Polymorphisms in exon 11 of the mptl-1 gene and monepantel resistance in *Haemonchus contortus*

Simone Cristina Méo Niciura1 · Cintia Hiromi Okino1 · Alessandra da Silva Nucci2 · Wilson Malagó Jr.1 · Magda Vieira Benavides3 · Sergio Novita Esteves1 · Ana Carolina de Souza Chagas1

Received: 29 August 2022 / Accepted: 27 September 2022 / Published online: 4 October 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Chemical treatments are the main strategy to control gastrointestinal nematodes in sheep, and the emergence of anthelmintic resistance, as consequence, results in control failures and leads to economic losses. Thus, molecular tests may constitute an excellent tool for the early detection of anthelmintic resistance-related mutations. Thus, a polymerase chain reaction (PCR)-based genotyping assay followed by polyacrylamide gel electrophoresis (PAGE) was developed to detect polymorphisms in exon 11 of the acetylcholine receptor monepantel-1 gene (mptl-1) that were previously associated with monepantel resistance through a genome-wide study in *Haemonchus contortus*. DNA samples recovered from individual and pooled third-stage larvae from two susceptible field-derived isolates and five (three in vivo-derived and two field-derived) resistant populations were used. New polymorphisms, including a 6-bp deletion and a 3-bp insertion, were detected in resistant individuals. These indels, confirmed using sequencing of cloned PCR products, are predicted to result in amino acid changes in transmembrane domain 2 (TMD2) of the MPTL-1 protein. The two susceptible isolates showed only the presence of the wild-type allele (100%), whereas lower frequencies of the wild-type allele were detected in monepantel-resistant populations (11.1 to 66.7%). These findings report new polymorphisms in the mptl-1 gene, validate the results obtained through genomic mapping for monepantel resistance, and provide a PCR-based assay to genotype indels located in exon 11 of mptl-1 in *H. contortus*.

Keywords  Amino-acetonitrile derivatives · Anthelmintic resistance · Genotyping · PCR

Introduction

Gastrointestinal nematode infections, especially those caused by *H. contortus* (the barber’s pole worm), are responsible for large economic losses in sheep production owing to decreased performance, treatment expenses, and anthelmintic resistance (Miller et al. 2012; Chagas et al. 2022). As anthelmintic resistance has become a worldwide problem, tests for resistance using molecular markers are an attractive alternative for the identification of early resistance emergence, supporting decisions on treatment management (Gasser et al. 2008). Furthermore, screening tools based on molecular markers can guide the rational use of anthelmintics in flocks to improve monitoring of resistance in the field. Thus, the widespread presence of resistance alleles can be avoided (Roos et al. 2004).

Considering the anthelmintic classes available for sheep treatment, molecular markers of resistance, as reviewed by Kotze et al. (2020), are only well-established for benzimidazoles (mutations in isotype 1 of the β-tubulin gene).
However, molecular markers for levamisole (mutations in the acr-8 gene) and amino-acetonitrile derivatives (AAD) (mutations in the mptl-1 gene) have only been partially elucidated (Kotze et al. 2020). In addition, molecular marker data are still inconsistent across studies of macrocyclic lactones in *H. contortus*; however, there is genomic evidence that a single major locus on chromosome V is associated with ivermectin resistance (Doyle et al. 2019). Genotyping assays based on sequencing or polymerase chain reaction (PCR) can be used to detect polymorphisms associated with anthelmintic resistance; the method of choice depends on the scale and resolution required (Kotze et al. 2020).

