Assessing the feasibility of targeted selective treatments for gastrointestinal nematodes in first-season grazing cattle based on mid-season daily weight gains

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1. Introduction

Infections by the gastrointestinal (GI) nematodes Ostertagia ostertagi and Cooperia oncophora are among the most costly constraints on the performance and productivity of first-season grazing (FSG) cattle in temperate regions (Corwin, 1997). Both of these nematode species are pasture-borne parasites found wherever cattle are grazing. Among other things, they have in common a simple direct life cycle with infective stages transmitted by the oral–faecal route through ingestion of grass on contaminated pasture (Andersson, 1992). Apart from losses due to sub-clinical GI infections, it has repeatedly been shown that heavy infections cause severe clinical signs and should therefore be regarded as an important animal welfare issue (for example Shaw et al., 1998a).
Problems caused by pasture-borne parasites of ruminants have been subjected to intense research activities over the past decade in Sweden. In two independent projects, it was demonstrated how low-grade infections with gastrointestinal parasites proceeded in calves grazed on permanent pastures in set-stocked paddocks (Dimander et al., 2000, 2003). Among other treatments, both trials included comparisons between animals that were completely unprotected against GI nematodes (negative control) and those that received an ivermectin bolus (positive control) at turnout, which protected them for 135 days on to pasture. At housing, the positive-control calves weighed on average 30 and 65 kg more than the negative-control calves, in the two trials (Dimander et al., 2000, 2003). Similarly, Larsson et al. (2006) showed that the weight gain penalties in set-stocked animals were on average ~40 kg at housing, compared to animals protected by regular injections of doramectin. Overall, these data clearly illustrate the importance of GI-nematode infections in FSG cattle under Swedish climatic conditions.

It is well known that good levels of nematode control can be achieved through the integrated prophylactic use of a range of highly effective anthelmintics in combination with grazing management. However, there are a number of concerns about whether the intensive use of anthelmintics could lead to long-term difficulties, and therefore be unsustainable. First, many consumers are sceptical about overdependence on biocides in livestock farming. For example, dependence on routine anthelmintic treatment is not generally accepted in organic livestock production in many European countries. In Sweden prophylactic treatment of cattle is prohibited in organic production systems (Anonymous, 2007).

Second, there is increasing evidence that GI nematode infections may become more difficult to control as a result of emerging problems with anthelmintic resistance (AR). Until recently, it was believed that problems with AR in nematode parasites of cattle were not likely to occur (Coles, 1988). However, recent surveys indicate that failure of anthelmintic treatment indeed is a problem in this sector. To date, the problem with GI nematodes in cattle that are refractory to anthelmintic treatment is widespread in the southern hemisphere, such as in New Zealand and in intensive cattle-rearing areas of Latin America (Waghorn et al., 2006; Anziani et al., 2001, 2004; Suarez and Cristela, 2007), but there is now increasing evidence that it is also an emerging problem in Europe (see this volume).

Accordingly, there is an urgent need to fine-tune the ways in which anthelmintics are used, to avoid escalating problems with AR. In this study, we have evaluated the conditions for individual targeted selective treatments (TST), and whether targeted treatment of individual FSG cattle can be based on weight gain in the middle of the grazing season. It should be feasible for most farmers to record individual weight gains in their cattle, making this a practicable marker for decision-making purposes. The long-term aim of TST is to minimise the numbers of whole-herd/flock anthelmintic treatments, by directing treatments towards only those animals/herds that are likely to suffer from disease and/or production loss. This will reduce the opportunities for any associated environmental and health risks, while maintaining agricultural productivity. However, in order to create low-input and sustainable programs for nematode control, TST strategies also need development and validation under practical farming conditions.

The aim of our work was to empirically assess the practicality of TST, using retrospective data on animal performance and parasitology collected from FSG steers in Sweden that had been exposed to various levels of GI nematode challenges. We tested whether it is possible to identify heavily infected animals based on their daily weight gains (Dwg) recorded up to 8 weeks after turnout. The following questions were addressed: (1) whether productivity losses at housing can be predicted from determinations of daily weight gain during the first half of the grazing season, and (2) to what extent GI nematodes are involved in variations in weight gain. The ultimate objective was to quantify the details of a possible TST for GI nematodes in FSG cattle in Sweden, along with an assessment of its probability for success.

