A 3-year field evaluation of pasture rotation and supplementary feeding to control parasite infection in first-season grazing cattle—Dynamics of pasture infectivity

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**Abstract**

A 3-year grazing trial (2002–2004) was conducted on a commercial beef cattle farm in south-central Sweden to assess different methods of parasite control. This paper focuses on the dynamics of the free-living larval stages, whereas data on performance and within-host parasitological variables are presented in a complementary paper. Each year in May, 4 groups of 10 first-season grazing (FSG) steers were turned out on to separate 2 ha paddocks and subjected to the following strategies: (1) spring turn-out on to pasture which had been grazed the previous year by second-season grazing (SSG) steers (paddock RT), followed by a move to aftermath (paddock AM) after 10 weeks (mid-July), (2) supplementary feeding with concentrate and hay for 4 weeks following turn-out (paddock FD), set stocked, (3) untreated control (paddock UT), set stocked and (4) anthelmintic treated control (paddock DO), set stocked. All paddocks were assigned a new set of FSG cattle each year whereas the treatments remained the same. Pasture infectivity were monitored partly by two tracer calves that grazed each paddock along with the FSG calves for 3 weeks after turn-out and prior to housing, partly by analysis of herbage samples for infective larvae (L3) that were collected from each paddock at monthly intervals between April and October. The predominant genera found were *Cooperia* and *Ostertagia*. Tracers grazing paddock RT overall harboured less worms, and in particular less *Ostertagia* spp., and tracers grazing paddock AM in mid-July harboured insignificant numbers of nematodes compared to tracers on the FD and UT paddocks. Although total worm counts varied between groups, smaller numbers were generally observed early in the grazing-season (May), compared to close to housing (September) when inhibited early L4 larvae were almost exclusively found. Results observed from herbage samples showed high numbers of L3 in spring before the time of turn-out, compared to around housing. In conclusion, the rotation control strategy showed promising results and provided a turn-out pasture that was ‘nematode safe’ to FSG cattle the following spring, whereas the feeding strategy failed as applied in this experiment.

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1. Introduction

Effective control of gastrointestinal (GI) parasites is not only a prerequisite to sustain animal welfare but also to optimise production in grazing cattle. Despite access to highly efficient anthelmintics, strategic prophylactic
use for nematode parasite control is not accepted in organic livestock production (KRAV, 2006). Thus, the move towards organic production necessitates scientific evaluation of sustainable control methods that are practically applicable for farmers.

In Sweden and other temperate regions, the most important species responsible for bovine parasitic gastroenteritis (PGE) are the trichostrongyloids Ostertagia ostertagi and Cooperia oncophora. These parasites are found on virtually all pastures where cattle have been grazing and animals become infected by ingesting the third stage infective larva (L3). Typically, the prepatent period is approximately 3 weeks, but completion to the adult stage may under certain conditions become delayed for some months due to arrested larval development, thereby preventing the shedding of eggs on to pasture at times of the year when conditions are unfavourable for external development (Armour and Duncan, 1987).

Various non-chemical approaches to nematode parasite control have been suggested to reduce the usage of anthelmintics to first-season grazing (FSG) cattle (Barger, 1997; Bransby, 1993). Strategies whereby susceptible animals are moved from an infected to a ‘safe’ pasture have showed promising results on both animal performance and pasture infectivity (Eysker et al., 1998), as well as where FSG cattle graze together with older and less susceptible age classes of cattle (Nansen et al., 1990). However, several studies on the dynamics of infective larvae on pasture in different geographical regions under various management practises are essential to understand the dynamics of parasite infections, and thereby enable the design of scientifically based and up-to-date control strategies. Although overwintered larval populations of O. ostertagi and C. oncophora have been shown to play a central role in the epidemiology of GI nematode infections in cattle in Sweden (Dimander, 2003), there is still a demand for more detailed epidemiological investigations.

In a previous report, data collected from this 3-year field study were used to evaluate the effects of (1) rotational grazing and subsequent grazing of the turn-out paddock by second-season grazing (SSG) cattle and (2) supplementary feeding after turn-out, on the performance and within-host parasitological variables of FSG cattle (Larsson et al., 2006). The present paper is complementary and presents information on the dynamics of the pasture infectivity of nematode parasites in relation to these treatments. Pasture samples and tracer tests were performed to assess the dynamics of pasture infectivity and to evaluate the exposure levels that followed by the use of these control strategies.