Monepantel, an AAD, is one of the most recently developed anthelmintic compound suitable for the control of sheep gastrointestinal nematodes that exhibited multiple resistance to previously introduced drug classes (Kaminsky et al. 2008, 2011). However, several years after its launch, monepantel resistance was reported in multiple flocks worldwide (Mederos et al. 2014; Van den Brom et al. 2015; Sales and Love 2016; Albuquerque et al. 2017). In *H. contortus*, monepantel acts on the acetylcholine receptor DEG-3-like protein (DEG-3) group of nematode-specific nicotinic acetylcholine receptors (nAChR), which comprise two receptors; one containing DEG-3 and the acetylcholine receptor DES-2-like protein (DES-2), and one containing MPTL-1 (reviewed by Holden-Dye et al. 2013). Monepantel and its metabolite, monepantel sulfone, are superagonists that open the MPTL-1 channel in an irreversible manner (Baur et al. 2015). As MPTL-1 is the main monepantel target receptor, several molecular changes in the mptl-1 gene, presumably leading to a non-functional protein, have been associated with monepantel resistance in *H. contortus* (Kaminsky et al. 2008; Rufener et al. 2009; Bagnall et al. 2017). In a previous extreme-quantitative trait loci (X-QTL) genomic mapping study, a selection sign on chromosome 2 in a region containing the mptl-1 gene was detected (Niciura et al. 2019). By comparing the allele frequency differences between samples collected before and after monepantel treatment, polymorphisms, mainly deletions, in exon 11 have been identified as potential causal mutations responsible for monepantel resistance (Niciura et al. 2019). Considering the large number and constant emergence of new polymorphisms reported in mptl-1, any assay for identification of polymorphisms related to resistance should address all possible mutations across the gene (Kotze et al. 2020) or, alternatively, fine-map regions identified by genome-wide approaches or investigate loci frequently affected in various populations.

Therefore, to validate previous results obtained using genomic mapping for monepantel resistance in *H. contortus*, the present study examined polymorphisms in exon 11 of mptl-1 and designed a PCR-based assay for genotyping the identified indels.

### Materials and methods

#### *H. contortus* strains

Seven *H. contortus* populations previously known for their monepantel resistance status were used. Two monepantel-susceptible field-derived (SFD) isolates, retrieved before the monepantel launch on the Brazilian market, were named SFD1 and SFD2. SFD1, recovered in 2010, is susceptible to monepantel but resistant to other drugs (Chagas et al. 2013), and was the parental susceptible isolate used for parasite crossing in an X-QTL study for monepantel resistance (Niciura et al. 2019), whereas SFD2, recovered in 1990, is susceptible to several drugs (Echevarria et al. 1991). Three resistant isolates, obtained in 2018–2019 through in vivo selection using monepantel subdosing administration (monepantel doses from 0.075 to 2.5 mg/kg in 19–26 rounds of selection at minimum 14-day intervals for 112–133 weeks) in three different sheep hosts after artificial infection with third-stage larvae (L₃) from the SFD1 isolate (Niciura et al. 2020), were referred to as RIV1, RIV2, and RIV3. The two resistant field-derived populations were named RFD1 and RFD2. RFD1 is a resistant isolate recovered in 2017 (Albuquerque et al. 2017) used as the resistant parent in the X-QTL for monepantel resistance (Niciura et al. 2019), and RFD2 is a population comprising 95.7% *Haemonchus* spp. and 4.3% *Trichostrongylus* spp. retrieved in 2022 from the Embrapa Pecuaria Sudoeste sheep flock, in which persistently high egg counts were observed despite treatment with 2.5 mg/kg therapeutic monepantel dose. After collection, *H. contortus* isolates were cryopreserved and maintained under nitrogen at −196 °C until passage through sheep hosts for larvae recovery. Pools and individual larvae from the six *H. contortus* isolates (SFD1, SFD2, RIV1, RIV2, RIV3, and RFD1) were used to investigate new polymorphisms in exon 11 of mptl-1. Additionally, individual larvae, using a larger sample size from the RFD2 population for validation, were used to assess the allelic and genotypic frequencies of polymorphisms.

