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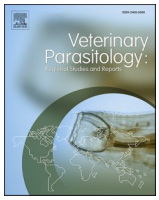

Original Article

The first survey of endoparasite infection in the brown rat (*Rattus norvegicus*) from a synanthropic environment in Hungary

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ABSTRACT

Urban rats are significant reservoirs of zoonotic endoparasites, posing serious health risks to humans. This study aimed to detect common endoparasites in wild brown rats (*Rattus norvegicus*) in Budapest, addressing the current lack of endoparasite surveys in Hungary. Carcasses of 131 rats collected following extermination were examined. Three zoonotic helminths were identified: *Hymenolepis nana* and *Hymenolepis diminuta* in the jejunum, and *Calodium hepaticum* in the liver. Additionally, non-zoonotic parasites were detected, including *Eimeria* spp., *Entamoeba muris*, *Heterakis spumosa*, *Nippostrongylus brasiliensis*, *Eucoleus gastricus*, *Aonchotheca annulosa*, *Syphacia muris* (intestine), and *Trichosomoides crassicauda* (urinary bladder). Helminth infection prevalence was 83.9 %, with no noticeable differences in prevalence or infection intensity between sexes. These findings highlight the potential public health risk posed by zoonotic parasites in urban rat populations, emphasising the importance of surveillance to mitigate possible human infection. This study demonstrates a practical and economical approach to monitoring urban rat populations. Further large-scale studies are recommended to better understand the parasitic landscape in Hungary's rat populations, leveraging data from rat control programs.

1. Introduction

Although numerous studies have explored parasites in wild animals, investigating parasites in non-domesticated mammals within synanthropic environments is crucial for public health and animal conservation (Esposito et al., 2023). Research has shown a strong correlation between urban immigration and the incidence of zoonotic diseases in humans (Coomansingh-Springer et al., 2019). Rodent species known to transmit pathogens are often found in high concentrations in urban areas (Blasdell et al., 2022). In particular, species from the genus *Rattus* spp. are significant contributors to environmental zoonoses, including bacterial (Dahmana et al., 2020), viral (Kurucz et al., 2018), and parasitic diseases (Mustapha et al., 2020; Galán-Puchades et al., 2021). Among synanthropic rodents, the brown or Norway rat (*Rattus norvegicus*) (Berkenhout, 1769), is a well-studied reservoir in urban areas (Tian et al., 2018).There have been limited surveys on zoonotic parasites of Norway rats conducted in major European cities. The majority of studies have focused on *R. norvegicus* in rural or wild environments (Webster and Macdonald, 1995; Meerburg et al., 2009; Himsworth et al., 2013; McGarry et al., 2015; Desvars-Larrive et al., 2017; Strand and Lundkvist, 2019). Common zoonoses transmitted by urban rats include giardiasis, hydatid disease, trichinellosis, and other helminthiasis caused by the tapeworms *Hymenolepis nana* (Von Siebold, 1852) and *Hymenolepis diminuta* (Rudolphi, 1819) (Acha and Szyfres, 1992; Coto, 1997; Hancke et al., 2011).To provide preliminary data for disease control in Hungary, carcasses of poisoned rats collected from various districts of Budapest were subjected to a rapid morphological examination for parasites commonly found in Europe. By regularly assessing parasite diversity in carcasses throughout the year, this cost-effective and straightforward method can be used for long-term monitoring of infection status.

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2. Materials and methods

2.1. Study area and study population

From September 2020 to September 2021, necropsies were performed on 131 rat carcasses obtained from eight districts in Budapest, Hungary (Fig. 1). The rats were poisoned using brodifacoum-based baits, and necropsies were conducted within 24 h post-mortem. On average, 10 rats were dissected monthly, with the highest numbers of carcasses (25) was received in May and December. The 131 rats were obtained through convenient sampling, rather than being poisoned specifically during the study period. This study was designed to apply a straightforward method aimed at detecting the most common parasites in these rats. While the sample size was limited, and the distribution of carcasses across months was uneven, this method is scalable for future large-scale investigations. The primary objective of this work was not to achieve statistical precision, but rather to demonstrate the utility of a simple detection technique. The sample included 67 male and 64 female rats, consisting of 119 adults and 12 juveniles. Age classification followed the criteria of Delattre and Le Louar (1981), with adult rats weighing over 130 g and juveniles under 130 g (Delattre and Le Louarn, 1981).

