

# BEHIND THE MUSCLES: SARCOCYSTIS PARASITES IN HORSES FROM LITHUANIA

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*Sarcocystis* is a genus of cosmopolitan protozoa that parasitizes birds, reptiles, and mammals. Infected animal meat causes significant economic losses each year, due to unsuitability for human consumption or physiological changes in the animals themselves. In Lithuania, a moderate prevalence of sarcocystosis has been previously reported, but the species diversity of parasites has not been studied. To date, five *Sarcocystis* species have been described in equids: *S. asinus*, *S. bertrami*, *S. equicanis*, *S. fayeri*, and *S. neurona*. However, scientific debate continues regarding the classification of *S. bertrami* and *S. fayeri* as some researchers consider them a single species, while others argue they are distinct. Given these uncertainties, more detailed molecular studies on equids are essential to determine the true species diversity and distribution.

This study aimed to investigate the species diversity of *Sarcocystis* in the muscle tissues of domesticated horses (*Equus ferus caballus*) in Lithuania and to assess the intensity and prevalence of these parasites. Muscle tissue samples from 107 horses were examined using both methylene-blue-stained and unstained muscle squash microscopy, followed by isolation of individual sarcocysts, genomic DNA extraction, PCR amplification of multiple genetic markers, electrophoresis, and sequencing.

Microscopical analysis revealed that 23.4% of the investigated samples were *Sarcocystis* positive. The parasite load varied from one to 12 sarcocysts per gram of tissue ( $\bar{x} = 3$  sarcocysts/g;  $n = 25$ ). Morphologically, sarcocysts observed in fresh squashed preparations were elongated and spindle-shaped, measuring  $400\text{--}6000 \times 30\text{--}225 \mu\text{m}$  ( $2036 \pm 1374 \times 108 \pm 51 \mu\text{m}$ ;  $n = 40$ ) in size. The sarcocyst wall was thin with finger-like protrusions, measuring  $1.0\text{--}1.9 \mu\text{m}$  ( $1.6 \pm 0.9 \mu\text{m}$ ;  $n = 40$ ) in length. Cysts were septated, and their inner compartments were filled with elongated, banana-shaped bradyzoites measuring  $10.0\text{--}16.0 \times 2.0\text{--}4.0 \mu\text{m}$  ( $12.0 \pm 1.2 \times 2.6 \pm 0.2 \mu\text{m}$ ;  $n = 20$ ) in size. Sequencing of the *cox1*, *18S* rRNA, and *28S* rRNA markers revealed the species present in Lithuanian horses as *S. bertrami*.

These findings confirm the presence of *S. bertrami* in horses in Lithuania and provide the first molecular characterization of this parasite in the country. However, the limited availability of comparative genetic data highlights the need for broader molecular investigations to resolve taxonomic uncertainties and better understand the diversity and distribution of *Sarcocystis* species in equids.