Lack of efficacy of monepantel against trichostrongyle nematodes in a UK sheep flock.

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Abstract

Monepantel resistance was diagnosed during routine monitoring of the effectiveness of a farm's roundworm control strategy. Weaned lambs had become ill thrifty and developed diarrhoea, despite the routine use of monepantel. This clinical presentation was caused by trichostrongylosis. The faecal egg count reduction was 76.7% (95% CI: 55.1 – 82.2%) following treatment with 2.5 mg/kg monepantel. Predominantly *Trichostrongylus vitrinus* along with small proportions of *Oesophagostomum venulosum* and *Trichostrongylus vitrinus* were identified by deep amplicon sequencing of pools of larvae recovered from pre and post monepantel treatment coprocultures and on postmortem examinations. The undifferentiated FECRT showed resistance to monepantel, but not to levamisole, ivermectin, or moxidectin. Examination of farm anthelmintic treatment and animal movement records suggested that treatments before movement onto silage aftermaths, putatively with low numbers of susceptible nematodes *in refugia*, may have placed a high selection pressure on monepantel resistance. Effective control of parasitic gastroenteritis using anthelmintic drugs is a prerequisite for sustainable sheep production. This case reiterates the need for care when combining anthelmintic treatments with movements to safe grazing, and the value of monitoring of anthelmintic efficacy as part of iterative planned animal health management.

1. Introduction

Parasitic gastroenteritis (PGE) in growing lambs is a significant global production limiting problem (Mavrot et al., 2015). The control of helminth parasites is being hampered by the development of resistance to many available pharmaceutical treatments. Anthelmintic resistance to the traditional benzimidazoles, imidazothiazoles and macrocyclic lactones is well documented in the United Kingdom (Glover et al., 2017; Thomas et al., 2015) as well as globally (Kaplan and Vidyashankar, 2012). In consequence there is a need to investigate strategies that protect the remaining efficacy of the traditional broad-spectrum anthelmintics, as well as incorporate the newer amino-acetonitrile derivative drug monepantel (4-AD), and the spiroindole drug derquantel (5-SI), given in combination with abamectin.

Monepantel represented the first new anthelmintic drug group to be marketed for use in sheep for over 25 years (Kaminsky et al., 2008). Monepantel became available first in New Zealand in 2009 and then in the UK in 2010. It was introduced with advice on how and when it should be used with reference to reducing the selection pressure for anthelmintic resistance. It was recommended that it should mainly be used as part of quarantine regimen and as a late-season wormer for lambs (Leathwick and Hosking, 2009).

The first report of resistance to monepantel came from New Zealand, two years after its release, with the diagnosis of resistance in *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Scott et al., 2013). Since then there have been multiple reports of monepantel resistance in *Haemonchus contortus* from across the world, including Uruguay (Mederos et al., 2014), Australia (Sales and Love, 2016; Lamb et al., 2017) and the Netherlands (Brom et al., 2015). The level of resistance in *H. contortus* was shown to be high in the majority of these investigations, with efficacy ranging from less than 50% to as low as zero. There have also been two reports of monepantel resistance from Brazil, implicating *T. colubriformis* (Cintra et al., 2016) and *Oesophagostomum* species (Ciuffa et al., 2017).

The development of resistance to monepantel is of concern to the global sheep industry and demonstrates the need for greater understanding of how to integrate knowledge of complex and multifactorial risk factors for anthelmintic resistance selection (Poulin, 1996; van Wyk, 2001; Waghorn et al., 2008; Kenyon et al., 2009; Leathwick and Besier, 2014) into practical farm management to allow the maintenance of effective pharmaceutical control of helminth parasites.

The aim of the current study was to assess monepantel efficacy on a farm using a faecal egg count reduction test (FECRT) and to link the findings with historical on-farm parasite control measures.

2. Materials and Methods

2.1. Background farm information

The investigation was undertaken on a beef cattle and sheep farm based in the UK, with a large breeding ewe flock grazed between March and December on a mixture of permanent and temporary grassland and forage crops, and housed over winter. Over the three year period from 2014 to 2017 'clean' (new leys and forage crops), or 'relatively clean' (previously harvested for hay/silage or grazed by cattle or dry ewes) grazing was prioritised for ewes with lambs or weaned ram lambs. Details of animal movements and anthelmintic treatments during 2016 are shown in Fig. 1; other years followed a similar pattern.