Our goal is to provide a proof of concept for the use of TSTs under these circumstances. Proof of concept is an (often incomplete) attempt to demonstrate the feasibility of a particular concept. The purpose is to verify that the concept is probably capable of exploitation in a useful manner, and to identify the initial practical details that will be involved. This is usually considered to be the first milestone on the way to a fully functioning system. Successfully passing this milestone justifies proceeding to the second milestone, which is a pilot experiment designed to verify that the concept does indeed have practical application under controlled conditions, and to further identify practical issues. If successful, this second milestone is then followed by a large-scale experiment under realistic conditions, which provides the final proof.

2. Materials and methods

2.1. Data collection

In this retrospective study, data were combined from three independent grazing trials conducted at different field sites during the past decade in Sweden. The experimental design and outcome of each trial have previously been described. Data from the following trials were combined into a common dataset: (1) the Nåntuna trial performed during 1997–1998 (Dimander et al., 2000), (2) the Kungsängen trial during 1998–2000 (Dimander et al., 2003), and (3) the Anhammar trial during 2002–2004 (Larsson et al., 2006).

Briefly, in each trial in each year 3–5 groups of 10 first-season grazing (FSG) calves were subjected to various levels of parasite control (a total of 60, 150 and 120 animals in the three studies, respectively). After the animals were turned out onto pasture in mid May, they were allowed to graze for approximately 20 weeks until housing. During the grazing season, the calves were weighed on an electronic scale and individual blood and rectal faecal samples were collected at intervals of 3 (Nåntuna) or 4
(Kungsängen and Änhammar) weeks. Dwgt was calculated as the difference in weight from the first sampling time divided by the relevant number of days.

In the laboratory, the numbers of trichostrongyloid nematode eggs (eggs per gram, Epg) were determined by a modified McMaster method (Anonymous, 1986), based on 3 g of faeces and a detection level of 50 Epg. Sera were prepared and stored at –20 °C until analysed for individual serum pepsinogen concentrations using a micro-determination method (Dorny and Vercruysse, 1998). In addition, serum antibodies to O. ostertagi infection were later determined according to the manufacturer’s instruction using a standardised ELISA-kit (SVANOVIR® O. ostertagi, Sweden).

2.2. Database construction

Information about animal performance, faecal egg counts expressed as Epg, serum pepsinogen levels expressed as units of tyrosine (Utyr) and Ostertagia antibody levels expressed as corrected optical-density ratios (ODR), together with general information about the trial, experimental treatment, sampling dates, etc., were compiled into a common database. Overall, the dataset contained individual information on Dwgt, Epg, Utyr and ODR for a total of 330 FSG steers, each recorded at 6–9 different time points.

Many other factors that are potentially important determinants of weight gain could not be included in the database, although their effects are likely to have been included in the experiments themselves, because the data came from three disparate trials and these data are either not available or are not directly comparable. These determinants include differences due to other infections, age and pasture availability.

In order to facilitate statistical comparisons between trials and years, the nematode-control levels were categorized into maximum (MAX), intermediate (INT) and minimum (MIN). MAX animals had been treated with an anthelmintic throughout the entire grazing season. The database thus contained animals with a wide range of levels of infection with GI nematodes.

Three sampling periods (i.e. Early, Middle and Late) were also defined in order to standardise the comparisons between trials. Early refers to samples collected 3–4 weeks post-turnout, Middle is defined as 6–8 weeks, and Late denotes the period around housing, when the animals had been on to pasture for 20–21 weeks.

2.3. Data analysis

Initially the relationships between daily weight gains observed at housing (Late) and at different time points (Early and Middle) during the grazing season were calculated, where the coefficient of determination, $r^2$, represents the proportion of variation held in common between the two variables.

Next, we assessed the relationships between the three parasitological variables (Epg, Utyr and ODR) and daily weight gains by the middle of the grazing season (Dwgt-M) using non-parametric Spearman rank correlation. We also graphically assessed each of the parasitological variables with respect to their within-season patterns of variation.

This allowed us to determine suitable cut-off values for discriminating presence/absence of biologically important levels of nematode infection. Cut-off values were evaluated with respect to the level of parasite control, so that the MAX animals were considered to have negligible levels of infection (i.e. were defined as being in the nematode-unaffected group), while the INT and MIN animals had variable levels. Many different cut-off values were evaluated, with respect to the differences between treatment levels and between trials. The final cut-off values were set so as to maximise the average weight difference between affected and unaffected animals.