2. Materials and methods

2.1. Experimental design

Data originate from a 3-year grazing trial with FSG cattle that was performed on naturally infected pastures on a commercial beef cattle farm in south-central Sweden. A detailed description of the experimental design is presented elsewhere (Larsson et al., 2006). Briefly, the trial was conducted during the grazing seasons 2002–2004, and each year 4 groups of 10 FSG steers were each turned out on to separate 2 ha paddocks which represent a normal stocking rate in Sweden of 5 animals per ha. These animals were 5–9 months old with an average weight each year of 189, 157 and 212 kg, respectively. Animals in the four groups were assigned to the following parasite control strategies:

(1) Turn-out on spring pasture that had been grazed during the late season of the previous year by SSG cattle (paddock RT), followed by one move after 10 weeks (mid-July) to an aftermath grazing paddock (paddock AM) where one silage cut had been taken in early-mid June. Thus, these animals each year used 4 ha (RT 2 ha + AM 2 ha). The SSG cattle were all treated with fenbendazole 7.5 mg/kg body weight (BW) (Axilur<sup>®</sup> susp 10%, Intervet, Igoville, France) prior to allocation.

(2) Supplementary feeding with 1 kg concentrate and hay ad libitum for 4 weeks following turn-out, set stocked (paddock FD).

(3) Untreated control, set stocked (paddock UT).

(4) Treated control, set stocked (paddock DO). Monthly injections with doramectin 10 mg/50 kg BW (Dec-tomax<sup>®</sup> vet., Pfizer, Amboise, France).

In 2003, all animals grazing paddock FD were salvage treated with doramectin 10 mg/50 kg BW (Dec-tomax<sup>®</sup>, Pfizer) after 7 weeks on pasture. In 2004, 3 animals in group UT were housed after approximately 4 weeks on pasture and treated with fenbendazole 7.5 mg/kg BW (Axilur<sup>®</sup>, Intervet). After 3 weeks of convalescence they were returned to the paddock UT.

2.2. Tracer animals

Tracer tests were conducted (1) on all paddocks prior to housing in September 2002, 2003 and 2004, (2) on paddocks RT, FD, UT and DO at turn-out in May in 2003 and 2004 and (3) on paddock AM at the mid-July move in 2003 and 2004. All tracer animals were indoor.
raised castrated males of the Swedish red and white breed, aged 6–9 months, and with an average weight of 181 (±S.D. 22) kg when turned out for grazing. Two animals were allocated to each pasture plot on each occasion and allowed to graze for 3 weeks together with the experimental animals. Following the 3 weeks grazing period, all tracer animals were housed in a common pen and fed silage and concentrate for additional 3 weeks, prior to being consigned to the abattoir for slaughter and viscera collection.

2.2.1. Sampling, slaughter and laboratory procedures

Prior to allocation to each of the paddocks individual rectal faecal samples were collected from the tracer calves for faecal egg counts (FEC), and pooled faecal cultures were prepared by mixing 10–20 g faeces with vermiculite and incubated for 2 weeks at 26–27 °C. Number of nematode eggs per gram faeces (epg) were analysed using a modified McMaster method (Anonymous, 1986), based on 3 g of faeces and with a sensitivity of 50 epg.

Following slaughter, the abomasum and approximately 10 m of the proximal small intestine were processed separately. This was done by collecting the bowel contents and washing of the mucosal surfaces in individual buckets and adjusted to a total volume of 4 l. In addition, the abomasal mucosa was scraped off and digested in a pepsin–HCl solution (10 g pepsin + 17 ml concentrated HCl dissolved in 1 l water). The final volume after digestion was adjusted to 2 l. Four 20 ml sub-samples were taken from each of the collected bowel contents and the abomasal digest, which gives a sensitivity of 200 and 100 worms, respectively, and stored at −20 °C until analysed. After thawing, worm counts of two sub-samples from each organ were examined. Nematodes were stained with Lugols iodine and identified, counted and differentiated into genera using the keys by Barth and Visser (1991).

