flotation method and *Serratospiculum* eggs were found. Adult parasites were cleared in lactophenol solution and morphological analysis of male reproductive organs (shape of male nematode spicules) indicated the species *Serratospiculum amaculata.* Scattered inflammatory cells were seen in the mucosal and submucosal layers of infected oesophagus and inflammatory foci were found mainly in the stroma of the air sacs.

The presence of the filariid nematode in the nonspecific species Great Tit *(Parus major*) common in Slovakia indicates the importance of monitoring of serratospiculiasis in the avian hosts. The parasite can cause serious health problems, even sudden death of their hosts, therefore suitable effective measures for their elimination should be implemented.

Keywords: air sacs; nematode; serratospiculiasis; Great Tit

Introduction

Serratospiculiasis is caused by the filariid nematodes infecting the air sacs of birds (*Serratospiculum* Skrjabin, 1915, *Dicheilonematinae* Wehr, 1938, *Diplotrianidae* Anderson, 1958, *Diplotriaenoidea* Anderson and Bain, 1976) (Anderson & Bain, 1976) and may cause serious health problems in their hosts. The host distribution of *Serratospiculum* is cosmopolitan, but to date majority of documented infections was described in birds of the order *Falconiformes*.

The life cycle of *Serratospiculum* is indirect, involving two hosts: intermediate insect host and definitive avian hosts. Intermediate insect hosts include the order *Orthoptera*, such as grasshoppers, locusts, beetles (Tarello, 2006) and wood lice (Samour & Naldo, 2001). Thin-shelled eggs containing fully developed first stage (L1) larvae are passed out by via the faeces of the infected bird and can be consumed at the nymphal stage or young adults by coprophagic beetles. Within four days the nematodes molt to become second stage larvae (L2). Within eight days after invading the fat bodies, they molt again to become third stage larvae (L3). The L3 larvae present in the intestine of an intermediate host are infective to the definitive host, the suitable avian species, which become infected after the ingestion of infected beetles. In birds, the L3 larvae are released from their capsule and penetrate the wall of the proventriculus and ventriculus (Mauritz & Cole, 2008). The migration of L3 *Serratospiculum* larvae to the air sac system is direct and not through the portal system. After reaching the air sacs, the L3 larva undergoes two molts to produce the final fifth stage larvae (L5) or immature adult filariid nematode. Adult females breed and produce the large numbers of embryonated eggs in the air sacs (Samour & Naldo, 2001). The eggs are coughed up, swallowed, and passed out via the faeces, thus completing the life cycle. Encapsulated larvae can survive as long as their intermediate hosts, with most transmissions occurring during the spring and summer (Mauritz & Cole, 2008). Adults can remain active and survive within the definitive host for several years (Cawthorn & Anderson, 1980). Female nematodes mature after four months and start laying eggs within the air sacs that move to the lungs through natural movement of air and mucus (Mauritz & Cole, 2008).

Serratospiculiasis is described as being not highly pathogenic by Zucca (2003), but other studies reported deaths in birds of prey (Kocan & Gordon, 1976; Ackerman *et al*., 1992). Pathological changes associated with larval stages, adult parasites and eggs include inflammatory lesions in the lungs, bronchitis caused by lymphoid hyperplasia, airways oedema, fibrotic thickening within the air sacs, periarteritis, endothelial swelling and vacuolation, the lung congestion, thrombi present in arteries, pulmonary inflammation, granulomatous pneumonia, and mucoid hyperplasia (Cawthorn *et al*., 1980). The majority of the pathological consequences of serratospiculiasis are associated with appearance and residence of adult parasites in the air sacs. The most documented cases of *Serratospiculum amaculata* infestation were found in birds of prey in North America (Smith, 1993) and *Serratospiculum seurati* infection in falcons was reported in the Middle East (Zucca, 2003).

Histopathologic findings of the infected birds normally demonstrate the presence of adult filariid nematodes, larvae, and embryonated ova within the tissues. The parasitic stages present in the lung periphery are associated with mild focal hemorrhages, focal necrosis, and mild to moderate macrophage infiltration (Samour & Naldo, 2001). Adult nematodes are commonly observed on both sides of the collagen-muscle fibre layer immediately below the epithelial or mesothelial section of the air sacs of the host. Once the larval stages reach the air sacs, an acute inflammatory reaction occurs around the nematodes, resulting in fibrotic thickening of the air sac as well as forming adhesions among the air sacs, the body cavity, and the internal organs (Cawthorn *et al*., 1980).

