A comparison of ITS and IGS sequences of ovine nematodes

B Conneely[1,2], B Good[2], E McCarthy[1], S Hanrahan[2], T deWaal[1]
[1]School of Agriculture, Food Science and Veterinary Medicine,University College Dublin, Dublin, Ireland
[2]Teagasc, Animal Production Research Centre, Athenry Co. Galway, Ireland
Email: brendan.conneely@ucdconnect.ie

Introduction Nematode parasitism of livestock is of economic importance worldwide. Accurate identification of nematode parasites is important in epidemiological studies as well and in selecting a control strategy. Traditional methods of identifying parasitic nematodes are based on morphological characteristics, which require expertise. DNA-based assays have the potential to rapidly and accurately identify the species infecting livestock. In recent years the intergenic spacer (IGS) and internally transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) have been exploited for the identification of parasitic nematode species. The aim of this study is to assess the feasibility of using the ITS and IGS regions as targets for a molecular assay based on species specific probes to identify parasitic nematode species relevant to sheep in Ireland**.**

Material and methods Eight species of parasitic sheep nematodes, belonging to the families Trichostrongylidae, Strongylidae, Trichonematidae, Molineidae, Chabertiidaeiidae and Trichuridae were used. DNA was extracted from a single adult worm from each species using a Roche High pure PCR template preparation kit according to the manufacturer's instructions. The entire ITS-1, 5.8s and ITS-2 region and IGS region of the rDNA were amplified using primer sets described either by Chilton *et al* (2004) or newly designed primers UniITS-F: 5'-GCGGGAAACAGTTAATCGC-3' and UniITS-R 5'-TCCCCGTTCACTCACTCGCCGTTA-3' for the ITS region and UniIGS-F 5'-ACCGTCGTGAGACAGGTTAG-3', UniIGS-R 5'-CTGCTCTAATGAGCCGTTCG-3'for the IGS region. PCR reactions were carried out in 50µl reaction volumes using 5µl genomic DNA, 250 µM of each dNTP, 2.5 µM $MgCl_2$, 0.6 µM of each primer and 1 U of *Taq* polymerase (Promega GoTaq®) with buffer supplied. Purified PCR products were then ligated into plasmids using a pGem ® Teasy vector system (Promega). The ligated plasmids were transformed into high efficiency competent JM109 *E.coli* cells for multiplication. Purified plasmids were then sequenced (GATC Germany). The Align tool using the Smith-Waterman algorithm was used to compare the sequences (EBI, 2009).

Results The sizes of the ITS-1 fragments ranged from 770bp (*Trichuris ovis)* to 368bp (*Chabertia ovina*) (Table 1). The GC content of the ITS-1 ranged from 40% to 57%. The sizes of the ITS-2 fragments ranged from 409bp (*Trichuris ovis)* and 231bp (*Haemonchus contortus*) (Table 1). The GC content of the ITS-2 for all species ranged between 32% and 62%. The levels of homology between the sequences of the different nematode species are shown in Table 1. Amplification of the IGS region was unsuccessful.

Table 1 Pairwise comparison of the percentage homology in the ITS-1 sequences (above diagonal) and ITS-2 sequences (below diagonal). ITS-1 sequence lengths are shown horizontally while ITS-2 sequence lengths are shown vertically.

	T. colu 387bp	*T. ovis* 770bp	*T. vitr* 390bp	*T. circ* 441bp	*O. venu* 376bp	*N. batt* 386bp	*H. cont* 405bp	*C. ovin* 368bp
Trichostrongylus colubriformis 238bp	-	40.2	97.7	84.7	66.5	70.8	80.9	66.7
Trichuris ovis 409bp	37.5	-	39.7	38.2	36.7	40.4	41.5	38.2
Trichostrongylus vitrinus 232bp	82.6	43.4	-	86.2	66.5	72.1	81.4	69.2
Teladorsagia circumcincta 246bp	81.1	44.6	78	-	62.3	68.8	79.9	68.5
Oesophagostomum venulosum 258bp	56.8	44.1	56.3	54.9	-	63.2	63.2	89.3
Nematodirus battus 231bp	65.1	38.9	62.6	63.8	52.6	-	64.6	68.5
Haemonchus contortus 231bp	75.1	43.1	88.4	74.4	54.2	58.9	-	65.7
Chabertia ovina 235bp	58.2	41.4	54.6	49.5	70.6	55.3	54.4	-

Conclusions Both ITS-1 and ITS-2 regions exhibit low levels of homology. The ITS-2 exhibited less levels in 22/28 (79%) of the pairwise comparisons These results are promising and may well provide the target region on which to base a rapid species identification assay.

References

Chilton, N.B., 2004. Animal Health Research Reviews 5, 173-187.
European Bioinformatics institute, 2009. Available http://www.ebi.ac.uk/Tools/emboss/align/ [accessed October 2009]

https://doi.org/10.1017/S2040470010004516 Published online by Cambridge University Press