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Investigating the perceived versus actual gastrointestinal nematode challenge on extensive sheep farms

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Abstract

Extensive farming systems form an integral part of sheep production systems across Europe. However, with innate production handicaps, declining sheep numbers and narrow economic margins, production is becoming increasingly challenging threatening the future sustainability of the industry. Gastrointestinal nematodes (GINs) are a significant cause of production losses to the global sheep industry, with well-established resistance to the major anthelmintic groups. Traditionally, extensive farming systems are not thought to have a significant parasite challenge compared with intensive farms, but there is a need to identify the scale and importance of GINs on extensive farms to inform the need for sustainable control strategies. In this study, a questionnaire of extensive farmers (n=34) was conducted and parasitological data were collected from nine study farms to investigate the perceived versus actual GIN and anthelmintic resistance challenge faced by extensive farms. The results showed a production-limiting challenge on most farms, with a higher GIN challenge observed on improved pastures. Furthermore, over half of the extensive farmers perceived anthelmintic resistance to be a greater problem for intensive farmers, with only 20% of respondents reporting known anthelmintic resistance. However, all study farms had evidence of resistance to at least one group of anthelmintics. Consequently, this study has demonstrated that despite the traditional perception of parasitism on extensive farms, there is a need to increasingly consider its impact and take a proactive approach to sustainable control, with solutions tailored to their unique management.

Keywords:

gastrointestinal nematode; anthelmintic resistance; sheep; Extensive hill and upland farms; Nemabiome

1. Introduction

Extensive farming systems are an integral part of the sheep industry across Europe and beyond representing over half of the utilised agricultural area (Eliasson et al., 2010). The small ruminant population (sheep and goats), in the European Union (EU) in 2015 was just over 98.5 million animals, of which 87% were sheep (Eurostat, 2022). Extensive farming systems face many innate production constraints including climate, topography and grassland quality, which limit the agricultural potential of the grazing to sheep and suckler cattle (Barnes et al., 2020). Despite the importance of extensive farming systems in maintaining communities and preventing land abandonment (MacDonald et al., 2000), the sheep flock in Europe has declined over the past two decades (Eurostat, 2022; SRUC, 2008). This has been attributed, at least in part, to narrow economic margins (Bohan et al., 2017), and further compounded by issues such as lack of labour and changing agricultural policies (Morgan-Davies et al., 2015; Reed et al., 2009). However, the delivery of livestock production in these areas remains a clear priority for stakeholders (Morgan-Davies and Waterhouse, 2010) thus the development of a resilient and efficient production system will be key to protecting the future of sheep production on extensive farms.

Gastrointestinal nematodes (GINs) are a leading cause of production loss in sheep worldwide, with the costs associated with treatment and productions losses exceeding £40 million per year in the United Kingdom (UK) alone (Charlier et al., 2020). This is compounded by increasing resistance to the major anthelmintic groups (benzimidazoles, imidazothiazoles and macrocyclic lactones) used to treat these infections, particularly on lowland (intensive) farms (Rose et al., 2015; Sargison et al., 2007; Vineer et al., 2020). This threatens the industry's future ability to effectively manage the impact of GIN infections in sheep. Consequently, recent research has focused on the development of sustainable control methods to preserve our long-term ability to manage these parasites through methods such as targeting anthelmintic treatments, managing larval exposure and the concept of maintaining populations in *refugia* (Greer et al., 2020; Kenyon et al., 2009a).

Traditionally, extensive farms were not regarded as having a significant GIN challenge (Hong et al., 1996; Mitchell et al., 1991), and thus research has focused on the development of strategies for more intensive production systems (Greer et al., 2020; Kenyon et al., 2009a). Extensive farms are primarily composed of rough hill and/or upland grazing, with limited improved pastures, which are used throughout the year for animal handling and lambing. It is often challenging for farmers to gather animals in for handling; therefore, sheep are often only gathered at set times of year for management events such as shearing, weaning, and mating.

Due to the lack of a perceived challenge, anthelmintic treatments on extensive farms largely coincide with other planned management events (Morgan-Davies et al., 2018). As suggested by Morgan et al. (2012), the first barrier to the adoption of sustainable practices may involve altering farmer's perceptions of anthelmintic resistance as a problem on their farm. Previous studies have demonstrated that awareness of sustainable worm control increases when farmers are facing a confirmed resistance issue (Cornelius et al., 2015). However, the uptake of solutions is slow, requiring many resources to overcome significant barriers (Jack et al., 2017) and facilitate a long-term behaviour change (Vande Velde et al., 2018).

With widespread anthelmintic resistance and climate change impacting parasite populations (Kenyon et al., 2009b), there is a need to identify the scale and importance of GINs on extensive farms to inform the need for more sustainable control strategies. This study aims to firstly understand the GIN challenge, as perceived by extensive sheep farmers, and describe the actual GIN challenge faced by extensive sheep farms through the evaluation of GIN burden, species composition, and anthelmintic efficacy. This was achieved using an initial questionnaire, followed by more detailed work with study farms.

2. Materials and Methods

2.1 Questionnaire

An online questionnaire hosted on the JISC online survey platform (JISC, 2023) was developed to investigate current gastrointestinal parasite control strategies and the perception of anthelmintic-related issues faced by extensive hill and upland sheep farmers in Scotland. The questionnaire underwent initial piloting by five farmers to ensure questions were easily understood and to calculate an average completion time.

The final questionnaire was launched in March 2021, comprising 63 questions relating to aspects of gastrointestinal parasite management (incorporating liver fluke management in addition to GINs). Data collected included farm demographics, current control strategies, anthelmintic use, perception of resistance and barriers to sustainable management. Dissemination of the questionnaire was predominantly performed on social media through multiple agricultural organisations and independent consultants, with further dissemination occurring in print from the farming press. The survey was closed after nine months. For the purposes of this study, a subset of 14 questions was analysed. The subset of questions is available in Supplementary Material 1.

2.2 Study farms

Nine extensive hill and upland sheep farms were recruited between May and June 2021 from both questionnaire respondents and adverts in the farming press. All selected farmers were based in Scotland, farmed a minimum 40 hectares, which comprised of predominantly rough grazing (obtained from questionnaire data that was self-reported by farmers), with a minimum of 100 breeding ewes to ensure flocks were commercially relevant. The recruited farms were geographically spread across Scotland (Figure 1), varying in scale and enterprise type (Table 1) and typical of extensive farming systems in Scotland. Flock sizes ranged from 120 to 2400 breeding ewes (mean = 869 ewes), and land areas from 50 to 3995 hectares (mean = 1019 hectares). As shown in Table 1, six of the farms had mixed land types and three farms were exclusively either hill or upland (with predominantly rough or semi-improved grazing, respectively). Eight farms had traditional UK hill breeds, either Scottish Blackface or North Country Cheviot, as their main breed type. As is commonplace, most of the study farms divided animals into multiple management groups. These groups were grazed in different areas within the same farm, but were not completely independent, changing throughout the year.

[Insert Figure 1 around here]

[Insert Table 1 around here]

2.3 Study farm sample collection & faecal egg count

Throughout one full grazing season (June to November 2021), farmers were asked to collect 15 freshly voided faecal samples from the ground from each group of lambs at point of treatment (pre-treatment) to monitor GIN burden (faecal sampling protocol available in Supplementary Material 2). Subsequently, to test anthelmintic efficacy, a further 15 samples from the same group were collected 7 days post-treatment for imidazothiazole treatments and 14 days post-treatment for treatments with the remaining anthelmintic groups (COMBAR, 2021). Due to management constraints, the pre- and post-treatment samples were unpaired (not originating from the same animals), but always originated from treated animals within the same group.

All samples were collected in polythene bags, tightly rolled for anaerobic storage, and mailed to the laboratory (Moredun Research Institute), typically arriving within 1-2 days (maximum = 7 days). When submitting faecal samples for testing, further information on management group, date of treatment, anthelmintic product used, and type of grazing (improved or rough) the sheep had grazed for the two to three weeks prior to sampling was also collected on the submission sheet (Supplementary Material 2).

At the laboratory, individual faecal egg counts (FECs) were carried out to identify strongyle and *Nematodirus spp.* eggs using a modified salt flotation cuvette method (Jackson and Christie, 1972), with a detection threshold of up to 1 egg per gram (epg). To increase the statistical power of the faecal egg count reduction test, in line with the current COMBAR (Combatting Anthelmintic Resistance in Ruminants; https://www.combar-ca.eu) guidelines (COMBAR, 2021), where less than 200 eggs were counted cumulatively across all 15 pre-treatment samples, a second subsample was counted for each sample within the submission (to reach a cumulative total exceeding 200 eggs). Where two subsamples had been counted pre-treatment, two subsamples were also counted for the corresponding post-treatment samples.