2.3. Herbage samples for infective larval counts

Two replicate herbage samples were collected according to Taylor (1939) from each experimental paddock before turn-out in May and thereafter at approximately monthly intervals until 4 weeks after housing in October. The first year (2002) herbage collection on paddock AM commenced in mid-July. Infective larvae (L3) were recovered according to a Baermann procedure originally outlined by Persson (1974). To remove fine soil particles, a single layer of Kleenex tissue was attached underneath the metal sieve that supported the herbage inside the funnel. Larvae were then identified and enumerated either from two sub-samples, or by counting all recovered L3 from each sample.

2.4. Meteorological data

Daily precipitation and temperatures were recorded at a meteorological station located 14 km from the experimental area. These data are presented as monthly means and expressed in relation to the long-term (1961–1990) averages (LTA).

2.5. Data analysis

The data were summarised using Microsoft Excel® 2000 spreadsheets. Worm counts from each tracer animal for each occasion are presented as means of 2–3 sub-samples, and infective larvae on herbage as means from two samplings per paddock each sampling occasion.

3. Results

3.1. Parasitology and worm counts of tracer animals

Before allocation to pasture all tracer calves had negative FEC, and no larvae were detected in the pooled faecal cultures. The number of worms found in the two tracer animals from each paddock on each occasion, and the proportion of inhibited early fourth stage larvae (EL4) are shown in Fig. 1. The predominant genera were Cooperia followed by Ostertagia. Generally only sporadic and low numbers of Nematodirus spp. were observed and are not included. However, in September 2004, one tracer on each of paddocks FD and DO had unusually high numbers Nematodirus helvetianus in the small intestine.

In September 2002, worm burdens >32,000 nematodes were observed in tracers FD and UT and approximately 19,000 in tracers RT, compared to a mean of 1700 nematodes in tracers AM, with the majority being Cooperia spp. The following year (2003) in May, tracers grazing paddocks FD and UT were heavily infected with worm burdens between approximately 55,000 and 87,000 nematodes and >25,000 were diagnosed as Ostertagia spp. In tracers RT, approximately 29,000 nematodes were found with the majority being Cooperia spp., and in tracers AM values were <1000 nematodes in mid-July of 2003. In September of the same year (2003), worm counts
between 50,000 and 176,000 nematodes were observed in tracers from all paddocks except DO, although within-group variations were considerable (Fig. 1). At spring turn-out in 2004, worm burdens in tracers grazing on paddock FD and UT had decreased compared to previous spring turn-out (2003), however the majority now being *Ostertagia* spp. in tracers UT. Tracers on the RT and AM paddocks harboured low to moderate numbers of nematodes and showed approximately the same pattern as the previous year in May and mid-July, respectively. At time for housing the last year (2004) tracers on paddock FD harboured approximately three times that of May-tracers, while total worm counts in tracers UT were comparable to May-tracers on the same paddock. Worm counts from RT and AM tracers were <40,000 and >52,000 nematodes, respectively.

Tracer calves that grazed on paddock DO harboured a maximum of 4300 nematodes throughout the trial, and on 4 out of 5 occasions both *Ostertagia* and *Cooperia* spp. were present in tracers from this paddock.

3.1.1. Inhibited larvae

In September all 3 years, virtually all tracer animals harboured inhibited EL4 of both *Ostertagia* and *Cooperia* spp. With tracers DO excluded, in September of 2002, 2003 and 2004, respectively, means of 30, 50 and 43% of the *Ostertagia* and 66, 78 and 64% of the *Cooperia* populations were found as EL4. In contrast, only a few May-tracers harboured EL4 (maximum 0.3% of worm population per animal) in 2003 and none in 2004. In mid-July tracers that grazed the aftermath paddock, a few inhibited larvae of both *Ostertagia* and
Cooperia spp. were found, however the total worm burden in these animals were low.

