



Pinworm infections in red squirrels at the urban-forest interface

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Abstract. The growing overlap between urban and forest environments increases the risk of cross-species parasite transmission, making the study of parasitic fauna in wild mammals highly relevant. The aim of this research was to investigate the prevalence and host specificity of oxyurid nematodes in Eurasian red squirrels (*Sciurus vulgaris*) inhabiting forested park areas of Polissia, Ukraine. It was analysed 191 faecal samples collected between September and December 2023 using an integrated approach that combined innovative monitoring methods, including video surveillance in the squirrels' natural habitat, systematic sample collection, and detailed

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microscopic examination of helminth egg morphology. Additionally, the YOLOv5 computer vision model was utilized for automated analysis of video recordings, enabling precise identification of individual squirrels. Three species of parasites from the superfamily *Oxyuroidea* were identified in this study. The squirrel-specific species *Trypanoxyuris sciuri* was the most prevalent, detected in 70.6% of squirrels (12 out of 17 individuals; 95% CI: 44.04-89.69). Parasites typically associated with murids were less common: *Syphacia obvelata* was found in 11.8% of squirrels (2 out of 17; 95% CI: 1.46-36.44), and *Aspicularis dinniki* in 5.9% (1 out of 17; 95% CI: 0.15-28.69). To further investigate these species, *S. obvelata* eggs were cultured under laboratory conditions, allowing for detailed characterization of L1 larvae morphology, while *A. dinniki* failed to develop, confirming its specific host requirements. These findings highlighted the high prevalence of *T. sciuri* among squirrels and indicated the potential transmission of *S. obvelata* and *A. dinniki* within their populations. The practical value of this study lies in expanding the understanding of helminth diversity and host-parasite interactions in transitional urban-forest ecosystems. The obtained results can be used to improve veterinary and sanitary measures, prevent parasitic diseases, and develop monitoring strategies aimed at preserving the health of wild populations and minimising risks to domestic animals and humans

Keywords: wildlife parasites; gastric nematodes; *Syphacia*; pinworms in squirrels

Introduction

With the expansion of urban areas and the reduction of natural habitats, the intensity of contact between wild mammals and animals living in close proximity to humans has increased. This creates favourable conditions for the circulation of parasitic agents and their potential transmission between different host species. One of the important directions in veterinary research is the monitoring of helminth infections in wild animal populations as part of the epizootic surveillance system. Helminths belonging to the family *Oxyuridae* attract particular attention due to their ability to rapidly adapt to new hosts and their potential involvement in interspecies transmission of pathogens. Timely detection of oxyurid nematodes in wild mammals, particularly in red squirrels (*Sciurus vulgaris*), is of great importance for veterinary parasitology, as it allows the assessment of potential risks of forming natural foci of parasitic infections.

In recent years, attention has increasingly focused on the interactions between parasites

and their hosts under conditions of urbanisation. A. Karvonen *et al.* (2020) demonstrated that the order and timing of co-infections can significantly influence the progression of parasitic processes: an initial infection may modify the host's immune response, either enhancing or suppressing subsequent infections. This mechanism helps explain the complexity of clinical manifestations observed in mixed helminth infections, which are common among wild mammals. A similar dynamic was described by G. Zilio & J.C. Koella (2020), who showed that the sequence of infections can affect the outcome of competition between helminth species, which is important for predicting the course of parasitoses and the development of immune memory.

Studies of behavioural aspects of parasitism in squirrels indicate that even in the absence of pronounced clinical signs, infected animals may exhibit notable behavioural changes. F. Santicchia *et al.* (2020) found that an invasive

nematode transmitted between species suppresses activity and social interactions in red squirrels, potentially affecting their viability and reproductive success. Comparable results were obtained by C. Romeo *et al.* (2021), who demonstrated that infection with *Strongyloides robustus* reduces the survival of red squirrels – a classic example of a “spillover” effect, where an invasive species transmits a parasite harmful to native fauna. K. Hamill (2023) emphasised that parasites may act as indirect biomarkers of population stress, reflecting disruptions in animal homeostasis under anthropogenic pressures.