Receiver operating characteristic (ROC) curves were then generated for each parasitological variable using the methods described by Fawcett (2006). The ROC curve plots the fraction of true positives and the fraction of false positives for a binary classifier, in relation to variation in the discrimination threshold. It thus shows the relationship between sensitivity and (the complement of) specificity. In our system the binary classification was based on the presence/absence of a nematode burden sufficient to affect the growth of the cattle (as determined by the parasitological measurements), with the response variable being Dwgt by the Middle of the season (Dwgt-M).

Finally, the ROC curves were used to quantify the sensitivity and specificity of using various values of Dwgt-M as the cut-off value for determining whether the cattle should receive anthelmintic treatment or not. Several combinations were tested, including and excluding different anthelmintic-treated groups and noting the differences between trials. The final analyses presented are those that maximized the area under the ROC curve (AUC), which estimates the probability that a randomly chosen nematode-affected animal (positive value) will be ranked higher than a randomly chosen unaffected animal (negative value).

Data analyses used either JMP™ 6.0 (SAS) or Excel X for Mac (Microsoft).

3. Results

3.1. Proof of concept for using ROC curves in designing TSTs

To achieve our overall goal, first we need to demonstrate that Dwgt by the middle of the season is a good predictor of weight gain by the end of the season (Section 3.2). Then, we need to demonstrate that variation in Dwgt...
is related to nematode infection, and that it is possible to
discriminate animals with a high level of infection using a
measurement of some parasitological parameter (e.g.
threshold FEC in the middle of the season) (Section 3.3).
Third, we will use these values to quantify the sensitivity
and specificity of using various values of weight gain as a
cut-off value for determining a target for when anthel-
mintic treatment should be administered (Section 3.4).
This will demonstrate that ROC curves can be used to
identify treatment thresholds based on mid-season daily
weight gains in FSG Swedish cattle, providing the required
proof of concept.

3.2. Relationships between Dwgt at different times

There was a positive relationship between the
individual daily weight gains at different times during
the grazing season (Fig. 1). However, the values observed
after 3–4 weeks post-turnout (Early) are not good
predictors of the values at the end of the grazing season
(Late), whereas those registered after 6–8 weeks on
pasture (Middle) are much better predictors (Fig. 1). We
therefore focussed the rest of our data analyses on the use
of daily weight gains by the middle of the season (Dwgt-
M), as a suitable criterion for decision-making with
regard to anthelmintic treatments.

The poor prediction by the Early daily weight gains
(Dwgt-E) arises because the early weight gains are much
more variable than are the later gains (Fig. 2). Up until
week 4 of the grazing season, the weight gains of
individual cattle can vary from being negative to strongly
positive, whereas after this time they are much more
consistent.

3.3. The parasitological variables

The seasonal fluctuations in the parasitological vari-
ables that were measured at more or less regular intervals
during the grazing season were quite repeatable between
trials and years (Fig. 3), although there were exceptions.
A dramatic increase in Epg was usually observed during
the first 4–6 weeks out on pasture, which was then
followed by a more or less steep decline. This pattern was
observed in all of the trials and years with the exception of
the second year at Kungsången, when the peak level was
somewhat delayed (Fig. 3). The maximum pepsinogen
(Utyr) levels were usually observed during the Middle of
the grazing season (Fig. 3), increasing from approximately
4–6 weeks post-turnout and onwards. However, in one
year for each trial there was no obvious decrease towards
the end of the season. The antibody (ODR) levels increased
somewhat more slowly than did the serum pepsinogen,
and the maximum levels were often observed late during the grazing season, around housing (Fig. 3).

Thus, we chose to investigate two possible criteria for using the faecal egg count as a criterion for discriminating a high level of parasite infection (i.e. whether an animal was considered to be nematode-affected or not): the observed Middle Epg for each animal, and the total Epg observed during the grazing season (i.e. the cumulative sum of the egg counts). For both Utyr and ODR we used the maximum observed value for each animal as the discriminator.

The Spearman rank correlations (Table 1) for each of these parasitological variables were statistically significant for daily weight gain by the Middle of the season (Dwgt-M). This indicates that parasite infection is a major contributor to variation in weight gain, and that they can potentially be used as indicators in any program of nematode control.