#### DNA extraction

The DNA of individual and pooled (1,000 individuals) *H. contortus* L₃ was extracted using an organic solvent and diluted in water (Niciura et al. 2012). Briefly, larvae were exsheathed with 0.15% sodium hypochlorite and incubated in digestion buffer with 0.4 mg/mL proteinase K at 56 °C overnight. DNA was then extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and washed with 100% isopropanol and 70% ethanol. The protocols used for individual and pooled L₃ differed only in reagent volume and final dilution in water (10 µL for individual and 50 µL for pooled L₃). For pooled L₃, DNA
concentration and purity were estimated using ultraviolet absorbance (NanoDrop™ 2000, Thermo Fisher). Because very low yields were obtained for individual L₃ (10 µL per sample), the DNA concentration was not measured.

**Genotyping of polymorphisms in exon 11 of mptl-1**

Polymorphisms in exon 11 of mptl-1 in *H. contortus* (HCON_00039360 in WormBase ParaSite, version WBPS16; Laing et al. 2013; Doyle et al. 2020), in a locus encoding transmembrane domain 2 (TMD2) of the MPTL-1 protein, were investigated using PCR. *H. contortus*-specific primers (forward: 5′-CGAATGGGACCATCATTCTAGC-3′ and reverse: 5′-GAAAGGTGCCTCGGAGTAAA-3′) were designed to flank a region from the introns 10–11/exon 11 junction (at positions 7,744,504–7,744,484 on chromosome 2) to introns 11–12 (at positions 7,744,347–7,744,328 on chromosome 2), leading to the amplification of a 177-bp fragment containing the entire exon 11 of mptl-1. PCR was performed using 1× buffer, 0.4 µM of each primer, 0.2 mM of each dNTP, 2 mM of MgCl₂, 1 U of Taq Platinum DNA polymerase (Thermo Fisher), and 50 ng of pooled L₃ DNA or 2 µL of individual L₃ DNA, in a final volume of 20 µL. The thermal profile was 94 °C for 2 min, followed by 40 cycles of 94 °C, 57 °C, and 72 °C for 30 s, and 72 °C for 10 min.

PCR products were subjected to both agarose and polyacrylamide gel electrophoresis. An adapted electrophoresis protocol using a 4% agarose gel (Bhattacharya and Van Meir 2019) with 1× TBE at 75 V for 130 min and ethidium bromide staining (0.5 µg/mL in gel followed by 2.0 µg/mL for 20 min in a bath) was used. In addition, denaturing 6% polyacrylamide gel electrophoresis (PAGE) with 1× TBE at 60 W for 2 h and silver staining was performed.

The susceptible homozygous genotype (named SS) presented two identical wild-type alleles, the resistant homozygous genotype (named RR) presented two copies of a mutated allele, and the heterozygous genotype (named RS) presented one wild-type allele and one mutated allele.

**Cloning and Sanger sequencing**

Pooled L₃ samples from each *H. contortus* isolate were amplified, cloned, and subjected to Sanger sequencing (5 or 6 clones per isolate). For amplification, a High-Fidelity Taq DNA Polymerase was used, and the PCR previously described was modified as follows: 0.2 µM of each primer and 1× of PCRBio Ultra Mix (PCR Biosystems) in a final volume of 25 µL. PCR amplification was confirmed using 1.5% agarose gel electrophoresis.

For cloning, PCR products were purified using Wizard® SV Gel and the PCR Clean-Up System (Promega), and subjected to A-tailing, ligation, and transformation procedures using pGEM®-T Easy Vector Systems (Promega). White colonies were picked, subjected to clone insert verification using colony PCR (Green and Sambrook 2019) with amplicon-specific primers, and visualized on a 1.5% agarose gel. Plasmid minipreps from recombinant colonies were generated using the PureYield™ Plasmid Miniprep System (Promega), and plasmids were quantified using a Qubit™ dsDNA HS assay (Thermo Fisher) in a Qubit fluorometer (Thermo Fisher).