A clinical diagnosis of serratospiculiasis could be based on radiographic evidence of filariid nematodes in the abdominal or thoracic air sacs, the occurrence of embryonated eggs at the oral cavity in falcons, especially if associated with small plaque-like lesions. Other signs of infection include the history of respiratory distress or signs of gastrointestinal upset and finding typical embryonated eggs in the faeces in infected birds (Ward & Fairchild, 1972).

The aim of present study was to describe the first case of serratospiculiasis using the clinical, pathological and histological methods in Great Tit, the nonspecific definitive host in Slovak Republic.

Material and methods

In February 2012, a dead adult female of the Great Tit was found in the urban area in Eastern Slovakia. The dead bird was transported to the Clinic for Birds and Exotic Animals at The University of Veterinary Medicine and Pharmacy where it was measured and necropsied. The endoscopic examination of the air sacs was performed and infection with nematodes suspected for *Serratospiculum* spp. was revealed. Individual parasite intensity was determined according to Bush *et al.* (1997). During the necropsy, the trachea, lungs, air sacs, kidneys, spleen, liver, heart, gallbladder, and the whole digestive tract of Great Tit (oesophagus, proventriculus, ventriculus, intestines) were examined. Adult nematode specimens subjected to morphological analyses were collected from the air sacs, and counted. Parasites were washed in saline solution and then fixed in 70 % ethanol, where they remained until clearing in lactophenol solution prepared according to the procedure described by Pritchard and Kruse (1982). Identification of parasites was based on the morphological features delineating species of the genus *Serratospiculum* showed by Skrjabin (1968), namely a length of small and large male nematode spicules. Morphometric measurements were performed using an Olympus Microscope BX51 and a Digital Analysis Imaging system „Analysis docu". The lung tissues, air sacs, liver, oesophagus, proventriculus, ventriculus and intestines intended for histological examination were fixed for 24 hours in 4 % paraformaldehyde in PBS. After fixation, the tissues were dehydrated in graded alcohols, cleared in xylene and embedded in the paraffin wax. The tissue sections (5 – 7 μm) were stained with Hematoxyline/Eosine (HE) and Toluidine Blue solutions (pH 0.5) in order to visualise connective tissue mast cells (CTMCs), the granules of which are seen in pink colour (Enerback, 1981). Intensity of accumulation and distribution of CTMCs were evaluated in the sections from all tissues using the light microscope as was described previously (Hrčková *et al*., 2006).

Results

Nematode infestation was identified post mortem and endoscopy revealed the presence of a number of nematodes in the air sacs of the bird. Following necroscopy,

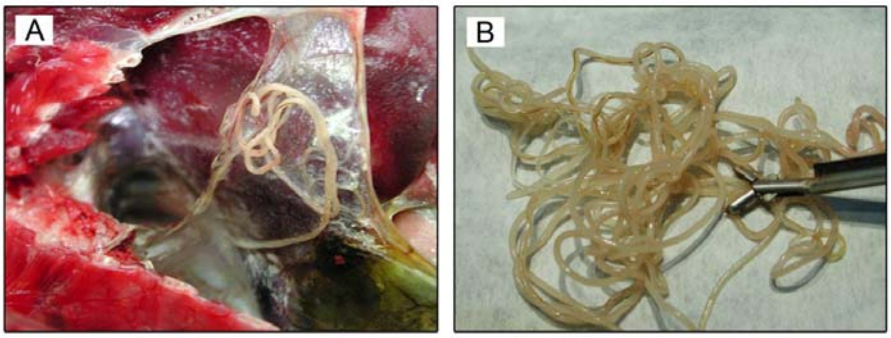

Fig. 1. A – necropsy of the air sacs in the Great Tit; B – adults of *Serratospiculum* collected from the air sacs of the Great Tit after the necropsy

