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Case report: Macrocyclic lactone resistance on new grass leys-the putative role of accidental “dose-and-move” strategies due to use of persistent macrocyclic lactone products

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Abstract

Nematodirosis and teladorsagiosis/trichostrongylosis were detected in 8-week old lambs on a Scottish lowland farm and treated with oral ivermectin. A drench check identified treatment failure in some pastures but not others. Ivermectin resistance was confirmed by faecal egg count reduction test. Grazing history suggested treatment of ewes with injectable moxidectin in the autumn and oral moxidectin post-lambing and movement onto clean grazing played a role in the selection for macrocyclic lactone resistance on this farm.

Introduction

Parasitic gastroenteritis can produce severe disease and debility in lambs and ineffective control can result in poor lamb growth rates and feed conversion efficacy, impacting on the profitability of sheep-rearing enterprises (Sargison 2012). A major challenge to the control of PGE is the emergence of strains of nematodes which are resistant to anthelmintic drugs. Surveys have suggested that up to 80% of lowland sheep farms in the UK have benzimidazole resistant worm strains (Bartley et al. 2003; McMahon et al. 2013). Resistance to the other commonly used classes of anthelmintics (levamisole and the macrocyclic lactones) is not uncommon (Bartley et al. 2006) and “triple resistance” is increasingly documented (Sargison et al. 2007; McMahon et al. 2013).

Moxidectin is a highly lipophilic macrocyclic lactone endectocide. It is many times more efficacious than ivermectin against ovine gastrointestinal nematodes, being effective against ivermectin resistant strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* (Pankavich et al. 1992; Gopal et al. 2001) and also has a prolonged

residence time (Alvinerie et al. 1998). Moxidectin is used both to suppress the periparturient rise in faecal worm egg counts in ewes at lambing time (Taylor et al. 1997; Sargison, Bartram & Wilson 2012) and, in the injectable formulation, to treat and control sheep scab (*Psoroptes ovis*) (O'Brien et al. 1996; Parker, O'Brien & Bates 1999). It is hypothesised that the persistent action of moxidectin may produce a high selection pressure for resistance both as the prolonged plasma concentration ensures a longer period over which resistant strains have a selective advantage and exposes gastrointestinal nematodes to a period of sublethal concentrations of moxidectin. Use of moxidectin to control the peri-parturient rise in faecal worm egg counts in ewes has been associated with the development of moxidectin resistance where this has occurred with low pasture *in refugia* nematode populations (Sargison et al. 2010).

Risk factors for the emergence and expansion of anthelmintic resistance in gastrointestinal nematodes of sheep relate to behaviours which produce a strong selective advantage for resistant strains. They include so-called "dose and move" strategies where animals are treated and then moved onto clean grazing i.e. grazing uncontaminated by nematode larvae (van Wyk 2001). As the only source of nematode larvae are any resistant worms which have survived the treatment the nematode population on the pasture will have a high frequency of resistant alleles (Waghorn et al. 2008). Frequent dosing gives a greater selective advantage to resistant strains (Barton 1983) while under-dosing or using expired or improperly-stored anthelmintic will expose the nematodes to sub-lethal doses which may increase selection for resistance (Smith et al. 1999).

As the veterinary profession and farming industry have become more aware of anthelmintic resistance advice on minimising further selection for resistance has become more widely publicised (www.scops.org.uk). One of the recommendations includes checking that any treatments administered have been effective by post-treatment faecal egg count (a "drench check"). Here we describe a case of ivermectin resistance which was initially suspected after drench checking and where the efficacy of ivermectin varied between pastures. The grazing history suggested the use of moxidectin for both sheep scab control and to suppress the periparturient rise may have played a role in the selection for ivermectin resistance in this case.