2.2. Dissection and parasite identification

Viscera were separated and dissected to identify endoparasites. The intestinal tracts were removed and placed in Petri-dishes. The organs were cut into pieces of 1–2 cm and the worms were washed out with tap water. Pathologic changes, potentially caused by parasites, were noted visually. Infected tissue samples were embedded in paraffin, sectioned, stained with haematoxylin and eosin, and examined by light microscope. The alimentary tract was split lengthwise, and gut contents were inspected. Spleen and lung touch slides were prepared to detect migrating larvae and protists. Baermann's larva isolation method was employed for lung samples to identify motile larvae of lungworms and roundworms. Muscle tissue from the diaphragm and thigh was examined for *Trichinella* sp. larvae using the Trichinoscope method. One gram of meat was diced and compressed between two glass plates, tightly sealed to form a thin, transparent layer. This sample was then examined under a binocular microscope (20× magnification) (Roepstorff and Nansen, 1998). To prevent cross-contamination between samples during necropsies, all dissection equipment was thoroughly cleaned and sterilised between each rat. Equipment were washed with absolute ethanol and water, and gloves were changed after each dissection to ensure that no contamination occurred between samples. Intestinal contents were sampled to obtain protozoa cysts and helminth eggs. Flotation techniques using zinc sulphate solution (specific gravity 1300 g/l) were applied to enrich and identify these parasites. Particles on the surface of the solution were picked up with a glass rod, smeared across the surface of a slide and examined for recognizable parasites by compound microscope following the protocols by Navone et al. (2005) (Navone et al., 2005). Rats were considered parasite carriers in the case of any worm or microscopic parasite detected by the methods employed in this study. Endoparasites were initially identified morphologically under a microscope, followed by routine histological techniques. Nematode and protozoa species were identified based on descriptions by Durette-Desset (1970) (Durette-Desset, 1970), Nemeséri and Holló (1972) (Nemeséri and Holló, 1972), Hugot and Quentin (1985) (Hugot and Quentin, 1985), del Rosario Robles et al. (2008) (del Rosario Robles et al., 2008), Anderson et al. (2009) (Anderson et al., 2009), and Ribas et al. (2013) (Ribas et al., 2013). Cestode identification referenced Khalil et al. (1994) (Khalil et al., 1994) and Makarikov and Tkach (2013) (Makarikov and Tkach, 2013).

