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Prevalence and Genetic Characterization of *Sarcocystis* parasites in Brains of Small Mammals from Lithuania

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INTRODUCTION

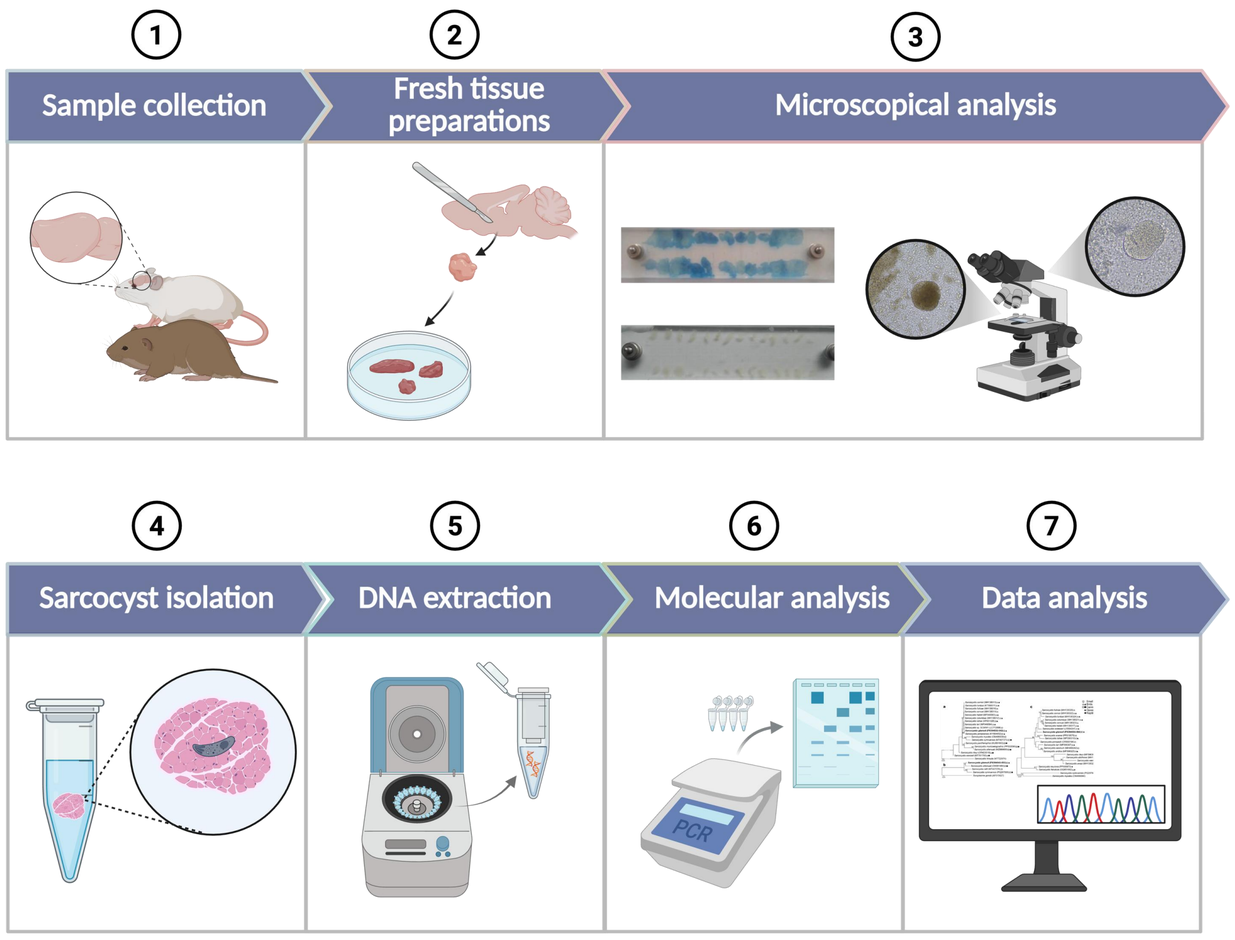
Protozoan parasites of the genus *Sarcocystis* (Apicomplexa) have a two-host life cycle based on predator–prey relationships. Sexual development occurs in the intestines of definitive hosts, where sporocysts are shed, while asexual stages form sarcocysts in intermediate hosts. Although sarcocysts are typically found in muscles, some *Sarcocystis* species develop in the brains of their intermediate hosts [1].

