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Precision worm control in grazing lambs by targeting group treatment based on performance of sentinels

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ABSTRACT

Given the economic impact of gastrointestinal nematode infection on livestock farming worldwide, and increasing anthelmintic resistance, it is imperative to develop practical, efficient and sustainable control strategies. Targeted selective treatment (**TST**), whereby anthelmintic treatments are administered to animals individually, based on selection criteria such as weight gain, has been shown to successfully maintain animal productivity whilst reducing the selection pressure for anthelmintic resistance and the economic cost of treatment in experimental and commercial settings. Despite the benefits of the TST approach, the equipment and time required to monitor animals individually make this strategy unsuitable for some farming enterprises. The sentinel group approach aims to maintain the benefits observed using TST whilst reducing these requirements. The study involved two experiments, each following a group of 80 lambs through their first grazing season. Anthelmintic treatment of the whole group was determined by monitoring the weight gain of identified sentinel lambs within it every 2 weeks: when 40% of the sentinel lambs failed to reach their weight gain targets, the whole group was treated. The sentinel lambs consisted of 45% of the group ($n = 36$) in experiment one and 20% ($n = 16$) in experiment two. A control group of 20 lambs was co-grazed with the main group during both experiments; in experiment one, the sentinel approach was compared with a TST approach, in which control lambs were treated on an individual basis in response to weight gain. In experiment two, the sentinel approach was compared with conventional prophylaxis, where all lambs in the control group were treated at strategic time points throughout the season (= strategic prophylactic treatment). The sentinel lambs were found to be representative of overall group performance regardless of the proportion of sentinels within the group: they recorded similar growth rates and reached weight gain targets simultaneously at each time point and overall. Live-weight gain was also similar between sentinel and control animals in both experiments. The findings of the current study suggest that monitoring sentinel lambs comprising 20% of a group of grazing lambs is sufficient to determine the need for anthelmintic treatment within the whole group, and that this approach maintains production in line with conventional or TST treatment regimes.

Implications

The 'sentinel group' approach is a novel monitoring strategy for grazing lambs, designed to target whole-group anthelmintic treatment based on weight gain of a proportion of the flock. Monitoring as few as 20% of lambs was sufficient to identify when the larger co-grazing group required treatment. This is a modification of weight-based targeted selective treatment, which maintains lamb production whilst reducing anthelmintic usage. Targeted selective treatment is best suited to high-throughput situations, using automated weighing and drafting facilities. The sentinel approach minimises the labour requirements, providing a more accessible monitoring method for targeting anthelmintic treatment.

Introduction

The growing worldwide threat of anthelmintic resistance (Kaplan, 2004; Rose et al., 2015; Ramunke et al., 2016) increases the need for more sustainable gastrointestinal nematode control. Use of ineffective anthelmintics was estimated to reduce live-weight gain of lambs by up to 9 kg over the course of a grazing season, compared with effective treatment (Miller et al., 2012). The timing of anthelmintic treatment is key to successful parasite control and influences the selection pressure placed on the population (Prichard et al., 1980; Van Wyk, 2001). Sustainable control relies on the maintenance of susceptible worm

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populations *in refugia* to dilute resistant alleles (Van Wyk, 2001; Hodgkinson et al., 2019). Several novel strategies have been developed for livestock in an attempt to maintain nematode populations *in refugia*, including targeting of treatments according to risk, at herd or individual levels (Kenyon et al., 2009; Charlier et al., 2014). Targeted selective treatment (**TST**) aims to leave a proportion of animals, and therefore worms, untreated (Van Wyk et al., 2006; Besier, 2008; Kenyon et al., 2009). Treatments are targeted to the individual animals that will benefit most from treatment, using parasitological or physiological indicators such as weight gain (Stafford et al., 2009; Kenyon et al., 2013), faecal egg count (**FEC**) (Leathwick et al., 2006; Cringoli et al., 2009) or anaemia (FAMACHA) (Vatta et al., 2001). Several studies have demonstrated the benefit of TST practices in a range of production scenarios (Van Wyk and Bath, 2002; Busin et al., 2014), with production maintained compared to frequent whole-group treatments. The maintenance of drug efficacy using TST has been monitored in a few studies (e.g. Kenyon et al., 2013), although more work is needed in this area (Gaba et al., 2010). Targeted selective treatment approaches have, therefore, been proven to provide considerable benefits in terms of productivity, cost-effectiveness and slowing of drug resistance. Despite evidence to support the benefits of TST practices (Kenyon et al., 2009), uptake remains low in commercial farming systems. Key barriers to uptake are the costs and time required to perform frequent whole-flock monitoring. Automated weighing and drafting equipment has been used to streamline whole-flock TST monitoring on some large-scale commercial farms, resulting in labour savings of four working days per year compared to conventional approaches (McBean et al., 2016; Morgan-Davies et al., 2018) but the initial cost of this equipment is often too great, especially for farms of modest size (Kaler and Ruston, 2019), possibly deterring uptake by the farming community.

Administering whole-group treatments is generally less labour-intensive than TST, but imposes higher selection pressure on parasites, increasing the risk of developing anthelmintic resistance (Kenyon et al., 2013). Targeted treatments (**TT**) are whole-group treatments that are administered once the risk of disease or presence of infection has been identified. The need for treatment is determined by monitoring physiological or parasitological indicators; this differs from more commonly used fixed-interval strategies, where treatments are given on pre-determined dates to suppress infection (Kenyon et al., 2009). Fixed-interval or 'strategic prophylactic' treatments (**SPT**) do not take into account temporal fluctuations in the size and species composition of active parasite populations. Employing such an inflexible approach to treatment may, therefore, produce good results in 1 year but result in productivity losses in other years if the opportune treatment date has been missed, particularly given the increasing variability in parasite epidemiology as a result of climate change amongst other factors (Van Dijk et al., 2010; Gethings et al., 2015). Since risk varies within and between years, and optimal responses can entail adapted whole group or individually TT (Kenyon et al., 2013), there is scope for designing more flexible strategies that combine the advantages of TT and TST while limiting farmer investment in monitoring.

Here, we tested a modified TT approach, to support evidence-based, pen-side treatment decisions on whole-group treatments. The weight gain of a proportion of lambs, assigned as 'sentinels', was monitored and the need for whole-group treatment inferred from the proportion of sentinels that were underperforming. The overall aim was to minimise costs and labour while maximising productivity and the effective life of anthelmintic drugs. This approach carries an inherent risk of misclassifying the need for whole-group treatment, if the sentinel lambs are performing better or worse than the group average. The present study also set out to investigate the extent to which the number of sentinel animals, as a proportion of total group size, influences misclassification risk, using a combination of statistical simulation and grazing trials in a realistic farm setting. This is needed to determine an efficient approach, in which monitoring effort is manageable, without causing undue errors in treatment decisions. The performance of groups treated in this way was compared with that of co-grazed lambs subjected to TST or SPT treatments, as defined above, to assess its viability against these more established strategies.

Material and methods

Experimental design

Field trials were carried out in 2010 and 2012 at the Moredun Research Institute, Scotland. All trials were examined and approved by the Moredun Research Institute Experiments and Ethics Committee and conducted under the legislation of a UK Home Office License (reference PPL 60/03899) in accordance with the Animals (Scientific Procedures) Act of 1986.