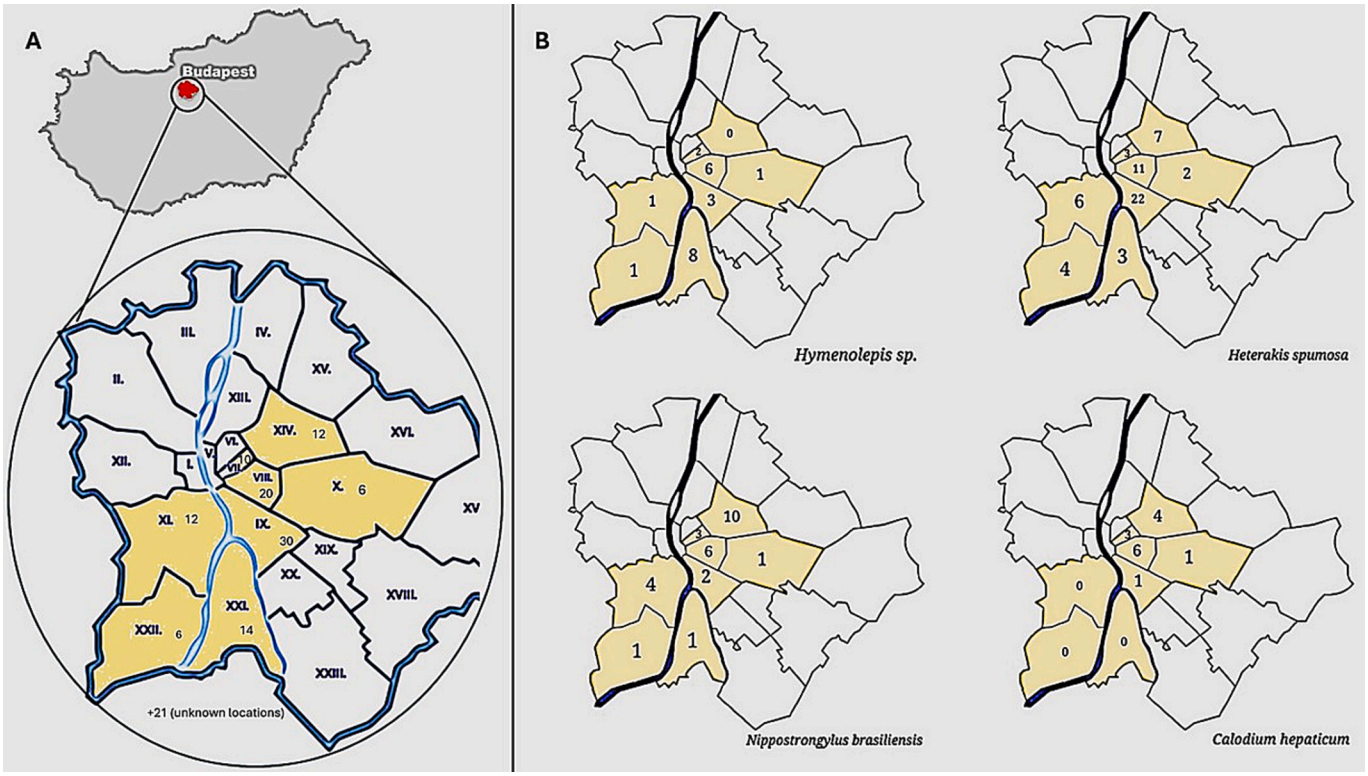

Fig. 1. Sampling area. A) Map of the surveyed districts in Hungary and Budapest with the number of analysed carcasses per district; B) Maps of study sites and the number of rat samples infected with four different parasite species from eight districts in Budapest, Hungary during the period of September 2020 to September 2021.

3. Results

3.1. Microscopic examination of rat carcasses

Of the 131 rat carcasses examined, 110 were found to harbour parasites, with nine helminth species and two protists identified (Table 1). Notably, *Calodium hepaticum* (Bancroft, 1893) Moravec 1982 (Syn.: Capillaria hepatica Bancroft, 1893) a significant zoonotic parasite, was identified in the liver of rats from four districts (VII, VIII, X, XIV), with the highest prevalence in district XIV where four out of 12 carcasses were positive. The infected livers contained numerous irregular yellowish-white patches or red tracts and streaks on the external surface. Next to the adult worms, egg mass was also visible in histological sections and in the smears of the tissue (Fig. 2A,B).

The *H. diminuta* and *H. nana* tapeworm species were noted under a collective name *Hymenolepis* spp. in Table 1. by the dissecting microscopic examination. As reliable identification of *Hymenolepis* spp. is only possible with a light microscopic examination of the scolex, which in many cases was missed, due to the decomposed state of the carcasses, where only the proglottids were identified (Fig. 3A). However, by carefully dissecting the scolex attached to the intestinal wall from some fresh carcasses, it became certain that both species can be found in rats, in Budapest (Fig. 5). The majority of tapeworms were found in samples from the XXI. district, where eight out of 14 samples were positive. The young rats were exclusively infected with *Hymenolepis* spp., with five out of 12 samples being positive, but no eggs were found in any of their intestinal contents.

Heterakis spumosa Schneider, 1866, the most common helminth, found in nearly half of the samples, with the highest prevalence in district IX. where out of 30 dissected carcasses 22 were infected. (Fig. 3D). This species was identified by adult specimens and their eggs were found in some cases together with adult worms.

Syphacia muris (Yamaguti, 1935) was detected only in January 2021 across three districts (VIII, XI, and one unknown location). Worms were identified by adult specimens.

Nippostrongylus brasiliensis (Travassos, 1914) was found in 66.7 % of carcasses from XIV. district, the second most common helminth (Fig. 3F), eight out of twelve carcasses were infected from XIV. district (Fig. 3F).

Trichosomoides crassicauda (Ballingham 1845), a urinary bladder parasite, was found in four out of 12 samples from XIV. district and present in all districts, which is the highest prevalence among the districts. At least one infected rat came from all eight districts, so this parasite seems to be very widespread (Fig. 3E).