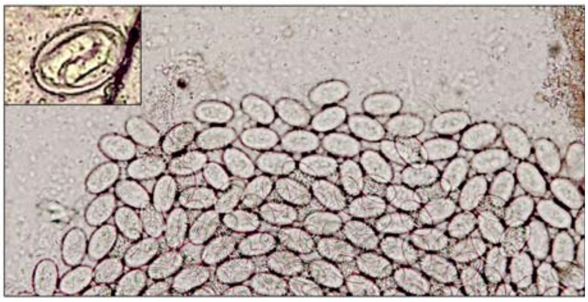

Fig. 2. Embryonated eggs of *Serratospiculum amaculata*

adults of *Serratospiculum* spp. were found in the air sacs with an intensity of infestation of 29 nematodes (Fig. 1A, B). Lesions associated with serratospiculiasis included cachexia, air sacculitis and pneumonia. Smears of necrotic material isolated from the crop and oesophagus contained large numbers of embryonated ova (Fig. 2). Faecal samples collected from the large intestine were also examined by the flotation method using Sheather solution and *Serratospiculum* eggs were found. The intensity of infection was assessed from the average number of eggs determined in 100 microscopic fields. We found more than 9 ± 3 eggs/field, which indicates very high (++++) intensity of infection.

Morphological criteria used for species identification were length of nematodes and shape and length of the male spicules, which were examined in the sample of 6 female and 9 male nematodes. The length of the females and males varied from 59 to 65 mm and from 26 to 29 mm, respectively, and width of all examined parasites was from 1.5 to 1.8 mm. The length of the large male spicules (780 – 1100 µm) varied with the mean value of 902 ± 135 µm, while length of the small male spicules varied in the range of 560 to 680 µm, the mean was calculated to be 625 ± 68 µm. The shape and length of male nematode spicules and the overall morphology corresponded to those of the species *Serratospiculum amaculata* (Wehr, 1938) (Fig. 3A – F) described in details by Skrjabin (1968).

The histopathological changes and the inflammatory lesions of the respiratory system (airsacculitis, pneumonia) were associated with the presence of larvae, which were