Plasmids were subjected to Sanger sequencing using M13 forward and reverse primers in two separate runs and a BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher), followed by analysis using an ABI Prism 3730XL DNA analyzer (Thermo Fisher). All nucleotide sequence data for full-length exon 11 of mptl-1 in *H. contortus* are available in GenBank (accession numbers: ON014540 to ON014573) (Supplementary Table 1). The resulting sequences from the forward and reverse runs were aligned using Bioedit to obtain contig sequences that were compared to the reference sequence (HCON_00039360) deposited on WormBase ParaSite (version WBPS16) and translated using Emboss Transeq and Emboss Sixpack software (Madeira et al. 2019).

**Results**

Initially, DNA from pooled L₃ of the six *H. contortus* isolates was subjected to PCR to amplify exon 11 of mptl-1, and different fragment sizes were observed after agarose gel electrophoresis. As further confirmed through sequencing, only the 177-bp wild-type fragment was amplified in the SFD1 and SFD2 susceptible isolates, whereas products of 171, 177, and 180 bp were observed for RIV1-, RIV2-, RIV3-, and RFD1-resistant isolates (Fig. 1A). An additional band larger than 180 bp was observed for RFD1 (Fig. 1A), but it was not retrieved from samples submitted for cloning and sequencing. This additional band was excised from agarose gels, purified, and subjected to Sanger sequencing, but no contig sequence could be generated due to the 6 bp-mutated allele presence in samples, leading to electropherogram peak superposition after sequencing.

After sequencing a 6-bp deletion (c.7,744,421_7,744,416delAAT GTCTp.M287_S288del), leading to a band of 171 bp, was detected in the field-derived resistant isolate RFD1, whereas a mutation involving four nucleotide substitutions together with a 3-bp insertion (c.7,744,396_7,744,393delTCCGinsAATGT TTp. S295_D296delinsNVY), leading to a band of 180 bp, was detected in the in vivo-selected resistant isolates (RIV1, RIV2, and RIV3) (Fig. 2). These two detected indels were predicted to cause non-synonymous amino acid changes (deletion of methionine and serine (MS) and substitution of serine and aspartic acid (SD) to asparagine, valine, and tyrosine (NVY) amino acids) in the TMD2 of MPTL-1 (Fig. 3).

In addition to the indels, several SNPs were detected in exon 11 of mptl-1 in both resistant and susceptible isolates (Fig. 2). However, the designed PCR-based assay followed by electrophoresis is not suitable for detecting these point mutations.
The association between the indels detected in exon 11 of mptl-1 and monepantel resistance was addressed through PCR-based genotyping using individual *H. contortus* L₃ from an independent resistant field-derived population RFD2. Plasmidial DNA samples were used as positive controls for 171 bp (RFD1-clone 4), 177 bp (SFD2-clone 4), and 180 bp (RIV2-clone 4) fragment sizes. While 4% agarose gel electrophoresis was able to detect polymorphic banding patterns when a few samples were subjected to electrophoresis on small (6 cm × 12 cm) gels (Fig. 1A), curving of the edges of the gel, leading to wrong genotype attribution, occurred when larger (15 cm × 25 cm) agarose gels were used. PAGE (Fig. 1B) was chosen as the post-PCR electrophoresis protocol to assess genotype frequencies in individual L₃ (Table 1). For all samples, each band appeared double in PAGE (Fig. 1B), even in homozygous individuals and clones, because of the denaturation protocol employed, highlighting the importance of using positive controls for accurate genotyping through banding patterns after electrophoresis.

*SFD1–2*, susceptible field-derived isolates 1 and 2; *RIV1–3*, resistant in vivo-derived isolates 1, 2, and 3; *RFD1–2*, resistant field-derived populations 1 and 2.