Capillariidae worms were identified by species in some cases if we could find adult males, specimens were considered to *Aonchotheca annulosa* (Dujardin, 1845) in the small intestine and *Eucoleus gastricus* (Baylis, 1926) in the stomach. In the small intestine, *A. annulosa* species was characterised by mass appearance if present. Interestingly, in the IX. district rats were not infected with *A. annulosa* despite this region having the largest sample size. This parasite was detected in all other examined districts, with the highest prevalence found in the XXII. district (50 %). In four individuals, *Eucoleus gastricus* helminths were identified in the stomach. These worms were also detected in XXII. district where the *A. annulosa* has the highest prevalence.

Entamoeba muris (Grassi, 1879) was detected in the spleen of one individual using Giemsa-stained smears (Fig. 3B).

The presence of *Angiostrongylus* and *Trichinella* species in inspected rats was not supported as larvae were not detected in lung and muscle samples.

3.2. Flotation method findings

Parasite eggs were detected from the whole intestinal contents using the flotation method. Recognised species and number of positive samples are presented in Table 1. The *H. diminuta* eggs occurred twice as often as *H. nana* eggs (Fig. 4 A,B). The eggs of the two *Hymenolepis* species were distinguishable by examining the embryonic membranes inside the eggs In the case of *H. nana*, two opposite thickenings and peaks are visible on the membrane that envelops the embryo, while in *H. diminuta* the membrane is smooth and rounded. Both species can be present in the same host individuals. Eggs were detected in carcasses from VIII. district only. *Heterakis spumosa* eggs were not observed in three districts (X, XI, XXII) but the 30 carcasses were positive for eggs from IX. district, where the highest, 73.3 % prevalence of this worm was found (Fig. 1B, Fig. 4C, Table 1).

Nippostrongylus brasiliensis eggs were found less frequently than adult worms (Fig. 4D). As strongylid eggs cannot always be verified to belong to this species, we considered these eggs to be *Nippostrongylus* eggs if they occurred together with the *Nippostrongylus* worms. These eggs were detected in the intestinal content of one rat originating from X. district and three rats from XIV. district.

Syphacia muris is the only worm detected in the adult stage during faecal flotation (Fig. 3C). In one of the IX. district samples, an adult female with eggs in the uterus was visible but free eggs were not observed in the sample.

Eggs of *Capillaridae* worms were usually identified with *Aonchotheca* specimens. In some cases these eggs were found without the presence of adult worms. Such helminth eggs were identified from carcasses in eight districts (Fig. 4E). Although neither *C. hepaticum* nor *T. crassicauda* normally release their eggs in the faeces, eggs of both species were identified in the intestinal content of one carcass after flotation.

Coccidia oocysts, most likely *Eimeria* spp., were detected in 13 carcasses (Fig. 4F) predominantly from the 12 young rats, 7 were positive in XXI. district. Fig. 1.B shows the distribution of the 4 most frequently detected parasitic species by district. These parasites were found in at least 14 rats during the survey, and numbers of infected rats varied in each district.

Table 1
Endoparasite species detected in rat carcasses through organ examination and the flotation method where N1 is the number of infected (n: male or female) rats detected from organ examination and N2 is the number of infected (n: male/female) rats identified using the flotation method. P% represents prevalence.

Endoparasite species		Infected organs	N1 (n:male/n: female)	P%	N2 (n:male/n: female)	P%
Hymenolepis sp.	*H. diminuta* *H. nana*	small intestine	21 (10/11)	16.03	8 (4/4) 4 (3/1)	6.11 3.05
Heterakis spumosa		small and large intestine	64 (34/30)	48.85	21 (10/11)	16.03
Syphacia muris		caecum and large intestine	3 (2/1)	2.29	1 (male)	0.8
Nippostrongylus brasiliensis		small and large intestine	27 (13/14)	20.61	4 (2/2)	3.05
Calodium hepaticum		liver	14 (7/7)	10.68	1 (male)	0.8
Trichosomoides crassicauda		bladder	20 (9/11)	15.26	1 (female)	0.8
Aonchotheca annulosa.		small intestine	15 (7/8)	11.45	24 (11/13)	18.32
Eucoleus gastricus.		stomach	4 (2/2)	3.05	n.a.	n.a.
Entamoeba muris		spleen	1 (male)	0.8	n.a.	n.a.
Eimeria spp.		n.a.	n.a.	n.a.	13 (7/6)	9.92