The diagnosis of *Teladorsagia circumcincta* resistance to benzimidazole (1-BZ), levamisole (2-LV), ivermectin (3-ML) and moxidectin (3-ML) in 2007 (Wilson and Sargison, 2007) led to a review of the farm's roundworm control strategies. These aimed to reduce further selection for resistance by minimising the requirement for anthelmintic use and attempting to maintain populations of susceptible helminths *in refugia* from treatment, within the constraints presented by practical farm management.

At all treatment events approximately 10% of the group were selected to act as carriers for susceptible worms *in refugia* by being given no anthelmintic treatment. These were animals that were in good body condition, without perineal faecal staining. The strategy had been developed by the farmer in an attempt to reduce the risk of further selection for anthelmintic resistance in a manner that was practical and could be integrated with management of a complex livestock and cereal cropping agribusiness. For some of the lamb treatment events these 'untreated' animals received a different class of anthelmintic, such as a benzimidazole, for the control of *Nematodirus battus* (Fig. 1A, paddocks 4 and 6). The one exception to this was in September 2016 when one group of lambs was treated with monepantel, following which the 10% that had been left untreated were also given monepantel four days later, coinciding with movement of the whole group onto a 'relatively clean' silage aftermath (Fig 1A, paddock 8). These lambs were moved again after ten days onto a new 'relatively clean' silage aftermath (Fig 1A, paddock 2). The second of these fields was grazed by pregnant ewes over the subsequent winter. These ewes were dosed with monepantel at lambing time and turned out onto newly reseeded 'clean' pasture with their newborn lambs. The ewes and lambs remained on this pasture until weaning and the ewe lambs were kept on the pasture for the rest of the summer. The movement of animals that resulted in this partial breakdown in the farm's planned *refugia* management arose as an unforeseen consequence of the need to balance other management priorities for grazing.

Monepantel was used on five occasions in 2014, six occasions in 2015 and 2016, and four occasions in 2017 in different groups of early and late lambing ewes, ram and ewe lambs, and stock rams.

Lamb growth rates had been judged satisfactory by the stockman until early July 2017, when most became ill thrifty and developed dark brown/green diarrhoea. Routine monitoring in mid-July, undertaken as part of the farms anthelmintic resistance mitigation strategy using a modified McMaster method with a sensitivity of 50 eggs per gram (epg) (Whitlock, 1948) on a pooled sample from eight lambs, showed a faecal strongylid egg count (FEC) of 800 eggs per gram (epg). A FECRT (Coles et al., 2006) was conducted using samples collected by the farmer in ten individually identified, five to six month old, ram lambs. The lambs were weighed and dosed orally with 2.5mg per kg bodyweight of monepantel (Zolvix; Elanco Animal Health). The mean pre-treatment FEC was 229 epg (range: 30 – 747 epg) and the post-treatment FEC from samples collected 14 days later was 30 epg (range: 15 – 99 epg). These results showed an efficacy of 81.4%, hence a need for further investigation.

2.2. Faecal egg count reduction test

A FECRT was conducted in August 2017 in a different group of four to seven month old ewe lambs. Faeces were collected from forty individually marked lambs on day zero. On day zero four groups of ten lambs were weighed and each group was orally dosed with one of: 2.5mg per kg monepantel (Zolvix; Elanco Animal Health); 200µg per kg ivermectin (Oramec oral 0.08%; Merial Animal Health Ltd); 7.5mg per kg levamisole hydrochloride (Chanaverm 7.5% oral solution; Chanelle UK); and 200µg per kg moxidectin (Cydectin oral 0.1%; Zoetis UK). All treatments were administered using a graduated syringe and dosages were calculated according to the individual bodyweight of each lamb (Appendix 1). Faecal samples were then collected from all lambs on day 14.

Faecal egg counts were performed using a salt flotation cuvette method (Christie and Jackson, 1982) with a sensitivity of up to one epg. Strongylid, *Nematodirus* and *Strongyloides* eggs were distinguished morphologically and enumerated separately. Pre- and post-treatment FEC data were entered into the eggCounts (Wang et al., 2017) package in R (R-Core-Team 2016). This program uses zero-inflated Bayesian hierarchical models to calculate the faecal worm egg count reduction and the 95% confidence limits.

2.3. Larval culture and identification

The remaining faeces from each group of ten lambs were incubated at 21^{o}C for 14 days to provide third stage larvae (L_3) for identification (MAFF, 1986).