The susceptible homozygous genotype, SS, was attributed to homozygous individuals harboring the wild-type 177 bp allele, the resistant homozygous genotype, RR, was attributed to both 171 bp and 180 bp homozygous individuals, and the heterozygous genotype, RS, was attributed to both 171 bp/177 bp and 180 bp/177 bp heterozygous individuals (Table 1). Heterozygous individuals with 171 bp/180 bp
indels were not detected. The 100% frequency observed for the SS genotype in susceptible SFD1 and SFD2 isolates decreased in the resistant RIV1 (66.7%), RIV2 (28.6%), RIV3 (62.5%), RFD1 (11.1%), and RFD2 (50.0%) populations (Table 1). RS and RR genotypes were detected at varying frequencies in monepantel-resistant RIV1 (22.2 and 11.1%, respectively), RIV2 (14.3 and 57.1%, respectively), RIV3 (0.0 and 37.5%, respectively), RFD1 (33.3 and 55.6%, respectively), and RFD2 (16.7 and 33.3%, respectively) populations (Table 1).
Discussion

Several polymorphisms in mptl-1 have been reported to be associated with monepantel resistance in *H. contortus* (Kaminsky et al. 2008; Rufener et al. 2009; Bagnall et al. 2017; Niciura et al. 2019). Among them, polymorphisms in exon 11 were considered as potential causal mutations in a genomic mapping study (Niciura et al. 2019) and were investigated using two monepantel-susceptible (SFD1 and SFD2) isolates and five monepantel-resistant (RIV1, RIV2, RIV3, RFD1, and RFD2) populations.

Using pooled L₃ from *H. contortus* isolates, PCR followed by 4% agarose gel electrophoresis showed amplification of a single fragment (177 bp) in the two susceptible isolates, whereas it resulted in banding patterns of various lengths (171 bp, 177 bp, and 180 bp) in resistant isolates. By sequencing cloned PCR products, a 6-bp deletion (leading to the 171 bp fragment) and a 3-bp insertion (leading to the 180 bp fragment) were detected. The two detected indels may potentially be responsible for the monepantel resistance status, as they were not detected in susceptible isolates, even in the SFD1 isolate that was resistant to other drugs than monepantel. Additionally, these indels result in amino acid changes in TMD2 of MPTL-1. In the nAChRs of free-living nematodes, TMD2 donates residues that line the ion channel (Jones and Sattelle 2003), thereby affecting protein function. Similarly, Bagnall et al. (2017) reported that most resistance-related mutations detected in mptl-1 led to TMD loss and truncated protein production, indicating that there may be a link between mutations in mptl-1, loss of MPTL-1 function, and monepantel resistance.

In addition, it is worth mentioning that the electrophoresis protocol on 4% agarose gels (modified from Bhattacharya and Van Meir 2019) following PCR detected fragments differing in only a few nucleotides (6 bp and 3 bp) in the pooled L₃ when using small-sized agarose gels. Thus, it may be useful for the initial screening of monepantel resistance status in *H. contortus* populations using a few samples of pooled larvae. However, owing to the curving of the edges of the gel, known as the “smile effect,” that occurred in the outer...
lanes of large agarose gels during electrophoresis, it was not a reliable post-PCR assay to determine genotypic frequencies at large-scale using larger-size gels. Furthermore, for correct genotype attribution and frequency determination, a PCR-based assay should be followed by a more sensitive and discriminatory electrophoresis protocol, such as PAGE, or, alternatively, high-resolution capillary electrophoresis. As the developed assay using PCR followed by PAGE is cost effective and able to quantify low allelic frequencies of mutations, it fulfills some of the requirements of an ideal molecular test to diagnose resistance (Kotze et al. 2020).

Based on the results presented here, we can confirm that several different mutations in mptl-1 in *H. contortus* may be associated with the phenotype of monepantel resistance in the same population (Bagnall et al. 2017) or in different populations, as reported for *Teladorsagia circumcincta* (Turnbull et al. 2019), indicating that monepantel resistance is likely a quantitative trait. However, exon 11 of mptl-1 was particularly responsive to the selective pressure imposed by monepantel, as two different indels in the same region were observed in *H. contortus*-resistant isolates from two different sources (field derived and in vivo-selected) and in an independent resistant field-derived population. In addition, the importance of the exon 11 region was previously reported in the literature. Kaminsky et al. (2008) described the absence of PCR amplification of intron 10 and exon 11 of mptl-1 in resistant individuals. Furthermore, downregulation of expression was detected in an AAD-mutant isolate by analyzing mRNA levels with primers directed to exons 11 and 12 of mptl-1 (Rufener et al. 2009). Thus, the developed assay was able to identify polymorphic patterns in exon 11 of mptl-1 potentially associated with the monepantel-resistant phenotype in *H. contortus*.