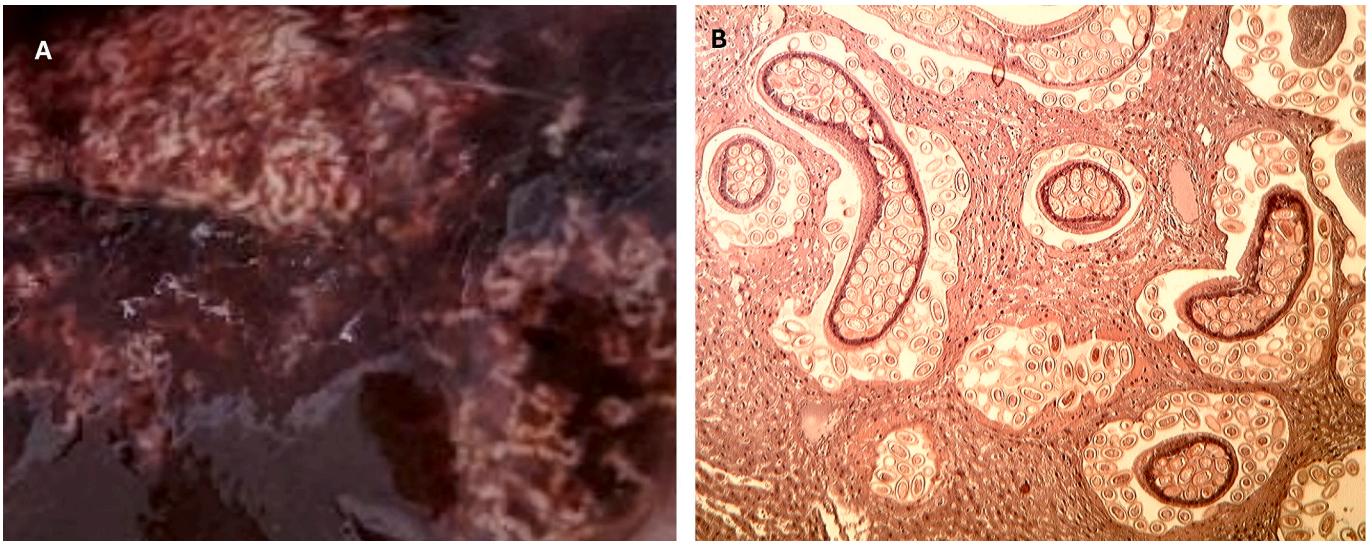

Fig. 2. A) Gross appearance of rat liver showing *Calodium hepaticum* infection. The organ contains many irregular yellowish tracts on the external surface of the liver. B) Cross section of adult *Calodium hepaticum* genital tubes illustrating the number of eggs. The eggs are laid in clusters within the parenchyma cells of the liver, stained with haematoxylin-eosin.