2.4 Retention of strongyle eggs for species identification

From each submission, strongyle eggs were retained for genomic DNA extraction and internal transcribed spacer-2 (ITS-2) species identification. If there were calculated to be more than 1000 eggs across the cuvettes counted for that submission, the contents of the cuvettes were passed across a 38 µm sieve. The retentate was rinsed with tap water then centrifuged at 203x g for 2 minutes. The supernatant was removed down to 1ml, then a 50 µl sub-sample was counted using the method in section 2.3. The number of eggs in 1 ml was calculated, and the eggs were stored as 1000 egg aliquots at -20°C in water. Where fewer than 1000 eggs were retained from cuvettes, a mass extraction of nematode eggs was performed as in Melville et al. (2020), with eggs collected on a 38µm sieve and stored as previously stated.

2.5 Genomic DNA extraction and ITS-2 deep amplicon sequencing

DNA lysates were prepared from retained strongyle eggs. Aliquots were first centrifuged for 4 min at 203x g, and excess water removed. These were then freeze-thawed in liquid nitrogen (lN_2) and transferred to 1.5 ml Eppendorf tubes using 50 µl of a 1:1 solution of MagMAX™ CORE Lysis buffer and 1x PBS. Proteinase K was added to reach a working concentration of 20 mg/µl, and the samples were incubated on a thermoshaker at 55°C, 450 rpm for 3.5 hours (Avramenko et al., 2015). Following the initial lysis step, purification was performed using the MagMAX™ CORE Nucleic Acid Purification Kit and MagMAX™ Express-96 Deep Well Magnetic Particle Processor, following manufacturer's instructions.

The initial PCR amplification of the rDNA ITS-2 region was performed using universal adapter primers prepared to 10 µM by creating an equal mix of the four forward and reverse adapter primers (Avramenko et al., 2015). The initial PCR used the following reagents: 5 µl 5X reaction buffer, 1.25 µl of each 10 µM forward and reverse adapter primers, 0.5 µl 10mM dNTPs, 0.25 µl 0.1 U/µl DNA polymerase enzyme, 14.75 µl nuclease-free water, and 2 µl genomic DNA. Thermocycling conditions were: 98°C for 30 s, then

40 cycles of 98°C for 10 s, 62°C for 15 s and 72°C for 25 s, before a final 2 min at 72°C. Successful amplification was confirmed using a 2% agarose gel. PCR products were subsequently purified using AMPure XP Magnetic Beads, following manufacturer's instructions.

The barcoded PCR amplification for sample identification was performed using the Illumina Nextera XT DNA Index Kit v2 adapters with 1.25 µl of each forward and reverse adapter 10 µM primer, 3 µl purified PCR product and 13.75 µl nuclease-free water. Thermocycling conditions were: 98°C for 45s, then seven rounds of 98°C for 20 s, 63°C for 20 s and 72°C for 2 min. PCR products were subsequently purified using AMPure XP Magnetic Beads, following manufacturer's instructions.

Library quantification was performed using the QuantiFluor ONE dsDNA System on the GloMax Discover microplate fluorometer. An equal concentration of purified PCR product was then pooled and submitted for Illumina MiSeq sequencing to Edinburgh Genomics, University of Edinburgh.

Sequences were analysed using a modified Command Prompt pipeline performed in Mothur v.1.46.1 as in Evans et al. (2021), and aligned to an ITS-2 reference library described by (Avramenko et al., 2015). Taxonomic levels with less than 100 reads per sample were removed, along with samples with fewer than 2000 total reads.

2.5 Data processing and statistical analysis

All data processing, visualisations and statistical analyses were compiled using R v4.2.1 and RStudio version 2022.07.2+576 "Spotted Wakerobin" (R Core Team, 2020). All visualisations were prepared using the packages 'ggplot2' v3.4.0 (Wickham, 2016) and 'cowplot' v1.1.1 (Wilke, 2020).

Questionnaire responses were initially screened, using the demographic information, to ensure all responses originated from extensive farms with predominantly hill and/or upland land types, located in

Scotland. Initially, descriptive statistics of the demographic information and current roundworm control were prepared. A Kruskall-Wallis test was then applied to compare how hill and upland farmers ranked the importance of anthelmintic resistance amongst the different farm types (hill/upland and lowland), compared to the perceived importance of anthelmintic on hill and upland farms only.

For the study farm data, the percentage reduction and bootstrapped 95% confidence intervals for the unpaired faecal egg count reduction test were calculated using the function 'fecrtCI' from the 'eggCounts' package version 2.3-2 (Torgerson et al., 2014; Wang, 2022). Due to the large variation in FEC data originating from different farms, a locally estimated scatterplot smoothing (LOESS) curve with 95% confidence interval (CI) was used to visualise trends in FEC data over time.

The species-assigned reads from the ITS-2 sequencing were multiplied by previously validated species-specific correction factors (Avramenko et al., 2017; Redman et al., 2019) prior to analysis. Species were presented as a proportion of FEC by multiplying the arithmetic mean FEC for each submission and then plotted using a LOESS curve. To calculate the species-specific percentage reduction, the proportion of each species present was expressed as eggs per gram (epg) of faecal material using the arithmetic mean FEC per submission, and these values were subsequently used to calculate the percentage reduction for each treatment. To improve the robustness of the calculations, only species which represented over 10epg of the pre-treatment FEC were included.

Using the 'glmmTMB' and 'lme4' packages (Bates et al., 2015; Brooks et al., 2017), a zero-inflated negative binomial generalised linear mixed model (ZINB GLMM) was used to measure the impact of the fixed effect of week on FEC, with a random effect of farm. In addition, a further ZINB GLMM was used to examine the effect of grazing type (rough, semi-improved and improved) on FEC. This model used fixed effects of both week and grazing type, with a random effect of farm. Management group was not included as a random effect to improve model convergence as groups were not consistently maintained throughout the sampling period. Furthermore, due to sparse data after October 2021, only data prior to October 2021 was included in the model to improve model convergence. The chosen model was selected using the Akaike information criterion (AIC), and the resultant model's performance and fit was examined using the 'DHARMa' and 'performance' packages (Hartig, 2022; Lüdecke et al., 2021).

3. Results

3.1 Questionnaire responses

In total, 34 questionnaire responses were received. Of these, three responses were excluded due to having only improved grazing or being located outside of Scotland. Of the 31 valid responses, there was a mix of respondents with solely hill, upland, or mixed hill/upland land types (Table 2). The average flock size was 864 breeding ewes (range: 30-3100) across an average of 1998 hectares (range: 6-12820). In total, 70% of respondents kept traditional Scottish hill breeds (Scottish Blackface and North Country Cheviot), with a smaller proportion (16.7%) keeping cross breeds (including mules). The type of sheep enterprise was largely commercial, selling lambs for meat production. This was split between respondents who sold 'finished' lambs (at slaughter weight) direct from the farm and 'store' lambs (sold-on to be finished to market weight by another producer) (Table 2).

[Insert Table 2 around here]

When asked about their current GIN treatment strategy for lambs, over half of respondents treated 'as required' based on clinical signs or FEC testing (Table 2), with a further 30% treating when animals were gathered for other management tasks (such as shearing). Four respondents used a combination of treatment strategies. Of those treating 'as required' or when gathering for other management tasks (n = 24), 54.2% (n = 13) based their decision to treat lambs on FEC results, a further 54.2% on dirty-tail ends, 25% (n = 6) on body condition scores and 12.5% (n = 3) on missed productivity targets. Eight respondents also specified they used other treatment criteria, including the SCOPS (Sustainable Control of Parasites in Sheep) *Nematodirus* forecast (n=2), general appearance of stock (n = 1), weather conditions (n=1) and proximity to other management events (n=1).

On average, respondents administered 3 anthelmintic treatments to lambs across one production year (range: 1-7). All respondents treated for roundworms, and 80% of farmers (n = 24/30) also treated specifically for *Nematodirus spp*. The anthelmintic groups and frequency of use in one production year are shown in Table 3. Benzimidazoles (1-BZ) were the most frequently used anthelmintic group, used by 87.1% (n=26) of respondents, followed by macrocyclic lactones (3-ML) which were used by 61.3% (n=19) of respondents. Imidazothiazoles (2-LV) were used by 45.2% (n=14) of respondents. The two newer groups, amino-acetonitrile derivatives (4-AD) and derquantel in combination with abamectin (5-SI), were utilised by 19.4% and 3.2% of respondents, respectively.