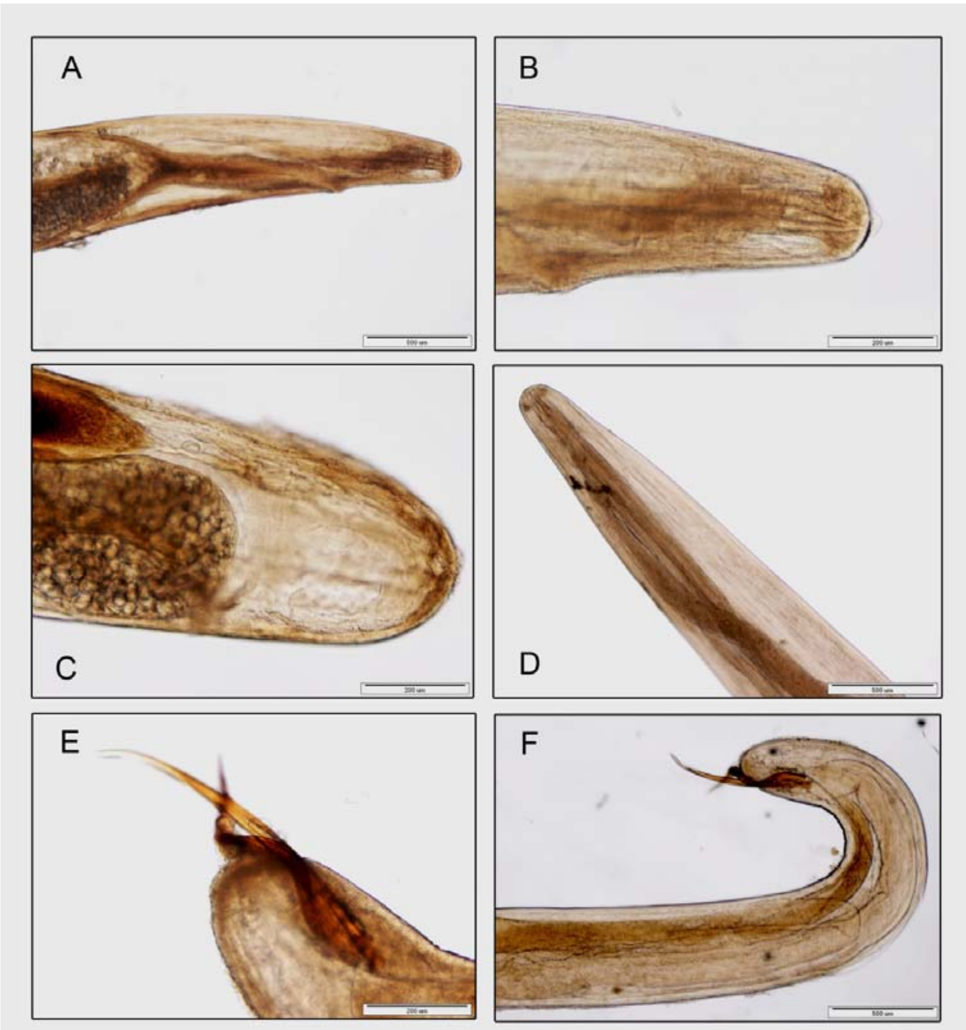

Fig. 3. Microscopic images of the adult nematodes of *Serratospiculum amaculata* after clearance in lactophenol. A, B – anterior part of the female *Serratospiculum amaculata* showing tube of oesophagus; C – caudal part of the female nematode showing a part of the uterus with eggs; D – a head region of the male nematode; E, F – caudal part of the male nematode with short spicule and long spicule localized ventrally along the body; Scale bars: A, D, F – 500 µm; B, C, E – 200 µm

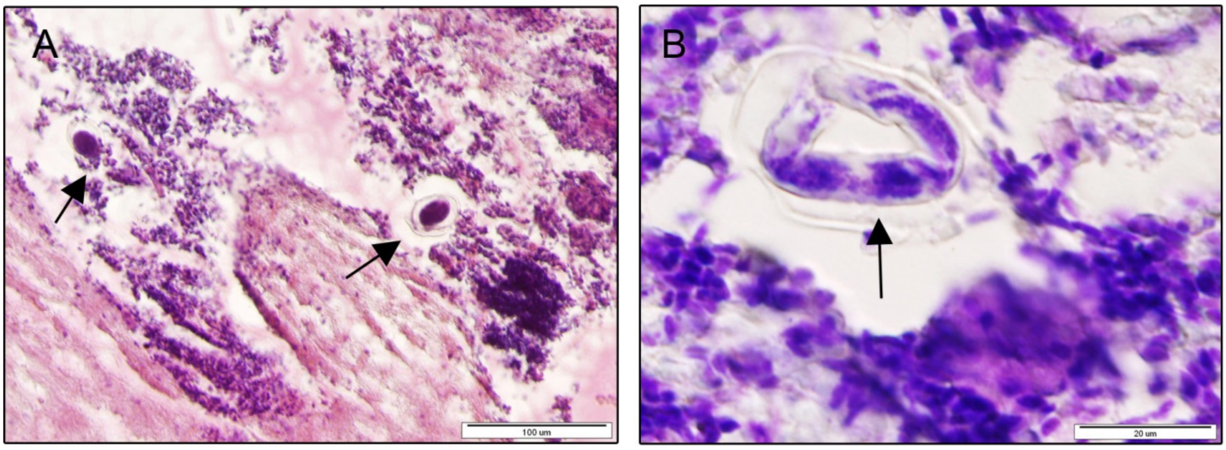

Fig. 4. *Serratospiculum* larvae (arrows) were embedded in the air sacs tissue from the infected Great Tit (Hematoxyline/Eosine staining) surrounded by massive inflammatory foci; Scale bars: A – 100 µm; B – 20 µm

identified after HE staining of histological sections of the air sacs (Fig. 4A, B). The most intense inflammatory reactions were elicited by larvae in the tissues, which were surrounded by inflammatory lesions situating in the attachment site. The inflammatory infiltrates consisted mainly of granulocytes and macrophages, however connective tissue mast cells (CTMCs) in the lesions close to the parasites were also scattered. The distribution of CTMCs was detected by the presence of enzyme tryptase in the mast cell granules deeply in the stroma. The highest number of CTMCs were found in the granulomatous inflammatory foci in the air sacs (Fig. 5A, B), which are characteristic for chronic inflammatory response. The scattered CTMCs in the mucosal and submucosal layers of infected oesophagus and ventriculus were also present, however in the large intestine, the occurrence of CTMCs was sporadic. In the liver, kidneys, spleen, heart, gallbladder and proventriculus of the Great Tit CTMCs were not observed.

