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PROTOZOOLOGY - SHORT COMMUNICATION

Survey of *Toxoplasma gondii* and *Trichinella* spp. in hedgehogs living in proximity to urban areas in the Czech Republic

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Abstract

Hedgehogs (Mammalia: Erinaceidae) are omnivorous nocturnal animals typically living in anthropogenic areas. They may be suitable as sentinels for a wide range of zoonotic infections. Only a few studies have investigated hedgehogs (and then as representative wildlife species) to establish their role in the life cycle of such tissue parasites with zoonotic potential as *Toxoplasma gondii* or *Trichinella* spp. Working with frozen hedgehog cadavers, we tested for these parasites using *T. gondii* DNA-specific magnetic capture isolation plus polymerase chain reaction and *Trichinella* spp. digestion assay. All of 50 examined hedgehogs were negative for *Trichinella* spp. larvae in their muscles, but brain tissue from 5 out of 26 *Erinaceus europaeus* (19.2%) and 4 out of 24 *E. roumanicus* (16.6%) tested positive for *T. gondii* DNA. Frequency of *T. gondii* for both hedgehog species was equal, as was distribution between males and females and across age categories. Although a few studies have suggested the possibility of *Trichinella* spp. infection in hedgehogs, the zero prevalence in the tested hedgehogs is not surprising in view of the generally low prevalence of *Trichinella* spp. in Central Europe. Our results show that hedgehogs are susceptible to infection by *T. gondii* and can be used as indicator wildlife animal species in anthropogenic ecosystems.

Keywords Zoonosis · *Erinaceus europaeus* · *Erinaceus roumanicus* · Wild mammals · Sentinel animal

Introduction

Zoonotic infections in wild animals are worthy of attention in both human and veterinary medicine. *Toxoplasma gondii* Nicolle et Manceaux, 1908 (Apicomplexa: Sarcocystidae) and *Trichinella* Railliet, 1895 (Nematoda: Trichinellidae) are typical parasites with low host specificity able to infect various host species, including humans (Polley 2005). The heteroxenous coccidium *T. gondii* is one of the most prevalent zoonotic parasites infecting warm-blooded animals. The life cycle includes felids as definitive hosts and a wide range of warm-blooded vertebrates as intermediate hosts. Traditionally, rodents were considered as constituting a typical reservoir of *T. gondii* and as a conventional indicator for *T. gondii* presence in an environment (Reperant et al. 2009). The parasitic roundworm *Trichinella* has been found in domestic and wild animals worldwide (Pozio and Murrell 2006). Two of the most common species, *T. spiralis* and *T. britovi*, are circulating in Europe, accompanied by the non-encapsulated species *T. pseudospiralis* that is found in various hosts, including birds. *T. britovi* is more widespread in sylvatic carnivores and *T. spiralis* is prevalent in wild boar, domestic pigs, and rodents (Pozio and Murrell 2006; Pozio et al. 2009). Sylvatic and domestic lifecycles blend into one another, and especially in anthropogenic areas (villages, urban and suburban regions) having specific ecosystems where wildlife can play an important role as a source of infections for domestic animals or humans (Kruse et al. 2004). Hedgehogs (Mammalia: Erinaceidae) are typical occupants of human-inhabited areas in Central Europe and may be suitable as sentinel species for a wide range of zoonotic infections (Földvári et al. 2014; Jahfari et al. 2017). The two hedgehog species *Erinaceus europaeus* Linnaeus, 1758 and *E. roumanicus* Barrett-Hamilton, 1900 are commonly present in all areas of the Czech Republic (Bolfíková and Hulva 2012). Only a few studies have investigated hedgehogs (and then as representatives of wildlife species) to establish their role in the life cycle of *T. gondii* (Havlík

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and Zástěra 1954; Havlík and Hübner 1960; Berengo et al. 1972; Orlandella et al. 1972; Sixl et al. 1989) or *Trichinella* spp. (Matov et al. 1969; Bakasejevs et al. 2012; Hosni et al. 2013). The present study is focused upon recent evaluation of *T. gondii* and *Trichinella* spp. infections in hedgehogs of Central Europe and assessment of hedgehogs as indicator wildlife animal species in urban areas to discover presence of these zoonotic parasites circulating within ecosystems near human settlements.