Anthelmintic resistance had been confirmed, using faecal egg count reduction tests, on 20% of farms (n=6). On four of these farms, resistance was confirmed to 1-BZ, and on the remaining two to 2-LV and 3-ML, respectively. No farms reported confirmed resistance to multiple groups.

[Insert Table 3 around here]

Figure 2 illustrates the importance of anthelmintic resistance on hill and upland farms as perceived by hill and upland farmers, ranked from somewhat important to very important. This was further grouped by the respondent's perception of the significance of anthelmintic resistance between different farm types (hill/upland and lowland), illustrating that farmers who ranked anthelmintic resistance as less important believed anthelmintic resistance was a more significant challenge on lowland holdings. However, farmers that perceived anthelmintic resistance as of greater importance were more likely to specify that anthelmintic resistance was of equal significance to both farm types ($p < 0.001$), with one respondent indicating they perceived anthelmintic resistance to be a more significant challenge on hill/upland farms.

[Insert Figure 2 around here]

3.2 Study farm roundworm challenge

A total of 484 samples from 31 pre-treatment submissions were received from the study farms across the 2021 grazing season. Generally, the strongyle FECs observed were below 500 epg, with good agreement between farms. An overall increase in the average FEC was observed across the season ($p < 0.001$), with one notable dip mid-season, around August (Figure 3). In July, the mean count was 169 epg (range: 0-909 epg), dropping to 142 epg (range: 0-1494 epg) in August, and subsequently increasing to a peak of 340 epg (range 7-2466epg) across October and November. *Nematodirus spp.* infections were observed on all farms, peaking at the start of sampling in late-May with a mean of 421 epg (0-1476 epg), which dropped to a mean of 48 epg (range: 0-468 epg) in August. Late-season, *Nematodirus* FECs were variable (range: 0-945 epg).

[Insert Figure 3 around here]

ITS-2 deep amplicon sequencing species identification was successfully performed for 57 submissions: all 31 pre-treatment, and 26 post-treatment. Detail of the individual submissions is shown in Supplementary Material 3. The strongyle species composition of pre-treatment submissions across the grazing season for all study farms is displayed in Figure 4. *Teladorsagia circumcincta* was the most abundant species between May and October. In May, *T. circumcincta* represented an average of 97.8% of the species composition (94.7%-99.9%). Later in the grazing season however, the average proportion of *T. circumcincta* decreased to 49.2% and 41.7% in September and October, respectively. Conversely, *Trichostrongylus vitrinus* represented an average of only 0.8% of the species composition in May, increasing to an average of 40.4% and 32.8% in September and October, respectively. In November, the average proportion of *T. vitrinus* surpassed that of *T. circumcincta*, 42.6% vs 38.7%, respectively. Like *T. vitrinus*, *Oesophagostomum venulosum* also increased in abundance across the season, from an average of 0.0 to 0.4% between May and July, increasing to 7.9%, 15.8% and 15.4% across September, October and November, respectively. *Chabertia ovina* was also frequently observed in pre-treatment submissions in August, representing an average of 3.7% (0-14.1%).

[Insert Figure 4 around here]

As a proportion of the total average FEC, similar patterns are visible (Figure 5). Early in the season, *T. circumcincta* represents the majority of the total average FEC, following a similar trend to the total mean epg. However, notably *Trichostrongylus colubriformis* and a decreasing proportion of *T. vitrinus* are also contributing. When the average decreased in late July to August, the species composition shifted and the mean epg of *T. circumcincta* remained relatively constant before decreasing, while *T. vitrinus* increased substantially, mirroring the trend of the total average epg from late-August to October. Due to their low abundance (Figure 4), the remaining species present represented little of the total proportional FEC.

[Insert Figure 5 around here]

3.3 Anthelmintic efficacy

Across the 2021 grazing season, 31 anthelmintic treatments were administered to lambs on the nine study farms between May and December 2021 (mean = 3.5 treatments; range 2-8). Most treatments (n = 21; 65.6%) were administered between July and September, with nine treatments occurring in August (28% of total treatments). The most frequently administered anthelmintic group were 1-BZs, totalling 40.6% of treatments (n = 13). All 1-BZ treatments occurred between May and August. 2-LV and 3-MLs were utilised throughout the grazing season, with six 2-LV treatments across three farms and nine 3-ML treatments across five farms. 4-ADs were utilised by three farms, with all treatments occurring in September.

The percentage reduction in FEC was calculated for 27 of the 31 anthelmintic treatments, as shown in Figure 6. No 1-BZ treatments gave a reduction of 95% or greater, with a maximum reduction of 86.6%. Four 1-BZ treatments did not reduce the FEC at all (Figure 6; shown by asterisks, *), and a further treatment only reduced the FEC by 0.8%. However, the remaining 1-BZ treatments all gave a reduction of over 50%, between 55.6-86.6%. The percentage reduction observed for 3-ML treatments was very variable, between 0-98.8%, with one treatment exceeding 95%. Four of the five 2-LV treatments (80%)

exceeded a 95% reduction, with all treatments providing a reduction of above 88.1%. All 4-AD treatments gave a reduction greater than 95%, between 97.8-99.8%.

[Insert Figure 6 around here]

As demonstrated in Figure 7, all anthelmintic groups provided a reduction of over 95% reduction for most species present. However, notably, no 1-BZ treatments gave a reduction in *T. circumcincta* of above 95% (range: 0-84.0%; mean: 39.0%). Similarly, the reduction in *T. circumcincta* only exceeded 95% on one occasion after 3-ML treatment (range: 0-98.6%; mean: 38.9%). The mean reduction in *T. circumcincta* after 2-LV treatment was 91.6% (range: 75.0-99.3%), and 98.5% after 4-AD treatment (range: 96.6-100.0%). Additionally, after 1-BZ treatment a reduction of 32.3% was observed for *C. ovina*.

Sufficient pre-treatment *Nematodirus spp*. eggs were counted to calculate the percentage reduction in FEC for 24 submissions. Excluding one submission, which was determined to be the result of treatment failure due to drench equipment failure, the mean percentage reduction in *Nematodirus* FEC across all anthelmintic groups was 93.4%. Five treatments gave a reduction of less than 95%, four 1-BZ treatments (21.6%-94.9%), and one 3-ML treatment (74.8%). All remaining treatments (n = 19) gave a reduction greater than 95% (97.8%-100%).

[Insert Figure 7 around here]

3.4 Impact of grazing type

Of the total pre-treatment samples, 139 samples from five study farms originated from lambs which had grazed rough grazing in the previous 2-3 weeks prior to treatment (and thus represented the contamination ingested from this grazing type). Similarly, 269 samples from improved grazing were received from eight study farms and 91 from semi-improved grazing on two study farms. Due to the low number of farms using semi-improved grazing it was excluded from the model to improve fit.

Using fixed effects of both grazing type and treatment week, grazing type was demonstrated to have a significant impact on the FEC burden of lambs at time of treatment ($p = 0.017$). Improved grazing resulted in a higher parasite challenge compared to the intercept, rough grazing (Figure 8). Treatment week also had a significant positive association with FEC ($p = <0.001$).

[Insert Figure 8 around here]

Discussion

To date, GIN control in the UK has largely focused on more intensive lowland farms, due to a well-established evidence base identifying both a significant GIN- and anthelmintic-related challenge (Evans et al., 2021; Vineer et al., 2020). This study has demonstrated that extensive hill and upland farming systems also face a GIN challenge that would have a production impact, with a higher parasite challenge experienced on improved pastures. Furthermore, it has highlighted that while over half of hill and upland farmers may still perceive anthelmintic resistance to be a greater problem for lowland farmers, with a low incidence of resistance, all study farms had evidence of anthelmintic resistance to at least one chemical group. This reinforces the need to incorporate extensive farming systems, with their unique management, in the design of sustainable control strategies.

Despite both management and climatic differences, the FECs of lambs on the extensive study farms demonstrated a similar seasonality to that described on intensive farms (Evans et al., 2021; Hamer et al., 2019), with peaks in late July and October. While the average magnitude of the FECs observed here may be less than on some lowland farms (Burgess et al., 2012; Evans et al., 2021), these counts would still likely be associated with a GIN burden that would impact lamb performance. This suggests that effective control of GINs to mitigate such production losses are also an important consideration for extensive farms.