Discussion

In the study, we confirmed the occurrence of the bird nematode *Serratospiculum amaculata* (subfamily *Dicheilonematinae)* in nonspecific definitive host, the Great Tit (*Parus major*). This is the first reported autochthonous case of serratospiculiasis in non-migrating birds in Slovakia. The host distribution of the species from *Dicheilonematinae* is cosmopolitan, however, the species of *Serratospiculum* have specific localization in the air sacs of carnivorous birds, mainly of the order *Falconiformes*. The first species of *Dicheilonematinae* in birds were described in Russia, later in Burma, India, Australia, China, Africa, North America (Mauritz & Cole, 2008). *Serratospiculum* species were for the first time identified in the Eurasian Kestrel (*Falco tinnunculus*) in Russia by Skrjabin (1915), who morphologically characterized eight species within this genus, of these *Serratospiculum tendo, Serratospiculum amaculata, Serratospiculum guttatum* are

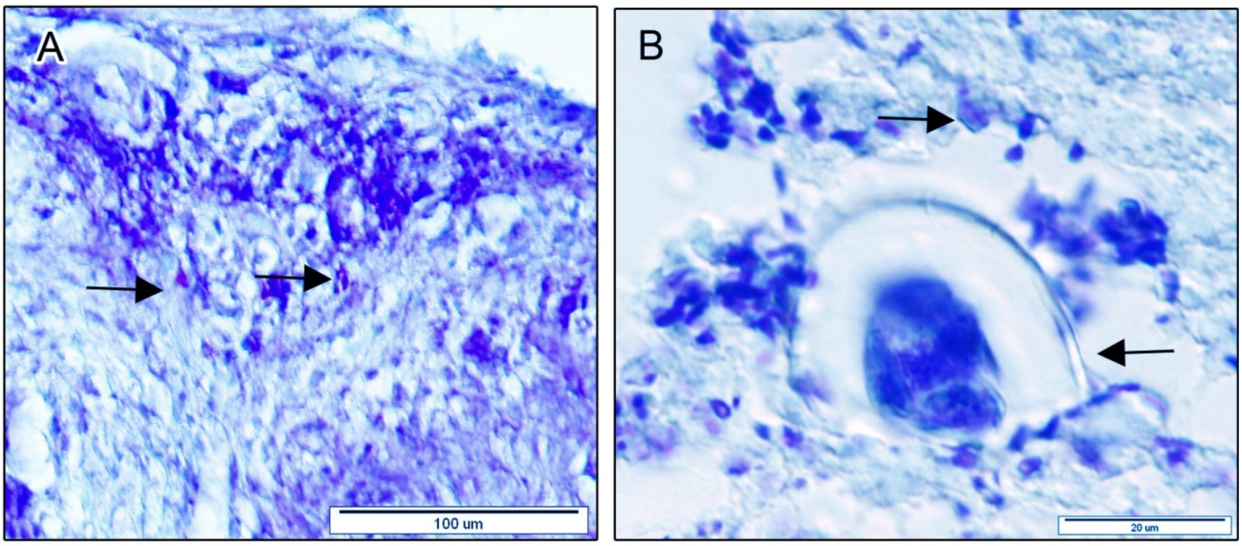

Fig. 5. Histological images of the infected tissues; A – granulomatous inflammatory lesions in the air sacs of the infected Great Tit comprised of granulocytes, macrophages and connective tissue mast cells (CTMCs) (arrows) during *Serratospiculum* infection (Toluidine Blue staining), which were seen mainly in the stroma of the air sacs; B – the inflammatory cells surrounding larva (arrowhead) with scattered CTMCs (arrow); Scale bars: A – 100 µm; B – 20 µm

