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Micromammals as a reservoir for the zoonotic nematode *Calodium hepaticum* (syn. *Capillaria hepatica*) in recreational areas of Slovakia

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ABSTRACT

The hepatic nematode *Calodium hepaticum* is a zoonotic parasite primarily parasitising small mammals, but it can infect a wide range of mammal species, including humans. Due to its specific life cycle and transmission pattern, it is one of the least studied helminths in the world. The only documented findings of *C. hepaticum* from Slovakia (former Czechoslovakia) come from the 60s and 70s of the 20th Century, including nine human cases of the infection reported *post-mortem*. The present study was conducted in the area of these original records in the Tatra National Park (TANAP) and the Košice Zoo. In TANAP, 484 small mammals of six shrew species (Insectivora: Soricidae) and eight rodent species (Rodentia: Muroidea) were collected. In the Košice Zoo, 163 rodents from 10 species were sampled. All specimens were examined for the presence of *C. hepaticum* eggs using the artificial digestion method. The parasite was recorded in two shrew species (*Sorex araneus* and *Neomys fodiens*) and five rodent species (*Arvicola amphibius*, *Microtus agrestis*, *Clethrionomys glareolus*, *Apodemus flavicollis*, and *Rattus norvegicus*) from TANAP, while in the Košice Zoo only a single individual of *R. norvegicus* was found to be infected.

1. Introduction

Calodium hepaticum (Bancroft, 1893) Moravec, 1982 (syn. *Capillaria hepatica*) is a nematode species with zoonotic potential distributed worldwide. The transmission of *C. hepaticum* depends on the death of a definitive host since adults of the parasite occupy and deposit eggs in the liver tissue, causing hepatic infection (hepatic capillariosis). The eggs are released from the liver parenchyma after death and decomposition of the host or through predation or cannibalism by ingestion and digestion of the infected liver and subsequent passage of the eggs into the environment, where, after a while – usually several weeks – they embryonate and become infectious to the next host (Spratt and Singleton, 2001). The eggs remain infective in the environment for approximately one year. After ingestion by the host, first-stage (L1) larvae are released in the intestine and migrate to the liver, where they mature and reproduce. Muroid rodents of the subfamilies Murinae and Arvicolinae are the primary hosts of *C. hepaticum*; however, due to low host specificity, the parasite has also been found in over 70 non-murid species, including humans (Fuehrer et al., 2011; Fuehrer, 2014a, b).

The zoonotic potential of *C. hepaticum* and the incidence of hepatic capillariasis in the human population are probably underestimated because of problematic diagnostics depending on liver biopsy or *post-mortem* examination (Spratt and Singleton, 2001). Several studies have reported the circulation of *C. hepaticum* in urban and residential areas of great agglomerations in Europe, including Milan, Italy (Ceruti et al., 2001), Liverpool, UK (McGarry et al., 2015), Marseille, France (Roqueplo et al., 2020), Barcelona, Spain (Millán et al., 2014), and Wroclaw, Poland (Buńkowska-Gawlik et al., 2017). Also, a newly

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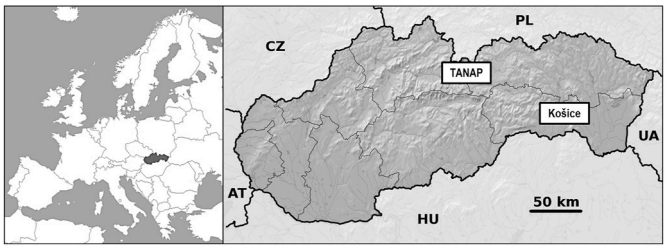

Fig. 1. The two localities in Slovakia where micromammal sampling was conducted: Tatra National Park (TANAP) and Košice Zoological Garden.

published study from Luzon Island, Philippines, revealed a two-fold prevalence rate of this parasite in residential localities compared to agricultural sampling sites (12.5% *vs* 6.7%, respectively) (Paller et al., 2024). Additionally, recent research has documented the circulation of *C. hepaticum* in wildlife ecosystems, specifically in the forest area near Dijon, France (Scandola et al., 2013). Considering the steadily increasing urbanisation and synanthropisation of wild animal species, the environment of frequently visited recreational areas represents a particular risk.

Despite this background, the only published findings of *C. hepaticum* from Slovakia (former Czechoslovakia) date back to the 1960s and 1970s, focusing on humans (Šlais, 1973). Therefore, our study aimed to investigate the prevalence of *C. hepaticum* in small mammals, considered its primary hosts, in specific regions of Slovakia.