From an epizootological standpoint, numerous studies have shown that rodents, particularly synanthropic species, can serve as persistent reservoirs of parasites hazardous to wild mammals. M.M. Islam *et al.* (2020) identified *Syphacia obvelata* as the most prevalent nematode species among urban rodent populations, while J. Jarošová *et al.* (2020) reported that the same species frequently occurs in pet and laboratory rodents, from which it may spread into wild populations. A. Grandón-Ojeda *et al.* (2022) demonstrated that co-infections are a typical feature of urban populations of *Rattus* and *Mus*, and that such complex parasitic assemblages contribute to the greater stability of parasite communities. In this context, *Sciurus vulgaris*, which often interacts both with humans and small rodents, may represent an intermediate link in the transmission chain of helminths. Additionally, F.R. Lima *et al.* (2024) highlighted the clinical aspect of treating oxyurid nematode infections in primates, emphasising that prompt diagnosis and anthelmintic therapy remain key components of preventive health management even in natural ecosystems.

Summarising the findings of these studies, it may be concluded that interspecies transmission of parasites among wild mammals is a widespread phenomenon with potentially serious veterinary implications. However, most available

data refer to Western Europe, while information on wild animal parasites in Eastern Europe, particularly in Ukraine, remains scarce. The role of oxyurid nematodes in squirrel populations adapted to urban environments, as well as the mechanisms of possible host-switching between squirrels and rodents, are still insufficiently understood. In view of this, it was hypothesised that in forest-park areas of a moderately urbanised city within the Polissya region, red squirrels could serve as reservoir hosts for various parasites, including certain nematodes of the family *Oxyuridae* capable of host switching. The aim of the study was to determine the species composition of parasites in this population and to assess infection prevalence within a landscape shaped by both natural and anthropogenic factors.

Materials and Methods

The study was conducted from September to December 2023 in forest-park areas within the city of Zhytomyr, northern Ukraine (50°16'N, 28°40'E), where stable populations of the Eurasian red squirrel (*Sciurus vulgaris*) are present. A combination of behavioural observation, non-invasive sample collection, and laboratory parasitological analysis was employed to ensure ethical and reproducible data acquisition. Four fixed feeding stations were established to attract squirrels for consistent monitoring (Fig. 1), while computer-assisted video analysis was used to identify individuals and document activity patterns.

The experiment was designed and implemented in two distinct stages. The preparatory phase (two months) involved regularly providing food (walnuts, raw sunflower seeds, spruce and pine cones) to the squirrels 2-3 times per week. Video surveillance was also implemented to allow squirrels to habituate to human presence. To facilitate faeces sample collection, the soil within a 2-meter radius around the feeders was covered with geotextiles (Mathis *et*

al., 2018). The second phase of the experiment, which was experimental in nature, lasted 60 days. The area was inspected daily, and faeces from squirrels ($n = 191$) were collected. Data on squirrel activity and abundance were recorded with a Stealth Cam G42 (Stealth Cam Inc., USA) video recorder, which remained operational throughout the experiment. The camera was

mounted at a height of 1.5 m to provide coverage of the feeder and the surrounding area. The camera settings included 10 MP resolution and night mode with infrared illumination. It automatically detected movement, resulting in the collection of over 2,000 images and videos over 60 days. Frames with clear images of the squirrels were selected for analysis.



Figure 1. Cartographic representation of the experiment

Note: top right: red dots indicate the locations of the feeding stations; below: feeder design (left) and a squirrel during feeding (right)

Source: created by the authors

Data processing was carried out using the YOLOv5 computer vision model to detect squirrels in the frames. Individual identification was performed using the DeepLabCut neural network, which analysed key features, including fur pattern and colour, tail shape and length, and body size. Based on these parameters, a digital profile was created for each squirrel, and identification was carried out using a re-identification algorithm to prevent data duplication. Algorithmic accuracy was validated through manual verification of 10% of randomly selected data, showing full concordance (Bochkovskiy, 2020). Individual monitoring of the squirrels allowed determination of the number of infested animals through subsequent parasitological analysis.