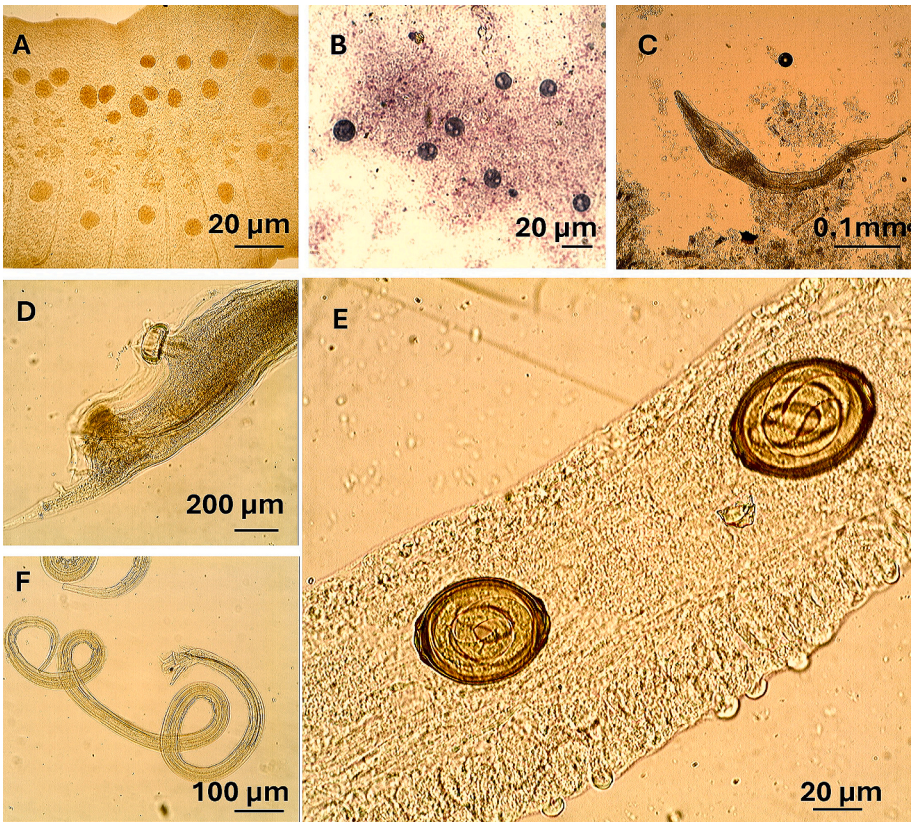

Fig. 3. A) Tapeworm of *Hymenolepis* sp. showing mature proglottids stained with haematoxylin B) *Entamoeba muris* trophozoites from spleen smear stained with Giemsa C) *Syphacia obvelata*, the adult female worm D) *Heterakis spumosa* posterior end of male, E) Ovoid, bipolar eggs inside of *Trichosomoides crassicauda* female uterus. F) Male *Nippostrongylus brasiliensis* worm isolated from the large intestine of a rat.

3.3. Seasonality

The seasonal analysis was limited by the uneven sampling across months, which restricts the reliability of conclusions regarding seasonal variation in parasite prevalence. However, we observed that the general prevalence of detected worms was higher in November, December, and May. *Eimeria* spp. were detected only in the spring months, while *Heterakis spumosa* were found throughout the year, with the highest detected intensity in May (Fig. 6).

4. Discussion

This study provides an initial report on the prevalence of intestinal parasites in urban brown rats from Budapest, Hungary. By making use of

Fig. 4. Parasite eggs detected by flotation A) *Hymenolepis diminuta* egg, B) *Hymenolepis nana* egg, C) *Heterakis spumosa* egg; D) *Nippostrongylus brasiliensis* egg, E) *Aonchotheca annulosa* egg, F) Unsporulated *Eimeria* sp. oocysts.