From the FECs conducted in this study, lambs grazed on improved pastures had higher FECs, suggesting higher larval contamination. This may be a consequence of the different management of the improved pastures, with the restricted area of improved pasture being used more intensively and at a higher stocking density than the rough hill grazing. In extensive farming systems, improved grazing would typically be used for lambing (when ewes are shedding more eggs due to the periparturient rise in egg output (Gibbs, 1986)) and as a holding area for sheep gathered off the rough hill grazing for management events such as shearing. Subsequently, lambs are then moved from the rough grazing to improved pastures at weaning to improve their growth rates and enable easy access for sale. As a result, there is little opportunity to rest these fields throughout the year, and the larval contamination deposited may pose a significant risk to lamb performance without careful management, particularly if they have not experienced a significant GIN challenge prior to being moved to allow them to develop a sufficient immune response in early life (McRae et al., 2015).

T.circumcincta is widely accepted as the predominant GIN species present on intensive farms in Scotland (Burgess et al., 2012; Evans et al., 2021; Melville et al., 2016). Similarly, here 100% of submissions from the study farms contained *T.circumcincta*, with *T.vitrinus* identified as the second most abundant species, but principally from August onwards. Furthermore, this work identified substantial variation in the number of species within a single submission, with results from single species up to 6 species in a single sample. The variation in species present is likely due to long-term anthelmintic treatments, management, climatic and other factors, occurring at a farm level (Bartley et al., 2003; Jackson and Miller, 2006). Due to the use of different grazing types (rough versus improved) that are subject to different management and stocking densities, it is also plausible that grazing type could be impacting the diversity seen here, however, this could not be explored here due to insufficient power.

The questionnaire study identified that over half of farmers that treated lambs 'as required' were using FEC diagnostics to support decision making (54%). This is a promising step towards a wider adoption of evidence-based decision making as part of sustainable parasite control, though this questionnaire did not differentiate between farms that occasionally use FEC testing and those that conduct routine monitoring. In addition, this may represent an increased over-representation from self-reporting. Furthermore, over a quarter of respondents (30%) specified that they treated either at set times or around times of other management events (e.g., shearing or weaning). For extensive flocks, it is often impractical to frequently gather animals due to the large area sheep are grazed across, and lack of labour (Morgan-Davies et al., 2015). Consequently, the uptake of evidence-based control, using tools such as FECs, will need to consider these management and topographical factors, which have been shown to discourage the uptake of solutions such as FEC testing (Jack et al., 2022).

Unfortunately, the questionnaire returned a relatively low response rate compared to the number of extensive hill and upland farms in the Scottish sheep sector. Dissemination was limited, due to an absence of agricultural events in 2021 because of COVID-19, but substantial efforts were made to advertise this questionnaire in the agricultural press online, on social media and in print to improve the response rate. It is likely that participants will have originated from a younger demographic of farmers than may be typical of this population and those with a greater interest or motivation surrounding this issue. However, despite this, results from the questionnaire still showed a vast underestimation of anthelmintic resistance compared to the study farms.

In this study, the number of anthelmintic treatments administered to lambs in one production year was slightly higher in the study farms compared to survey respondents (3.5 versus 3 treatments, respectively). This figure is similar to a previous UK-level survey, reporting 3.55 treatments per lamb per year (Morgan et al., 2012), with previous work in Europe showing a variable number of treatments, ranging from 2-5 treatments per lamb per year (Bartley et al., 2003; Chartier et al., 1998; Maingi et al., 1996). The higher number of treatments in the survey farms was likely due to multiple management groups being tested throughout the season to obtain a wider picture of the on-farm challenge (Supplementary Material 3), while the survey asked about lamb treatments without explicit mention of multiple management groups.

Historical studies conducted in the UK by Mitchell et al. (1991) and Hong et al. (1996) suggested that anthelmintic resistance was more prevalent on intensive lowland farms rather than extensive hill and upland farms. Recent evidence from Northern Ireland has, however, shown that upland farms had a higher prevalence of both 3-ML and 2-LV resistance compared with lowland farms (McMahon et al., 2013). It is, therefore, encouraging to see that over half of survey respondents (58%) considered anthelmintic resistance to be of high importance, and of equal significance between both lowland and hill/upland farms in Scotland, but those that did not see anthelmintic resistance as important on hill farms often perceived this to be more of an issue on lowland farms.

The study farm work performed identified that all farms had demonstrable anthelmintic resistance to at least one anthelmintic class. This finding concurs with McMahon et al. (2013), in that anthelmintic resistance is also a significant problem facing extensive farming systems. However, these results contrast with the questionnaire results, where only 20% of farmers reported confirmed resistance to at least one anthelmintic (through faecal egg count reduction tests), a figure similar to that reported in Morgan et al. (2012), 19% . The significant disparity between reported and actual anthelmintic inefficacy is likely due to a lack of testing.

It is also important to note that as the work conducted in this study relied upon the farmers performing the anthelmintic treatments themselves, it is possible that lack of efficacy may be due to treatment failure, as opposed to resistance. This was identified on one occasion, on farm A, where it was later recognised that, due to equipment failure, all animals received a sub-therapeutic dose of anthelmintic, which did not cause the anticipated reduction in FEC. While a disadvantage of such studies, this represents a true reflection of how anthelmintic resistance may develop in a practical setting and reinforces the importance of mitigating potential confounders in interpreting faecal egg count reduction test results through correct anthelmintic administration (Morgan et al., 2022). There is, however, a balance to be struck between mitigating such confounders and ensuring that testing remains practical for farmers. In this instance, the study used unpaired samples to negate the need for additional handling and individual identification of extensively grazed animals. This accommodation was simple to account for in the final analysis but made the sampling protocol substantially more accessible for the study farmers in terms of both time and practicality.

It is well established that resistance to 1-BZs is the most prevalent on farms worldwide (Kaplan, 2004; Ramünke et al., 2016; Rose et al., 2015). Therefore, it is unsurprising that no treatments administered here gave a reduction greater than 84%. Despite widespread resistance, 1-BZs are still used on almost all farms, particularly in the control of *Nematodirus spp.* in lambs in spring, due to its high safety index (Lacey and Gill, 1994) and low cost, and only a low prevalence of resistance detected in *Nematodirus* populations the UK to date (Melville et al., 2020). This may account for the high number of 1-BZ treatments administered here, despite the lack of efficacy against strongyle species, with all 1-BZ use contained within the first half of the grazing season.

Similarly, 3-MLs only gave a reduction of >95% on one occasion. It is hypothesised that the significant increase in 3-ML resistance in the UK over the past two decades is a consequence, at least in part, of the repeated use of long-acting 3-MLs (McMahon et al., 2013; Sargison et al., 2010). In addition, 3-MLs are often used to treat the ectoparasitic disease sheep scab (Jones et al., 2022), which is now endemic in the UK. This may occur without farmers acknowledging the potential impact on GI nematodes, and mostly occurs in wintertime, where there may only be a small population of nematodes *in refugia*, thereby unknowingly selecting for resistant genotypes (Kenyon et al., 2009a; Sargison et al., 2010; Van Wyk, 2001).

In contrast to the 1-BZs and 3-MLs, 2-LV was effective in 80% of treatments, with only one treatment outcome falling below 95% reduction, at 88.1%. Despite having been on the market since 1970, generally lower levels of resistance have been reported to 2-LV in the UK compared with the 1-BZs and 3-MLs (McMahon et al., 2013; Vineer et al., 2020). Interestingly, the use of 2-LV appears to be lower than of 1-BZs and 3-MLs, with only two study farmers using it in 2021, so this may be a contributing factor. Similarly, only 46% of questionnaire respondents used 2-LV in 2020, compared with 84% and 61.3% for 1-BZ and 3-ML, respectively.

The 4-ADs were only used by three of the study farms in 2021, with all use occurring in September. This concurs with current guidance in place in the UK, which advocates the use of 4-AD for quarantine purposes and as a mid-season 'break drench' (Stubbings et al., 2020). Both 4-AD and 5-SI were reclassified in 2017, making them available on prescription from pharmacists and registered animal medicines advisors (RAMAs), in addition to veterinary clinicians. However, the high cost per dose of these groups, difficulty to obtain, along with many farmers believing their existing anthelmintics are working well, means few farmers have integrated these products as part of their routine parasite management. This is echoed in the questionnaire responses, with only 19% and 3% of survey respondents using 4-AD and 5-SI, respectively.