Quantitative parasitological analysis of faeces (performed within 12 hours of collection) was conducted using the McMaster method (Ministry of Agriculture, Fisheries and Food, 1986). Qualitative analysis was carried out through flotation, with results recorded as “present/absent” (Gerwin *et al.*, 2017; World Health Organization, 2019). Microscopy was performed using a KERN OBE digital microscope (KERN, Germany) at magnifications of $\times 40$, $\times 100$, and $\times 600$. Eggs were cultivated in laboratory conditions within a thermostat, and placed in Petri dishes at a temperature range of 23–27°C for 28–30 days, with developmental progression examined every two days. Egg development was monitored microscopically, and the stage of embryonic development was

assessed based on the morphology of the embryo. The taxonomic position of the nematodes was determined according to the current systematics of helminths and specialised identification guides (World Health Organization, 2019; Lynnggaard *et al.*, 2020).

All procedures in this study were conducted in accordance with the principles of animal welfare and international ethical standards for the use of animals in research. Ethical approval for this study was obtained from the Ethics Committee of Polissia National University (Approval No. 57, dated 29 June 2023). The research complied with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and adhered to the quality management principles outlined in DSTU EN ISO/IEC 17025:2019 (2019). No animals were captured, restrained, or subjected to invasive procedures during the study. All observations and faecal sampling were carried out non-invasively, using field feeders and passive video monitoring. The research design ensured minimal disturbance to the animals and their habitats, maintaining full conformity with ethical and biosafety regulations.

Statistical analyses were performed to assess spatial differences in infection parameters and to verify potential associations between squirrel infestation rates and the locations of feeding stations. The dependence of oxyurid infection prevalence on feeder sites was evaluated using Pearson's chi-square (χ^2)

test. Differences in the number of *Trypanoxyuris sciuri* eggs among samples collected from different feeding stations were analysed using the non-parametric Kruskal-Wallis test owing to non-normal data distribution. Infection prevalence was expressed as the percentage of infected individuals, with 95% confidence intervals (CIs) calculated according to the Clopper-Pearson method. The intensity of infection was presented as the arithmetic mean \pm standard error of the mean (S.E.M.). All statistical procedures were performed using R version 4.3.1 (R Core Team, 2023), with the significance threshold set at $p < 0.05$.

Results and Discussion

During the 60-day observation period, four feeding stations were regularly visited by 17 individual red squirrels, each of which was recorded at least three times. The most active individuals were observed visiting almost daily, with up to three squirrels occasionally present simultaneously. The parasitological examination of the collected faecal samples revealed the presence of helminths belonging to the superfamily *Oxyuroidea*. The obtained results (Table 1) demonstrated distinct infection patterns and notable spatial variability among the study sites. Differences in infestation rates across the feeding stations indicate that local environmental conditions and host behavioural activity may play an important role in shaping the transmission dynamics of oxyurid nematodes in semi-urban squirrel populations.

Table 1. Squirrel feeder visits and fecal parasitological analysis

Feeder No.	Squirrels (total)	Fecal samples collected	Total infected fecal samples	Infected squirrels			Total infected squirrels
				<i>Trypanoxyuris sciuri</i>	<i>Syphacia obvelata</i>	<i>Aspicularis dinniki</i>	
1	4	59	49	4	2	-	4
2	7	39	11	2	-	-	2
3	3	46	38	3	-	-	3
4	3	47	36	3	-	1	3

Source: created by the authors

Infestation of red squirrels with oxyurids varied significantly depending on feeder location ($\chi^2 = 10.12$, p -value = 0.0176), indicating significant differences between study points. At Feeder 1, the highest prevalence of *Trypanoxyuris sciuri* was observed, with 100% of the squirrels infected. Additionally, 50% of the individuals also had a simultaneous infestation of *Syphacia obvelata*. At Feeders 3 and 4, all squirrels (100%) were also infected with *Trypanoxyuris sciuri*. However, a single case of co-infestation with *Aspicularis dinniki* was recorded at Feeder 4. Feeder 2 exhibited the lowest infestation rate, with only 28.6% of squirrels infected

with *Trypanoxyuris sciuri*, and no other oxyurid species were found at this site.

No significant differences in the number of *Trypanoxyuris sciuri* eggs were found in faecal samples ($n = 134$) from squirrels ($n = 12$) collected at different locations in the city ($P = 0.71$). In general, the most common species in the urban squirrel population was *Trypanoxyuris sciuri*, with an infestation rate of 70.59% of all individuals (Table 2). The species *Syphacia obvelata* and *Aspicularis dinniki*, which are less common in squirrels, were characterized by low infestation intensity in a small number of individuals.