The aim of the trial was to assess: first, if monitoring weight gain in sentinel lambs fairly reflects the weight gain in the whole Test group, and second, whether performance of the sentinels can effectively determine the need for anthelmintic treatment of the whole Test group. Different numbers of sentinels were used between trials as a means of optimising whole-group anthelmintic treatments while limiting monitoring effort.

In each of two experimental trials, 100 weaned lambs were co-grazed on a single paddock, naturally infected with *Nematodirus* and various other trichostrongylid species, from June until October each year. Lambs were approximately 3 months old at the start of the experiment. The breed of lambs differed between experiments, with mule × Texel lambs being used in experiment one and mule × Lleyn in experiment two, due to a farm management decision. Within each experiment, animals were divided into two groups: a Control group ($n = 20$) and Test group ($n = 80$), which were balanced for lamb weight and sex at day 0. A proportion of the Test lambs was randomly identified at the start of each trial and assigned as sentinels (Fig. 1). Individualised target growth rates were calculated using the 'Happy Factor' model (Greer et al., 2009; Kenyon et al., 2013) for all sentinel lambs every 2 weeks, and anthelmintic treatment of all Test group lambs, including the sentinels, was triggered when over 40% of the sentinel lambs failed to reach their live-weight targets. This proportion was determined from analysis of the use of the 'Happy Factor' TST in field trials (Kenyon et al., 2013, data not shown) and was the average peak

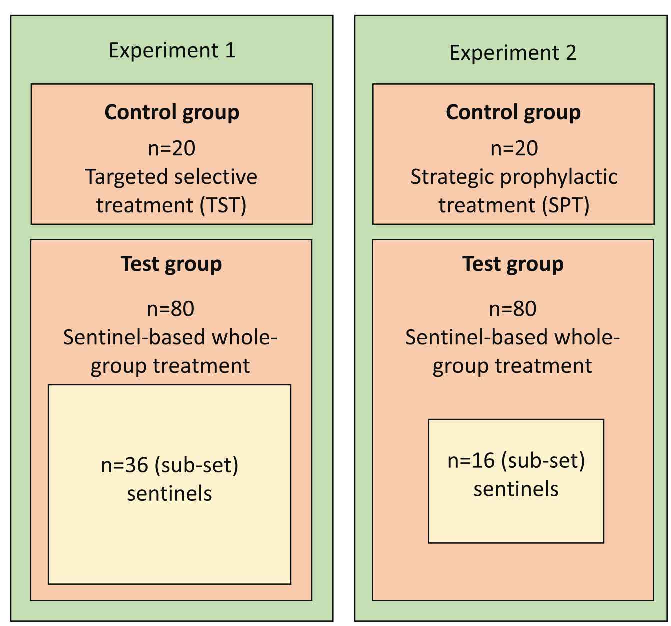

Fig. 1. Schematic showing the breakdown of lambs in each experimental group. The sentinel lambs comprised 45 and 20% of the Test group in each experiment.

percentage of lambs treated in each year of that 5-year trial. Target weights were also determined for the non-sentinel animals, but they were only used retrospectively to determine if animals had reached their weight targets and evaluate whether the sentinel lambs reflected the overall average weight gain in their corresponding group.

The treatments administered in each Control group differed, in order to compare the sentinel approach to different commonly used treatment strategies. In experiment one (run in 2010), the Control group followed a performance-based TST approach. Lambs were assessed on their ability to reach individualised target live-weight predictions ('Happy Factor', Greer et al., 2009) every 2 weeks; animals failing to reach these targets were treated. Control group animals in experiment two (run in 2012) followed a SPT regime: these lambs were treated at strategically appropriate times (at weaning and 4 weeks post-weaning), to prevent impacts on performance from trichostrongylid nematodes (Gascoigne et al., 2018), as commonly practised on UK farms (Burgess et al., 2012).

All treatments consisted of ivermectin oral drench (Oramec, Merial Animal Health Ltd., UK) at the manufacturer's recommended dose rate of 0.2 mg/kg live weight. The efficacy of ivermectin was 79% (95% CI 78–80%) for the experimental flock. In experiment two, all lambs (including the Control group) received an additional drench on day 22 to control *Nematodirus* spp. infection before the implementation of the different treatment regimes, due to high risk under the weather conditions that year.

Measurements and sampling

Live weight of all lambs was recorded every 2 weeks throughout the experimental period, and faecal samples collected *per rectum* from each lamb at the same time. Faecal egg counts were conducted following a modification of the salt flotation method (Jackson, 1974), with a sensitivity of 1 egg per gram (**EPG**). Food availability was estimated in the intervening weeks between weight and faecal sampling by measuring pasture biomass using a Grassmaster II probe (Novel Ways, New Zealand) whilst walking through the paddock in a 'W' pattern. Pasture biomass, animal weights and climatic data were included in the 'Happy Factor' model to calculate production targets (Greer et al., 2009).

Number of sentinels

A misclassification approach was used to determine the effect of sentinel numbers on group treatment decisions. Individual lamb performance varies, and average performance of a number of sampled lambs is, therefore, more likely to reflect that of the whole group when more individuals are monitored. Conversely, as the number of sentinels decreases, the chance that their performance does not accurately represent that of the whole group increases. This could lead either to false positive results (the sentinels perform less well than the group, by chance, and the group is erroneously classified as needing treatment) or false negative results (a group in genuine need of treatment is not recognised as such because the sentinels perform better than average). The chance of such errors is highest for a single sentinel animal, and zero if all group members are monitored. Intermediate probabilities of misclassification were estimated by Monte Carlo simulation.

In experiment one, 45% ($n = 36$) of lambs in the Test group were assigned as sentinels. The data collected during this first trial were used to determine whether monitoring a smaller number of sentinel lambs could still accurately indicate the need for anthelmintic treatment at group level, while reducing monitoring effort. At each time point, the proportion of the Test group underperforming was used to simulate the probability, *P*, that 40% of the sentinel lambs would also underperform, using the binomial distribution and different sentinel sample sizes (Hilborn and Mangel, 1997) (10 000 Monte Carlo repetitions per parameter set, with replacement). For time points at which < 40% of the Test group underperformed, *P* is the false positive misclassification rate. When ≥ 40% of the Test group underperformed, the false negative rate is 1-*P*. The proportion of simulations leading to misclassification of true group status was plotted against number of sentinels, to determine the cost of reduced monitoring for the reliability of treatment indications.

Results

Statistical analysis

Comparison of performance between sentinel and non-sentinel lambs in the Test group

We conducted separate analyses on the data for each of the two experiments. All analyses were conducted in R 3.6.1. First, we assessed whether the distribution of BW differed between sentinel versus non-sentinel animals, where the sentinels comprised 45 or 20% of the Test group. We compared the distribution of data for each measurement day separately using Kolmogorov–Smirnov tests.

Next, we tested for weight differences between sentinel and non-sentinel animals across the whole experimental period using linear mixed-effects models in the 'glmmTMB' package (Brooks et al., 2017). All models included a random effect of individual identity to account for multiple sampling of the same individuals across time. We fitted a 'null' model (model 0) where the only fixed effect was the intercept; a model (model 1) where the only fixed effect was assigned status (sentinel or non-sentinel); model 2, where the only fixed effect was the experimental day as a categorical variable; model 3, with both the categorical fixed effects of day and assigned status; model 4, which was the same as model 3 with the addition of an interaction between assigned status and day. Models were compared using likelihood ratio tests (**LRTs**), where the χ^2-distributed test statistic is calculated as $-2^*(\text{LogLik}_{\text{model1}} - \text{LogLik}_{\text{model2}})$ in order to test each of the fixed effects in turn. As such, we tested models 1 and 2 against model 0, in order to test for each fixed effect in the absence of any other fixed effects; model 3 against models 1 and 2, in order to test each of the fixed effects in the presence of the other fixed effect; and model 4 against model 3 in order to test for the interaction.