Small mammals (including rodents, tree shrews, and eulipotyphlans) play a key ecological role and serve as natural intermediate hosts for nearly one quarter of the more than 200 described *Sarcocystis* species [2]. Despite this importance, the epidemiology of *Sarcocystis* in small mammals remains poorly understood. Research is limited by low parasite prevalence, difficulties in obtaining sufficient sample sizes from wild and often threatened species, and the very small body size of many hosts, which restricts the amount of material available for analysis [3].

The aim of this study was to investigate the prevalence of *Sarcocystis* in the brains of small mammals from Lithuania and performed a comprehensive analysis of their genetic diversity.

MATERIAL AND METHODS

Between 2023 and 2025, neural tissue samples from 687 small mammals representing 11 species were collected across Lithuania.



RESULTS

Sarcocysts were detected by light microscopy in three rodent species (*A. flavicollis*, *C. glareolus*, *M. arvalis*) and one shrew species (*S. araneus*).

Overall prevalence in examined small mammals was 2.2% (15/687) (Table 1).

Table 1. Prevalence of *Sarcocystis* spp. in the methylene blue-stained brains of small mammals from Lithuania.

INVESTIGATED SPECIES	INFECTED/EXAMINED	PREVALENCE
Apodemus agrarius	0/41	0
Apodemus flavicollis	3/223	1.4%
Clethrionomys glareolus	10/77	13.0%
Microtus agrestis	0/39	0
Microtus arvalis	1/61	1.6%
Micromys minutus	0/23	0
Mus musculus	0/50	0
Alexandromys oeconomus	0/20	0
Neomys fodiens	0/15	0
Sorex araneus	1/58	1.7%
Sorex minutus	0/80	0

In fresh preparations, the detected cysts greatly varied in shape and size (Figure 1).

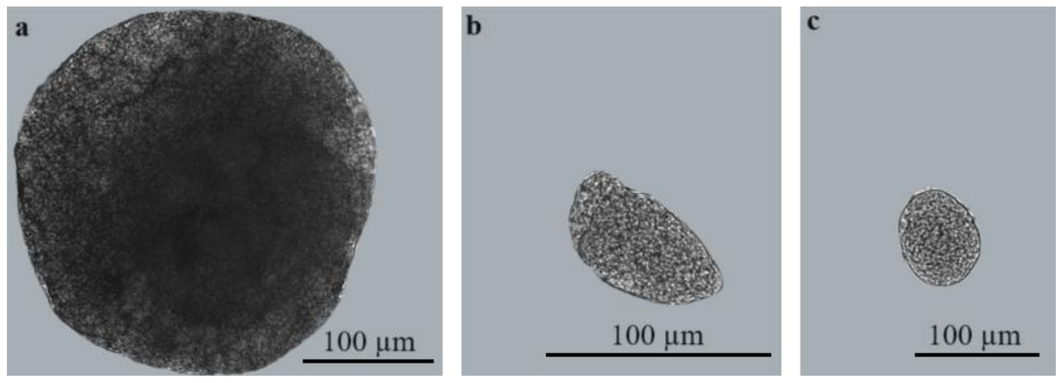


Figure 1. Sarcocysts in brain tissues of *C. glareolus*: (a): round form, (b): oval form, (c): irregular round form.

All 15 microscopy-positive samples were confirmed as *Sarcocystis glareoli* by DNA sequencing (Figure 2). Intraspecific variation was detected only in *ITS1* and *ITS2*, while the other loci analyzed (*18S* rRNA, *28S* rRNA, *cox1*, *cytb*, *rpoB*) showed no variability compared with previously reported isolates.

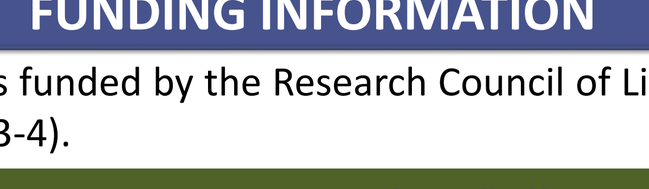


Figure 2. The phylogenetic tree depict relationships between *Sarcocystis glareoli* and other *Sarcocystis* species using multiple nucleic loci. The trees were constructed based on *ITS1* sequences.

CONCLUSIONS

- This study provides the first molecular confirmation of *S. glareoli* in *M. arvalis* and the first global record in a shrew (*S. araneus*).
- Genetic diversity of *S. glareoli* was low as variation was observed only in *ITS1* and *ITS2*, indicating these regions are the most informative markers for species identification.
- Host range and transmission patterns remain incompletely understood, but available data indicate that *S. glareoli* is widespread in Europe, primarily cycling between small mammals and raptorial birds, while non-vole hosts likely act as incidental intermediate hosts.

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