Table 2. Prevalence and mean intensity of oxyurid infection in squirrels, $n = 17$

Species	Prevalence (%) with 95% CI	Mean intensity \pm S.E.M.
<i>Trypanoxyuris sciuri</i>	70.59 (44.04-89.69)	75.99 \pm 3.72
<i>Syphacia obvelata</i>	11.76 (1.46-36.44)	23.91 \pm 1.32
<i>Aspicularis dinniki</i>	5.88 (0.15-28.69)	12.5 \pm 2.9

Source: created by the authors

Following the summarised prevalence data presented in Table 2, detailed morphological examination and laboratory cultivation of the detected oxyurid eggs were carried out to confirm species identification and assess developmental viability. Microscopic analysis focused on the distinguishing structural features of the three helminth species, while incubation under controlled laboratory conditions provided additional information on their embryonic development and survival potential.

The morphology of *Trypanoxyuris sciuri* eggs was consistent with species-specific diagnostic characteristics. The eggs were symmetrical and oval, measuring up to 70 μm in length, with a smooth, transparent shell and regular contours. No distinct surface ridges, grooves, or polar thickenings were observed (Fig. 2). These features fully corresponded to the description of *T. sciuri* as a host-specific oxyurid of the red squirrel. Given the well-documented laboratory

model status of this species, experimental incubation was not performed, and species identification was based on morphological criteria alone.

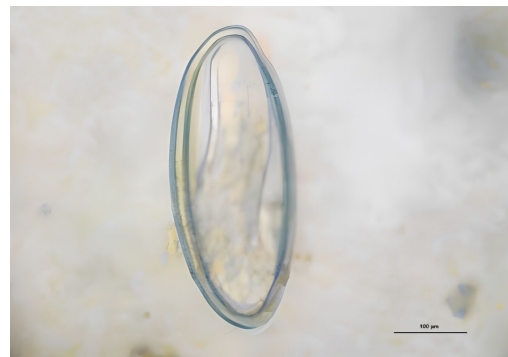


Figure 2. External morphology of *Trypanoxyuris sciuri* egg ($\times 450$)

Source: photo taken by the authors

Eggs of *Syphacia obvelata*, reaching up to 60 μm in length, were oval with slightly flat-

tened poles and a thin, smooth shell exhibiting faint transverse striations (Fig. 3). The subtle texture gave the surface a finely lined appearance characteristic of this species. Laboratory cultivation of *S. obvelata* eggs under controlled conditions revealed active embryonic development, with up to 90% viability observed throughout the incubation period. A typical feature of the L1 larvae was the oval anterior end without a vesicle and an oesophagus with a distinct bulb and well-defined cuticular plates (Abdel-Gaber *et al.*, 2018) (Figs. 4-5).



Figure 3. Eggs of *Syphacia obvelata* at the initial stage of incubation ($\times 450$)
Source: photo taken by the authors



Figure 4. The anterior end of *Syphacia obvelata* at the L1 stage ($\times 600$)
Source: photo taken by the authors



Figure 5. Morphology of the esophageal bulb in *Syphacia obvelata* L1 larvae. ($\times 600$)
Source: photo taken by the authors

Eggs of *Aspicularis dinniki* (Figs. 6-7) were similar in general shape to those of other members of the genus, with a smooth, transparent shell and a clearly visible embryo in the early stages of development. Cultivation did not yield positive results. During the first few days, germ cell division was observed, indicating initial egg activity and favourable conditions for development. However, by days 4-5, the developmental process abruptly stopped, and despite further observation, no viable larvae were obtained. This suggests species-specific sensitivity to cultivation parameters or limited adaptability to artificial incubation conditions.