### 3.4. Sensitivity and specificity

ROC analyses are intended to evaluate different discrimination levels (in our case, different cut-off values for receiving an anthelmintic treatment) in relation to the assessed infection status (nematode-affected versus unaffected). That is, the sensitivity (ability to detect affected animals) and specificity (ability to avoid unaffected animals) are evaluated in relation to variation in treatment cut-off values. In our

<table>
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<th>Variable</th>
<th>Correlation</th>
<th>Probability</th>
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<tr>
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case, the cut-off values were different values of Dwgt-M, and sensitivity/specificity were defined using one of the four discriminators: total Epg, Middle Epg, Utyr or ODR. Total Epg proved to be ineffective, and so the results are not presented here.

On the ROC graph (Peterson et al., 2008), the origin (0,0) is where the classification procedure never falsely identifies absences but also fails to identify every known presence. The top-right corner (1,1) is where the procedure correctly identifies every true presence but also misidentifies all of the absences. The top-left corner (0,1) indicates that the classification procedure identifies all true positives and never misclassifies any of the true absences. By varying the threshold of the cut-off values (discriminating animals to be treated), a curve in ROC space is traced. A straight line from bottom left to top right indicates a procedure that does no better than random. The space above such a line represents the degree to which the classification procedure is better than random, with success increasing towards the top left. The area under the ROC curve is thus a quantitative measurement of success.

ROC curves for Dwgt-M were constructed using each of the four potential discriminators based on a variety of cut-off values. The final cut-off values were chosen to be those that produced the best sensitivity and specificity: 200 Epg for the total FEC, 250 Epg for the Middle Epg, 3.5 Utyr, and 1.0 units for ODR. These cut-off values were then used to discriminate parasite-affected from "unaffected" animals.

Several of the ROC curves (Fig. 4) have an area under the curve of ~0.6, which we consider to be satisfactory for the three combined field trials which were evaluated retrospectively. The AUC is largest for the two Epg, with the counts in the Middle of the season producing the best sensitivity values in the range relevant for our purposes. We have therefore used this variable in the final step of our analyses.

We then evaluated the sensitivity and specificity of three possible criteria for choosing a Dwgt-M cut-off value (Fig. 5), to be used as a means of determining which individual animals should be given anthelmintic treatment in the Middle of the season. The first criterion was a sensitivity of 0.8, which would provide good protection for a herd. This yields a Dwgt-M of 0.86 kg/day and a specificity of 0.35. The second criterion was the average weight gain of those animals that were defined by our analyses as unaffected. This Dwgt of 0.75 kg/day yields a sensitivity of 0.71 and a specificity of 0.48. Finally, we used a Dwgt-M of 0.7, as being a satisfactory practical goal for a farmer. This criterion yields a sensitivity of 0.65 and a specificity of 0.55.

![Fig. 4. ROC curves for daily weight gain by the Middle of the season (Dwgt-M) based on four parasitological measurements: (a) total faecal egg counts (FECtot), (b) faecal egg count in the Middle of the season (FEC-M), (c) serum pepsinogen levels (Utyr) and (d) Ostertagia antibody levels (ODR). Also shown is the area under the ROC curve (AUC), which estimates the probability that a randomly chosen positive value will be ranked higher than a randomly chosen negative value.](image-url)
suitability of targeted selective treatments in dealing with veterinary parasites. Our goals were to provide some evidence that TSTs are more than just a theoretical possibility, and to identify some of the practical issues that will potentially be involved. We have not provided any experimental proof of the effectiveness of TSTs, nor have we examined the magnitude of the effect that TSTs might have.

Using historical data from several Swedish farms over several years, we assessed the feasibility of using mid-season weight gains to identify which animals will potentially benefit from anthelmintic treatment. We will not be interpreting the data in terms of a manipulative experiment that tests the effectiveness of TSTs, but merely whether TSTs are a method that is worth pursuing. Issues such as the use of TST in worm control management and its utility by farmers will not be addressed, nor will we specifically comment on the possible applicability of our data to other areas of Sweden or to anywhere else.

Animal performance is the factor of main interest to cattle farmers, and in particular the live weight at the end of the grazing season. Because of this, and the fact that daily weight gain can be determined rather easily in field situations, we decided to evaluate whether this variable is of any value as a marker of gastrointestinal parasite infection on which to base treatment decisions. We conclude that, indeed, measurement of Dwgt by the middle of the grazing season is likely to provide a simple and practicable means by which a farmer could decide which animals should be given anthelmintic treatment, and that improvement in final weight will likely result from such a treatment.