Finally, we tested for differences between sentinel and non-sentinel lambs in their ability to meet a weight gain target or not (as assessed using the 'Happy Factor' method) using binomial generalised linear mixed-effects models (**GLMMs**). We fitted the same five models as described above for BW and tested fixed effects with LRTs. For both models of BW and ability to meet weight gain targets, we also tested for linear effect of day and for day fitted with a non-parametric smooth function (generalised additive mixed-effects model), but these did not improve model fit over day as a categorical variable.

Comparison of performance between Test and Control groups

We first compared the performance of animals in the Test and Control groups (TST in experiment one and SPT in experiment two, see Fig. 1) at the end of the experimental period. Final BW from animals in each of the two groups was compared using a *t*-test, while total weight gain across the experiment (from day 0 to the last day) was compared using a Mann–Whitney *U* test. Next, we tested for differences in changes in BW across the experimental periods in the Test versus the Control groups using the same five models described above. Once again, we tested for differences in changes in ability to meet weight gain targets using binomial GLMMs as described above.

Impact on pasture contamination

We next calculated a total FEC for each animal as the sum of all FECs collected from animals which had FEC assessed at every sampling day in 2010 (Test group $N = 31$; TST group $N = 11$) and 2012 (Test group $N = 46$; SPT group $N = 6$) and tested for differences between the Test and TST/SPT groups using a Mann–Whitney *U* test. Finally, we tested for differences in changes in strongyle FEC across the experimental period. We fitted all five GLMMs as described above, with FEC modelled using the 'nbinom1' error structure in 'glmmTMB', which is a negative binomial error structure where the variance is equal to the dispersion parameter multiplied by the mean. Models were assessed for heterogeneity of residuals, overdispersion and homoscedascity using the 'DHARMa' package (Hartig, 2019), and this model proved superior to log-transformed FEC with Gaussian errors, Poisson errors, a zero-inflated Poisson model, an alternative parameterisation of the negative binomial ('nbinom2') and zero-inflated negative binomial models. Once again, fixed effects were tested using LRTs.

Number of sentinels

Simulation using the binomial distribution showed that as the number of sentinels decreases, so the risk of misclassification increases (Fig. 2). This was most marked at intermediate levels of true group failure. When only 19% of the Test group underperformed, sentinels comprising 20% of the group would erroneously indicate that treatment is required only 2% of the time (= 200 of 10000 Monte Carlo repetitions), compared to never for 45% sentinels, and 8% of the time if only 10% of the group were monitored as sentinels. When 51% of the test group underperformed, the decision was to treat in almost all cases, regardless of the number of sentinels sampled. When 30% of the group underperforms, however, treatment would be triggered around 17% of the time if 45% of the group were sentinels (30% of the time with 20% sentinels and 45% with 10% sentinels). Misclassification risk is highest close to the underperformance threshold for treatment, that is, 40% of the whole group: at 39% group underperformance, treatment is indicated in most cases regardless of the proportion monitored as sentinels. When the group is very close to the threshold requiring treatment, however, the consequence of misclassification is arguably less serious, since performance is in any case borderline. Given the limited increase in misclassification risk as sentinels are reduced from 45 to 20% of the group, experiment two proceeded with 20% of the flock assigned as sentinels.

Comparison of performance between sentinel and non-sentinel lambs in the Test group

The distribution of live weight of lambs was similar between sentinel and non-sentinel lambs throughout the course of both experiments (Supplementary Figure S1). Kolmogorov–Smirnov tests revealed that the distribution of live weights was comparable between groups both when pooling weight data collected across all sample points and at each individual time point (Supplementary Table S1).

Change in BW across the course of the experiments was modelled testing for an effect of status (sentinel or non-sentinel), day of measurement or their interaction. The results from both experiments were very similar: there was support for the fixed effect of day in both the experiments where the sentinels comprised 45% of the group ($\chi^2_6 = 1167.40$, $P < 0.001$) and where the sentinels comprised 20% of the group ($\chi^2_7 = 893.05$, $P < 0.001$); in both cases, weight increased across the experiment (Fig. 3). Sentinel status was not supported as a factor in either experiment one (estimate = 0.65 ± 1.04SE, $\chi^2_1 = 0.40$, $P = 0.529$) or experiment two (estimate = 0.49 ± 0.97SE, $\chi^2_1 = 0.25$, $P = 0.616$). There was also no support for the interaction between status and day in either experiment (45%: $\chi^2_6 = 7.37$, $P = 0.288$; 20%: $\chi^2_7 = 4.44$, $P = 0.728$), suggesting that BW changed in the same manner across the experiment in sentinels and non-sentinels alike and that the sentinels were consistently representative of the whole group throughout both experiments (Fig. 3). Weight was not comparable between years due to the change in the breed of lambs used in the experiment.

The ability of lambs to reach individual weight gain targets across the course of each experiment was modelled to test for the impact of sentinel status, day and any interaction between them. As for BW, there was support for the fixed effect of day in both experiment one ($\chi^2_6 = 31.72$, $P < 0.001$) and experiment two ($\chi^2_7 = 87.22$, $P < 0.001$).

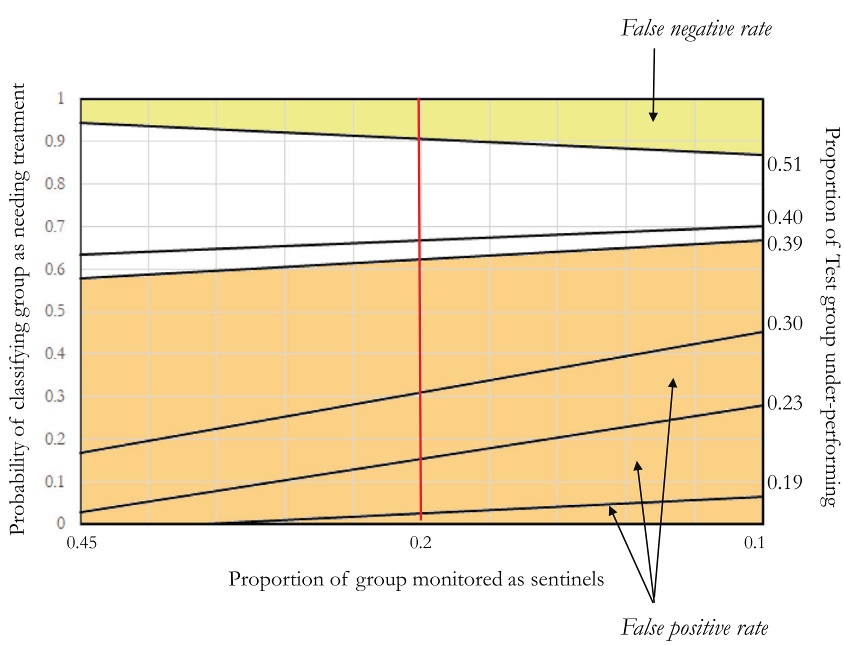

Fig. 2. Effect of the number of sentinels on potential classification error. The proportion of simulations generating false group classifications is shown for different sentinel sample sizes, at each monitoring point in experiment 1 (see methods). Each black line shows how misclassification risk increases as fewer sentinel lambs are sampled, from 45% of the group (= experiment 1, y-intercept) down to 10% of the group. The proportion of the Test group that actually underperformed is shown against each line, on the right-hand axis. As fewer sentinels are monitored, the chance of misclassifying the need for treatment increases. Vertical red line at x = 0.2 indicates the predicted misclassification rate if 20% of the group is monitored as sentinels, as in experiment 2. Shading denotes false negative versus false positive misclassification risk. Misclassification is less likely if the true proportion of the group underperforming is far from the critical boundary of 40%, and if more sentinels are monitored. Results of Monte Carlo simulation using the binomial distribution.