Material and methods

A total of 50 hedgehog carcasses were obtained as frozen cadavers from rescue centers or as road kills and held at − 20 °C prior to necropsy. The hedgehogs originated from three districts surrounding the three largest cities (Prague, Brno, Ostrava) of the Czech Republic. Cadavers were thawed 12–24 h at room temperature before dissection. Species (*E. europaeus* and *E. roumanicus*), sex, and age categories were morphologically determined for each animal according to Dickman (1988) and Aulagnier et al. (2009). Brains and muscle tissue were removed under sterile conditions, placed individually into plastic vials, weighed, then stored at − 20 °C until further analyses.

DNA was isolated using *T. gondii*-specific magnetic capture protocol (Opsteegh et al. 2010) with modification for small sample size as described by Juránková et al. (2014). Samples of 560–2820 mg of hedgehog brains were homogenized in 5-mL tubes with cell lysis buffer (100 mM Tris HCl, pH 8.0; 5 mM EDTA, pH 8.0; 0.2% SDS; 200 mM NaCl; and 28.8 mg/l proteinase K 41.8 mAnson U/mg) in a ratio of 2.5 mL of buffer per 1 g of tissue. A crude extract was obtained and 500 μL of the crude extract from each sample was transferred to a clean 1.5-mL tube, the remainder was frozen. Removal of free biotin was performed by adding streptavidin sepharose (1 μL/mL; binding capacity 300 nmol/mL; GE Healthcare, Amersham, UK). Capture oligonucleotides Tox CapF and Tox CapR (10 pmol of each) were added to each sample for the sequence-specific magnetic capture (Opsteegh et al. 2010). Afterwards, the DNA denaturation (95 °C for 15 min) and hybridization between capture oligonucleotides and *T. gondii* DNA (55 °C for 45 min) were followed by magnetic capture using 80 μL of washed M-270 Streptavidin Dynabeads (Invitrogen, USA) and 100 μL of 5 M NaCl per sample. Finally, the *T. gondii* DNA was isolated using a DynaMagTM-2 magnet (Invitrogen, USA) and dissolved in 30 mL of polymerase chain reaction (PCR) water. PCR amplification on a 529-bp repeat element for *T. gondii* detection was performed in total volume of 20 μL reaction mixture consisting of 10 μL Combi PPP Master Mix (Top-Bio, Czech Republic), 0.01 μM of each primer (Tox 9F and Tox 11R), and 5 μL of template DNA. The reaction included initial incubation at 95 °C for 10 min, followed by 45 amplification cycles consisting of denaturation (95 °C, 10 s), annealing (58 °C, 20 s), and extension (72 °C, 20 s). Finally, the samples were cooled to 40 °C for 5 s. Each PCR run included positive (*T. gondii* DNA) and negative (PCR water) controls. PCR products were visualized in 1.5% TAE electrophoretic gel stained with GoodView nucleic acid stain (Ecoli, Bratislava, Slovak Republic).

Trichinella spp. digestion assay was performed using diaphragm and skeletal muscles (mainly forelimbs) from each hedgehog. The total amount of tissue used from each was 0.5–5 g, depending upon the hedgehog size. Part of muscle samples from each hedgehog was stored at − 20 °C for later determination of positive individuals. A mix of muscle tissue was combined from 2 to 10 hedgehogs to obtain final weight 5 g or 10 g. The mixed muscle samples were thoroughly homogenized manually with sterile scissors and examined by digestion assay in accordance with World Organisation for Animal Health recommendations. Minced muscle samples were digested in digestive solution (200 mL water, 1.6 mL 25% HCl, 1 g pepsin for 10 g of muscle tissue) at 45 °C for 30 min in 1-L beakers using a magnetic stirrer. The digested fluid was poured through a 180-μm sieve into a 2-L separating funnel for 30 min of sedimentation. Then, 10 mL of fluid was drained into a Petri dish and systematically examined at × 40 magnification for the presence of *Trichinella* spp. larvae.

