A comparative study of *Echinococcus granulosus* and *Echinococcus ortleppi* extracellular vesicles in metacestode infection

Edileuza D da Silva, Maria Eduarda Battistella, Guilherme B dos Santos, Henrique B Ferreira, Arnaldo Zaha.
Programa de Pós-Graduação em Biologia Celular e Molecular, Centro de Biotecnologia, UFRGS, Porto Alegre, Brazil.

Hydatidosis is a parasitic disease caused by the larval stage (hydatid cyst) of *Echinococcus granulosus* and *Echinococcus ortleppi*. A long-term growth is characteristic of the chronic infection caused by *Echinococcus* spp metacestodes, suggesting that different molecular mechanisms are employed by the parasite to ensure its survival and development in the host microenvironment. The hydatid cyst is filled by the hydatid fluid, which contains secretory products from the parasite as well as from the host. Secretory products have an important role in the host-parasite interplay, because they participate in process such as immunoevasion, nutrient uptake and cell communication. Recent reports have highlighted the importance of secreted extracellular vesicles (EVs) in these processes in helminth parasites. The objective of this study is to provide a comparative analysis of *E. granulosus* and *E. ortleppi* EVs. To that, *E. granulosus* and *E. ortleppi* hydatid fluid was collected by punction and aspiration and sequentially centrifugated at 500 x g, 2.000 x g and 10.000 x g to remove cell debris and denaturated proteins. EVs were isolated from precleared hydatid fluid by ultracentrifugation (100.000 x g). The presence of EVs in the ultracentrifugation pellets were confirmed by transmission electron microscopy and nanoparticle tracking analysis. Immunoblotting was performed to search for common EV proteins. *E. granulosus* and *E. ortleppi* EVs show typical spherical structures that, besides showing variable sizes, had a predominant diameter around 100 nm. Using specific *Echinococcus* spp antibodies produced in our laboratory, we were able to detect enolase and 14-3-3, which had already been described in literature as EV cargo, in both *E. granulosus* and *E. ortleppi* EVs. The next steps in this study are to perform proteomic analysis on EVs isolated from *E. granulosus* and *E. ortleppi*, to identify and compare EV proteins in these species. Financial Support: CNPq.