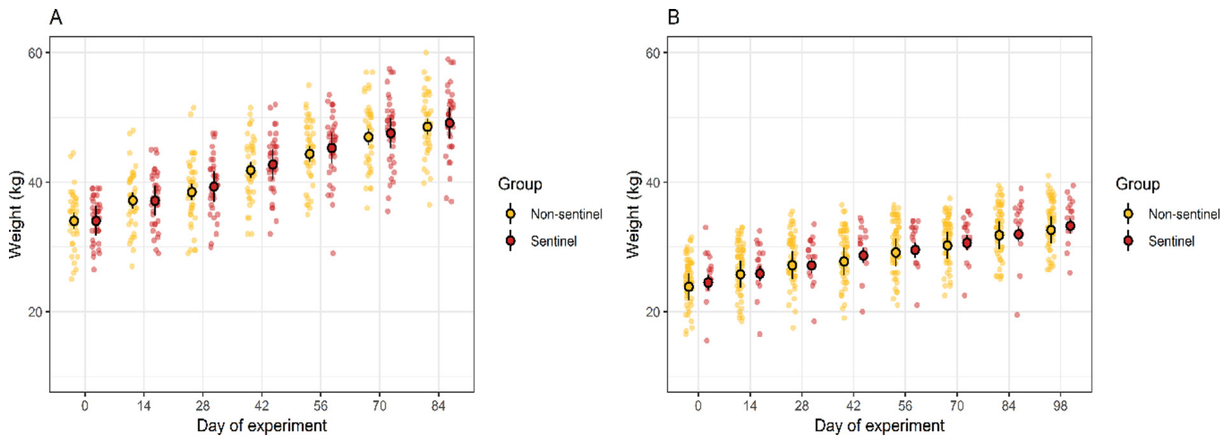

Fig. 3. The changes in BW across the course of experiments conducted where the sentinels comprised (A) 45% and (B) 20% of the Test group. Coloured points show the raw weights collected from sentinel and non-sentinel animals; large points with error bars show estimates changes in BW ± 95% confidence interval as estimated by model 4 for both experiments. The estimates show that the interaction is not supported and that the change across time is the same in lambs assigned as sentinels and non-sentinels.

A difference between the sentinels and non-sentinels was not supported in either experiment one (estimate = 0.18 ± 0.18SE, $\chi^2_1 = 0.01$, $P = 0.905$) or experiment two (estimate = − 0.12 ± 0.20SE, $\chi^2_1 = 0.34$, $P = 0.562$). There was also no support for the interaction between assigned status and day in either experiment (45%: $\chi^2_6 = 2.67$, $P = 0.849$; 20%: $\chi^2_7 = 3.22$, $P = 0.863$), suggesting that sentinels were consistently representative of the whole group throughout both experiments (Fig. 4). In both experiments, the ability to make weight gain targets fluctuated across the experimental period, but did so to the same extent in both sentinels and non-sentinels. Weight targets were more consistently met in experiment two than experiment one, but this cannot be ascribed to the differing proportion of sentinels, since breed and possibly other factors such as weather also differed.

The proportion of the Test group designated as sentinels did not impact the ability of the sentinel performance data to determine the need for treatment in the group as a whole. Fig. 4 shows the probability of lambs reaching their individual target weights throughout the course of the experiments in both years. Whether monitoring sentinels comprising 45 or 20% of the group, anthelmintic treatment was successfully triggered by the sentinel data at each time point when 40% of the whole group failed to reach their weight targets.

Comparison of performance between Test and Control groups

The sentinel approach was compared to two alternative, established strategies for determining the timing of anthelmintic treatment in grazing lambs. In experiment one, the sentinel approach was compared with TST, with lambs treated on an individual basis in response to performance against target weight gain (= Happy Factor). In experiment two, the comparator (Control) was a conventional SPT approach, with all lambs treated at strategic time points throughout the season. The performance of the Test group was compared to the TST and SPT approaches to assess whether production efficiency was maintained using this novel strategy.

Comparison of lamb weight at the end of the experiment found no significant differences between the Test and the TST group in experiment one (*t*-test; $t = 0.00$; $P = 0.998$; Fig. 5A) or between the Test and SPT lambs in experiment two (*t*-test; $t = 0.54$; $P = 0.597$; Fig. 5B). Comparison of total weight gain between the Test and the respective alternate treatment groups also identified no significant differences in either experiment one (Mann–Whitney *U* test; $P = 0.260$; Fig. 5C) or experiment two (Mann–Whitney *U* test; $P = 0.528$; Fig. 5D). Indistinguishable weight gain between the two paired treatment strategies indicates similar production efficiency.

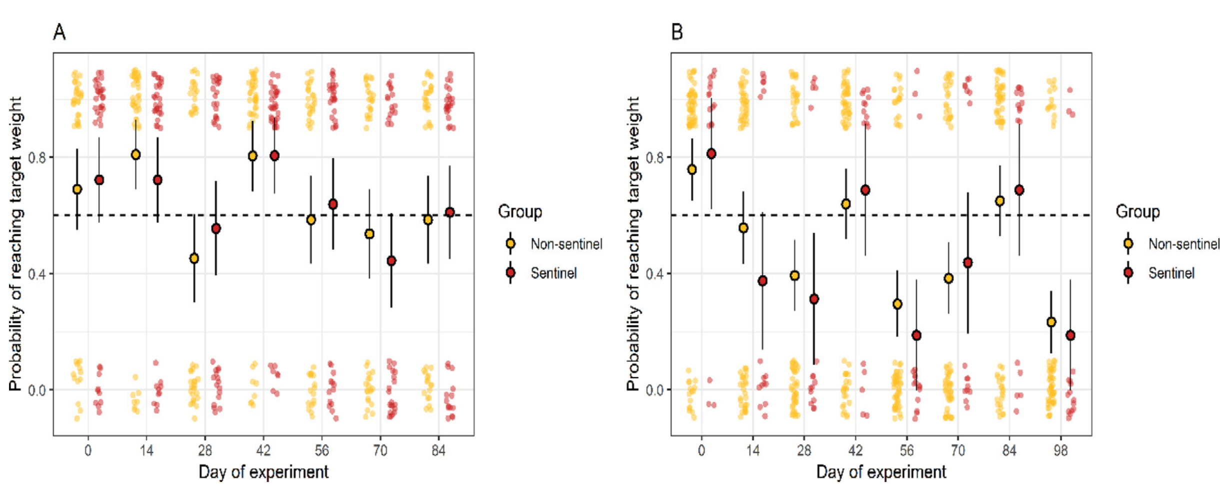

Fig. 4. The changes in probability of meeting target weight across the course of the study where sentinels comprised (A) 45%, experiment one, and (B) 20%, experiment two, of the Test group. Coloured points show whether or not each animal succeeded (1) or failed (0) in making their weight target; random vertical and horizontal jitter has been added to points to make data easier to visualise. Large points and lines show estimated changes in probability of meeting weight gain target ± 95% confidence interval as estimated by model 4 for both experiments, illustrating that there are no significant differences between animals assigned to be sentinels or non-sentinels across time points. The broken horizontal line signifies the treatment threshold: when more than 40% of sentinel lambs failed to reach the weight gain target (i.e. when fewer than 60% succeeded), the whole group was treated.

