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Research paper

The development of anthelmintic resistance with best practice control of nematodes on commercial sheep farms in the UK

Jane Learmount*, Nathalie Stephens, Valerie Boughtflower, Alba Barrecheguren, Kayleigh Rickell

Animal and Plant Health Agency, Sand Hutton, York YO41 1LZ, UK

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ABSTRACT

Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections. The widespread development of anthelmintic resistance is a major global issue affecting the effective control of parasite diseases in grazing livestock production. Sustainable control strategies that reduce dependence on antimicrobials have the potential to slow the further development of resistance but there is little data on the effect of control strategies on resistance development in the field. This report documents a study undertaken to measure the temporal effect of the UK sustainable control of parasites in sheep (SCOPS) guidelines on the development of anthelmintic resistance. Farms carrying out SCOPS or traditional worm control (TRADITIONAL) were tested for resistance to the benzimidazole and imidazothiazole anthelmintics *in vitro* using a discriminating dose (dd) larval development test (LDT) in year 1 and then 7 years later. In years 5 and 7, resistance was also measured using a dose-response LDT assay. There was a significant increase in *Teladorsagia* survivors at the benzimidazole dd assay between year 1 and year 7 for both treatment groups, but the increase in survivors was greater for the farms carrying out their traditional worm control compared to the SCOPS farms. There was also a significant difference between benzimidazole dd results generated across years for *Trichostrongylus*, but the year and treatment interaction was not significant. Only one of the farm *Teladorsagia* populations had survivors in the imidazothiazole dd assay in years 1 and 7 and none of the *Trichostrongylus* populations survived in year 1 compared to isolates from three of the farms in year 7. Dose-response data showed a significant effect for time for both the benzimidazole and imidazothiazole anthelmintics and the increase was again significantly higher for the *Teladorsagia* populations in the TRADITIONAL group compared to the SCOPS group. This data suggests an increased sensitivity both to detect and to measure changes in response to anthelmintics with the dose-response assay compared to the dd and this is important particularly when allele frequencies are low as might be the case when novel compounds are released to the market. Anthelmintic use across years 5–7 was significantly lower for the farms in the SCOPS group compared to the TRADITIONAL group and farmers in the SCOPS group had selected products from the benzimidazole group less often than farmers in the TRADITIONAL group. Both groups had made minimal use of the imidazothiazole anthelmintic classes and the majority of ewe treatments were selected from the macrocyclic lactone class. Further research is required to determine the effect of these anthelmintic choices on the development of resistance to the macrocyclic lactones.

1. Introduction

Antimicrobial resistance now ranks as one of the most important global health concerns of our age and threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi (WHO, 2014). Parasite diseases are widely acknowledged as a major economic threat to grazing livestock production worldwide and so the widespread development of anthelmintic resistance (dos Santos et al., 2014; Geurden et al., 2014; Karrow et al., 2014) is a major global issue severely affecting their control. This is of particular concern due to the need to produce more protein in a world with a rapidly expanding human population. Developing and monitoring the effectiveness of interventions is critical to the global effort to slow further resistance and will contribute to ensuring the sustainability of our livestock production. Developing and optimising sustain-

* Corresponding author.
E-mail address: jane.learmount@apha.gsi.gov.uk (J. Learmount).

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able control strategies is particularly critical when promoting the use of much needed novel drug classes that may be discovered now and in the future. Research, particularly in the UK, Australia, South Africa and New Zealand, has resulted in the recognition of potential resistance-delaying strategies for anthelmintics that can be used on farms (Coles, 2002, 2003; Gaba et al., 2006; Michel, 1985; Van Wyk, 2001) and high and low risk practices have been identified. National guidelines for sustainable control of parasites in sheep (SCOPS) were produced in 2004, incorporating all available evidence for best practice control, with the aim of slowing the development of anthelmintic resistance on UK sheep farms (Abbott et al., 2012). Promotion and revision of the guidelines is ongoing and facilitated by the SCOPS committee, with broad membership representing industry and researchers in the field. This kind of coordinated national approach is critical to ensure maximum uptake and to minimise the potential for the spread of resistance alleles, for example through movement of sheep between farms via breeding stock or common grazing. However, the guidelines are based on data often derived from experimental infections and largely with single species in controlled environments. There is little data on the effect of the guidelines on resistance development in the field in the many commercial settings that exist in the UK. Such data is essential not only to better understand how resistance is selected for in the farm environment, which in turn should allow the optimisation of resistance delaying strategies, but also to convince farmers of the benefits and to guide policy on the safe and effective use of veterinary medicines.

This report, therefore, documents a study undertaken to measure the temporal effect of the SCOPS guidelines on the development of anthelmintic resistance. Sixteen farms were engaged to the study with 14 of these being part of a previous study carried out between 2007 (year 1) and 2010 when resistance to the benzimidazole and imidazothiazole drugs were tested as part of an extension study to promote and monitor the uptake of the SCOPS guidelines on UK sheep farms. Half of the farms carried out SCOPS control and half carried out the traditional worm control that had been used on the farms for many years. In 2012 (year 5), the farms were engaged on a second 3-year study to evaluate the effect of the SCOPS guidelines in practice and the SCOPS guidance was intensified, while resistance to the benzimidazole and imidazothiazole anthelmintics was further investigated.