2. Materials and methods

2.1. Study areas

The study was carried out in rural and urban areas of the Tatra National Park (TANAP), Slovakia (Fig. 1). This highest part of the Carpathian Mountains forms the natural border between Slovakia and Poland. TANAP covers an area of 738 km^2 and offers around 600 km of marked hiking and bike trails, making it the most popular tourist destination in Slovakia.

For comparison, the second, geographically distant, and ecologically different sampled locality was selected in the Zoological Garden in Košice, the second-largest city in Slovakia (Fig. 1). The Zoo is about 10 km from the city centre and covers an area of 288 ha, making it one of the largest in Europe. The vast terrain comprises a typical Carpathian habitat with semi-natural *Fagus-Carpinus* forests and meadows.

2.2. Animals and samples

In total 484 small mammals belonging to 6 shrew species (*Crocidura suaveolens*, *Neomys fodiens*, *Sorex alpinus*, *Sorex araneus*, *Sorex minutus,* and *Talpa europaea*) and 8 rodent species (*Arvicola amphibius*, *Microtus agrestis*, *Microtus arvalis*, *Clethrionomys glareolus*, *Apodemus agrarius*, *Apodemus flavicollis*, *Mus musculus,* and *Rattus norvegicus*) were collected from the territory of the TANAP between 2018 and 2023. Of them, 205 individuals came from rural areas, and 279 were sampled in residential localities of the TANAP. The animals were sampled in the framework of the research project focused on zoonotic tapeworm *Echinococcus multilocularis* and were mainly found dead, road-killed, or captured within the pest control operation. The sampling was performed by employees of the TANAP Museum and Research Station in Tatranská Lomnica; the carcasses were kept frozen at −20 °C and subsequently delivered to the Institute of Parasitology of the Slovak Academy of Sciences (IP SAS) in Košice.

In the Košice Zoo, a total of 163 free-living rodents belonging to 7 species (*Cricetus cricetus*, *Microtus arvalis*, *Clethrionomys glareolus*, *Apodemus agrarius*, *Apodemus flavicollis*, *Mus musculus*, *Rattus norvegicus*) were trapped on the plots in 2023. Standard live traps set in lines, with seeds and nut mixture, applied as baits, were used for sampling. The traps were spaced approximately 5 m apart, exposed for two nights, and checked each morning. The captured rodents were humanely euthanised before the carcasses had been handled following the authorisation by the Ministry of Environment of the Slovak Republic under permit No. 498/2018-6.3.

2.3. Parasitological examination

During the necropsy, the livers of the sampled micromammals were examined macroscopically for the presence of cysts and other lesions. The liver tissues with macroscopic changes were squashed, washed with phosphate-buffered saline (PBS), and investigated under the microscope. To avoid underestimating the prevalence, all livers were subsequently processed using the artificial digestion method with an acidified pepsin solution of final pH 2.0. The clarified digest was examined in thin layers on the Petri dish under the stereomicroscope for typical bi-polar *Capillaria*-type eggs (Gamble et al., 2000; McGarry et al., 2015).

2.4. Statistical analysis

The prevalence values with a 95% Wilson Score confidence interval (CI) was calculated using a web application (https://www.statskingdom.com/).

3. Results

Macroscopic examination of liver tissues from 484 small mammals in the TANAP area revealed lesions consistent with hepatic capillariosis in only six animals. Subsequent homogenisation and artificial digestion of the samples confirmed the presence of *C. hepaticum* eggs in animals with pathologically altered livers and revealed the pathogen in 16 additional animals whose livers showed no macroscopic pathologies. The overall prevalence of *C. hepaticum* was 4.5% (95% CI: 3.0–6.8%) among rodents from the TANAP. Of 163 micromammals from the Košice Zoo, only a single individual of *R. norvegicus* was infected with the parasite, indicating a somewhat lower prevalence of 0.6% (95% CI: 0–3.1%) in the locality (Table 1).