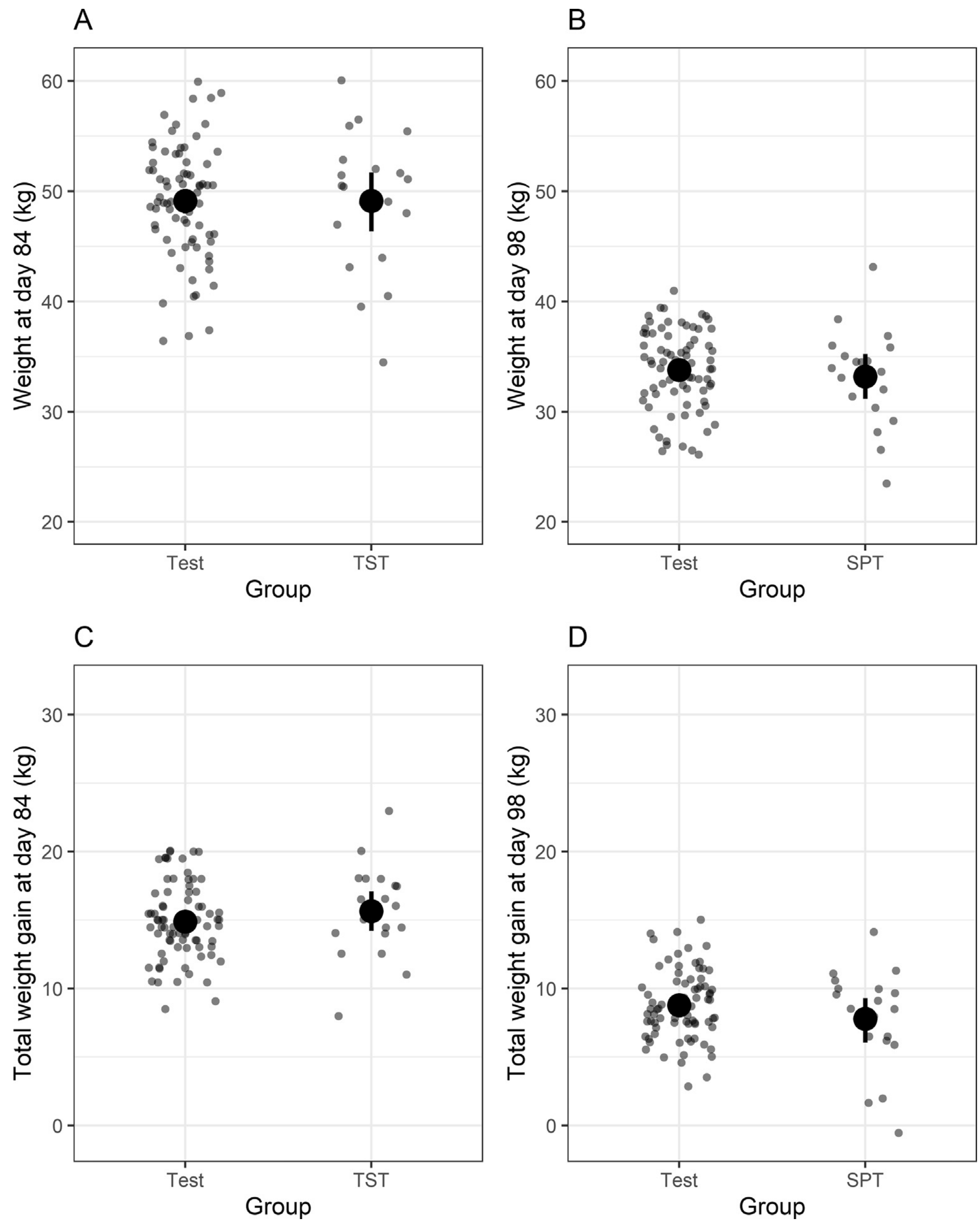

Fig. 5. Comparison of performance between lambs in the Test groups and Control groups using targeted selective treatment (Texel-cross lambs) or strategic prophylactic treatment (Lleyn-cross lambs) regimes. (A) BW at the end of experiment one; (B) BW at the end of experiment two; (C) total weight gain at the end of experiment one; (D) total weight gain at the end of experiment two. Small points show raw data; large points show raw means ± 95% confidence interval.

The changes in BW during experiments one (comparing Test and TST groups) and two (comparing Test and SPT groups) were modelled to assess the influence of 'group', 'day' and an interaction between these factors. Body weight varied with day in experiment one ($\chi^2_6 = 1482.30$, $P < 0.001$) but not between the Test and TST groups (estimate = 0.06 ± 1.17SE, $\chi^2_1 = 0.00$, $P = 0.957$), and there was no interaction between day and group ($\chi^2_6 = 6.72$, $P = 0.347$). Similarly, in experiment two, where 20% of the Test group were sentinels, weight varied between days ($\chi^2_6 = 1036.50$, $P < 0.001$) but not between the Test and SPT groups (estimate = − 0.18 ± 0.91SE, $\chi^2_1 = 0.04$, $P = 0.845$), and there was no interaction between day and group ($\chi^2_6 = 9.38$, $P = 0.226$). Thus, in both experiments, weight increased across time, but there were no detectable differences between the groups in either year (Fig. 6).

Impact on pasture contamination

Total FEC over each experiment was used to compare pasture contamination between groups. In experiment one, total FEC was higher in the TST group (mean 62, range 0–486 EPG) than the Test group (mean 46, range 0–558 EPG) (Mann–Whitney *U* test; $P = 0.034$). However, when analysing individual time points (Table 1), FEC differed significantly between groups only on days 42 and 84, 2 weeks after whole group treatment of the Test group. Similar results were observed in experiment two: FEC differed significantly between Test and SPT groups at several time points, mostly following whole group treatments. However, there was no difference in total FEC between the Test and SPT groups in experiment two (Mann–Whitney *U* test; $P = 0.920$): mean