At a species level, resistance was almost exclusively observed in *T.circumcincta* for all anthelmintic groups. It is widely established that *T.circumcincta* has developed resistance to the three older anthelmintic groups (1-BZ, 2-LV and 3-ML) across Europe (Rose et al., 2015), but it is interesting to note that *T.circumcincta* was the only species in which resistance was observed, apart from one 1-BZ treatment possibly indicating *C.ovina* resistance, most likely the result of a low pre-treatment FEC. This may be due to the timing of treatments of certain groups of animals, where *T.circumcincta* was the dominant species present (i.e. 1-BZ treatments were largely in spring and early summer before the increase in *T.vitrinus* occurred), but also may indicate that resistance has not yet developed in these species on extensive sheep farms in Scotland to the same degree that it has elsewhere in the UK (Rose et al., 2015). Consequently, this work highlights the important role that species information will play in anthelmintic resistance management once this technology is commercially available at a farm-level, allowing farmers to optimise the timing the use of anthelmintic groups that may otherwise be ineffective at certain times of year, to help maximise their longevity.

In addition to species composition impacting the efficacy of individual anthelmintic treatments, there is likely a significant within-farm variability in efficacy that is present on all farms. This is most likely due to many different GIN populations existing on one farm, a consequence of multiple distinct management groups, stocking rates, grazing intensity, anthelmintic treatments and climate (Bartley et al., 2003; Jackson and Miller, 2006). Consequently, farms with multiple management groups will require to undertake multiple faecal egg count reduction tests to develop a more complete picture of the anthelmintic challenge present on-farm.

Conclusion

The use of study farms, complemented by an industry-level questionnaire, has provided a unique insight into the perceived versus actual GIN challenge facing extensive sheep farms in Scotland, which could be extrapolated to similar farming systems in Europe and beyond. This work demonstrated that extensive farms do face a significant GIN challenge that is higher on improved pastures, with all farms experiencing resistance to at least one anthelmintic group. Ultimately, with extensive farms representing a significant proportion of sheep farms across Europe, these farms will need to recognise their role and take a proactive approach to slow the development of anthelmintic resistance, with solutions tailored to their unique management practices.

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Ethical Approval

Ethical approval for the questionnaire study was obtained through the University of Edinburgh's Human Ethical Review Committee (ref: HERC667). Ethical approval for the study farm work was obtained through the Moredun Research Institute's Animal Welfare and Ethical Review Body (ref: NARF03/23).

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Tables

Table 1: Overview of the nine (n=9) recruited study farm enterprises and their management.

Farm	Type of farm	Number of breeding ewes	Area (hectares)	Primary enterprise type*	Main sheep breed
A	Upland	120	70	Commercial – store	Texel cross
B	Hill/Upland/Lowland	800	533	Commercial – finished	Scottish Blackface
C	Hill/Upland	350	50	Commercial – mixed	North Country Cheviot
D	Hill/Upland	1500	865	Commercial – finished	North Country Cheviot
E	Hill	300	1001	Commercial – store	North Country Cheviot
F	Hill/Upland	850	3995	Commercial – finished	Scottish Blackface
G	Hill	900	768	Commercial – store	North Country Cheviot
H	Hill/Upland	2400	1680	Pedigree breeding	Scottish Blackface
I	Hill/Upland	600	215	Commercial - finished	Scottish Blackface

*Primary enterprise types: Commercial – finished: Lambs bred for meat production being sold at slaughter weight. Commercial – store = Lambs bred for meat production being sold before reaching market weight to a second producer to fatten the lambs to market weight. Pedigree breeding: Production of lambs to sell as breeding ewes and/or rams.

Table 2: Demographic details of all included responses (n = 31)

Demographics	**Responses (total n = 31)**	
	Proportion of respondents (%)	**Number of respondents (n)**
Land type		***31***
Hill	29.0%	9
Upland	35.5%	11
Hill/Upland	32.2%	10
Mixed*	3.2%	1
Sheep enterprise type		***31***
Commercial – finished	38.7%	12

Commercial – store	48.4%	15
Pedigree breeding	6.5%	2
Other	6.5%	2
GIN treatment strategy†		***30***
Regular intervals	20%	6
Same as previous years	13.3%	4
When gathered for other management tasks	30%	9
As required	56.7%	17

*Mix of hill, upland, and lowland land types. †Respondents could select >1 answer to this question

Table 3: Number of anthelmintic treatments administered to lambs in 2020, grouped by the anthelmintic group administered

Anthelmintic group	**Number of uses**	**Number of respondents (n)**	**Percentage of respondents (%)**	**Valid percentage* (%)**
Benzimidazole (1-BZ)	0	5	16.1%	
	1	14	45.2%	53.8%
	2	8	25.8%	30.7%
	3	4	12.9%	15.3%
Imidazothiazole (2-LV)	0	17	54.8%	
	1	9	29.0%	64.3%
	2	4	12.9%	28.6%
	3	1	3.2%	7.1%
Macrocyclic Lactone (3-ML)	0	12	38.7%	
	1	11	35.5%	57.9%
	2	5	16.1%	26.3%
	3	2	6.5%	10.5%
	4	1	3.2%	5.3%
Amino-acetonitrile derivatives (4-AD)	0	25	80.6%	
	1	6	19.4%	100%
Derquantel (5-SI)†	0	30	96.8%	
	2	1	3.2%	100%

*Percentage excluding 0 values.
†only available in the UK as a dual-active with abamectin

Figure captions

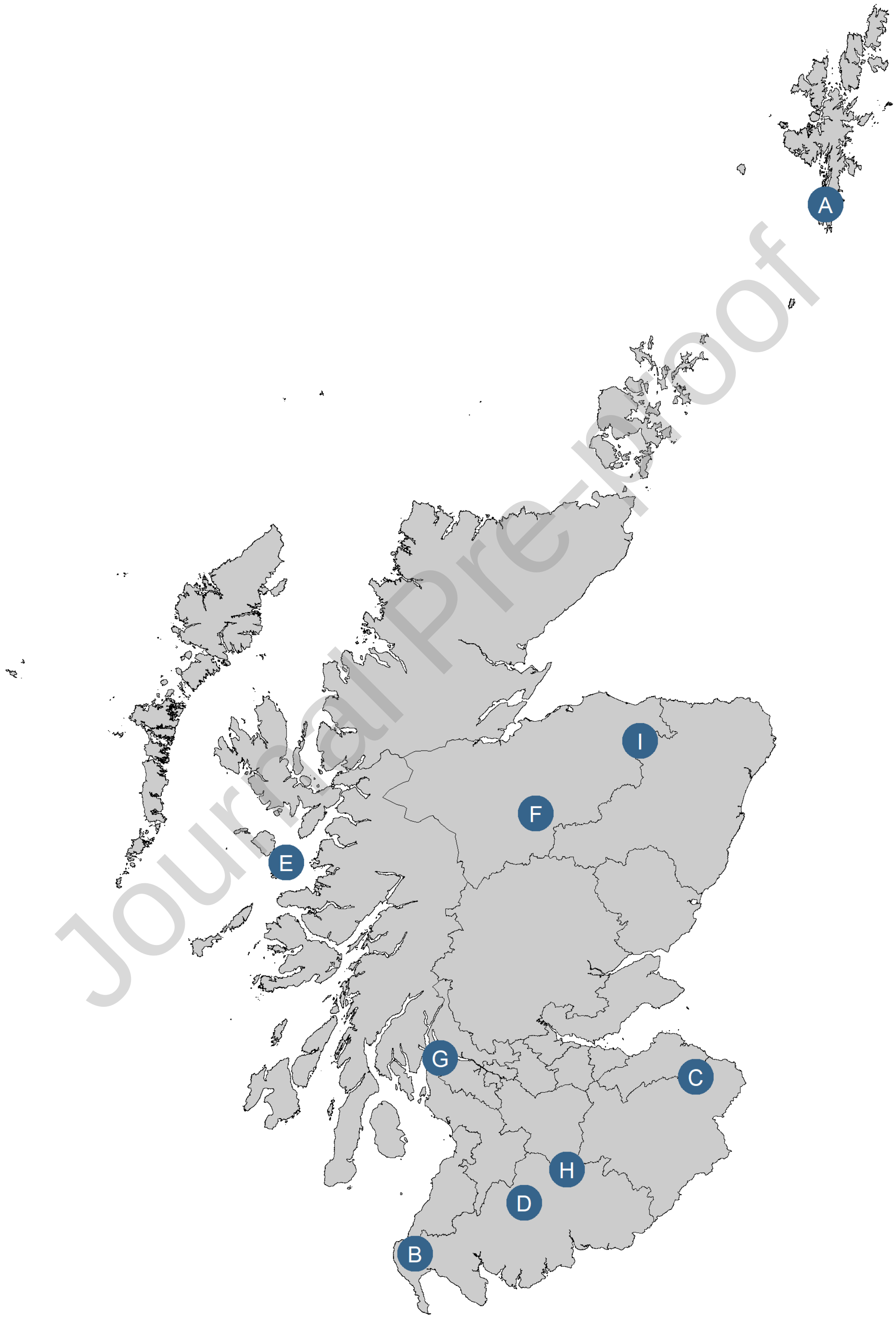
A
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Figure 1: Map of study farm locations. Each dot represents a single study farm location.