**Conclusion**

In conclusion, previous results obtained through genomic mapping for monepantel resistance were validated and a suitable PCR-based assay was designed for detecting indel polymorphisms in exon 11 of mptl-1 in *H. contortus*. Considering the use of Brazilian-derived isolates and the high genetic diversity of *H. contortus* observed worldwide, additional studies using *H. contortus* populations from other regions should be performed to verify the associations of the described indels in exon 11 of mptl-1 with the phenotype of monepantel resistance and thus validate them as molecular markers.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00436-022-07682-6.

**Author contribution** S.C.M.N., C.H.O., and M.V.B.: conceptualization; S.C.M.N., C.H.O., A.S.N., and W.M.J.: methodology and investigation; S.N.E. and A.C.S.C.: resources. All authors have read and approved the final manuscript.

**Funding** This study was supported by the São Paulo Research Foundation, FAPESP (grant number 2019/02967–2).

**Data availability** Nucleotide sequence data reported in this paper are available in GenBank under accession numbers ON014540 to ON014573.

**Declarations**

**Ethics approval** This study was performed in strict accordance with the relevant guidelines and regulations of animal welfare in experimental science and with approval from the Animal Ethics Committee of Embrapa Pecuária Sudeste (Permit Number: 03/2019).

**Consent to participate and to publish** Not applicable.

**Conflict of interest** The authors declare no competing interests.

**References**

Albuquerque ACA, Bassetto CC, Almeida FA, Amarante AFT (2017) Development of Haemonchus contortus resistance in sheep under suppressive or target selective treatment with monepantel. Vet Parasitol 151:112–117. https://doi.org/10.1016/j.vetpar.2017.09.010

Bagnall NH, Ruffell A, Raza A, Elliott TP, Lamb J, Hunt PW, Kotze AC (2017) Mutations in the Hco-mptl-1 gene in a field-derived monepantel-resistant isolate of Haemonchus contortus. Int J Parasitol Drugs Drug Resist 7:236–240. https://doi.org/10.1016/j.ijpddr.2017.05.001

Baur R, Beech R, Sigel E, Rufener L (2015) Monepantel irreversibly binds to and opens Haemonchus contortus MPTL-1 and Caenorhabditis elegans ACR-20 receptors. Mol Pharmacol 87:96–102. https://doi.org/10.1124/mol.114.095653

Bhattacharya D, Van Meir EG (2019) A simple genotyping method to detect small CRISPR-Cas9 induced indels by agarose gel electrophoresis. Sci Rep 9:4437. https://doi.org/10.1038/s41598-019-39950-4

Chagas ACS, Katiki LM, Silva IC, Giglioti R, Esteves SN, Oliveira MC, Barioni Júnior W (2013) Haemonchus contortus: a multiple-resistant Brazilian isolate and the costs for its characterization and maintenance for research use. Parasitol Int 62:1–6. https://doi.org/10.1016/j.parint.2012.07.001

Chagas ACS, Tupy O, Santos IB, Esteves SN (2022) Economic impact of gastrointestinal nematodes in Morada Nova sheep in Brazil. Braz J Vet Parasitol 31:e008722. https://doi.org/10.1590/S1984-29612022044

Doyle SR, Illingworth CJR, Laing R, Bartley DJ, Redman E, Martinelli A, Holroyd N, Morrison AA, Rezansoff A, Tracey A, Devaney E, Berriman M, Sargison N, Cotton JA, Gilleard JS (2019) Population genomic and evolutionary modelling analyses reveal a single major QTL for ivermectin drug resistance in the pathogenic nematode, Haemonchus Contortus BMC Genomics 20:218. https://doi.org/10.1186/s12864-019-5592-6