Case presentation

A pooled faecal sample from 8 week old lambs on a lowland farm in south-east Scotland was submitted to the Royal (Dick) School of Veterinary Studies Farm Animal Practice on 22nd May 2013 for faecal worm egg count (FWEC). This was performed by the modified McMaster technique and a count of 450 eggs per gram (epg) trichostrongyle type eggs and 150 epg *Nematodirus battus* eggs obtained. The farmer was thus advised to treat the lambs with either an macrocyclic lactone or levamisole based drench given the widespread presence of benzimidazole resistance among trichostrongyles in lowland flocks in the UK. The farmer was instructed to submit samples 10 days post-treatment to check treatment effectiveness.

Pooled faecal samples were submitted 18 days after treatment with 0.2 mg/kg liveweight ivermectin (Depidex Drench 0.08% w/v Oral Solution for Sheep, Novartis Animal Health UK Ltd) from two groups of lambs- those on permanent leys ("old grass") and those on the "new grass" which had been undersown under barley the previous year. The counts obtained were:

	Trichostrongyle (epg)	<i>N.battus</i> (epg)
Old grass	50	250
New grass	400	0

These results suggested that the drench had been effective on the "old grass" but not on the "new grass". *N.battus* has a pre-patent period of 14-16 days so the presence of *N.battus* eggs 18 days after treatment is not unexpected (Taylor, Coop & Wall 2007). The history of the "new grass" was established more clearly: it had been undersown under spring barley the previous year and had first been grazed by ewes the previous October. Prior to turnout onto this pasture the ewes were prophylactically treated against sheep scab (*Psoroptes ovis*) with injectable moxidectin at 0.2mg/kg (Cydectin 1% w/v Solution for Injection for Sheep, Zoetis UK Ltd.) .The ewes had been removed from the pasture in December and it had then been ungrazed until ewes with 2 week old twin lambs at foot were moved onto it. All twin-bearing ewes were treated on exit from the lambing shed with oral moxidectin at 0.2mg/kg (Cydectin 0.1% w/v Oral Solution for Sheep, Zoetis UK Ltd.) and turned onto permanent pastures near the farm stading, some were then moved onto the new grass pasture. At first these animals had access to another field which had been "new grass" (with an identical usage history) the year before; they were shut off from this field at the end of May.

Investigation

Differential diagnoses for positive trichostrongyle worm egg counts 18 days post-treatment include:

- anthelmintic resistance
- *Haemonchus contortus* infection (as the pre-patent period of *H.contortus* can be as low as 18 days)
- ineffective drenching technique
- underestimation of weights
- use of improperly stored or expired product

To investigate the possibility of *H.contortus* infection purified trichostrongyle eggs from the faecal sample from the "new grass" lambs was submitted to the Moredun Research Institute for fluorescent peanut agglutinin assay (Jurasek et al. 2010). No *H.contortus* eggs were detected.

The calibration of the dosing gun used for the administration of the ivermectin was checked and found to be accurate. The product was within date and had been stored according to the manufacturer's instructions.

A faecal egg count reduction test was performed on the "new grass" lambs. On 17th June 2013 10 lambs were individually identified, faecal samples taken from the rectum and they were dosed to individual bodyweight by syringe with 0.2 mg/kg ivermectin (Depidex Drench 0.08% w/v Oral Solution for Sheep, Novartis Animal Health UK Ltd). This process was repeated with a further 10 lambs which were drenched with levamisole at 7.5 mg/kg bodyweight (Levacide Drench 3% Oral Solution, Norbrook Laboratories). Individual FWECs were performed. The farm was revisited 10 days later and the lambs sampled again. Individual FWECs were repeated. The mean pre- and post-treatment counts for the two groups are displayed in Figure 1. The percentage reduction in trichostrongyle worm egg count for each animal was then calculated using the formula $(T1-T2)/T1 \times 100$ where T1= count pre-treatment and T2= count post-treatment. The

arithmetic mean for each treatment was then calculated. These are displayed below and in Figure 2:

	Mean % reduction	95% confidence interval
Ivermectin	8.5	65.6, -48.6
Levamisole	98.8	104.7,92.8

Results

The threshold for resistance is considered to be 95% efficacy, with the 95% confidence interval including 90% (Coles et al. 1992). In this case the mean percentage reduction and confidence interval of levamisole do not suggest resistance, while the reduction in FEC in the ivermectin treatment group was not statistically significantly different to zero ($p=0.555$, 1-sample t-test, Minitab 16).