On the territory of TANAP, *C. hepaticum* infection was confirmed in two insectivore species, in particular, *S. araneus* (9/60) and *N. fodiens* (1/3) and five rodent species, including *A. amphibius* (1/1), *M. agrestis* (1/8), *R. norvegicus* (1/18), *A. flavicollis* (8/187) and *C. glareolus* (1/84) (Table 1). Twelve infected micromammals came from rural localities, while the other ten were sampled in residential zones. The following macroscopic pathologies were found in the six abovementioned specimens: multifocal white-yellow nodules with irregular contours ranging from less than 1 mm to 3 mm on the liver surface were present; the lesions were grouped in patches and covered about one-third of the liver parenchyma. The eggs had the characteristic morphological features of *C. hepaticum* with typical barrel-shaped morphology, asymmetrical bi-polar plugs, and radial striations in the thick shell (Fig. 2). No adult parasites were found in the infected livers.

Table 1
Host species parasitised by *Calodium hepaticum* in the study areas of the Tatra National Park and Zoological Garden, Košice, Slovakia.

Study area		Tatra National Park			Košice		
Host species		*N*	*n*	Prevalence (%) (95% CI)[a]	*N*	*n*	Prevalence (%) (95% CI)[a]
Scientific name	Common name						
Insectivora							
Crocidura suaveolens	Lesser white-toothed shrew	1	0	0	–	–	–
Neomys anomalus	Southern water shrew	–	–	–	–	–	–
Neomys fodiens	Eurasian water shrew	3	1	33.3 (6.1–79.2)	–	–	–
Sorex alpinus	Alpine shrew	1	0	0	–	–	–
Sorex araneus	Common shrew	60	9	15.0 (8.1–26.1)	–	–	–
Sorex minutus	Eurasian pygmy shrew	16	0	0	–	–	–
Talpa europaea	European mole	4	0	0	–	–	–
Muroidea							
Arvicola amphibius	Water vole	1	1	100	–	–	–
Cricetus cricetus	Common hamster	–	–	–	1	0	0
Microtus agrestis	Field vole	8	1	12.5 (2.2–47.1)	–	–	–
Microtus arvalis	Common vole	4	0	0	1	0	0
Clethrionomys glareolus	Bank vole	84	1	1.2 (0.2–6.4)	40	0	0
Apodemus agrarius	Striped field mouse	30	0	0	34	0	0
Apodemus flavicollis	Yellow-necked field mouse	187	8	4.3 (2.2–8.2)	51	0	0
Mus musculus	House mouse	67	0	0	8	0	0
Rattus norvegicus	Brown rat	18	1	5.6 (1.0–25.8)	28	1	3.6 (0.6–18.0)
Total		484	22	4.5 (3.0–6.8)	163	1	0.6 (0.1–3.4)

Abbreviations: *N*, number of examined hosts; *n*, number of infected hosts.
[a] 95% CI Wilson Score Interval.

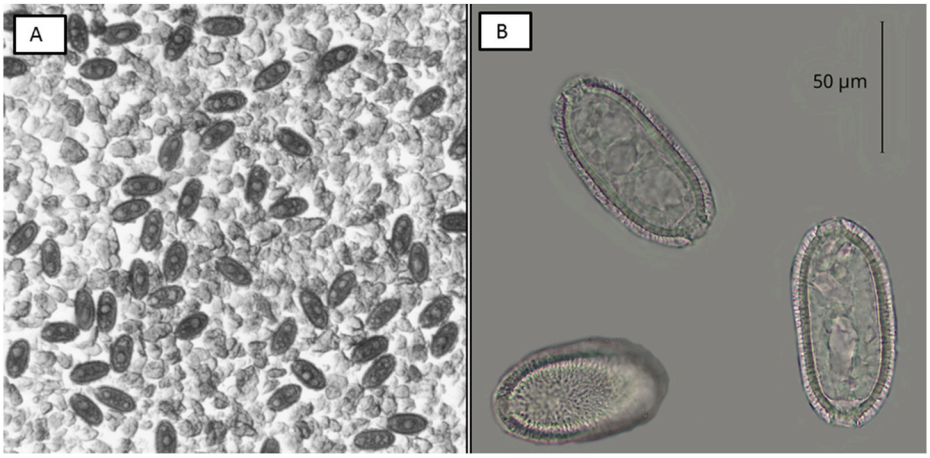

Fig. 2. A cluster of *Calodium hepaticum* eggs in the liver tissue of infected *Sorex araneus* (**A**) and parasite eggs (cleared in water solution of lactic acid, phenol and glycerol) isolated by digestion method from the liver of *Apodemus flavicollis* (**B**).

