**Holobiont responses to multiparasitism on the mosquito-*Dirofilaria*-microsporidia model**

Blood-sucking insects are important vectors of pathogens that cause a wide range of serious diseases worldwide, with a significant impact on human and animal well-being. Among them, mosquitoes are major vectors of protists, nematodes, and microsporidians. Thus, mosquito gut microbiota consists of many different species, mainly belonging to bacteria, although archaeans, fungi, micro- and macroparasites, including nematode filarial worms, may also be present. Overall, billions of people are exposed to the risk of contracting mosquito-borne pathogens. According to the World Health Organisation (WHO), nearly 900 million people are at risk of filariasis and require preventive chemotherapy to stop the spread of this parasitic infection. Dirofilariosis, a zoonotic disease caused by nematodes of the *Dirofilaria* genus, is one of the most common causes of filariasis.

There are about 27 species in the *Dirofilaria* genus of which *D. immitis* and *D. repens* are the most common throughout the Europe. Dogs, cats, and other carnivores (e.g. foxes, wolves, and coyotes) are hosts for these *Dirofilaria* species, but an increasing number of infections in humans are being reported. Considering climate warming, these numbers may increase. *Dirofilaria immitis* adult stages occur in the pulmonary arteries and right heart chambers and causes heartworm disease, while adult *D. repens* are typically located under the skin, the subcutaneous tissue. The intermediate hosts of dirofilarians are mosquitoes of the family Culicidae with nearly 70 species susceptible to the parasite and thus considered potential vectors. Female mosquitoes consume first-stage larvae (L1) present in the host's bloodstream of infected vertebrates during their blood meal. Following ingestion, microfilariae migrate from the single layer of epithelial cells midgut to the Malpighian tubules via the haemocoel of the insect where they molt into the second (L2) and third (L3) infective larval stage. The L3 then actively leave the Malpighian tubules to migrate through the body cavity and the thorax to the head and finally the proboscis, where they await transmission to the next host.

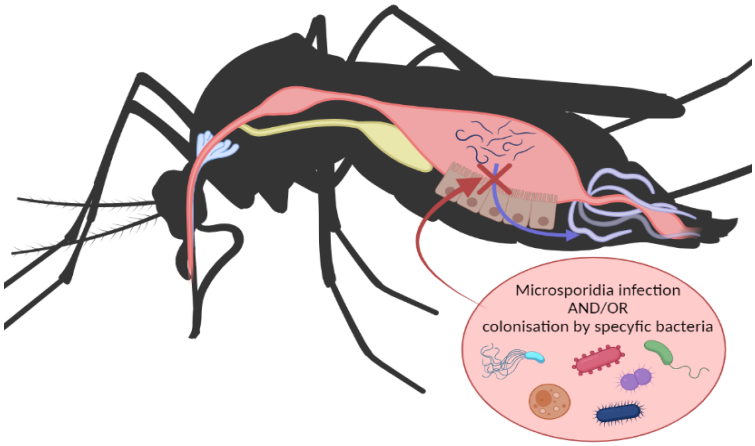


*Figure 1. Possible inhibition of Dirofilaria spp. transmission on the mosquito midgut epithelial cells by microbes, including microsporidians. Image generated in BioRender.*

Mosquitoes are often hosts of microsporidians (Microsporidia), the obligate intracellular parasites. Among the 1700 described microsporidian species, over 250 belonging to 34 genera have been reported in mosquitoes. Microsporidians can infect a diverse range of mosquito organs, including the gut, ovarioles and muscles. Nevertheless, the epithelial cells of the midgut are commonly colonised. These parasites may manipulate both the composition of the microbiome and the immune response of mosquitoes. These changes, especially regarding the lowering of pH, could potentially affect the growth of other pathogens, especially dirofilarians.

The main aim of the project is to assess the feasibility of using microsporidians as a biological control agent for *Dirofilaria* spp. The project will enable the following to be determined: (1) prevalence and diversity of *Dirofilaria* and microsporidian spp. in different western European countries; (2) relationship between microsporidians and the development of *Dirofilaria* spp.; (3) changes in the mosquito microbiome during microsporidian, dirofilarial, and mixed infections; (4) characterisation of the transcriptomic response of the host microbiome to the infection; (5) evaluation of the effect of microsporidians on the number of *Dirofilaria*-competent mosquitoes.