

Insights into the exoerythrocytic development of *Haemoproteus attenuatus* in the Thrush nightingale *Luscinia luscinia*

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Abstract

Haemoproteus parasites (Haemosporida, Haemoproteidae) are cosmopolitan blood pathogens. Despite their importance for avian host fitness and health, little is known about the exo-erythrocytic development of most described species. Recent discoveries based on molecular markers showed that tissue stages of haemoproteids damage various internal organs, sometimes resulting in severe and even lethal avian haemoproteosis, including cerebral pathologies. This study aimed to identify and describe the exo-erythrocytic (tissue) stages of *Haemoproteus* parasites in *Luscinia luscinia* (Muscicapidae). Blood and tissue samples of 8 infected individuals were examined under a microscope and using PCR-based methods. Organs were examined for exo-erythrocytic stages by histology methods and application of in situ hybridization tools using genus-specific and lineage-specific oligonucleotide probes targeting the 18S ribosomal RNA of the parasites. Exo-erythrocytic meronts of *H. attenuatus* (lineage ROBIN01) were found and described for the first time in 6 bird individuals. Most meronts were located in the lungs; a few also were found in the liver, heart and pectoral muscle. Mature meronts contained numerous roundish merozoites. Megalomeronts were observed in the gizzard and the heart of two individuals, which also contained meronts. Based on the morphology and site of infection, these megalomeronts likely belong to parasites of *Haemoproteus majoris* group, which present in co-infection with *H. attenuatus*. This study reports new pathological aspects of haemoproteosis during infection of *H. attenuatus* and probable co-infection with *H. majoris*, opening new directions for better understanding pathology during haemoproteosis. This study was funded by the Research Council of Lithuania (S-PD-22-71).

Key words: Avian haemosporidians, *Haemoproteus*, Phylogeny, In situ hybridization, Meront, Megalomeront