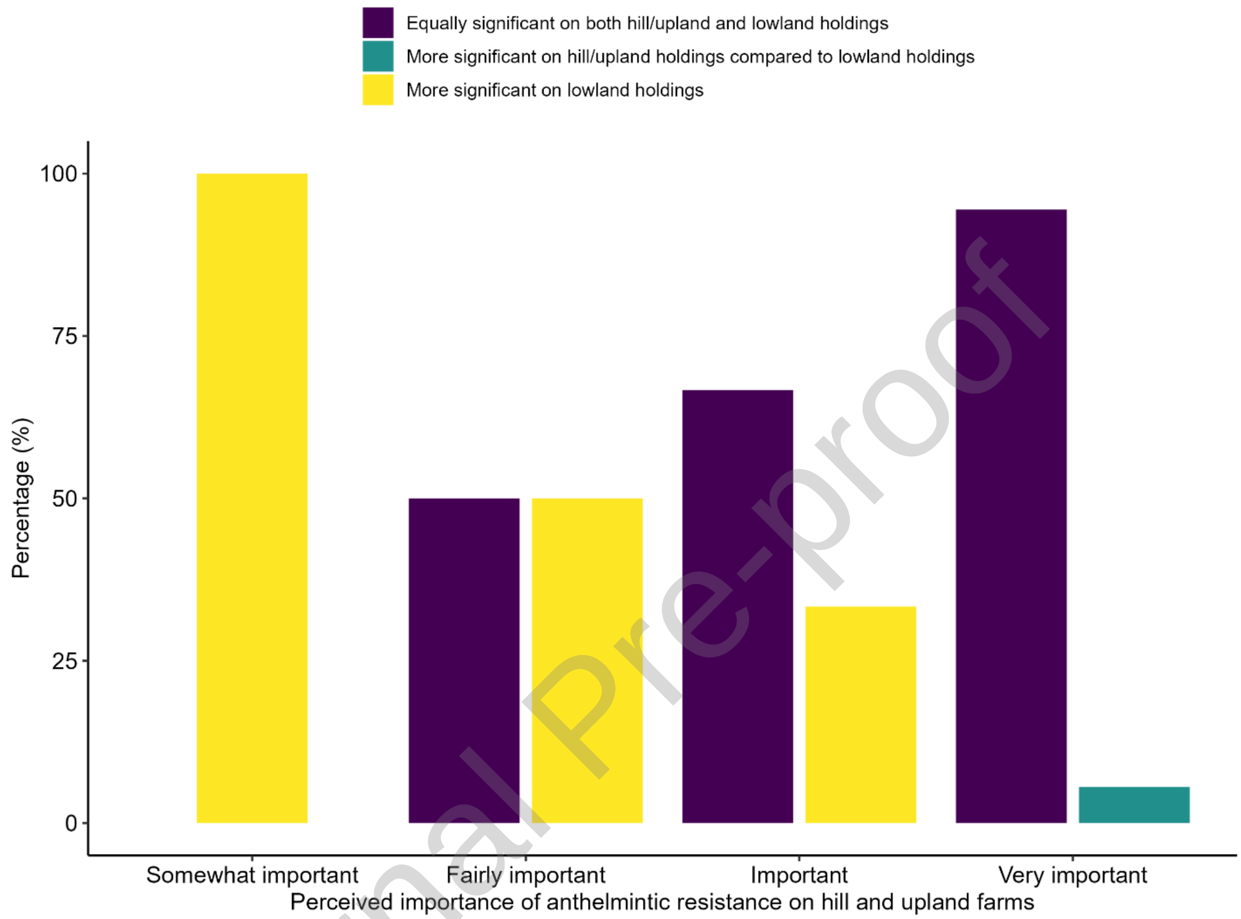

Figure 2: Responses to the question "Do you believe anthelmintic resistance for roundworm control is...?", with responses grouped according to respondents' answers to "How important do you perceive anthelmintic resistance to be for roundworm control on hill and upland sheep farms?". Equally important = Equally important on both hill/upland and lowland holding. Hill/upland = More important on hill/upland holdings. Lowland = more important on lowland holdings. % calculated as no. per answer/n in group (n= Somewhat: 3; Fairly: 4; Important: 6; Very: 18).

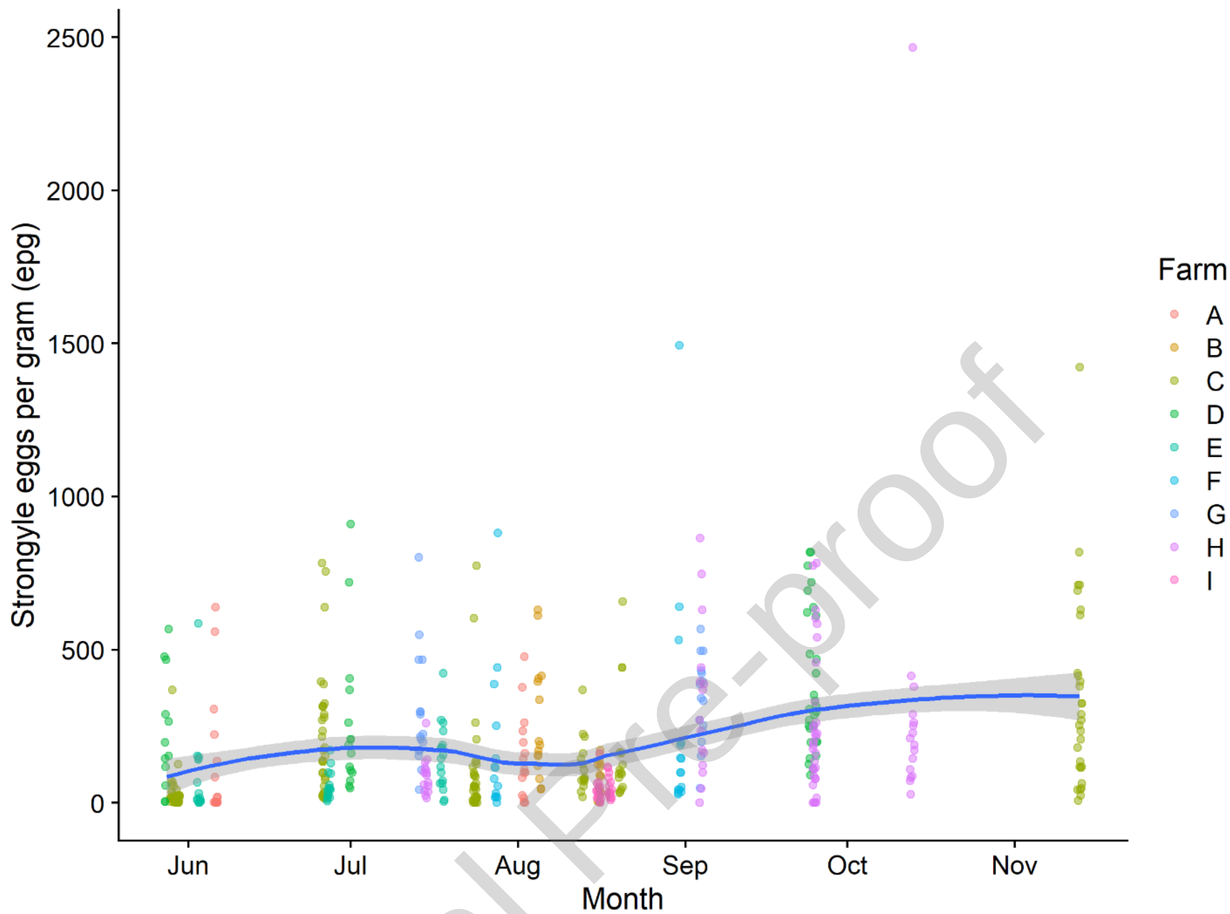

Figure 3: Strongyle-type faecal egg counts at time of treatment across 2021 in lambs. Trend visualised using a LOESS curve.