First, we have demonstrated that Dwgt by the middle of the season is a good predictor of weight gain at the end of the season ($r^2 = 0.54$; Fig. 1). Weight gain earlier in the season is too variable among individuals to be a useful predictor (Fig. 2). Second, we have demonstrated that variation in Dwgt is related to nematode infection (Table 1), and that it is also possible to discriminate animals with a high level of infection using a threshold $E_{pg}$ in the middle of the season (Fig. 3). Third, we have used this $E_{pg}$ to quantify the sensitivity and specificity of using various values of weight gain as a cut-off value for determining a target for when anthelmintic treatment should be administered (Fig. 4). Our evaluation indicates that using a mid-season Dwgt of 0.75 kg/day will involve treating about 70% of those animals with $>250$ Epg (i.e. sensitivity = 0.7) as well as treating twice as many animals as necessary (i.e. specificity = 0.5) (Fig. 5). These values can be used as the criteria for a targeted selective treatment.

Consequently, this study shows that ROC curves can be used to identify treatment thresholds based on mid-season daily weight gains in FSG cattle. As such, this is a novel application of this statistical method to quantify the TST approach. A clear advantage of the ROC approach is that it was possible to obtain unbiased and precise estimates of both the treatment threshold and the fraction of the herd that will be treated. Until now, the usage of ROC curves in the area of veterinary medicine has been limited to comparing the accuracy of different test methods, such as the outcome from various immunological diagnostic tools (e.g. Reichel et al., 2005), with only limited use in clinical decision-making (Garner and Greiner, 2006). In this context, it is important to note that AUC values of 0.7–0.9 are considered to be acceptable in laboratory investigations (Garner and Greiner, 2006), whereas in field trials the values may often be lower. With historical data, pooled from experiments conducted under different conditions, and AUC of 0.6 is quite acceptable for a proof of concept. It would, however, be relatively poor for a single controlled trial or for a laboratory experiment; and we anticipate larger values in future experiments.

Although it is well known that GI nematodes cause significant weight losses in FSG cattle, there are only a few studies available that include detailed analyses of the effects on weight gains in individual animals, which is the approach that we have taken in our analyses. In two papers, Shaw et al. (1998a,b) scrutinized the general patterns of GI nematode infections in FSG calves and their effects on the physical performance of the host. Although there was a significant positive correlation between mean weight gains and the duration of prophylaxis (Shaw et al., 1998b), it was concluded that it was not possible to predict weight gains obtained at the end of the season from any of the parasitological factors that were measured. This apparently contradicts our results. However, this outcome should be interpreted with caution as the data were obtained mostly from imprecise estimates, and apply to various set-stocked conditions on Western European farms (as explained by Shaw et al., 1998a).

Similarly, Ploeger et al. (1994) made quantitative estimations of the growth performance of FSG calves following experimental exposure to different levels of GI nematodes, and the same parasitological variables as those measured in our study were evaluated as estimators of the weight gains. In agreement with the results from our study.
(Table 1), it was found that measurements of FECs during the first part of simulated grazing seasons were significantly correlated with the final weight gains. Although these FECs also reflected the initial infection levels, problems arose after the onset of immunity. It was later concluded by Eysker and Ploeger (2000) that the mid-season FECs could be used as a valuable monitoring tool to predict initial infection levels. At the same time, they argued that it may be problematic to estimate the effects of using FECs for cattle in temperate regions, particularly as the fecundity of the more pathogenic species *O. ostertagi* is lower than that for *C. oncophora*. Nevertheless, it is evident that the pattern of faecal egg excretion observed in our study is not unique, and that there is a negative association between FECs and mid-season weight gains.

Furthermore, serum pepsinogen was also investigated in response to different levels of nematode exposure, by both Shaw (1998a,b) and Ploeger et al. (1994). In agreement with the results of our analysis (Fig. 3), the pepsinogen values in most instances increased more slowly than did the FECs. Although it has been shown that pepsinogen levels can be used as a tool to discriminate between animals with clinical and subclinical levels of ostertagiosis around housing (Hildgen et al., 1989; Berghen et al., 1993), a general problem is the lack of standardisation of the diagnostic techniques used (see this volume). This occurs despite a simplified micro-determination test having been available for some years (Dorny and Vercruysse, 1998). Eysker and Ploeger (2000) concluded that pepsinogen levels should always be used in conjunction with clinical and parasitological observations. Interestingly, although we observed a significant negative correlation between mid-season pepsinogen levels and weight gains, the ROC curve based on this factor indicated lower sensitivity and specificity compared to the one based on the FEC.

