

TESTING THE EFFECT OF PLANT COMPOUNDS ON *ASCARIDIA GALLI* WITH AN *IN VITRO* LARVAL MOTILITY ASSAY

Rotovnik, R.¹; Williams, A.R.¹; Pan, G.¹; Raza, A.¹; Permin, A.²; Kjærup, R.B.³; Dalgaard, T.S.³; Mejer, H.¹; Thamsborg, S.M.¹

¹ Parasitology and Pathobiology, Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark

² Værløse Dyreklinik IVS, Denmark and Unibrains IVS, Denmark

³ Department of Animal and Veterinary Sciences, Aarhus University, Denmark

Corresponding author: Rosalina Rotovnik, rosalina.rotovnik@sund.ku.dk

Keywords: ascariidiasis, poultry, plant-based anthelmintics

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 incubated in saline or RPMI with and without antimicrobials (Penicillin-Streptomycin and Amphotericin B) at 37.5°C with 5% CO₂ for three days with daily egg collection and change of incubation medium. Eggs were embryonated in 0.5M H₂SO₄ at 22°C for three weeks and stored at 5°C until use. Initial testing of selected plant extracts was performed as follows: Eggs were hatched using chlorine (30% in water), washing and overnight incubation in HBSS, followed by centrifugation and Baermannization with HBSS. Larvae were transferred to a 96-well plate with RPMI (~50 L3 per well). Two-fold dilutions of plant compounds, in DMSO or water, were added in duplicates. Negative (1% DMSO or 50% water) and positive (50 ug/mL Levamisole or Ivermectin) controls were included. Plates were incubated at 37.5°C with 5% CO₂. Motile larvae were counted before adding compounds and after 24 and 48 hours.

Results

Egg yield and embryonation suggested that saline without antimicrobials was most favourable for worm cultures. Larvae yield from hatching was >50% of embryonated eggs. The full 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 two compounds so far. Variation was observed in positive controls (motility loss 54-98%), which could be due to limited drug suspension stability.

Conclusion

The larval motility assay represents a simple method with low equipment requirements. Further validation of the method and investigations on various plant compounds are needed, but the method shows promise for initial screening of new treatment options for *A. galli*.