the most prevalent. Nematodes in this genus have been reported in birds in South America, Australia, Asia, the Middle East and in Europe (Great Britain, Poland, Italy) (Bain & Mawson, 1981; Quentin *et al*., 1983; Gomez *et al*., 1993; Lierz & Remple, 1997; Samour & Naldo, 2001; Lloyd, 2003, Kalisinska *et al*., 2008; Santoro *et al*., 2012) and the most of reported cases of infection were found in the various species of falcons, including Prairie Falcon (*Falco mexicanus*), Peregrine Falcon (*Falco peregrinus*), Gyr Falcon (*Falco rusticolus*), Bald Eagle (*Haliaeetus leucocephalus*) and Cooper's Hawk (*Accipiter cooperii*) (Mauritz & Cole, 2008; Jones, 2006; Santoro *et al*., 2010; Santoro *et al*., 2012). Interestingly, they have also been recorded in accipiters in North America (Sterner & Espinosa, 1988; Taft *et al*., 1993). Our morphological analysis of male nematodes isolated from the Great Tit and a shape of short and long male spicules indicated *Serratospiculum amaculata,* however, their lengths were proportionally smaller than those measured in specimens described in raptors by Skrjabin (1968). Accordingly, mean body length of male and females were shorter due to small size of the host. *Serratospiculum amaculata* resides within the air sacs of birds of prey and infection is the most common in falcons native to Australia. Although this species seems to be primarily parasite of falcons, the infection may also occur in other species of raptors. For example, *Serratospiculum amaculata* has been reported from both Peregrine Falcons and Prairie Falcons, the Bald Eagle (*Haliaeetus leucocephalus*), and Cooper's Hawk (*Accipiter cooperii*) (Jones, 2006; Santoro *et al*., 2010; Santoro *et al*., 2012). In the birds in Australia, two other species of the nematode were identified, namely *Serratospiculum guttatum* in *Falco longipennis* and *Falco peregrinus* and *Serratospiculum tendo* in *Falco peregrinus* (Rose, 2005). To date, in the European countries, *Serratospiculum* spp. was morphogically described only in the carnivorous birds. *Serratospiculum tendo* was found in *Falco peregrinus* (Furmaga, 1957; Kalisinska *et al*., 2008) and *Falco cherrug* by Furmaga (1957) in Poland and *Serratospiculum* spp. has been reported in *Falco eleonorae* in the Mediterranean area in Greece (Diakou *et al*., 2007).

Our results confirmed the occurrence of *Serratospiculum amaculata* in the nonspecific host Great Tit (*Parus major major*), which belongs to the *Paridae* family. It is a widespread species occurring in Europe, the Middle East, Central and Northern Asia, and parts of North Africa, and is common in Slovakia. Similarly to other members of *Paridae* family*,* in the summer, birds are predominantly insectivorous and feed on wide range of insects, caterpillars, and beetles, but in the winter their diet is remarkably varied (Estók *et al*., 2010). Species of *Paridae* often live in the same habitats as the carnivorous birds. Therefore they can feed on the same spectrum of intermediate insect hosts, which may harbour *Serratospiculum* infection. To our knowledge, this infection has not been detected in the species belonging to *Falconiformes* in Slovakia so far, either in live birds or post mortem. Nevertheless, our case indicates that this infection can circulate locally in the Eastern Slovakia. The possibility to regularly monitor the infection prevalence in the species of *Passeriformes* is limited and parasitostatus in the individual specimens is usually examined accidentally post mortem. In our case, the bird was found dead in the urban area of Eastern Slovakia, where birds from the order *Falconiformes* are common also in the sub-urban areas. In this study we highlighted the fact that *Serratospiculum amaculata* can complete their life cycle also in *Paridae* species despite much smaller size of birds and their air sacs.