2. Materials and methods

2.1. Selection of study farms

All study farms were those previously reported by Learmount et al. (2015). In year 1, data were from 14 of the farms, which were part of a wider cohort (n = 30) of farms engaged to a study that aimed to provide a qualitative evaluation of the practicality and effectiveness of the SCOPS guidelines in practice by deploying them across a network of representative farms. Farms were assigned to one of two experimental treatments: 1. SCOPS, for farms that were already using or were willing to implement the SCOPS guidelines; and 2. TRADITIONAL, for farms that wished to continue employing their traditional worm control without regard to SCOPS guidance. The farms were self-selecting to treatment group: farmers were given information about the trial and then, if they wished to participate, selected whether they did or did not wish to carry out worm control using SCOPS guidance. The SCOPS guidelines advocate a 'toolbox' of resistance delaying control methods, with their deployment dependent on individual farm requirements. Hence, evolving strategies were devised for each farm based on veterinary advice. A network of veterinarians was, therefore, established at the start of the project, with each vet visiting and monitoring their assigned farms at least ten times across a three-year period. Results of this study demonstrated some reduction in anthelmintic use and no significant difference in infection levels in the lambs between the two groups. However, data clearly demonstrated that farmers had not used all of the potential resistance delaying strategies advocated by the SCOPS guidance. Fourteen of the farms (as well as an additional two) were then engaged to a second study (Learmount et al., 2015) in 2012, which aimed to intensify the intervention and collect robust evidence of outcomes relevant to policy makers and industry (years 5–7). The farms were selected based on treatment group, farm type and region to allow a balanced factorial design. Farmers in the SCOPS treatment group pro-actively adopted low-risk management practices while farmers in the TRADITIONAL treatment group were known to have adopted several high-risk management practices during the first study. Further detail is described by Learmount et al. (2015). As before, all study farms had a private veterinarian responsible for animal welfare, and sample and data collection who also developed a formalised farm plan for worm control and advised on diagnostic results for each of the SCOPS farms. As two other factors (Region and Farm Type) might have affected the epidemiology of gastrointestinal worms (Coyne et al., 1991; Crofton, 1965; Gibson et al., 1981), these were equally represented in SCOPS and TRADITIONAL treatment groups. Regional (South west or North east) grouping was carried out to account for the possible effects of climate on the measured effects and farms were divided for type (Lowland or Upland) using the criteria previously described (Learmount et al., 2015).

2.2. Evaluation of anthelmintic resistance

In year 1, discriminating dose (dd) larval development tests (LDT) were conducted using 0.1 μg/ml thiabenzidole or 1 μg/ml levamisole as these doses are reported to be minimum inhibitory concentrations (MIC's) for susceptible *Teladorsagia* and *Trichostrongylus* (Taylor et al., 2009). In years 5 and 7, dose response assays were carried out as evidence gathered during the study suggested that this may be a more sensitive method for determining smaller changes in drug sensitivity over time. In both cases, the LDT used a protocol based on the method originally described by Taylor (1990). Eggs used in the assays were harvested from faecal samples collected from ewes prior to treatment each season. Where possible, the same samples from each farm were used for the tests with levamisole and thiabenzidole at each of the time points. For each sample, larvae were exposed to thiabenzidole or levamisole, as well as left untreated (controls), using the following protocol. Stock solutions were prepared for the dd tests by dissolving drugs in methanol to give a final exposure concentration of 0.1 μg/ml thiabenzidole or 1 μg/ml levamisole. In years 5 and 7, a range of appropriate concentrations for each farm population, with the aim of killing between 5 and 10% at the lowest and 90–99% of the worms at the highest concentration, were prepared for the dose response assays. Doses ranged between 0.013 and 0.8 μg/ml for thiabendazole and 0.003 and 0.4 μg/ml for levamisole. The discriminating dose (dd) of 0.1 μg/ml was incorporated into the dose ranges for thiabenzidole, and an additional dose of 1 μg/ml used for levamisole for all respective dose response tests, to allow comparison of data over time. A 0.075% solution of lyophilized *Escherichia coli* (Sigma-Aldrich) was mixed with an equal volume of sieved sterile, worm free sheep faecal material in solution (25 g faeces: 85 ml water) and 1 ml of water containing the harvested eggs at the appropriate concentration to give 50–60 trichostrongyle eggs per assay. Aliquots of 190 μl of the solution were added to each well of a 24-well plate, shaking the egg suspension well between each aliquot to ensure even dispersal of the eggs. A 10 μl aliquot of each prepared drug solution was then added to each of the wells and methanol alone was added to the control wells. For each assay, 4 replicates were prepared for