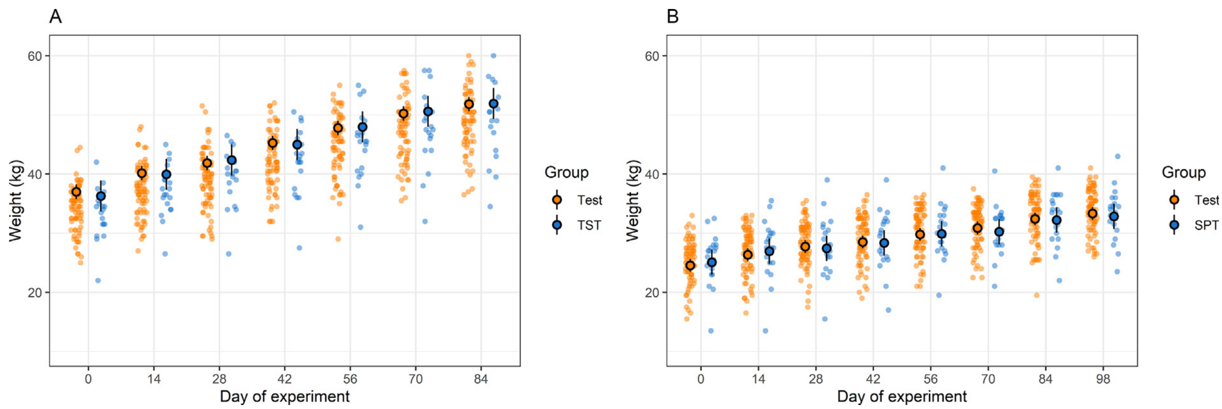

Fig. 6. Body weight of lambs across the course of (A) experiment one, 45% of the Test group monitored as sentinels, and (B) experiment two, 20% of the Test group monitored as sentinels. Coloured points show the raw weights collected from the Test and targeted selective treatment (TST) groups (experiment one) or the Test and strategic prophylactic treatment (SPT) groups (experiment two); large points with error bars show estimates changes in BW ± 95% confidence interval as estimated by model 4 for both experiments. The estimates show that the interaction is not supported and that the change across time is the same in both groups in both experiments.

FEC was 100 (range 0–954) EPG in the Test group and 105 (0–2781) EPG in the SPT group.

The change in FEC over the course of the experiment was modelled to determine the influence of 'group' (Test versus TST in experiment one and Test versus SPT in experiment two), 'day' and their interaction. Change in FEC across both experiments was influenced by 'group', 'day' and their interaction. In experiment one, FEC varied across the days ($\chi^2_6 = 250.92$, $P < 0.001$) and was higher overall in the TST group (estimate = 0.38 ± 0.12SE, $\chi^2_1 = 9.40$, $P = 0.002$), but not once day was also included in the model (estimate = 0.18 ± 0.14SE, $\chi^2_1 = 1.48$, $P = 0.224$). The interaction between day and group was, however, supported ($\chi^2_6 = 47.02$, $P < 0.001$). Similar results were apparent in experiment two: FEC varied between days ($\chi^2_6 = 404.39$, $P < 0.001$), and while FEC did not vary between groups when group was fitted alone in the model (model 1, estimate = − 0.19 ± 0.13SE, $\chi^2_1 = 2.45$, $P = 0.118$), group was supported once day was accounted for in the model (model 3, estimate = − 0.43 ± 0.14SE, $\chi^2_1 = 9.65$, $P = 0.002$), suggesting that FEC was lower in the SPT group compared to the Test group. The interaction between day and group was also supported ($\chi^2_6 = 71.32$, $P < 0.001$). Overall, the results suggested that the change across time differed between groups in both experiments (Fig. 7). Variation in FEC across time was expected between groups due to differences in the timing of treatments and the proportion of animals treated at each time point. In experiment one (45% sentinels), this difference was derived from whole-group treatments administered to the Test group on days 28 and 70 compared with TST group treatments, where animals were treated on an individual basis. In experiment two (20% sentinels), the interaction arose because of the different days on which anthelmintic was administered in the two groups, being on days 0, 28 and 56 in the Test animals and days 0, 42 and 70 in the SPT animals (Fig. 7).

Numbers of anthelmintic treatments administered

Anthelmintic usage was similar between groups, within each experiment. In experiment one, two whole-group anthelmintic treatments were administered to the Test group, on experimental days 28 and 70. Meanwhile, animals in the TST group received 0–3 treatments (mean 1.5), with on average 21.4% of the TST group (0–30%) treated at each sampling period. In experiment two, both the Test and the SPT groups received two drenches throughout the experiment (excluding the initial drench that both groups received on day 0). Treatments in the Test group were triggered on days 28 and 56 and although the proportion of lambs that failed to reach their weight gain targets was over 40% on days 14 and 70, no drench was administered as this was within the withdrawal period of the treatments administered in the previous 2 weeks. Treatments were given to the SPT group 2 weeks later than to the corresponding Test group, on days 42 and 70.

Discussion

Monitoring of a proportion of lambs from a large co-grazing group was sufficient to determine the need for anthelmintic treatments at group level in the present study. Such sentinel lambs were found to be

Table 1
A comparison of the faecal egg count of Test and Control group lambs across the two experiments. Groups were compared per time point using a Mann–Whitney test and the Bonferroni-corrected *P*-value for each experiment was also calculated. In grey are highlighted statistically significant differences between the Test and targeted selective treatment groups (experiment one) and the Test and strategic prophylactic treatment groups (experiment two).

Experiment	Day	Test group N	TST/SPT N	Test group mean (SE)	TST/SPT mean (SE)	Mann-Whitney test P	Bonferroni P
One (45% sentinels)	0	75	19	46.6 (7.64)	63.9 (13.9)	0.126	0.007
	14	68	17	35.8 (7.17)	29.2 (11.4)	0.732	0.007
	28	63	19	81.9 (10.7)	71.0 (19.0)	0.397	0.007
	42	69	19	0.8 (0.2)	37.1 (11.3)	<0.001	0.007
	56	71	19	35.6 (8.29)	29.4 (9.92)	0.98	0.007
	70	63	18	67.1 (12.7)	72.1 (16.0)	0.691	0.007
	84	67	18	2.01 (1.22)	92.3 (33.4)	<0.001	0.007
Two (20% sentinels)	0	72	17	271 (23.7)	214 (41.7)	0.2776	0.006
	14	69	15	5.6 (1.71)	14 (6.68)	0.674	0.006
	28	73	17	122 (15.5)	60.1 (24.1)	0.021	0.006
	42	70	12	26.8 (4.00)	127 (41.1)	0.002	0.006
	56	73	18	102 (12.4)	19.5 (9.28)	<0.001	0.006
	70	73	17	23.6 (5.00)	314 (173)	0.071	0.006
	84	72	17	154 (17.1)	63.1 (16.6)	0.002	0.006
	98	72	19	118 (8.92)	54.3 (8.49)	<0.001	0.006

Abbreviations: N = the number of animals in each group for which faecal egg count (FEC) data was available; TST = targeted selective treatment; SPT = strategic prophylactic treatment.