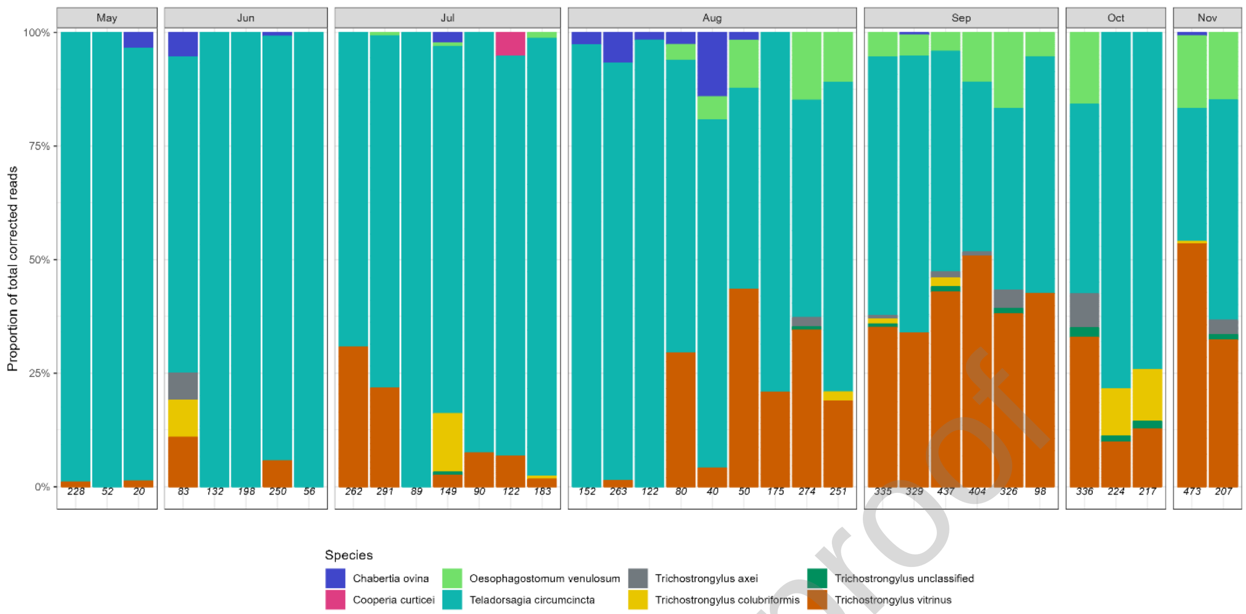

Figure 4: Species composition (%) of pre-treatment submissions, expressed as the proportion of the corrected total reads. Each panel represents a treatment month, and within each panel the samples were ordered by treatment date. The mean eggs per gram (epg) for each submission is displayed under the corresponding bar.

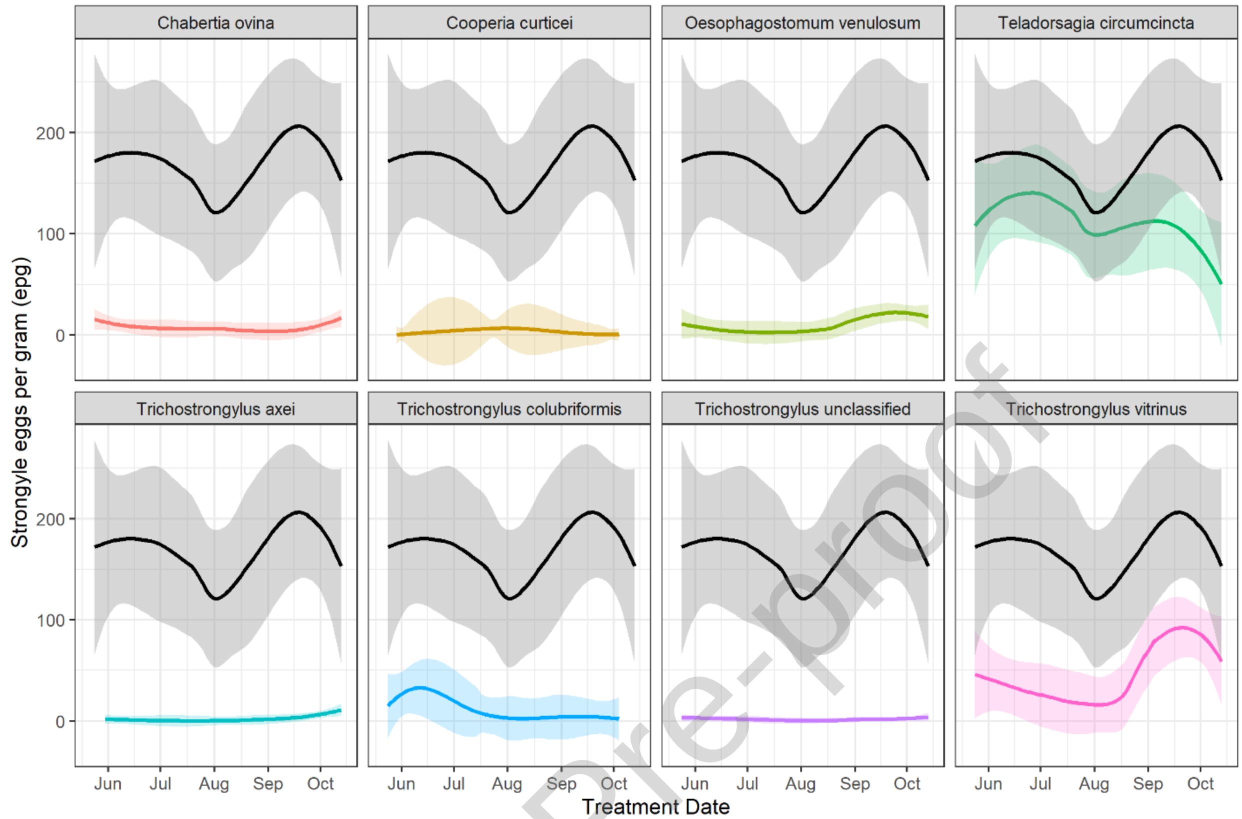

Figure 5: Proportional faecal egg counts over time. Black line: total mean eggs per gram (epg). Coloured line: proportional mean FEC value per species.

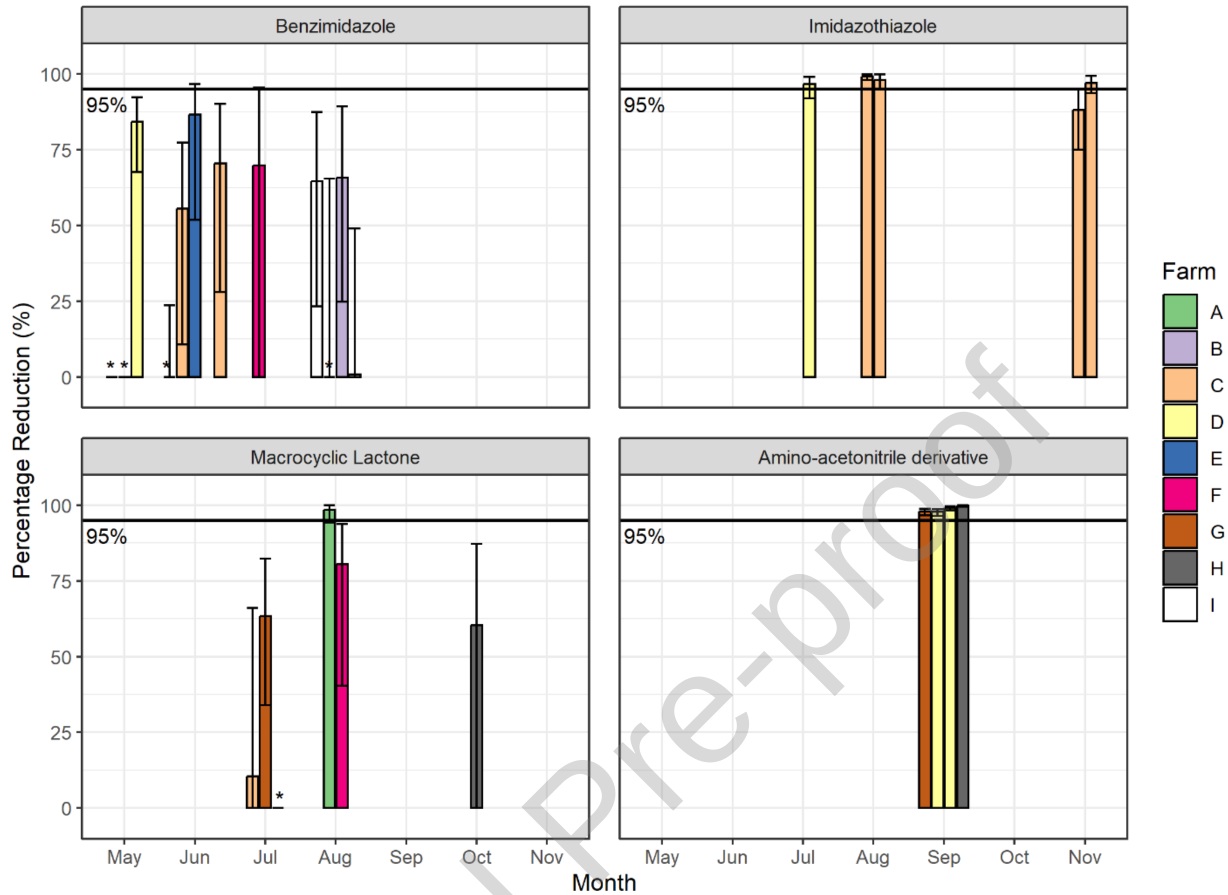

Figure 6: Percentage reduction in strongyle FEC per anthelmintic group, over time. Black horizontal line = 95% reduction (previously defined threshold, above which, with a lower confidence interval of above 90%, is considered an 'effective' treatment). Asterisks (*s) represent a percentage reduction of 0% or below. Bar colours represent different farms. Error bars represent the bootstrapped 95% confidence interval.