The farmer was advised to use levamisole for treatment of the lambs on the new grass and most importantly to treat them with levamisole before they left the new grass for pastures elsewhere on the farm at weaning to avoid the spread of the highly ivermectin resistant strain of trichostrongyle nematode to other pastures on the farm. The farmer's attention was drawn to the SCOPS principles (www.scops.org.uk) and the farmer was advised that any future new grazing should in the first instance be grazed by untreated animals or that at least 10% of animals turned onto the grazing should be left untreated.

In this case a strain of trichostrongyle highly resistant against ivermectin (and by inference other macrocyclic lactone anthelmintics) was found after a post-treatment faecal egg count indicated treatment failure highlighting the need to monitor the performance of anthelmintics.

Discussion

Given the very low efficacy of ivermectin it seems likely that this resistant strain is the sole contaminant of the pasture. This strain is likely to have been highly selected as any L3 on the pasture have their origin in nematodes which either survived treatment with injectable moxidectin in the autumn or which survived oral moxidectin treatment in spring and were deposited after the ewes were moved onto the pasture 1-2 weeks post-lambing. As ivermectin and moxidectin share mode of action (Shoop, Mrozik & Fisher 1995) and moxidectin is widely considered the more potent of the two against the common gastrointestinal nematode parasites of sheep, selection for moxidectin resistance would result in ivermectin resistance. Ivermectin resistance in a moxidectin resistant *Teladorsagia circumcincta* population has been previously reported (Sargison et al. 2010)

Use of endectocides for sheep scab prophylaxis requires that the entire flock be treated to avoid leaving reservoirs of scab mites in untreated animals which could then re-infect the flock (O'Brien et al. 1996; Williams and Parker 1996). This does not leave a within-animal refugia population i.e. a population of nematodes unexposed to the anthelmintic. Thus the on pasture *in refugia* population is the only unexposed population and must be maintained (Waghorn et al. 2008, 2009). In this case there was no on pasture *in refugia* population. Even on permanent pasture the on pasture *in refugia* population may be low depending on prior grazing history and climactic conditions; sheep scab prophylaxis is

often administered in the autumn/winter when pasture larval levels are falling (Taylor, Coop and Wall 2007). Due to the persistent nature of moxidectin (and, indeed, doramectin) animals must remain on contaminated pasture for the entire period of the persistence of the product in order not to be passing resistant worm eggs onto clean grazing. Even at the end of the period of product action the within-animal susceptible population may be low as ewe immunity is high (Vlassoff, Leathwick and Heath 2001) and infection pressure from the pasture is low so the establishment of large numbers of susceptible nematodes in the sheep is unlikely. In this case the ewes had been turned onto the new grass shortly after scab treatment.

Drenching of ewes with a persistent product to suppress the peri-parturient increase in worm egg counts and so reduce pasture contamination to reduce larval challenge to lambs later in the season is common practice (Taylor et al. 1997, Wilson et al. 2008, Sargison, Bartram and Wilson 2012). In order to reduce selection pressure it is recommended that single-bearing ewes (which usually have lower worm egg counts due to better immune status) are not treated and that this procedure only be done when ewes are turned out onto "dirty" pasture. In this case the farmer had followed all this advice- only the twin-bearing ewes had been treated with moxidectin and all went out onto dirty pasture. However a group of twin lambs and their mothers were moved shortly after onto the new grass. Due to the persistent action of moxidectin these ewes had not acquired any susceptible nematodes and so once again the only source of nematodes eggs were resistant worms.

