Death Assay Evaluation for Angiostrongylus cantonensis Nematode

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An increasing dilemma in tropical climates is that of Rat Lungworm disease, caused by human infection by A. Cantonensis larvae. These larvae typically survive within a rat or slug host, the latter of which poses a risk in garden vegetable consumption. If an appropriate assay for accurately determining the vitality of these larvae could be found, many experiments could be done with more definitive results. To determine vitality, mobility tests are efficient; however, distinguishing immobile live from dead larvae is difficult, because they are similar in appearance. Staining using Eosin-Y stain is another possible option, because the stain reportedly can only perforate the larvae epithelium once the larvae is dead. Larvae were obtained from infected Parmarion Martensi slugs collected from a tropical area and digested in an HCl-Pepsin solution. Live larvae were divided into "live" and "dead" categories and the "dead" larvae were frozen for at least 12 hours at -80° C. Three trials each of live and dead larvae samples were conducted for both the staining and mobility tests. Larvae were isolated and Eosin-Y stain was added in a 1:1 ratio with the extraction solution. The sample was observed over a period of two days, and over the same period, a group of unstained nematodes were observed for mobility. The mobility test was deemed more accurate for immediate and long term results, though immobile live larvae could still affect experimental data.