**PCR-RFLP GENOTYPING OF A PUTATIVE P-GLYCOPROTEIN POLYMORPHISM RELATED TO MULTIDRUG RESISTANCE IN HAEMONCHUS CONTORTUS**

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The economic importance of sheep production has increased worldwide simultaneously to the emergence of parasite resistance, especially in Haemonchus contortus. Alterations in the gene encoding the membrane P-glycoprotein (Pgp) have been associated with multidrug resistance. In previous experiments we identified single nucleotide polymorphisms (SNP) in the Pgp gene and here we describe the methods for genotyping the SNP878 (C>G) possible associated with resistance in H. contortus. DNA of single L3 larvae was extracted and the SNP878 was genotyped by nested-PCR followed by RFLP-PCR. Each PCR consisted of 1X buffer, 0.25 µM each primer, 0.2 mM dNTP, 1.5 mM MgCl2 and 1 U Taq DNA polymerase in a final volume of 20 µL. Forty cycles of 30 sec of denaturation at 94°C, annealing and extension at 72°C were performed. The primers RF4f2 (5'-GATCAGATTTTCGTTTTC-3') and MX-D (5'-AGACAAAGACATTCAGAG-3'), 2 µL of DNA (equivalent to 0.2 larvae) and annealing temperature of 51.2°C were used for the first reaction. In the second reaction, the primers PGP414F (5'-ACTCGGCGACTTCAACCAAT-3') and PGP962R (5'-ACACTTCGCTACTGGTTCCG-3') and 1 µL PCR product from the first reaction were used, with annealing at 60°C. In PCR-RFLP, 6 µL of the PCR product from the second reaction were incubated with 1U of the SpeI restriction endonuclease (A^CTAGT) in 1X buffer at 37°C for 3 hours. After electrophoresis in 2.5% agarose gels the genotypes were determined according to the banding pattern: CC with 567 bp, GG with 347 and 220 bp, and CG with 567, 347 and 220 bp. The results obtained with PCR-RFLP were confirmed by Sanger sequencing. The C allele and the CC genotype were previously associated to multidrug resistance and further studies employing the methods described here should be used to confirm the SNP878 in Pgp gene as a molecular marker of multidrug resistance in other isolates of H. contortus.