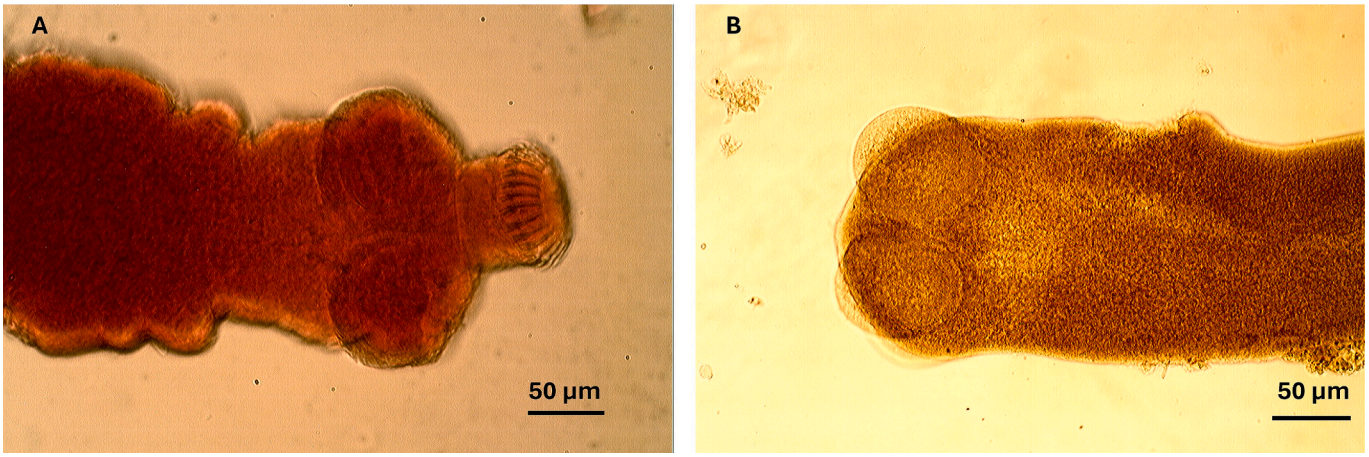

Fig. 5. The scolex of the A) *Hymenolepis nana* and B) *Hymenolepis diminuta.*

exterminated rat carcasses this study effectively identified the prevalence of endoparasites across different districts of Budapest, proving to be a cost-effective method for parasite surveillance. Carcasses of exterminated rats were examined within 24 h, however the degradation of some carcasses affected the detectability of *Hymenolepis* spp. The high prevalence (83.9 %) of parasitic infection aligns with findings from other cities such as 85 % from Barcelona, Spain (Galán-Puchades et al., 2018), 84 % from Dhaka, Bangladesh (Barman et al., 2020), and 98.60 % from Palermo, Italy (Milazzo et al., 2010). No noticeable gender-specific distribution of parasites was observed in this study (Table 1). Zoonotic endoparasites, including *Calodium hepaticum* (87.9 %) and *Hymenolepis* spp. (34.4 %) were present in Baltimore, Maryland, USA (Easterbrook et al., 2007).

In the present study, *H. spumosa* appears to be a common non-pathogenic parasite species in urban rats. *Heterakis spumosa* was found throughout the year, in all surveyed districts of Budapest (Fig. 1.,6.). However, the prevalence of infection is variable in different studies. It was found in 48.9 % of animals in the present work compared to 82.5 % of brown rats in Palermo (Milazzo et al., 2010), 76 % of animals in Liverpool, UK (McGarry et al., 2015), 61 % in Chanteraines park, in France (Desvars-Larrive et al., 2017)(Desvars-Larrive et al. 2017). *Heterakis* sp. can spread easily as infection occurs through oral ingestion of embryonated eggs from soil (Šnábel et al., 2014) and the wide host species range (rats, mice, and even occasionally hedgehogs (Klimpel et al., 2007)) further facilitates its spread. The rate of infection with *N. brasiliensis* in brown rats in this study (20.6 %) is consistent with

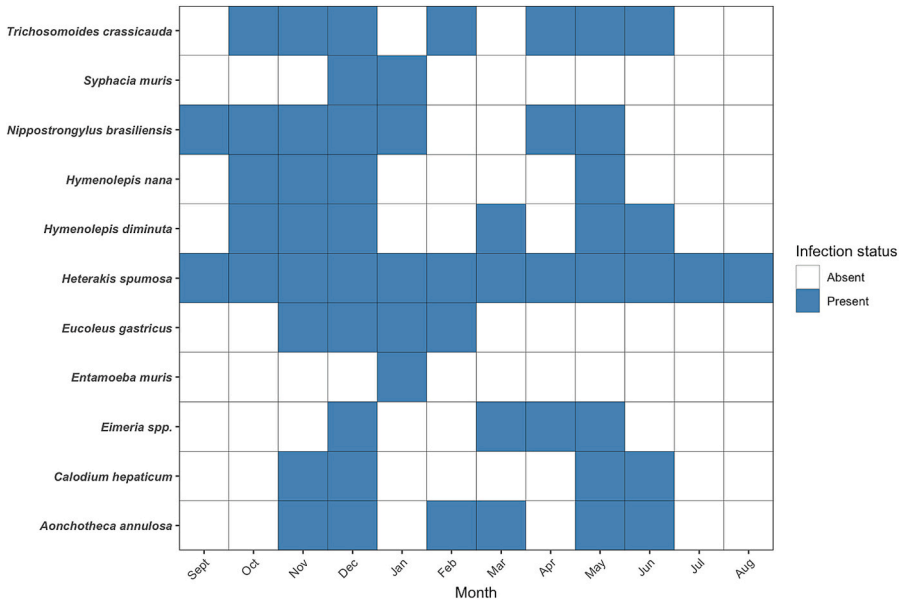

Fig. 6. Tile plot for seasonal change in infection status of urban brown rats with detected endoparasite species.

studies 17 % from Kuala Lumpur (Mohd Zain et al., 2012) and 23 % from UK farms (Webster and Macdonald, 1995). In the case of *N. brasiliensis*, there was the greatest difference between the detection of the number of infected rat's carcasses with adult worms (20.6 %) and the eggs (3.05 %) from the faeces.

Hymenolepis diminuta eggs (6.11 %) occurred twice as often as *H. nana* eggs (3.05 %) identified via the flotation method, in agreement with the results of previous research conducted in other countries, according to which *H. diminuta* is more common (Ito and Kamiyama, 1987; Abu-Madi et al., 2001). No eggs were detected in young animals which suggests that by the time the worms begin to produce eggs, the animal is already an adult. These zoonotic parasites highlight the risk posed by urban rats in spreading pathogens.

