Structural response of the cestode tegument to the host blood serum in incubation experiments

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Cestodes may be pathogenic to some farmed fish as well as other species important to the fishing industry. Therefore, it is crucial to understand the relationships among these parasites and their hosts. The goal of our study was to reveal the response how cestodes from marine and freshwater fish to the presence of various factors from the host blood serum. We conducted experiments with plerocercoids of *Triaenophorus nodulosus* infecting the livers of *Perca perca*, plerocercoids of Pyramicocephalus phocarum infecting the livers of Gadus morhua and plerocercoids of Ligula intestinalis residing in the Abramis brama body cavity. Parasites from experiment 1 ("Ringer") were incubated with the physiological saline solution, which matched the host blood salt composition. Parasites from experiment 2 ("Blood serum") were incubated with the mixture of the same saline solution and the host blood serum (up to 50%). After the incubations, experimental and control animals were fixed and prepared for the examination under the scanning and transmission electron microscope. We have found that the cestode tegument exhibits an active response to the experimental conditions. Some features of this response are unique for plerocercoids from the "Blood serum" group; other reactions are similar in both experimental groups but differ in their expression intensity, Specifically, in the presence of the host blood serum: 1) the secretion of various membrane structures is intensified (fig. 1B – arrows); 2) glycocalyx is thicker and its globular particles are more prominent; 3) there are more extracellular vesicles in the space between microtriches; 4) plerocercoids of L. intestinalis produce specific membrane lamellas, which are not found in control and "Ringer" animals. Additional layer composed of vesicles, membrane structures and extracellular matrix is also formed on top of the border of microtriches as a result of exposure to experimental conditions of both types.

We have demonstrated for the first time that the plerocercoids of the three studied cestode species respond to the factors from the host blood serum to a different degree. Plerocercoids of *T. nodulosus* exhibit extremely acute reaction which includes the complete loss of their tegument to the extent that circular muscles become the outer layer of the worm. The response of *L. intestinalis* is the strongest at the anterior body end, where most microtriches are lost (fig. 1A). Nevertheless, some microtriches persisted and others formed circles (fig. 1A – arrowheads). By contrast to the two species mentioned above, we did not observe significant changes in the tegument structure of *P. phocarum* in the presence of the host blood serum compared to the incubation with pure saline solution. In both experimental groups as well as in the control animals we observed small vesicles (from 20 to 150 nm diameter) originating from the tegumental plasma membrane. We suppose that these vesicles are extracellular vesicles that may play an important

role in parasite-host interactions. Our result correlates well with the data on the secretion of extracellular vesicles in cestodes *Taenia crassiceps*, *Mesocestoides corti* and *Echinococcus multi-locularis* (Ancarola *et al.*, 2017).