Use of a PCR to identify the main gastrointestinal nematodes resistant to anthelmintics in cattle farms, in Uruguay. Preliminary results

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Introduction

The prevention and treatment of gastrointestinal nematodes based mainly on the use of antihelminthic drugs, producing increasingly the phenomenon of anthelmintic resistance (AR) in ruminants.

The fecal egg reduction test (FECRT) is one of the main diagnostic method to detect AR and the resistant GIN are phenotipically classified. Molecular techniques are currently being studied to identify NGI resistant to different chemical groups. The objective of this work was to set up molecular tests to typify the main (GIN) identified as resistance by coprocultures when performing the FECRT in cattle herds.

Methods

This work is part of a study conducted to obtain information on AR in cattle farms in the northern of Uruguay during 2018-2019. Third stage larvae (L3) were obtained from the FECRT coprocultures from 10 farms. Morphological and molecular identification was performed using L3 obtained for the evaluated drug groups: ivermectin 1% (IVM), levamisole (LEV), ricobendazole (RBZ), fenbendazole (BZ) and untreated control.

A commercial kit NucleoSpin® Soil DNA (Macherey-Nagel) was utilized to extract DNA from a mixed pool of L3 from each FECRT group. Uniplex PCR reactions were conducted with primer pairs described in the literature specific for regions in the Internal Transcribed Spacer 2 (ITS 2) of *Haemonchus* sp., *Cooperia oncophora*, *Ostertagia ostertagi* and *Trichostrongylus* spp. (Table 1).

Table 1. Description of specific primers characteristic for the cattle gastrointestinal nematodes used in the study

Name	Target	Sequence	Ta[°C][1]	Length [bp][2]
Hc-SH-for2 Hc-SH-rev	*H.contortus ITS-2*	CCATATACTACAATGTGGCTA ATTTCTACAAATGATAAAAGA ACATCGTCGC	62	226
Tricho-2-Multi-83F Tricho-2-Multi-187R	*T.Columbriformis ITS-2*	CTTACGTCTGGTTCAGGGTT GACTGAAATGGGAATCATCA CAATATTT	53	106
Coop-SH-For2 Coop-SH-Rev2	*C.Oncophora ITS-2*	ATGGCATTTGTCTACATCTGT TTAAATGATAACGAATACTAC TATCTCCA	62	192
Ost.ost-SH-For Ost.ost-SH-Rev	*O.Ostertagi ITS-2*	TAACATTGTTAACGTTACTGA ATGATACTGATATAAATGATA CATCGAATATACAATAC	50	124

Adapted from Demeler et al., 2013
1=temperature annealing; 2=base pair

The Cohen´s kappa statistics was applied to estimate the agreement beyond chance between phenotipical and molecular typification of resistant GIN.

Results

The coproculture results indicated that Cooperia spp was the main GIN genera identified as developing resistance to al the studied drugs (Fig. 1).

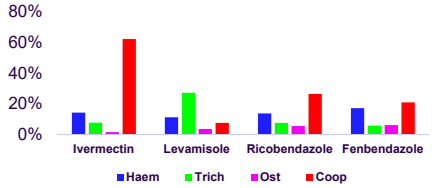

Figure 1. Result from coprocultures presenting the mean percentage of each of the GIN genera identified from 10 farms and by drug groups.

Results

From 38 L3 cultures where *Haemonchus* spp was morphologically classified, 20 of them gave an amplicon of 226 base pairs (bp) corresponding to the expected size for this parasite. From 34 samples where *Trichostrongylus* spp. was typified, 29 revelead amplicons of 106bp reported for this genus.

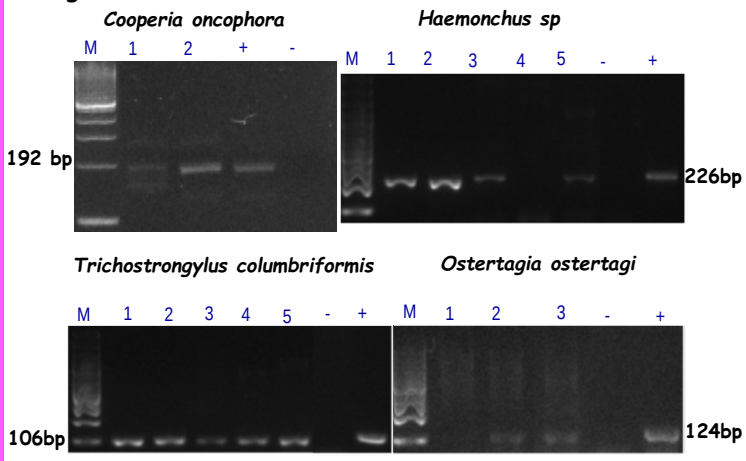

Figure 3. Compatibility of PCR with samples from L3. Lanes with different numbers represent field samples with positive (+) and negative (-) controls.

From 38 samples where *Cooperia oncophora* was typified, 20 gave amplicons of 192 bp described for the species and out of 21 samples where *Ostertagia ostertagi* was typified, four gave amplicons of 124 bp expected for this species.

Table 2. Results of the Cohen`s Kappa test for the agreement between the morphological identification of the L3 recovered from coprocultures and PCR identification.

Gastrointestinal nematode	Kappa agreement	P value
Haemonchus sp	0,29	<0.01
Ostertagia ostertagi	0,12	0.11
Cooperia oncophora	0,14	0.10

The agreement results between L3 morfological classification and PCR for detecting resistant nematodes is presented in Table 2. The kappa value indicates fair agreement for *Haemonchus* sp and slight and non significant agreement for *Ostertagia ostertagi* and for *Cooperia oncophora*. There was no agreement for *Trichostrongylus* spp.

Conclusions

The preliminary results indicate that the applied PCR protocols successfully identify L3 from mixed GIN coproculture samples. The study will continue to increase sample size to improve the agreement between both morphological and molecular tests under our working conditions.