Differences in the numbers of positive findings between hedgehog species (*E. europaeus* and *E. roumanicus*), between sex (males and females), and among age categories (juveniles, subadults, adults) were analyzed statistically using Fisher's exact test and an online website for statistical computation (http://vassarstats.net/).

Results and discussion

A total of 50 hedgehogs were examined and determined as 26 *E. europaeus* and 24 *E. roumanicus*. The set contained 25 males and 25 females. Age categories included 28 juveniles (age 0–6 months), 14 subadults (7–12 months old), and 8 adults (> 1 year old). Of the 50 animals examined, 9 (18%) were positive for *T. gondii*. The relation between *T. gondii* positivity and amount of brain tissue tested was not observed; weight of positive samples was 560–2060 mg. Five out of 26 *E. europaeus* (19.2%) and 4 out of 24 *E. roumanicus* (16.6%) were *T. gondii* positive. That prevalence is lower than the 25% prevalence revealed in a serological investigation performed in 64 *E. europaeus* by Sixl et al. (1989) in Austria. Our statistical analysis showed no significant difference between the tested hedgehog species ($p = 1$). We revealed more positive juveniles (5 animals) than subadults (2) and adults (2), but the difference between age categories was not statistically significant ($p = 0.776$). Regarding gender-based *T. gondii* positivity (4 females and 5 males), the difference was statistically insignificant ($p = 1$). It is difficult to evaluate an influence of age on *T. gondii* positivity within such a small set of samples, but the results do show that hedgehogs can become infected at a young age up to 6 months. Historically, *T. gondii* in hedgehogs had been reported based upon serological tests in the former Czechoslovakia (Havlík and Zástěra 1954; Havlík and Hübner 1960), central Italy (Orlandella et al. 1972), and Austria (Sixl et al. 1989). Berengo et al. 1972 found no positives among 40 hedgehogs tested serologically in Teramo Province, Italy. Due to the inability of *T. gondii* serological detection to determine the actual *T. gondii* infection, we chose a molecular method based on sequence-specific magnetic capture isolation of *T. gondii* DNA, which previously had been tested in wild house mice (Juránková et al. 2014). The method allows determination of *T. gondii* presence in hedgehogs with high sensitivity of detection. A previous study using magnetic capture *T. gondii* DNA isolation revealed low prevalence of *T. gondii* (± 1%) in wild house mice (*Mus musculus musculus*, *M. m. domesticus*, and their hybrids) trapped in or near human settlements or farms from two segments of the hybrid zone across the Czech–German border (Hůrková-Hofmannová et al. 2014; Juránková et al. 2014). On the other hand, our present study suggests that hedgehogs might be suitable as sentinel species for indicating the presence of *T. gondii* circulating within ecosystems near human settlements.

Our study revealed no positive sample among 50 hedgehogs using digestive assay for detection of *Trichinella* spp. In 10 animals, low amount of tissue 0.5 g was used due to small size of hedgehogs and little amount of muscle tissue from *Trichinella* predilection sites. Investigations of hedgehogs as *Trichinella* host species have been conducted only sporadically. In Bulgaria, *T. spiralis* was found in an autopsied hedgehog (Matov et al. 1969). In Latvia, three hedgehogs were included into a study of *Trichinella* in wildlife and no positive hedgehog was detected (Bakasejevs et al. 2012). A larger study was carried out in Libya by Hosni et al. (2013), where *Trichinella* larvae were found in 5.7% (4/70) of North African hedgehogs (*E. algirus*). Although generally the role of hedgehogs in the *Trichinella* spp. life cycle within Central Europe has been insufficiently explored, the zero prevalence in the tested hedgehogs is not surprising in consideration of the generally low prevalence of *Trichinella* spp. in Central Europe, especially in the Czech Republic (International *Trichinella* Reference Centre, ITRC, https:/trichinella.iss.it/Trichinella).

In conclusion, this study shows that hedgehogs are susceptible to *T. gondii* infection and can be used as indicator wildlife animal species in areas located near human settlements.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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