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Concurrent Session 8.1

A: Drugs and drug resistance

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**Detection of anthelmintic resistance in gastrointestinal nematodes from Spanish sheep flocks comparing the egg hatch assay and the presence of resistant alleles by pyrosequencing**

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Anthelmintic resistance (AR) in ovine gastrointestinal nematodes (GIN) to benzimidazole (BZs) is associated with single nucleotide polymorphisms (SNPs) in the isotype 1 of  $\beta$ -tubulin, at codon 200 principally, but also at codons 167 and 198. In this study we compared the levels of AR of GIN field strains by the Egg Hatch Assay (EHA) and the percentage of BZ resistance alleles obtained after pyrosequencing. Throughout the years 2012 and 2013, variation in resistance was checked by EHA in two sheep flocks (A and B). Resistance was measured by the proportion of eggs hatched at discriminating dose of 0.1 g/ml thiabendazole. DNA extraction was carried out from a pool of larvae and eggs from faecal samples. Prior to pyrosequencing, the fragments including the SNPs198/200 were amplified for *Trichostrongylus* spp, *Teladorsagia circumcincta* and *Haemonchus contortus*, independently for each species; a fragment for the SNP167 in *T. circumcincta* was also amplified. Flock A had a high level of hatching throughout the study, with a mean of 77.74% (57.21-90.64%); in flock B, the resistance level increased over time, with values of 33.25% in 2012 (15.81-57.46%) and 52.02% in 2013 (46.06-55.19%). Pyrosequencing analysis detected the presence of the BZ resistance allele at codon 200 in *Trichostrongylus* spp in both flocks; the percentage of the resistant allele was 81.28% (19.53-100%) in flock A and 21.37% (1.57-51.48%) in B. However, for codon 198, the resistant allele in this species was at a low level in both flocks, 2.74% in A and 5.04% in B, respectively. In *T. circumcincta*, the resistant allele incorporating SNP200 was not present in any flock, with a mean of 0.32%; these low levels were very similar at codons 198 and 167, with values of 0.87 and 1.61%, respectively. In *H. contortus*, pyrosequencing was only carried out in flock B, where this species was detected after morphological identification; the proportion of the BZ resistance allele was 6.33% (0-22.77%) at codon 200 and 1.35% (0-4.93%) at codon 198. When comparing the results of EHA and the frequency of BZ resistance alleles at codon 200 in *Trichostrongylus* spp, in both flocks and throughout the experiment, a significant correlation between the two techniques ( $r=0.6$ ;  $p<0.01$ ) was found. These results suggest that *Trichostrongylus* spp is the main contributor to the development of AR in these Spanish GIN field strains. Study funded by Spanish INIA(RTA2013-00064-C02-02)

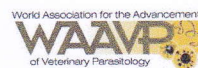
ABSTRACT  
BOOK



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