Doyle SR, Tracey A, Laing R, Holroyd N, Bartley D, Bazant W, Beasley H, Beech R, Britton C, Brooks K, Chaudhry U, Maitland K, Martinelli A, Noonan JD, Paulini M, Quail MA, Redman E, Rodgers FH, Sallé G, Shabbir MZ, Sankaranarayanan G, Wit J, Howe KL, Sargison N, Devaney E, Berriman M, Gilleard JS, Cotton JA (2020) Genomic and transcriptomic variation defines the chromosome-scale assembly of Haemonchus contortus, a model
gastrointestinal worm. Commun Biol 3:656. https://doi.org/10.1038/s42003-020-01377-3

Echevarria FMA, Armour J, Duncan JL (1991) Efficacy of some anthelmintics on an ivermectin-resistant strain of Haemonchus contortus in sheep. Vet Parasitol 39:279–284. https://doi.org/10.1016/0304-4017(91)90044-v

Gasser RB, Bolt NJ, Chilton NB, Hunt P, Beveridge I (2008) Toward practical, DNA-based diagnostic methods for parasitic nematodes of livestock — bionomic and biotechnological implications. Biotechnol Adv 26:325–334. https://doi.org/10.1016/j.biotechadv.2008.03.003

Green MK, Sambrook J (2019) Polymerase chain reaction. Cold Spring Harbor Protocols 6:pdb-top095109. https://doi.org/10.1101/pdb.Prot095224

Holden-Dye L, Joyner M, O’Connor V, Walker RJ (2013) Nicotinic acetylcholine receptors: a comparison of the nACHRs of Caenorhabditis elegans and parasitic nematodes. Parasitol Int 62:606–615. https://doi.org/10.1016/j.parint.2013.03.004

Jones AK, Satelle DB (2003) Functional genomics of the nicotinic acetylcholine receptor gene family of the nematode, Caenorhabditis elegans. BioEssays 26:39–49. https://doi.org/10.1002/bies.10377

Kaminsky R, Ducray P, Clover R, Rufener L, Bouvier J, Weber SS, Wenger A, Wieland-Berghausen S, Goebel T, Gauvry N, Pautrat F, Skripsi T, Frollich O, Komoin-Oka C, Westlund B, Sluder A, Mäser P (2008) A new class of anthelmintics effective against drug-resistant nematodes. Nature 452:176–180. https://doi.org/10.1038/nature06722

Kaminsky R, Bapst B, Stein PA, Streihau GA, Allan BA, Hosking BC, Rolfe PF, Sager H (2011) Differences in efficacy of monepantel, derquantel and abamectin against multi-resistant nematodes of sheep. Parasitol Res 109:19–23. https://doi.org/10.1007/s00436-010-2216-0

Kotze AC, Gilless JD, Doyle SR, Prichard RK (2020) Challenges and opportunities for the adoption of molecular diagnostics for anthelmintic resistance. Int J Parasitol Drugs Drug Resist 10:119–125. https://doi.org/10.1016/j.jpddr.2020.10.005

Laing R, Kikuchi T, Martinelli A, Tsai IJ, Beech RN, Redman E, Holroyd N, Bartley DJ, Beasley H, Britton C, Curran D, Devaney E, Gilabert A, Hunt M, Jackson F, Johnston SL, Kryukov I, Li K, Morrison AA, Reid AJ, Sargison N, Saunders GI, Wasmuth JD, Wieland-Berghausen S, Goebel T, Gauvry N, Pautrat F, Skripsi T, Frollich O, Komoin-Oka C, Westlund B, Sluder A, Mäser P, Rufener L, Mäser P, Roditi I, Kaminsky R (2009) Haemonchus contortus acetylcholine receptors of the DEG-3 subfamily and their role in sensitivity to monepantel. PLoS Pathog 5:e1000380. https://doi.org/10.1371/journal.ppat.1000380

