

## Genetic characterization of *Sarcocystis* species in opossums *Didelphis aurita* from Brazil

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*Sarcocystis* species have a two-host life cycle with herbivores as intermediate hosts and carnivores as definitive hosts. Most species of *Sarcocystis* are host specific for their intermediate hosts but not for the definitive host. This study was done to characterize molecularly and phylogenetically *Sarcocystis* species that infect opossums, *Didelphis aurita*. Sporocysts were obtained from intestinal tracts of 8 of 13 *D. aurita* opossums trapped near Campos dos Goytacazes city in Rio de Janeiro state, Brazil. The small intestinal epithelium was scraped off and digested in 10% bleach and processed to obtain sporocysts. These sporocysts were orally inoculated into budgerigars (*Melopsittacus undulatus*) and nude mice (Balb/c, *Mus musculus*) to determine if they were infective for birds, mammals or both. Some birds developed clinical signs and died 6, 7, 13, 16 and 24 days after inoculation (DAI). All other birds and all mice were euthanized 60 DAI. Tissues were stored in absolute alcohol for PCR using primers JNB 33/54 (*S. falcatula*/*S. neurona* complex) and ITS (all *Sarcocystis* species). None of the inoculated mice developed clinical signs and sarcocysts were not found in their muscles by PCR. African green monkey, *Cercopithecus aethiops*, kidney cells were used as host cells and amplification products produced by JNB 33/54 primers were subjected to RFLP using restriction endonucleases *Dra*I and *Hinf*I. Sequencing for phylogenetic analysis was done using ITS primers for the 18S rRNA gene. The phylogenetic tree was constructed under the criterion of Maximum Parsimony and Maximum Likelihood with the parameter model Kimura 2 (G + I) using the MEGA7 program. Bootstrap analyzes (1000 replicates) were performed to evaluate the reliability of the branch and species of the genus *Eimeria* were defined as external group. *Sarcocystis* asexual stages were isolated in cell cultures inoculated with sporozoites. Parasite DNA isolated from bird tissues and cell cultures demonstrated that *S. falcatula*-like parasites were present in all samples derived from positive opossums. Asexual stages molecularly characterized as *S. lindsayi* were isolated in cell culture from one opossum with an apparent multiple infection. The sequences of tissues and cultures were deposited at GenBank with accession numbers KX470742, KX470743, KX470744, KX470746, KX470745, KX577781 e KX577782. This study demonstrated that genetically *S. falcatula*-like parasites can be considered a species of *Sarcocystis* different from others found in opossums and indicates that *S. lindsayi* can be found in coinfections of this opossum.

Key words: *Sarcocystis falcatula*-like, *Sarcocystis lindsayi*, RFLP, sequencing