

**β -tubulin Genes and
Benzimidazole Resistance in
*Trichostrongylus colubriformis***

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Preface

I certify that the substance of this thesis has not already been submitted for any other degree and is not being submitted currently for any other degree.

I certify that the work for this study was carried out solely by the candidate. All assistance received in the preparation of the thesis and all the sources used have been acknowledged herein.

Lisa J. Mascord
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Abstract

This thesis describes the characterisation of the β -tubulin gene family in *Trichostrongylus colubriformis* and the role of a particular β -tubulin locus (*tcb-1*) in BZ resistance and the subsequent development of a PCR (Polymerase Chain Reaction) based assay that can be used to genotype individual *T. colubriformis* for a marker associated with a BZ resistance allele. This is the first genotypic assay for the detection of a drug resistance allele in this species of nematode and one of only two (the other detects BZ resistance in *Haemonchus contortus*) genotypic assays for the detection of a drug resistance gene in parasitic nematodes of sheep. The assay detects a polymorphism in a β -tubulin allele shown to be involved in BZ resistance in an RFLP analysis. Sequence analysis of this allele revealed striking sequence homology to a β -tubulin allele shown to be involved in BZ resistance in the closely related nematode *H. contortus* suggesting that these genes have functional homology. Comparison of these sequences with other nematode β -tubulins suggested that the gene responsible for BZ resistance in these parasitic nematodes was closely related to the *ben-1* gene in the free-living nematode *Caenorhabditis elegans*, the product of which has been shown to be responsible for BZ susceptibility in that nematode.

When the PCR assay was applied to a BZ naive strain (McMaster susceptible) the resistance allele could be readily detected and the allele frequency of the resistance allele was shown to be in Hardy-Weinberg equilibrium. This suggests that the resistance allele was present in the unselected population as a natural polymorphism and that no deleterious effects were associated with the resistance allele. The assay was also applied successfully to a number of independently selected BZ resistant populations implying that the same allele was selected in each population.

Clones for an additional three *T. colubriformis* β -tubulin genes were obtained. Sequence analysis of the variable 3' ends of these genes showed that the sequence of the loci vary within *T. colubriformis* but are highly conserved between closely related nematodes species. This degree of homology between species has only been reported for vertebrates and this is the first example of conservation of isotypic classes for β -tubulins in invertebrates.

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