Aliquots of 1,000 L_3 were stored in 70% ethanol, before using the nemabiome metabarcoding method precisely as described by Avramenko et al. (2015) to identify the proportions of specific sequences in the ITS-2 region of the ribosomal DNA cistron amplified by PCR. In brief, lysates were made from the ethanol stored L_3 pools, from which a 311 – 321 bp fragment encompassing the rDNA ITS-2 sequence was amplified using Clade V nematode-specific conserved primers (Avramenko et al., 2015). PCR products were purified and labeled with molecular bar codes before being added to pooled libraries. Pooled libraries were run on an Illumina MiSeq sequencer to generate FASTQ sequence reads that were identified, separated and analysed according to their molecular bar codes.

2.4. Estimation of lamb performance

The weight of each of these forty lambs was measured on each faecal sampling occasion, using an electronic weigh-scale. A note was made of whether each lamb had faecal staining of the perineum as an indicator of the presence or absence of diarrhoea. Lambs were subjectively visually classified as 'dirty' if staining was significant, or 'clean' if not. These criteria had previously been used by the farmer to identify 'clean' animals not requiring anthelmintic drug treatment.

2.5. Postmortem examintations

Post mortem examinations were carried out on two lambs that died during this investigation. One died from *Pasteurella* pneumonia 10 days after treatment with monepantel. Using warm isotonic saline samples of worms were collected from abomasal and small intestinal washings, and classified morphologically (MAFF, 1986) by the Scottish Rural College Veterinary Services. The second lamb died from aspiration pneumonia several weeks after treatment. Samples of adult nematodes from the abomasum and small intestine were examined for morphological classification (MAFF, 1986).

3. Results

3.1. Parasitology

Faecal egg counts in the lambs pre-treatment ranged from 270 – 5,049 epg with all groups averaging over 1,500 epg. Post-treatment the group mean FECs were 68, 7, 275 and <1 epg for levamisole,

ivermectin, monepantel and moxidectin, respectively. The faecal egg count reductions for levamisole, ivermectin, monepantel and moxidectin, were 95.7%, 99.6%, 76.7%, and 100% (Fig. 2). The FECRT showed resistance to monepantel, but not to levamisole, ivermectin, or moxidectin (Table 1).

The proportions of ITS-2 sequence reads for *Oesophagostomum venulosum*, *T. circumcincta* and *Trichostrongylus vitrinus* generated by the nemabiome metabarcoding method are shown in Table 2. For each species, the proportions of ITS-2 sequence reads were similar in the pre- and post-treatment coprocultures, with *T. vitrinus* predominating.

Nematodes from the lamb examined post mortem ten days after monepantel treatment were all located in the small intestine and reported to be *Trichostrongylus* species based on the morphology of the adult males. The second lamb had a mixture of larval and adult nematodes in the abomasum and small intestine. Those in the abomasum were reported as being morphologically identified as *T. circumcincta*, whereas those in the small intestine were *Trichostrongylus* species.

3.2. Lamb performance

Lamb performance results are shown in Appendices 1 and 2. An impact on flock productivity was suggested by the reduced mean DLWG seen in the lambs treated with monepantel, when compared with those treated with effective anthelmintics, but this was not supported by statistical analyses.

4. Discussion

The diagnosis of monepantel resistance in *T. vitrinus* within seven years of the introduction of the drug to the UK, highlights the need for better understanding of the principles of judicious anthelmintic use (Abbott et al., 2012). The finding is similar to those seen in New Zealand where monepantel resistant *T. circumcincta* and *Trichostrongylus colubriformis* were detected in goats following treatment at one and a half times the sheep recommended dose rate (Scott et al., 2013). Previous reports of monepantel resistance show efficacies of less than 50% against *H. contortus*, in one case following the use of monepantel on a farm on just four occasions (Sales and Love, 2016). However in the case we are reporting here, monepantel had been used on at least 21 separate occasions on the farm, in different age categories, over a three year period. This may reflect different mechanisms of resistance development in different nematode species (Wolstenholme et al., 2004), albeit we do not provide experimental evidence in support of this concept. Alternatively there may have been a protective effect of maintaining an unexposed population *in refugia* at each

191 treatment event, by leaving some animals untreated (van Wyk, 2001). More data would be needed
192 to assess the relationship between anthelmintic resistance and the risk factors here.

193 Without the application of correction factors to account for between species variation in the ITS-2
194 DNA content, extraction efficiency, amplification rate, and, or, copy numbers (Avramenko et al.,
195 2015; 2017), the nemabiome deep amplicon sequencing method was only valid in describing the
196 presence or absence of L_3 in the pre- and post-treatment coprocultures. When supported by albeit
197 crude postmortem parasitology, the approach was nevertheless informative in identifying *T. vitrinus*
198 as the predominant monepantel resistant species, while also demonstrating treatment survival of *O.*
199 *venulosum* and *T. circumcincta*. This latter aspect requires further experimental study. The manner
200 in which pre- and post-treatment proportions of each species were similar, shows selection of the
201 pre-treatment populations through previous monepantel treatments.

