Testing the Effect of Plant Compounds on *Ascaridia Galli* with an *In Vitro* Larval Motility Assay

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Introduction
Ascaridia galli infections have a major impact on poultry health and productivity. *In vitro* assays can be used to screen compounds for anthelmintic activity, reducing the need for *in vivo* studies. We present a method for assessing the effects of plant compounds on the motility of third-stage *A. galli* larvae (L3).

Methods
Live adult *A. galli* were cultured for egg collection. Eggs were embryonated in 0.5M H_2SO_4 at 22ºC for three weeks and then stored at 5ºC. Eggs were hatched using chlorine (30% in water), washing and overnight incubation in HBSS, centrifugation and Baermannization with HBSS. Larvae were transferred to a 96-well plate with RPMI (~50 L3 per well). Two-fold dilutions of compounds, in DMSO or water, were added in duplicates. Negative (1% DMSO or 50% water) and positive (50-100 ug/mL Levamisole or Ivermectin) controls were included. Plates were incubated at 37.5ºC with 5% CO_2. Motile larvae were counted before adding compounds and after 24 and 48 hours.

Results
Effect of compounds was observed after 48 hours, where negative controls showed <10% motility loss, whilst plant compounds caused up to 100% motility loss. Dose-responses were observed for *Trifolium pratense* ethanol extract and a commercial plant compound (ASQARI). Variation was observed in positive controls (motility loss 54-98%), possibly due to limited drug suspension stability.

Conclusion
The larval motility assay is simple and requires minimal equipment. Further validation and investigations on various compounds are needed, but the method shows promise for initial screening of new treatment options for *A. galli.*