There have been reports that nematodes from the genus *Serratospiculum* can cause serious and sometimes lethal disease (serratospiculiasis) in the falcons from the various parts of the world. This parasite does not appear to be particularly pathogenic when intensity of infection is low; however, frequent mortalities in Prairie Falcons as a result of this infection have been described by both Bigland *et al.* (1964) and Kocan and Gordon (1976). Ward and Fairchild (1972) reported several cases of suspected *Serratospiculum* spp., infection in Prairie Falcons where air sacs of one dyspneic bird were filled with hundreds of adult nematodes. In the study in which *Serratospiculum amaculata* infection was considered the major contributing cause of death in the Prairie Falcons, necrotic foci in the liver, spleen, heart, in the crop and in oesophagus contained the large numbers of embryonated ova or free larvae Bigland *et al.* (1964). Santoro *et al.* (2010) observed that falcons which suffered from the frequent cachexia harboured at least 10 and more nematodes of *Serratospiculum tendo* in the thoracic and abdominal air sacs, indicating correlation between intensity of infestation and severity of clinical signs. Parasitic infestation in raptors appear to cause little or no distress to healthy individuals, but parasites can lead to serious health problems with clinical signs when combined with other factors or at times of stress (Krone & Cooper, 2002). Clinical signs of infestation include dyspnoea, weight loss to cachexia, anorexia, lethargy, pneumonia and air sacculitis. Serratospiculiasis may representant an important cause of morbidity and mortality in free-ranging falcons, but the prevalence of infestation in Europe is unknown (Santoro *et al*., 2010). However, no publish data describe to the correlation between intensity of serratospiculum infestation and severity clinical signs in *Paserinae*. In our case report on *Parus major*, 29 nematodes were collected from the air sacs, which were nearly completely filled with parasites. As no other parasitic infection was found in the bird and no visible injury was present, we suppose that cachexia due to the presence of relatively massive infection in comparison with the size of the air sacs could significantly contribute to the death of this non-specific definitive host.

The clinical symptoms in infected birds could involve not only respiratory difficulties due to pathological changes in the lungs and air sacs, but also gastrointestinal problems possibly due to invasion or irritation of the proventricular mucosa by larvae or ova (Ward & Fairchild, 1972; Santoro *et al*., 2010). The inflammation of the gastrointestinal tract and vomiting also contribute to deterioration of the overall health status of birds (Ward & Fairchild, 1972). Samour and Naldo (2001) reported that in several birds with severe infections, serratospiculiasis was associated with pneumonia, airsacculitis and early lesions of aspergillosis. Damage caused to the air sacs by serratospiculiasis predisposes infected birds to secondary infections with species of *Aspergillus*, *Pseudomonas* and *Klebsiella* (Samour & Naldo, 2001). Additional clinical signs of this type of infection may include general unthriftiness, lethargy, decreased body weight and poor plumage. As the number of larvae increase within the lungs, tissue swelling and presence of the nematodes can cause blockage and congestion of the air passages. In case of massive air sacs and lung nematode infestation, infected birds may die (Ward & Fairchild, 1972).

It has been suggested that *Serratospiculum* spp. might not be either host-specific or host-opportunistic throughout the parasite's geographical range (Krasnov *et al*., 2008, 2011). Similarly, the composition of parasite communities may change as a function of latitude because of the availability of species specific intermediate hosts (Poulin & Leung, 2011). *Serratospiculum* spp. use insects as first and intermediate hosts, respectively, therefore host agility, a broad host diet, and selective feeding on invertebrate hosts, represent the main determinants influencing infection prevalence in individual countries (Poulin, 1997). Urbanisation causes significant changes to species composition and important consequences of urbanisation are changes in host-parasite interactions (Delgado-V & French, 2012).

The other factor which should be emphasized is that infected Great Tit in our study was found in the urban area, not excluding a possibility that habitat where bird became infected could also be sub-urban or rural areas. Food-orientated migratory behaviour of this *Paridae* species represents potential risk of dissemination of embryonated nematode eggs. Transmission route of this species to Slovakia is not clear and it is well known that migratory birds serve as reservoirs and/or mechanical vectors for numerous parasites and play a significant role in the dispersion of pathogens (Sehgal, 2010). In this respect, infection could be transmitted from Poland, where *Serratospiculum tendo* was found in *Falco peregrinus* (Furmaga, 1957; Kalisinska *et al*., 2008) and *Falco cherrug* by Furmaga (1957).

In conclusion, this case report revealed that *Serratospiculum* infection circulates in Eastern Slovakia and therefore represents risk for various species of avian hosts. Our finding of *Serratospiculum amaculata* infection in an unusual host *Parus major* suggests that more attention should be paid to monitoring and correct diagnosis of serratospiculiasis.

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