Figure 6. Egg of *Aspicularis dinniki* at the onset of incubation ($\times 400$)
Source: photo taken by the authors



Figure 7. Egg of *Aspicularis dinniki* 24 hours after the start of incubation ($\times 400$)

Source: photo taken by the authors

Three species of helminths from the superfamily *Oxyuroidea* were identified in the analysed squirrel faecal samples. A total of 191 faecal samples were analysed, of which 134 (70.16 %) contained oxyurid eggs. Infestation of red squirrels with oxyurids varied significantly depending on feeder location ($\chi^2 = 10.12$, $P = 0.0176$), indicating significant differences between study points. The uneven distribution of infected individuals across locations may be due to local differences in ecological conditions (e.g., proximity to a river, lower urbanization), squirrel population density, and closeness to residential areas. The location with the highest infection rate was situated near a private sector, where synanthropic micromammals – natural hosts of some detected nematodes – are potentially present. However, no direct ecological or quantitative assessment was carried out during this study, and the proposed explanations should be regarded as hypothetical. Future research should consider the use of trapping surveys or genetic identification of potential parasite reservoirs in the environment.

The pinworm *Trypanoxyuris sciuri*, a species characteristic of the Eurasian red squirrel, was identified as the dominant helminth in the examined population. Its prevalence confirms the

high degree of host adaptation and effective transmission within the population. The absence of significant differences ($P = 0.71$) in egg numbers across faecal samples from different urban sites suggests that infestation intensity is not influenced by local environmental factors. This pattern is consistent with the view that *T. sciuri* exhibits strong host specificity and well-developed mechanisms for maintaining stable transmission cycles within squirrel populations (Santicchia *et al.*, 2020; Romeo *et al.*, 2021). Such intra-population persistence may be supported by frequent social contact among individuals and overlapping feeding territories, both of which facilitate passive transmission through contaminated substrates.

Of particular note are the rare occurrences of two atypical oxyurid species, *Syphacia obvelata* and *Aspicularis dinniki*, detected in two and one individuals, respectively, from distinct feeder sites. These findings likely reflect occasional spillover infections originating from murid rodents inhabiting the same urban parks. Similar cross-infections were reported by J.M. Behnke *et al.* (2015), who demonstrated that parasite dispersal in rodents is often linked to overlapping habitats and interspecific contact zones, and by K.E. Galbreath *et al.* (2019), who emphasised that shared ecological niches promote the exchange of parasitic fauna among sympatric mammal species. The detection of *S. obvelata* in squirrels may therefore indicate the existence of environmental reservoirs enriched with parasite eggs, possibly resulting from contamination of feeding areas or shared nesting materials.

The occurrence of *S. obvelata* in non-typical hosts has also been documented in previous research. S. Chawla *et al.* (2015) identified this nematode as a common parasite of murid rodents such as *Mus musculus* and *Apodemus sylvaticus*, while S. Koirala *et al.* (2016) demonstrated its ability to survive under favourable

conditions in a wider range of rodent hosts. The present observation of viable egg development in laboratory culture is consistent with the findings of R. Abdel-Gaber (2016), who described the morphological and genetic adaptability of *S. obvelata*, and P.M. Gerwin *et al.* (2017), who confirmed the species' capacity to persist under experimental conditions. Together, these studies highlight the ecological flexibility of *S. obvelata* and its potential to establish temporary infections in secondary hosts when interspecific transmission routes are available.

In contrast, *Aspicularis dinniki* is typically associated with arvicoline rodents, particularly the grey vole (*Microtus nivalis oseticus*), as reported by M. Leblanc *et al.* (2014). Its detection in squirrels may represent either accidental ingestion of infective eggs or an early stage of host-range expansion. However, the unsuccessful cultivation of *A. dinniki* eggs observed in the present study may be explained by the species' limited physiological tolerance. V.O. Yevstafieva *et al.* (2020) demonstrated that oxyurid egg development is highly dependent on environmental parameters such as temperature, humidity, and aeration. Even minor deviations from optimal conditions can inhibit embryogenesis, as reflected by the cessation of cell division in the cultured samples. These findings suggest that *A. dinniki* exhibits lower environmental plasticity than *S. obvelata*, reflecting its adaptation to more stable and specific microhabitats.

The detection of murid-associated nematodes in red squirrels highlights the ecological permeability of urban environments. According to C. Lynnggaard *et al.* (2020) and K.E. Galbreath *et al.* (2019), urbanisation promotes increased overlap among synanthropic and wild rodent populations, creating opportunities for parasite transmission across species barriers. The sporadic appearance of *S. obvelata* and *A. dinniki* in squirrels thus supports the hypothesis of limited but ecologically relevant

cross-species infections. Given the high mobility of red squirrels and their frequent use of anthropogenic feeding sites, such incidental parasitism may have epidemiological significance, particularly in densely populated urban landscapes.