Finally, fluctuations in serum antibodies were also measured, both in our study and by Shaw (1998a,b) and Ploeger et al. (1994). Although various ELISAs using crude *Ostertagia* antigens have been used repeatedly in seroepidemiological investigations, the presence of antibodies is not always an accurate indicator of infection or impact on productivity, partly due to a lack of sensitivity and specificity (Berghen et al., 1993) and partly to wide between-host variation (Eysker and Ploeger, 2000). Consequently, the ROC curve based on this factor was difficult to interpret, mainly due to different baseline levels in different individuals.

For the foreseeable future it can be assumed that anthelmintics will constitute the cornerstone of most parasite control programmes for grazing livestock. However, to preserve the efficacy of available drugs and to achieve a wider acceptance of reduced drug use, particularly in relation to organic farming, it will be necessary to refine the ways in which anthelmintics are used. One possibility is to replace current treatment regimes with targeted selective treatments. Today in Sweden, anthelmintics against GI-nematodes are administered at strategic times to all first-season grazing animals that are at risk. The concept of TST is clearly different from an ordinary prophylactic programme, as it treats individual animals rather than whole herds. Thus, it is simple and easy to accept, especially in cases where animals with a high worm burden are easily identified, for example by showing clinical signs such as diarrhoea. However, it is well recognised that the greatest losses associated with GI nematode parasites in cattle in Sweden are sub-clinical, and reduced productivity is more likely to be observed (Dimander et al., 2000, 2006; Larsson et al., 2006).

For any TST approach it is crucial to choose a practicable indicator, and ideally one with a high sensitivity and specificity. It is also essential to identify treatment thresholds. Of the many potential TST indicators, we decided to focus on daily weight gains, which are a somewhat crude indicator. Thus, our suggested TST has a sensitivity of only 0.7. This means that 30% of the animals with a biologically important level of infection will remain untreated. If we define a reasonable final weight gain as greater than 0.7 kg/day (Dwgt-L >0.7), then our data indicate that 55% of the animals that we defined as affected by nematodes (i.e. >200 Epg) can potentially maintain their productivity in spite of the nematode infection (i.e. are resilient), while 90% of those with good weight gains by the middle of the season (Dwgt-M >0.75) are resilient. We thus conclude that our suggested TST will produce good weight gains for most of the animals, because they will either be resilient (i.e. they will produce high daily weight gains even if they are infected with GI nematodes) or they will receive an anthelmintic treatment. It needs to be emphasized that control of GI nematodes is a fundamental requirement to optimise production even if animals have parasites, because resilient animals are going to propagate infection and thereby contribute to further pasture contamination. This needs to be tested in a real-life situation, and it is an obvious component to be examined in the field studies that will follow on from our successful proof of concept.

In order to create low-input and sustainable programs for nematode control, TST strategies must not only be further developed but also validated under practical farming conditions. The long-term aim with TST is to minimise the numbers of whole herd/flock anthelmintic treatments by directing treatments towards only those animals/herds that are likely to suffer from disease and production loss. This will reduce the opportunities for any associated environmental and health risks, while maintaining agricultural productivity. Our study shows that it is possible to decide whether FSG cattle should be treated or not, provided that it is accepted that some animals will still be dewormed unnecessarily.

Our analysis is in the nature of a retrospective proof of concept using historical data collected under Swedish field conditions. We do not yet have sufficient information to determine the precise effects of potential confounding factors, such as breed, age, climate and (particularly) pasture availability. Our data were pooled from a number of disparate trials involving several different types of nematode-control treatment, so that these sources of variation were included in the experiment but their individual effects cannot be determined. So, we cannot assess the effects of geographical variation or farm management, for example. The next stage is to validate
our conclusions in a controlled field trial; that is, a pilot experiment designed to verify that the concept does indeed have practical application under more carefully controlled conditions.

Conflict of interest

There are no conflicts of interest.

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