In vitro exploration of interactions and stressors in Caco-2 cell cultures versus the infective L3 larva stage of *Toxocara canis* and *Parascaris univalens*

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Background: The recent increase in anthelmintic resistance of parasitic nematodes, for example in the equine roundworm *Parascaris univalens,* calls for further research to understand the complex interactions between host and parasite. Although host immune modulation via parasite excretory-secretory products have been described in different experimental models, most host-parasite species-specific and zoonotic interactions remain unexplored. This is largely due to the complex and species-specific infective life cycle and a lack of proper *in vitro* models to effectively test these interactions.

In vitro model: To this end, we have begun investigating the feasibility of cell culture models during interactions with the infective L3 stages of *Toxocara canis* and *Parascaris univalens*. To help quantify our methods, we used the resazurin-resorufin assay. Resazurin is a low molecular weight, non-toxic dye, which during normal metabolism in the cell is non-reversibly reduced to resorufin. Under excitation, resorufin produces a distinct red fluorescence signature, giving a direct readout of metabolic levels. The resazurin-resorufin assay gives robust and repeatable results in a variety of cell types and under a variety of cellular stressors. In addition to metabolic readout, thrashing assays via the WormAssay program, were also performed to judge the effect of stressors on the L3 larva in a non-biased way. Our main stressor was thiabendazol, an actively used anthelmintic of the benzimidazole family know to selectively target beta-tubulin.

Results: When infective L3 larva of *Toxocara canis* or *Parascaris univalens* were co-cultured for 48-hours with adherent human intestinal epithelial cells (Caco-2) we have seen significant changes in overall cellular metabolism (change in resorufin fluorescence), in comparison to control incubations with either L3 larva or adherent cells. When in contact with host cells a significant decrease in thrashing rates in *Toxocara canis* L3 larva, as well as a shift in thrashing behavior was also observed. Furthermore, we observed a significant decrease in overall metabolic rate in co-cultures of *Toxocara canis* and Caco-2 when exposed for 48-hours to 100µM thiabendazol, a decrease that was not seen in control Caco-2 cells when they were cultured alone under the same thiabendazol concentration. Our results indicate that the early phase *in vitro* co-culture model is resulting in metabolic changes for both host and parasite and parasite behavioral changes which indicates a potential model for further research.