Trichosomoides crassicauda is a strictly rat species-specific parasite of the lumen of the urinary bladder. We identified *T. crassicauda* in 15 % of rats, which is less than previous studies in Liverpool, UK in which 31 % of rats were infected (McGarry et al., 2015).

The prevalence of *C. hepaticum*, was 10.68 % in this survey, and was found in carcasses through the year, whereas autumn was at lowest risk of infection in Belgrade, Serbia (Kataranovski et al., 2011). Farm rats 23 % were *C. hepaticum* positive in a UK study conducted 30 years ago (Webster and Macdonald, 1995). Eggs of *A. annulosa* were identified in the intestine (18.32 %) more often than the adult form (11.45 %).

Syphacia muris was present in small numbers in the samples as free eggs were not detected, but one female worm was detected with immature eggs indicating potentially successful reproduction in the host. Protozoan parasites were the least abundant in the study, though again this does not necessarily mean that the infection rate is low, as these parasites are more difficult to detect with the flotation method due to their size (Galán-Puchades et al., 2021).

Entamoeba muris is a parasite species that occurs in the intestine but can also enter the bloodstream, from where it can reach parenchymatous organs. This unicellular parasite was identified in this study in stained smears of a spleen. Of the 13 carcasses that contained *Eimeria* spp. oocysts, seven were young rats. This means that more than half of the dissected 12 young rats were infected with *Eimeria* species, probably with *E. nieschulzi* and/or *E. separata*. As a result of shrinking of unsporulated oocysts in the flotation solution during the flotation method the two species couldn't be identified. The detected 9.9 % rate of coccidial infection, it can therefore be assumed that coccidiosis primarily affects younger animals as stated by Ernst et al. 1968 (Ernst et al., 1968).

The same parasites were often found in the carcasses originating from the same district at the same time, suggesting that the rats lived in close proximity to each other and likely fed together. With regard to endoparasitic nematodes (with the exception of *E. gastricus*) more worms were detected during dissection than eggs by the flotation method (Table 1.). This can be explained by the periodic egg production of different worms. However, for most species, only the eggs could be detected from each carcass, so it can be stated that neither method alone is sufficient to confirm infection. The reliance on poisoned rat carcasses may impose certain limitations on the detection of parasites. Even with necropsy conducted within 24 h post-mortem, decomposition can compromise the identification of the more delicate parasites and eggs. Furthermore, poisoning may influence the presence of ectoparasites, as they could abandon the host carcass, making their detection challenging. Conducting larger-scale research would also enhance our understanding of the geographic and temporal patterns of zoonotic parasites in urban environments, providing critical data for public health interventions (Mustapha et al., 2020; Galán-Puchades et al., 2021; Acha and Szyfres, 1992; Coto, 1997; Hancke et al., 2011).

The potential transmission routes for zoonotic parasites such as *Hymenolepis diminuta*, *Hymenolepis nana*, and *Calodium hepaticum* are of particular concern. These parasites can be transmitted to humans through direct contact with contaminated soil, water, or food, or via intermediate hosts such as arthropods. Poor sanitation and close proximity between rats and human populations in urban areas increase the risk of zoonotic transmission, underscoring the public health relevance of these findings (Mustapha et al., 2020; Acha and Szyfres, 1992; Coto, 1997).

5. Conclusion

This study is the first to report the endoparasite fauna of urban brown rats in Hungary, highlighting the presence of zoonotic species such as *H. diminuta*, *H. nana*, and *C. hepaticum* in Budapest. These findings support the role of rats in maintaining and spreading zoonotic pathogens, posing a potential threat to public health. The results demonstrate that parasites can be effectively detected in poisoned rat carcasses, suggesting this method of monitoring urban rat populations is both practical and economical. Enhanced surveillance and detailed inspection of culled rats could inform future public health strategies and

improve rodent management programs.

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Ethical standards

No experiments on animals.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Alexandra Juhász: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Tamás Tóth:** Writing – review & editing. **Camilla J.L. Eldridge:** Writing – review & editing, Visualization. **Gábor Majoros:** Writing – review & editing, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare there are no conflicts of interest.

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