Overall, the results confirm that *T. sciuri* remains the dominant helminth species in urban red squirrel populations, maintaining stable transmission independent of location. The presence of atypical oxyurids suggests occasional host-switching events, likely facilitated by habitat overlap with murids. These findings align with broader observations on the effects of urbanisation on parasite ecology, which emphasise both increased exposure to environmental infective stages and the emergence of new host – parasite relationships. Further molecular analysis of the detected species would help clarify the extent of such interspecific transmission and its potential role in the evolution of urban parasite communities.

Conclusions

Over a 60-day observation period, 191 faecal samples were collected from individually identified squirrels. Parasitological examination revealed three nematode species from the superfamily *Oxyuroidea*: *Trypanoxyuris sciuri*, *Syphacia obvelata*, and *Aspicularis dinniki*. The dominant parasite, *T. sciuri*, was found in 70.6% of examined individuals and demonstrated a uniform distribution across the study area, indicating stable transmission within the squirrel population. The detection of *S. obvelata* and *A. dinniki* in a small number of squirrels represents rare and potentially transient infections, suggesting possible spillover from murid rodents that share the same urban habitats. Laboratory cultivation confirmed the high viability of *S. obvelata* eggs under controlled conditions, while *A. dinniki* development ceased at early embryonic stages, reflecting differences in ecological tolerance between the two species.

The study demonstrates that urban red squirrels can serve as sentinel hosts for monitoring parasitic agents circulating at the wild-life-human interface. The presence of atypical oxyurid species in this population highlights the permeability of ecological boundaries in urban ecosystems and underscores the need for continued veterinary surveillance of wild mammals living in proximity to humans. Future research should focus on molecular identification of parasite isolates to clarify host-parasite relationships and potential routes of interspecies transmission. Expanding the geographical coverage of sampling and assessing immunological responses in infected squirrels would provide a more comprehensive

understanding of helminth dynamics in urbanised environments.

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Conflict of Interest

None.

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Інфекції гостриками у рудих вивірок на межі міста та лісу

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Анотація. Зростання взаємопроникнення міських і лісових екосистем підвищує ризик міжвидової передачі паразитів, що визначає актуальність вивчення паразитофауни диких ссавців. Метою дослідження було з'ясувати поширеність та видовий склад оксюрідних нематод у популяції рудих вивірок (*Sciurus vulgaris*), які мешкають у лісопаркових зонах Полісся (Україна). Було проаналізовано 191 зразок фекалій, зібраних у період з вересня по грудень 2023 року, із застосуванням інтегрованого підходу, що включав відеоспостереження у природному середовищі, систематичний відбір проб і мікроскопічне дослідження морфології яєць гельмінтів. Для автоматизованої ідентифікації вивірок використовували модель комп'ютерного зору YOLOv5. У результаті виявлено три види паразитів із надродина Охууроідеа. Найпоширенішим був *Трупаохурус sciuri*, типовий для вивірок, який зафіксовано

у 70,6 % тварин (12 із 17 особин; 95 % CI: 44,04–89,69). *Syphacia obvelata* виявлено у 11,8 % (2 із 17; 95 % CI: 1,46–36,44), а *Aspicularis dinniki* – у 5,9 % (1 із 17; 95 % CI: 0,15–28,69). Культивування яєць *S. obvelata* у лабораторних умовах дало змогу описати морфологію личинок L1, тоді як *A. dinniki* не розвивалась, що підтвердило її специфічні вимоги до хазяїна. Ці результати підкреслили високу поширеність *T. sciuri* серед білок і засвідчили потенційну передачу *S. obvelata* та *A. dinniki* в популяціях цих тварин. Практична цінність роботи полягає у розширенні знань про різноманіття гельмінтів і міжвидові зв'язки паразитів у перехідних місько-лісових екосистемах. Отримані результати можуть бути використані для вдосконалення ветеринарно-санітарних заходів, профілактики паразитарних захворювань і розробки стратегій моніторингу, спрямованих на збереження здоров'я диких популяцій і мінімізацію ризиків для свійських тварин та людини

Ключові слова: паразити дикої природи; шлункові нематоди; *Syphacia*; гострики у білок