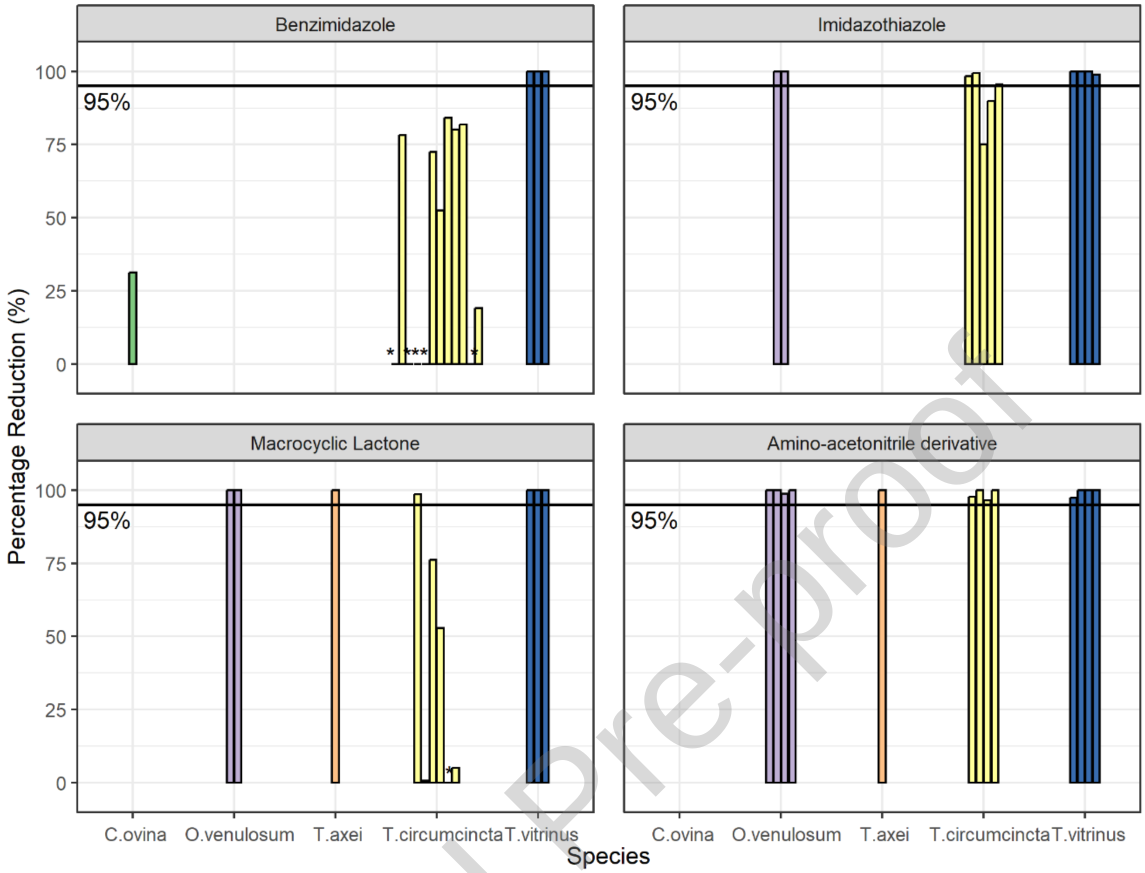

Figure 7: Species-wise percentage reduction in strongyle FEC per anthelmintic class. Black horizontal line = 95% reduction (previously defined threshold, above which, with a lower confidence interval of above 90%, is considered an 'effective' treatment). Asterisks (*s) represent a percentage reduction of 0% or below. Bar colour = species. Not all species were present in all samples, hence the number of bars differs across each panel.

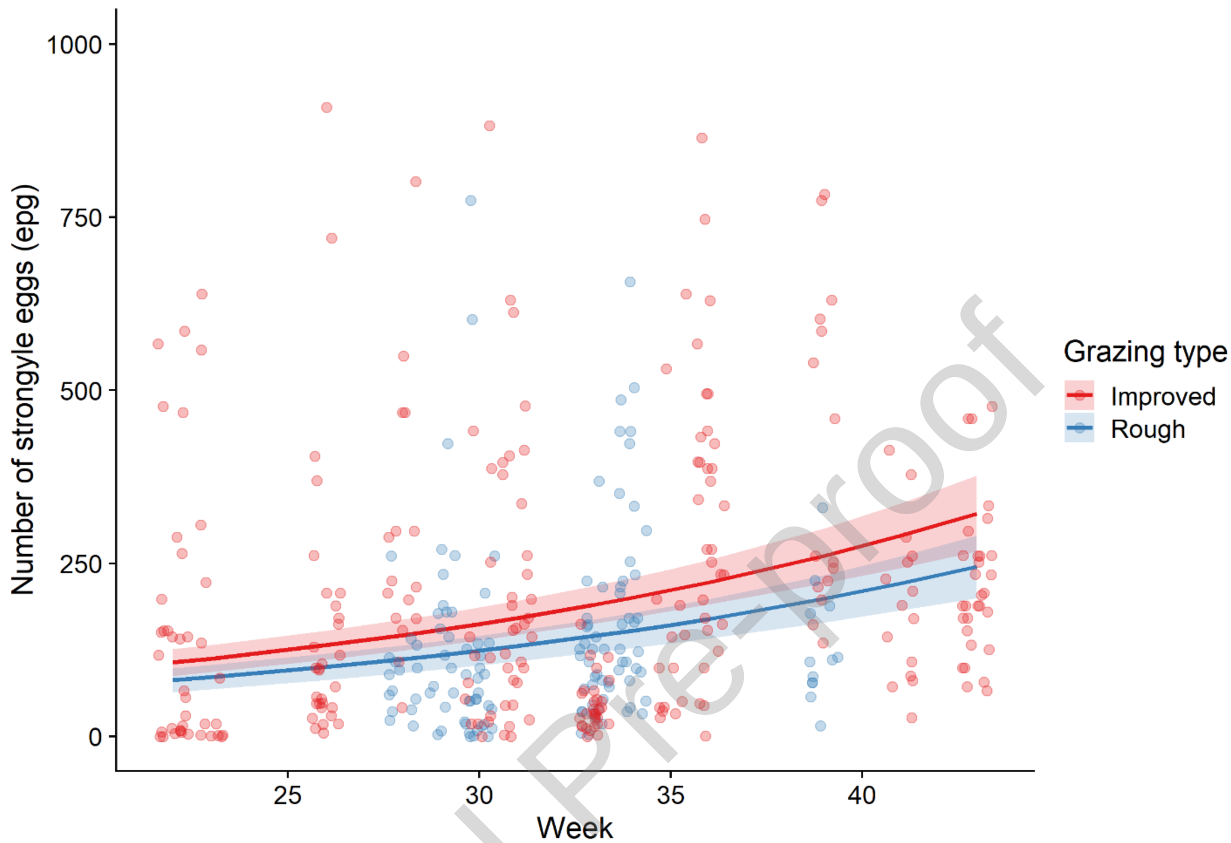

Figure 8: Zero-inflated negative binomial generalised linear model output demonstrating the impact of grazing type on the FEC burden of lambs at time of treatment across the 2021 grazing season. Each point represents an individual FEC result, coloured by the grazing type. The lines represent the model prediction, with a 95% confidence interval, also coloured by grazing type. For visualisation, two outliers (at 1494epg and 2466epg) were removed, however, were included in the model fit.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Few surveyed extensive farmers perceived an anthelmintic resistance issue
- All study farms demonstrated evidence of anthelmintic resistance
- Improved grazing tended to have a higher parasite challenge than rough grazing
- Farm management differences will need to be considered for future parasite control