In both cases the initial treatment may have eliminated all adults from the gastrointestinal tract but the dose-limiting stage for many nematodes species is the L3 larvae so re-infection with a resistant strain which was subsequently transported onto the clean pasture is possible (Dobson, Lejambre and Gill 1996).

Reseeded pastures will initially have very low levels of parasitic nematode contamination (though survival of nematode larvae on unploughed field margins is possible). As such they have the potential for maximising production from grazing alone (both because of the absence of a parasite challenge and because the reseeding allows for the optimisation of the species composition of the sward). However the absence of a nematode population on the pasture means there is no pasture *in refugia* population. Thus care is needed to avoid selecting for anthelmintic resistance. Options to avoid this include:

- Not treating the sheep at all when moving onto the pasture. If nematode populations in the animals are low and the residence time on the pasture will be limited this is a viable option as pasture larval levels will not have built up to a growth limiting level by the time the sheep are moved off.
- Delaying drenching the sheep until after they have grazed the pasture for several days. This establishes a pasture *in refugia* population prior to treatment. The number of days required depends on how large the nematode egg output is and how effective the anthelmintic is (as the aim is to ensure the "unselected" nematode population dwarfs the nematodes which survived treatment).
- Leaving a percentage of animals untreated when moving onto the pasture, again the percentage required to be left untreated will vary with the effectiveness of the anthelmintic used (a figure of 10% is given for wormers assumed to be near 100% effective). (See www.scops.org.uk for more details).
- Drenching the sheep and leaving them where they are to be come reinfected by "unselected" larvae before moving to the reseeded pasture. The number of days delay before movement will be dependent on the level of larval contamination on the prior pasture i.e. how rapidly the sheep are reinfected, and the effectiveness

of the anthelmintic. This approach will not work with persistent products e.g. moxidectin.

There are several limitations to this case report- no control group was included in the faecal egg count reduction test, the effectiveness of ivermectin on the permanent pasture was assumed from the drench check and not investigated by faecal egg count reduction test and the species composition before and after treatment in the FECRT was not established so the resistant species(es) were not identified. While desirable these further investigations were unacceptable to the farmer due to the further time and cost involved, especially as the limited investigation described above had already answered all the questions the farmer had- does the product work and, if not, which alternative does?

Conclusion

This case highlights the failure in the application of general guidelines to a specific farm. It also illustrates the importance of considering different pastures in a single farm as potentially different as regards the resistance profile of the gastro-intestinal parasitic nematodes on them depending on their grazing history. Advice to leave single-bearing ewes undosed will not preserve an *in refugia* population in every field if twins and singles are managed separately- as on most farms. Turning sheep onto dirty grazing post-treatment before moving onto clean grazing will only establish susceptible as well as resistant worms on the pasture if the product used has no persistence. Drench checking can serve as an early warning of resistance problems on pastures allowing their further investigation and the implementation of more nuanced control measures. Use of injectable macrocyclic lactone endectocides is a potential risk factor for the development of anthelmintic resistance and farmers must be made aware that dose and move strategies whether deliberate or accidental should be avoided wherever possible due to the high selection pressure for resistance they produce. Ultimately chemotherapeutic measures alone are unsustainable for the control of nematode parasites and more sophisticated strategies are required.

Key Points

- Anthelmintic resistance profiles may differ between pastures on the same farm.
- Persistent anthelmintics can result in accidental "dose and move" incidents unless close attention is paid to animal movements.
- Use of injectable macrocyclic lactones for sheep scab control may contribute to the development of macrocyclic lactone resistance in the nematode population.
- "Drench checks" are valuable tools for the early detection of potential anthelmintic resistance problems.
- Establishing the efficacies of different anthelmintic classes allows for informed planning of control strategies.

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Legends

Figure 1.

This figure shows the mean faecal worm egg counts (eggs per gram) for the two test groups before treatment and 10 days post-treatment.

Figure 2.

This figure shows the mean percentage reduction in FWEC following treatment with ivermectin and levamisole. The error bars denote the 95% confidence intervals.