202 It is noteworthy that resistance to monepantel emerged in *T. vitrinus*, a species that had not
203 previously, or concurrently been shown to be resistant to any other anthelmintic drug groups on the
204 farm. Previously documented reports of monepantel resistance all involve nematode species that
205 were resistant to multiple other drugs (Scott et al., 2013; Mederos et al., 2014; Sales and Love, 2016;
206 Lamb et al., 2017; Ciuffa et al., 2017), or at least to macrocyclic lactones (Brom et al., 2015). In these
207 cases, the rapid development of resistance to monepantel and poor efficacies achieved against
208 resistant populations might suggest a shared resistance mechanism with one or more of the other
209 drug classes. However, our report differs in that resistance has occurred in an otherwise susceptible
210 species, has taken longer to emerge, and has attained a lower level. This might implicate an
211 independent genetic basis for resistance and is a candidate for future research.

212 In this case we hypothesise that significant selection pressure for resistance in *Trichostrongylus*
213 occurred in the autumn of 2016, when lambs were treated with monepantel and then moved to
214 'relatively clean' grazing without a sufficient susceptible population *in refugia*. The pregnant ewes,
215 which grazed this pasture after the lambs, may have perpetuated or amplified the resistant
216 population during the unusually mild winter of 2016/17. The ewes were then treated with
217 monepantel at lambing and turned out onto 'clean' pasture with their lambs, potentially affording
218 an opportunity for the resistant *Trichostrongylus* to establish as the dominant gastrointestinal
219 nematode population in the ewes, and subsequently contaminate the post-lambing pasture for their
220 lambs. Once the resistant parasites had been selected, there may have been heavy selection
221 pressure for these to survive and outcompete susceptible parasites due to the almost exclusive use
222 of monepantel on the farm. It must be emphasised that this breakdown in *refugia* management did
223 not arise as a result of the farmer's lack of knowledge of risk factors for anthelmintic resistance, or

failure to attempt to implement known mitigation strategies, but was a result of attempting to balance different management priorities within a complex agribusiness.

Trichostrongylosis causes characteristic signs of diarrhoea (Martin and Aitken, 2000), hence targeted anthehelmintic treatments based on signs of diarrhoea or perineal staining might have contributed to the selection of resistance in *T. vitrinus*. This criteria for targeted treatment proved to be flawed in the case reported here, as lambs that were classed as having 'clean' perineal regions had consistently high pre-treatment FECs, all over 1000 epg (Appendix 1).

Helminth species identification is useful for the interpretation of faecal egg count reduction tests, as on each farm different species of helminth have varying levels of resistance to each class of anthelmintic drug (Taylor et al., 2009). Consequently, drugs to which resistance is diagnosed may still be of use when the implicated species is not the dominant cause of disease. For example, in the UK *T. circumcincta* is most often found in late spring and early summer, while *Trichostrongylus* species tend to dominate late summer through to winter (Boag and Thomas, 1977). Therefore, if monepantel is still effective against *T. circumcincta* on a farm, it may still be a useful product for early summer treatments.

This case reiterates the known need for care when combining the use of safe grazing with anthelmintic treatments. There is a need for consideration of the risks this poses, attention to detail and continual, on-going farm health planning. When using 'new' anthelmintic drugs, care must be taken when applying pre-defined strategies for resistance prevention. Monitoring the efficacy of these anthelmintics on a farm is essential to avoid undetected loss of production due to resistance. When performing FECRTs to detect resistance it is important to be aware of which species of worms are present to inform future use of anthelmintics on each individual farm.

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Figures

Fig. 1: Schematic diagram of movements of January born lambs in 2016 (A), Sheep movements and anthelmintic treatments during 2016 (B).

Fig. 2. Faecal egg count reduction for each treatment group. Bars show 95% confidence intervals.

Tables

Table 1: Faecal egg count reduction test results and daily live weight gains.

Table 2: Molecular speciation pools of 1,000 L_3 recovered from of pre and post monepantel treated coprocultures.

Appendices

Appendix 1: Raw data from the faecal egg count reduction test.

Appendix 2: Estimation of lamb performance after treatment with ivermectin, levamisole, monepantel or moxidectin.