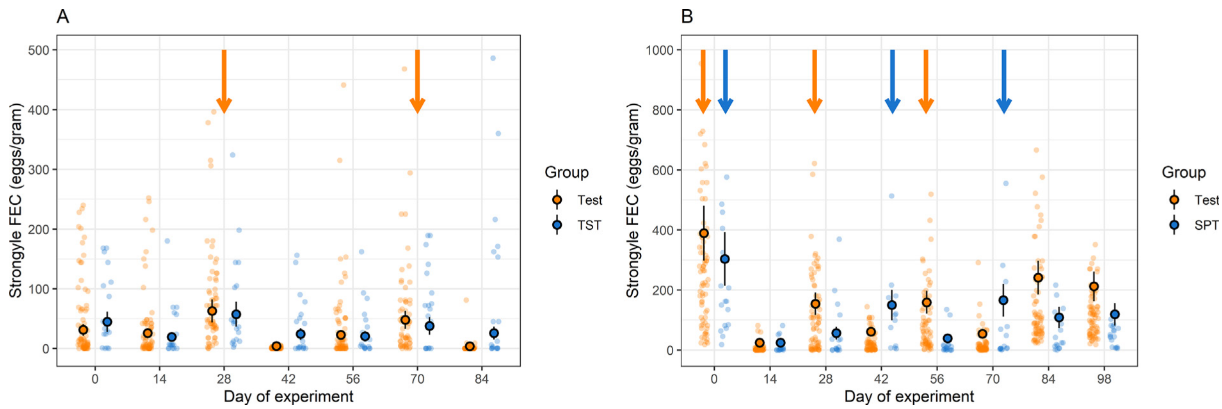

Fig. 7. The changes in strongyle faecal egg count (FEC) of lambs over time in (A) experiment one, and (B) experiment two. Small coloured points show the raw numbers of strongyle eggs recovered from faeces in the Test and targeted selective treatment (TST)/strategic prophylactic treatment (SPT) groups; large points with error bars show estimates changes in FEC ± 95% confidence interval as estimated by model 4 for both experiments. Arrows indicate the timing of treatments in the Test and SPT groups.

representative of the overall group in the key production indicator, weight gain, and in levels of parasite egg output estimated by FEC, and successfully identified time points when a critical number of the grazing group failed to reach their weight targets. The sentinel strategy maintained production efficiency of the group in line with TST and conventional SPT anthelmintic treatment strategies, without increasing pasture contamination, and is a viable alternative to those strategies.

The aim of this study was to explore a simplified version of performance-led TT, monitoring only a proportion of the group in order to determine the need for treatment in all animals. This method could be used to target whole-flock treatments, as demonstrated here, or to identify time points when further performance measurement in support of TST would be most useful. In either case, the lower labour requirements of a sentinel approach should encourage farmers to monitor more frequently than might be possible if all lambs are to be tracked. Here we demonstrated the method using weaned lambs, but the same protocol could be used pre-weaning, similar to previous TST studies (Kenyon et al., 2013). Monitoring the performance of grazing animals more closely may have additional benefits, such as detection of other disease problems, and information on growth rates to enable optimal finishing. This can be achieved by selecting randomly from the group. Because of individual factors affecting growth, however, more precise targets can be applied to individual lambs, and therefore underperformance identified more accurately.

Inevitably, the ability of sentinels to accurately indicate the state of the whole group increases with their number, as a proportion of total group size. However, the proportion of lambs that were over the threshold for treatment at each point in the study was similar in the sentinel and non-sentinel animals, whether they comprised 45% or 20% of the Test group. This approach, even with the smaller number of sentinels, was found to be sufficient to trigger treatments correctly (Fig. 4) and to maintain productivity in the flock (Figs. 5 & 6). Monitoring 20% rather than 45% of the group would greatly reduce labour costs and time requirements in a commercial setting. Monte Carlo simulation was useful for predicting the effect of sentinel numbers on misclassification risk and could be extended to consider other monitoring and intervention strategies, for example, the use of sentinels to trigger optimal timing of individual performance-based treatments across the group, in a combined TT-TST approach. Moreover, the viability of selecting lambs randomly for monitoring versus tracking the performance of assigned sentinel individuals could be investigated by simulation, and results used to design informative field trials.

Pasture contamination is an important factor to consider in any management regime, and total FEC indicated that the TST group contributed more to pasture contamination compared with the Test group animals in experiment one, an observation that was attributed to the proportion of animals left untreated at each time point. Although seemingly counter-intuitive, the increased pasture contamination as a result of leaving a proportion of animals untreated can provide benefits in terms of parasite refugia (Van Wyk, 2001; Hodgkinson et al., 2019). Contributing parasites un-selected by anthelmintic treatment to the overall population can dilute resistant alleles, potentially reducing the selection pressure for anthelmintic resistance on the population as a whole. Despite the difference in pasture contamination between TST and Test group animals being statistically significant, the effect size was small and therefore we can conclude that overall pasture contamination was comparable between the treatment strategies. FEC was not statistically or biological different between the Test and SPT groups in experiment two. Combining sentinel and TST approaches might provide opportunities to further increase refugia while ensuring productivity, with limited monitoring effort.

The number of anthelmintic treatments was similar between groups, but the timing varied by monitoring strategy. In experiment two (20% sentinels), the Test group was treated 2 weeks earlier than the SPT lambs. In the current study, this delay in treatment timing did not impact the overall weight or FEC of the groups, but in other circumstances delaying treatment could lead to production loss and increased pasture contamination. Coop et al. (1982) demonstrated that growth rate in lambs is reduced by gastrointestinal nematode infection; weight gain will recover when the animal is treated, but not to the weight of an uninfected lamb. Production recovery is dependent on the severity of the growth check and therefore early intervention can minimise the impact of parasitism and reduce the production deficit. Thus, monitoring a flock to ensure that lambs are treated at optimal time points could produce production benefits for farmers as lambs can be finished quicker, with the associated protection of pastures, both in terms of grazing and reducing pasture contamination for subsequent animals.

To determine the impact on production of using sentinels to make treatment decisions, the approach was compared with the ‘Happy Factor’ TST approach on co-grazed lambs, a method that offers sustainable and optimised worm control, and an SPT approach, for comparison with a treatment strategy commonly used by farmers (Burgess et al., 2012). Weight gain in lambs was similar between groups (Fig. 6); thus, the sentinel approach maintained production in line with other commonly used treatment protocols. Test group and control animals were co-grazed throughout both experiments to ensure that all lambs were subject to the same parasite challenge; however, given this experimental design, pasture contamination was influenced by the anthelmintic treatments administered to each treatment group. The number of treatments administered was similar between groups, albeit at different times, and the overall pasture contamination attributed to each group was comparable and as such the impact of individual group treatments on pasture contamination was considered to be relatively similar, supporting the validity of production comparisons between groups. Thus, the sentinel approach appears to provide an attractive alternative for farmers unable to conduct an extensive TST approach.

The sentinel approach used here has been shown to be able to monitor group performance effectively and act as a pen-side decision support system upon which treatment timing can be determined. While further refinements are possible, the reduced labour requirement of this approach increases the viable options available to farmers who wish to engage with sustainable parasite management in the face of growing threats to production from anthelmintic resistance.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100176.

Ethics approval

All trials were examined and approved by the Moredun Research Institute Experiments and Ethics Committee and conducted under the legislation of a UK Home Office License (reference PPL 60/03899) in accordance with the Animals (Scientific Procedures) Act of 1986.

Data and model availability statement

None of the data were deposited in an official repository.

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Declaration of interest

The authors had no competing interests.

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References

Besier, R.B., 2008. Targeted treatment strategies for sustainable worm control in small ruminants. Tropical Biomedicine 25, 9–17.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Neilsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9, 378–400.

Burgess, C.G., Bartley, Y., Redman, E., Skuce, P.J., Nath, M., Whitelaw, F., Tait, A., Gilleard, J.S., Jackson, F., 2012. A survey of the trichostrongylid nematode species present on UK sheep farms and associated anthelmintic control practices. Veterinary Parasitology 189, 299–307.

Busin, V., Kenyon, F., Parkin, T., McBean, D., Laing, N., Sargison, N.D., Ellis, K., 2014. Production impact of a targeted selective treatment system based on liveweight gain in a commercial flock. Veterinary Journal 200, 248–252.

