CHEMICAL CHARACTERIZATION AND 3D RECONSTRUCTION OF *Trichuris muris* EGGS: EXPLORING THEIR ULTRASTRUCTURE WITH EDS-SEM AND FIB-SEM

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Trichuris trichiura – the etiological agent of the Neglected Tropical Disease trichuriasis – affects millions of people worldwide from underserved communities. Infection occurs through the ingestion of embryonated eggs present in contaminated soil and food, therefore, the first structures to establish contact with the host. *Trichuris* eggs possess a rigid eggshell, which provides resistance to environmental variations, and two polar plugs, that interact with the host's microbiota and play a crucial role in promoting the hatching of the first-stage larva (L1). Given their fundamental role in the parasite's life cycle, studying *Trichuris* eggs may provide insights into novel strategies for controlling and mitigating trichuriasis. Based on this, the present study aims to perform a chemical and morphological analysis of *Trichuris muris* eggs – an experimental model for trichuriasis – employing state-of-the-art techniques such as Energy-Dispersive X-ray Spectroscopy (EDS-SEM) and Focused Ion Beam Scanning Electron Microscopy (FIB-SEM). The samples were cryofixed by high-pressure freezing (HPF) and chemical fixed through a freeze-substitution process using Bal-Tec 010 HPF machine and a Leica EMP apparatus, respectively. Subsequently, the eggs were embedded in Polybed 812 epoxy resin and the solid blocks were trimmed. The analyses were performed by ThermoFisher Helios NanoLab 660 dual-beam microscope (LNNano/CNPEM). FIB-SEM serial images provided a 3D view of the whole egg and its main structures, including: the three major eggshell's sublayers – *Pellicula ovi*, Chitinous layer, and Electron-dense parietal coating –, and the L1 larva and its granules, while the EDS-SEM technique mapped chemical elements present on those egg's structures, including Cr, S, Cl, P, and Ca elements. Both microscopy methods provided new insights into the morphology and chemistry of trichurid eggs.

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