Miller CM, Waghorn TS, Leathwick DM, Candy PM, Oliver AM, Watson TG (2012) The production cost of anthelmintic resistance in lambs. Vet Parasitol 186:376–381. https://doi.org/10.1016/j.vetpar.2011.11.063

Niciura SCM, Veríssimo JC, Gromboni JGG, Rocha MIP, Mello SS, Barbosa CMP, Chiebao DP, Cardoso D, Silva GS, Otsuk IP, Pereira JR, Ambrosio LA, Nardon RF, Ueno THE, Molento MB (2012) F200Y polymorphism in the beta-tubulin gene in field isolates of Haemonchus contortus and risk factors of sheep flock management practices related to anthelmintic resistance. Vet Parasitol 190:608–612. https://doi.org/10.1016/j.vetpar.2012.07.016

Niciura SCM, Tiizito PC, Moraes CV, Cruvinel GG, Albuquerque ACA, Santana RCM, Chagas ACS, Esteves SN, Benavides MV, Amarante AFT (2019) Extreme-QTL mapping of monepantel resistance in Haemonchus contortus. Parasit Vectors 12:403. https://doi.org/10.1186/s13071-019-3663-9

Niciura SCM, Cruvinel GG, Moraes CV, Chagas ACS, Esteves SN, Benavides MV, Amarante AFT (2020) In vivo selection for Haemonchus contortus resistance to monepantel. J Helminthol 94:e46. https://doi.org/10.1017/s0022183419000221

Roos MH, Otsen M, Hoekstra R, Veenstra JG, Lenstra JA (2004) Genetic analysis of inbreeding of two strains of the parasitic nematode Haemonchus contortus. Int J Parasitol 34:109–115. https://doi.org/10.1016/j.ijpara.2003.10.002

Rufener L, Mäser P, Roditi I, Kaminsky R (2009) Haemonchus contortus acetylcholine receptors of the DEG-3 subfamily and their role in sensitivity to monepantel. PLoS Pathog 5:e1000380. https://doi.org/10.1371/journal.ppat.1000380

Sales N, Love S (2016) Resistance of Haemonchus sp. to monepantel and reduced efficacy of a derquantel/abamectin combination confirmed in sheep in NSW, Australia. Vet Parasitol 228:193–196. https://doi.org/10.1016/j.vetpar.2016.08.016

Turnbull F, Devaney E, Morrison AA, Laing R, Bartley DJ (2019) Genotypic characterisation of monepantel resistance in historical and newly derived field strains of Teladorsagia circumcincta. Int J Parasitol Drugs Drug Resist 11:59–69. https://doi.org/10.1016/j.jpddr.2019.10.002

Van Den Brom R, Moll L, Kappert C, Vellema P (2015) Haemonchus contortus resistance to monepantel in sheep. Vet Parasitol 209:278–280. https://doi.org/10.1016/j.vetpar.2015.02.026

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under applicable law. This journal article may be used for personal research and study purposes. Permission must be obtained from the publisher for further uses, such as: photocopies, republication, further distribution and all forms of storage and transmission, and for adapted versions of the journal article including for commercial purposes, in any form or by any means, whether electronic or mechanical, including photocopy, print on Demand, and microfilm to be sold or distributed to the public.

Springer Nature or its licensor holds exclusive rights to this article under applicable law. This journal article may be used for personal research and study purposes. Permission must be obtained from the publisher for further uses, such as: photocopies, republication, further distribution and all forms of storage and transmission, and for adapted versions of the journal article including for commercial purposes, in any form or by any means, whether electronic or mechanical, including photocopy, print on Demand, and microfilm to be sold or distributed to the public.

Springer Nature or its licensor holds exclusive rights to this article under applicable law. This journal article may be used for personal research and study purposes. Permission must be obtained from the publisher for further uses, such as: photocopies, republication, further distribution and all forms of storage and transmission, and for adapted versions of the journal article including for commercial purposes, in any form or by any means, whether electronic or mechanical, including photocopy, print on Demand, and microfilm to be sold or distributed to the public.