Charlier, J., Morgan, E.R., Rinaldi, L., Van Dijk, J., Demeler, J., Hoglund, J., Hertzberg, H., Van Ranst, B., Hendrickx, G., Vercruysse, J., Kenyon, F., 2014. Practices to optimise gastrointestinal nematode control on sheep, goat and cattle farms in Europe using targeted (selective) treatments. The Veterinary Record 175, 250–255.

Cringoli, G., Rinaldi, L., Veneziano, V., Mezzino, L., Vercruysse, J., Jackson, F., 2009. Evaluation of targeted selective treatments in sheep in Italy: effects on faecal worm egg count and milk production in four case studies. Veterinary Parasitology 164, 36–43.

Gaba, S., Cabaret, J., Sauve, C., Cortet, J., Silvestre, A., 2010. Experimental and modeling approaches to evaluate different aspects of the efficacy of targeted selective treatment of anthelmintics against sheep parasite nematodes. Veterinary Parasitology 171, 254–262.

Gascoigne, E., Morgan, E.R., Lovatt, F., Rose, H.R., 2018. Controlling nematode infections in sheep: application of HACCP. Practice 40, 334–347.

Gethings, O.J., Rose, H., Mitchell, S., Van Dijk, J., Morgan, E.R., 2015. Asynchrony in host and parasite phenology may decrease disease risk in livestock under climate warming: Nematodirus battus in lambs as a case study. Parasitology 142, 1306–1317.

Greer, A.W., Kenyon, F., Bartley, D.J., Jackson, E.B., Gordon, Y., Donnan, A.A., McBean, D.W., Jackson, F., 2009. Development and field evaluation of a decision support model for anthelmintic treatments as part of a targeted selective treatment (TST) regime in lambs. Veterinary Parasitology 164, 12–20.

Hartig, F., 2019. DHARMa: residual diagnostics for hierarchical (multi-level / mixed) regression models. R package version 0.2.3. Retrieved on 12 December 2019 from. https://CRAN.R-project.org/package=DHARMa.

Hilborn, R., Mangel, M., 1997. The ecological detective: confronting models with data. Princeton University Press, Princeton, NJ, USA.

Hodgkinson, J., Kaplan, R.M., Kenyon, F., Morgan, E.R., Park, A.W., Paterson, S., Babayan, S. A., Beesley, N., Britton, C., Chaudry, U., Doyle, S.R., Ezenwa, V.O., Fenton, A., Howell, S. B., Laing, R., Mable, B.K., Matthews, L., McIntyre, J., Milne, C.E., Morrison, T.A., Prentice, J.C., Sargison, N.D., Williams, D.J.L., Wolstenholme, A.J., Devaney, E., 2019. Refugia and anthelmintic resistance: concepts and challenges. International Journal for Parasitology: Drugs and Drug Resistance 10, 51–57.

Jackson, F., 1974. New technique for obtaining nematode ova from sheep faeces. Laboratory Practice 23, 65–66.

Kaler, J., Ruston, A., 2019. Technology adoption on farms: unisng normalisation process theory to understand sheep farmers' attitudes and behaviours in relation to using precision technology in flock management. Preventitive Veterinary Medicine 170, 104715.

Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends in Parasitology 20, 477–481.

Kenyon, F., Greer, A.W., Coles, G.C., Cringoli, G., Papadopoulos, E., Cabaret, J., Berrag, B., Varady, M., Van Wyk, J.A., Thomas, E., Vercruysse, J., Jackson, F., 2009. The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. Veterinary Parasitology 164, 3–11.

Kenyon, F., McBean, D., Greer, A.W., Burgess, C.G., Morrison, A.A., Bartley, D.J., Bartley, Y., Devin, L., Nath, M., Jackson, F., 2013. A comparative study of the effects of four treatment regimes on ivermectin efficacy, body weight and pasture contamination in lambs naturally infected with gastrointestinal nematodes in Scotland. International Journal of Parasitology: Drugs and Drug Resistance 3, 77–84.

Leathwick, D.M., Waghorn, T.S., Miller, C.M., Atkinson, D.S., Haack, N.A., Oliver, A.M., 2006. Selective and on-demand drenching of lambs: impact on parasite populations and performance of lambs. New Zealand Veterinary Journal 54, 305–312.

McBean, D., Nath, M., Lambe, N., Morgan-Davies, C., Kenyon, F., 2016. Viability of the happy factorTM targeted selective treatment approach on several sheep farms in Scotland. Veterinary Parasitology 218, 22–30.

Miller, C.M., Waghorn, T.S., Leathwick, D.M., Candy, P.M., Oliver, A.M.B., Watson, T.G., 2012. The production cost of anthelmintic resistance in lambs. Veterinary Parasitology 186, 376–381.

Morgan-Davies, C., Lambe, N., Wishart, H., Waterhouse, T., Kenyon, F., McBean, D., McCracken, D., 2018. Impacts of using a precision livestock system targeted approach in mountain sheep flocks. Livestock Science 208, 67–76.

Prichard, R.K., Hall, C.A., Kelly, J.D., Martin, I.C., Donald, A.D., 1980. The problem of anthelmintic resistance in nematodes. Australian Veterinary Journal 56, 239–251.

Ramunke, S., Melville, L., Rinaldi, L., Hertzberg, H., de Waal, T., von Samson-Himmelstjerna, G., Cringoli, G., Mavrot, F., Skuce, P., Krucken, J., Demeler, J., 2016. Benzimidazole resistance survey for *Haemonchus*, *Teladorsagia* and *Trichostrongylus* in three European countries using pyrosequencing including the development of new assays for *Trichostrongylus*. International Journal of Parasitology: Drugs and Drug Resistance 6, 230–240.

Rose, H., Rinaldi, L., Bosco, A., Mavrot, F., De Waal, T., Skuce, P., Charlier, J., Torgerson, P.R., Hertzberg, H., Hendrickx, G., Vercruysse, J., Morgan, E.R., 2015. Widespread anthelmintic resistance in European farmed ruminats: a systematic review. The Veterinary Record 176 (21), 102982.

Stafford, K., Morgan, E.R., Coles, G.C., 2009. Weight-based targeted selective treatment of gastrointestinal nematodes in a commercial sheep flock. Veterinary Parasitology 164, 59–65.

Van Wyk, J.A., 2001. Refugia - overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. The Onderstepoort Journal of Veterinary Research 68, 55–67.

Van Wyk, J.A., Bath, G.F., 2002. The FAMACHA((c)) system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Veterinary Research 33, 509–529.

Van Wyk, J.A., Hoste, H., Kaplan, R.M., Besier, R.B., 2006. Targeted selective treatment for worm management–how do we sell rational programs to farmers? Veterinary Parasitology 139, 336–346.

Van Dijk, J., Sargison, N.D., Kenyon, F., Skuce, P.J., 2010. Climate change and infectious disease: helminthological challenges to farmed ruminants in temperate regions. Animal 4, 377–392.

Vatta, A.F., Letty, B.A., van der Linde, M.J., Van Wijk, E.F., Hansen, J.W., Krecek, R.C., 2001. Testing for clinical anaemia caused by Haemonchus spp. in goats farmed under resource-poor conditions in South Africa using an eye colour chart developed for sheep. Veterinary Parasitology 99, 1–14.