4. Discussion

Although *C. hepaticum* has been recorded worldwide, including in the Slovak Republic, no up-to-date studies have been conducted on the occurrence of this species in this country (Fuehrer, 2014a, b). The most recent data on *C. hepaticum* in micromammal species in Slovakia is available from a study conducted over fifty years ago (Mituch, 1970). It is important to note that these data were not published in a scientific journal but are only available as an annual report in the library (archive) of the Institute of Parasitology of the Slovak Academy of Sciences. The study recorded the presence of the helminth in several host species (*S. araneus*, *S. minutus*, *N. fodiens*, *A. flavicollis*, *A. sylvaticus*, *Microtus nivalis*, and *C. glareolus*) in the territory of TANAP. Our paper, therefore, presents the first available scientific data on insectivore and rodent host species of *C. hepaticum* in Slovakia.

Our study in TANAP and Košice Zoo, two geographically distant areas, has demonstrated a notable variability in the prevalence of *C. hepaticum*. Except for a single specimen of the brown rat from the Košice Zoo, the parasite was recorded exclusively in TANAP. Also, comparing our data with the archived reports from TANAP (1966–1970; Mituch, 1970), allows us to assume the existence of a persistent focus of parasite circulation in the TANAP region. An additional argument supporting this hypothesis is that Mituch (1970) reported *C. hepaticum* infection not only in small mammals but also in four carnivorous species, *Vulpes vulpes* (1/43), *Lynx lynx* (1/4), *Mustela nivalis* (1/17), and domestic *Felis catus* (1/73). This evidence suggests that the TANAP region may serve as a long-term endemic area for the parasite, where parasite circulation is sustained within the populations of both small mammals and carnivorous species for at least 50 years. It should also be noted that other researchers have also observed the phenomenon of localised foci of *C. hepaticum* (Stojčević et al., 2002; Reperant and Deplazes, 2005).

According to the review article of Fuehrer et al. (2011), 163 cases of human hepatic capillariosis were documented worldwide; most cases (78) represented spurious infections, 72 cases had clinical manifestations, and 13 cases were confirmed serologically. This review also includes nine cases of *C. hepaticum* infection reported by Šlais (1973). These cases were confirmed retrospectively *post-mortem* in the former Czechoslovakia (at present, Czechia and Slovakia). Following these data, it should be concluded that the incidence of human hepatic capillariosis is extremely low. However, given that the infection is rare, characterised

by non-specific clinical manifestations and complex diagnostics, it is conceivable that the number of human cases may be seriously underestimated. The available epidemiological studies show that humans, especially children, living in poor sanitary conditions and possible contact with synanthropic rodents, rats and mice have more chance of being infected (Fuehrer et al., 2011; Gonçalves et al., 2012; Wang et al., 2019). These reports align well with Fuehrer's (2014a) conclusion that rats seem to be the most important host of this parasite, with reported prevalences above 50% on several continents.

5. Conclusions

Our paper presents the first findings of the zoonotic nematode *C. hepaticum* in insectivore and rodent hosts from Slovakia since Mituch's unpublished data over five decades ago. The results show that *C. hepaticum* circulates in natural ecosystems and urban areas. The study provides evidence of a persistent focus in the Tatra National Park, where the helminth species circulates for at least 50 years. Despite the low incidence of human cases, the pathogen's prevalence in rats, its global distribution, and the potential underestimation of its prevalence in humans highlight the importance of regular monitoring, primarily in synanthropic micromammal species, for the presence of this parasite. Further efforts should be made to monitor micromammal populations for zoonotic helminths to understand better the current epidemiological distribution of *C. hepaticum* in Slovakia and beyond.

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Ethical approval

The captured rodents were humanely euthanised before the carcasses had been handled following the authorisation by the Ministry of Environment of the Slovak Republic under permit No. 498/2018-6.3.

Statement on the use of AI-assisted technologies

During the preparation of this article, the authors used Grammarly (https://www.grammarly.com/) to correct grammatical errors and improve readability. After using this tool, the authors reviewed and edited the content as needed. The authors take full responsibility for the content of the published article.

CRediT authorship contribution statement

Martina Miterpáková: Conceptualization, Resources, Supervision, Visualization, Writing – original draft. **Zuzana Hurníková:** Resources, Investigation, Writing – review & editing. **Petronela Komorová:** Resources, Investigation, Writing – review & editing. **Michal Stanko:** Resources, Writing – review & editing. **Gabriela Chovancová:** Resources, Writing – review & editing. **Yaroslav Syrota:** Resources, Visualization, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the outcomes of this study.

Data availability

The data supporting the conclusions of this article are included within the article.

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