Efficiency of a genetic test to detect benzimidazole-resistant *Haemonchus contortus* nematodes on sheep farms in Québec

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Introduction

Benzimidazole-based drugs have been used to treat *Haemonchus contortus* and other gastrointestinal nematode infestations in sheep. However, numerous cases of benzimidazole-resistance in *H. contortus* have been reported worldwide. The fecal egg count reduction test (FECRT), which consists of comparing parasite egg counts in feces before and after treatment, is a technique commonly used to assess anthelmintic resistance. Unfortunately, this method is not very sensitive and is dependent on factors that can distort the results. Veterinarians and farmers have been seeking a method for detection of anthelmintic resistance which would be faster and cheaper than the FECRT, and used prior to anthelmintic treatment. Mutations called single nucleotide polymorphisms (SNP) on the α-tubulin isotype 1 gene of *H. contortus* have been associated with benzimidazole resistance. These mutations are located at positions 200 (a TTC codon in susceptible parasites and a TAC codon in resistant ones), 167 (TTC for susceptible parasites and TAC for resistant parasites), and 198 (GAA for susceptible parasites and GCA for resistant parasites). A molecular method that uses pyrosequencing has been developed to measure the frequency of the resistant allele of these three markers of benzimidazole resistance. The female nematode releases eggs, which are shed through the feces of the sheep. We can apply the pyrosequencing protocol on nematode eggs isolated from sheep feces to assess the benzimidazole resistance of the adult nematodes that colonize the host's abomasum.

Materials and Methods

The study was conducted on five sheep farms in Quebec. From each farm, 20 sheep were selected; 10 were allocated to an untreated control group and 10 were allocated to a treated group (Panacur® 10% orally administered). From each sheep, fecal samples were collected directly per rectum before and after treatment. Additionally, on two farms, three composite fecal samples were randomly obtained from the pasture. Fecal egg counts (FEC) for *H. contortus* were performed on all fecal samples. For each sheep, the pre- and post-treatment FECs were compared, and benzimadazole resistance was defined as an egg reduction rate (ERR) between pre- and post-treatment fecal samples of < 90%. The DNA of *H. contortus* eggs was extracted and used as a template for DNA amplification of -tubulin isotype 1. The percentage of resistant parasites was estimated with the frequencies of susceptible and resistant alleles at the three markers of benzimidazole resistance detected by pyrosequencing. On the basis of the results obtained from the genetic test, farms were classified as having benzimidazole-resistant *H. contortus* when the percentage of resistant parasites was > 10% (definition of resistant population).

Results

The minimum FEC to perform the FECRT is 150 eggs per gram. Two of five sampled farms had sheep with sufficient FECs to perform the FECRT; the ERR for those two farms was 0% and 52%, respectively. Only 20 *H. contortus* eggs are required for pyrosequencing. The genetic test was performed on *H. contortus* eggs obtained from fecal samples of all five farms. Among all eggs analyzed in this study, no SNPs were observed at position 198. On the basis of results obtained from the genetic test, the mean percentage of resistant parasites within farms was 71%. The farm with the ERR of 0% had 79% resistant parasites, and the farm with the ERR of 52% had 60% resistant parasites. The frequencies for the resistant allele obtained from composite fecal samples collected from the pastures were similar to those obtained from fecal samples collected per rectum.

Significance

Results of this study indicate that Quebec sheep farms have benzimidazole-resistant *H. contortus*. Because some farms lacked sheep with sufficient *H. contortus* FECs, we could not compare the results of the genetic test with the results of FECRT on those farms. However, we can propose a protocol on the basis of the genotype of the parasite population which can be used on farms for routine analysis to detect benzimidazole resistance. When the genetic test is used before anthelmintic treatment and results suggest the presence of benzimidazole resistance, a more appropriate anthelmintic can be chosen for treatment of the flock. Our results also suggest that pyrosequencing performed on random composite fecal samples collected from a pasture can provide an accurate estimate of benzimidazole resistance at the

farm level. Compared with the FECRT, which requires fecal samples obtained from large number of sheep, the genetic test used in this study is faster, more accurate, and results regarding a herd's benzimidazole resistance status can be obtained with only two to three composite fecal samples obtained from pastures. These samples can be collected by the producer, and no animal handling is required. Use of an ineffective anthelmintic can result in contamination of the pasture with drug-resistant parasites, as well as drug residues in the pasture and sheep. These issues could be avoided if veterinarians knew resistance status of the parasites prior to drenching so that an appropriate treatment could be used.