

Association between P-glycoprotein gene polymorphisms and multidrug resistance in *Haemonchus contortus* - Mello S.S.¹, Rocha M.I.P.¹, Giglioti R.², Chagas A.C.S.³, Oliveira M.C.S.³, Esteves S.N.³, Niciura S.C.M.^{3*}

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Gastrointestinal nematodes are considered the main obstacle to small ruminant production in tropical areas. Among the parasites of sheep, *Haemonchus contortus* is the most prevalent and pathogenic and results in economical losses. Despite the ease and usefulness of the fecal egg count reduction test (FECRT) to determine the anthelmintic efficacy in herds, it is only reliable when more than 25% of the worms are resistant, making difficult to reverse the resistance status. Thus, molecular techniques are excellent tools for anthelmintic management since they can find resistance in parasite populations with less than 25% of resistance promoting an early diagnosis. In a previous project ("Diagnosis of genetic resistance to benzimidazole in nematodes of sheep in São Paulo State") in 34 sheep flocks, it was observed multidrug resistance to five anthelmintics that was not explained by the F200Y polymorphism in the β -tubulin gene. For this reason, using the same samples, this project aims to develop and validate a molecular test for early detection of multidrug resistance to anthelmintics in *H. contortus*, and also to associate the molecular resistance status to management practices in herds aiming to identify risk factors and to develop strategies to control the resistance. Two sheep were experimentally infected with a sensible and a multidrug-resistant isolate, and adults of *H. contortus* were collected from abomasum after slaughter. DNA extraction of worms was performed with organic solvents and PCR are being optimized to amplify a fragment of P-glycoprotein gene for further sequencing and SNP identification.

Key-words: drug resistance, management practices, PCR

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Association between P-glycoprotein gene polymorphisms and multidrug resistance in *Haemonchus contortus*



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Introduction

Gastrointestinal nematodes are considered the main obstacle to small ruminant production in tropical areas. Among the parasites of sheep, *Haemonchus contortus* is the most prevalent and results in great economical losses.

Despite the ease and usefulness of the fecal egg count reduction test (FECRT) to determine the anthelmintic efficacy in herds, it is only reliable when more than 25% of the worms are resistant, making difficult to reverse the resistance status.

Thus, molecular techniques are excellent tools for anthelmintic management since they can find resistance in parasite populations with less than 25% of resistance promoting an early diagnosis. In a previous project ("Diagnosis of genetic resistance to benzimidazole in nematodes of sheep in São Paulo State") in 34 sheep flocks, it was observed multidrug resistance to five anthelmintics that was not explained by the F200Y polymorphism in the β -tubulin gene.



Objective

This project aims to develop and validate a molecular test for early detection of multidrug resistance to anthelmintics in *H. contortus*. It also aims to associate the molecular resistance status to management practices in flocks aiming to identify risk factors and to develop strategies to control the resistance

Material and Methods

Two sheep were experimentally infected with a sensible and a multidrug-resistant isolate, and adults of *H. contortus* were collected from abomasum after slaughter. DNA extraction of worms was performed with organic solvents and PCR are being optimized to amplify a fragment of P-glycoprotein gene for further sequencing and SNP identification.

Results

It was possible to confirm the expected P-glycoprotein gene fragment amplification in agarose gel and by sequencing of PCR products.

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