### 3.2. Infective larvae on pasture

At the commencement of the trial in May 2002, the number of L3 was <500 L3/kg DM on all paddocks (Fig. 2). In the beginning of July, 8 weeks after turn-out, larval counts increased somewhat, followed by a gradual decline to below or at detection level by late August. At the time of housing, a slight increase was observed in herbage samples from paddock FD and 4 weeks after housing the number of larvae was elevated in all paddocks except RT and DO. The larval count on paddock DO was approximately 200 L3/kg DM at turn-out in May but then declined and remained low throughout the grazing season. In April 2003, up to 9000 L3/kg DM were observed on paddock FD, however numbers subsequently decreased in all paddocks, and by turn-out in May levels were approximately 3300 L3/kg DM on paddocks FD and UT and <500 on RT, and by July levels were <1000 L3/kg DM in all paddocks. An increased number of larvae were observed on paddocks UT and AM in late August, however as with the previous year, a large increase was only seen following housing. Four weeks before turn-out in 2004, overwintered numbers of approximately 9000 and 17,000 L3/kg DM were found on paddocks UT and AM, respectively, whereas numbers of approximately 1000 L3/kg DM were found on paddock RT. However, at turn-out only paddock UT had >1000 L3/kg DM, and after another 4 weeks <600 L3/kg DM were found on all paddocks. A slight increase was observed on all paddocks towards the end and around housing in 2004. Four weeks after housing the numbers of L3 on all paddocks were <1600 L3/kg DM herbage.

### 3.3. Meteorology

The monthly precipitation (mm) and mean temperature (°C) during 2002, 2003 and 2004 are presented in Fig. 3. June and July of 2002 were wet with at least twice the amount of rainfall compared to the LTA. In contrast, August 2002 was dry with <4 mm rain. Temperatures during the 2002 grazing season were above the LTA. The following autumn and winter was cold, although only intermittent snowfall was registered until the end of January in 2003. During February and beginning of March 2003, the ground was covered with snow. In 2003, April to June had more rain compared to the LTA, whereas September and October were relatively dry. As the previous season, July to September was warmer than the LTA and October cold. November to December in 2003 and February to May in 2004 were comparatively warm with a persistent snow cover from January to March. Grazing season 2004 commenced with a dry spring and above normal temperatures, followed by cool and wet conditions in June and July. August and September 2004 were warmer than normal, and September dryer compared to the LTA.

### 4. Discussion

This trial showed that a mid-summer move of FSG cattle in combination with late summer grazing by SSG cattle provided a spring turn-out pasture the following
year that is acceptably nematode safe to FSG cattle. This was confirmed both by moderate worm burdens in tracers and the low numbers of L3 in herbage on the RT paddock at spring turn-out each year.

The benefit of moving FSG cattle to a parasite ‘safe’ pasture before the build up of new generations of infective larvae has been demonstrated in previous studies, both in Sweden (Dimander et al., 2003a) and Denmark (Henriksen et al., 1976; Kristensen et al., 2006; Nansen et al., 1988). In the present trial, the same aftermath pasture was successfully used for 3 consecutive years and yet found to be virtually free from nematode parasite larvae by mid-July, thus providing an excellent late season pasture for FSG cattle. This effect is achieved partly by the reduction of overwintered larvae from mid-May to mid-June (Nansen et al., 1987), partly by the silage cut or mowing in early-mid June, as demonstrated in Sweden (Dimander et al., 2003a) and in the Netherlands (Eysker et al., 1988), respectively.

Overwintering of infective larvae is regarded as an important epidemiological factor of nematode parasitism in FSG cattle under Scandinavian conditions (Dimander, 2003; Tharaldsen and Helle, 1984), and in this trial indeed resulted in exposure levels that exceeded the threshold for clinical PGE in the FSG cattle on paddocks FD and UT early in the season (Larsson et al., 2006). However, elevated levels in pasture infectivity due to translation of early-season contamination were not observed until early September. This is in agreement with findings by Dimander et al. (2003a). Nevertheless, this late-season increase in pasture infectivity obviously resulted in high levels of overwintered larvae in spring the following year as observed on paddocks FD and UT, compared to the moderate numbers on paddock RT. Thus, the strategy of moving the FSG cattle from their spring turn-out paddock, followed by grazing of SSG cattle in that same paddock during the latter part of the grazing season, resulted in relatively parasite ‘safe’ turn-out pastures the following year. The beneficial diluting effect on pasture infectivity by grazing dams alongside their calves was suggested already by Michel et al. (1972). Grazing with different age classes has later been found successful in controlling nematode infections of FSG cattle, either by pasture alternation (Axelsen et al., 1986; Dimander et al., 2003a), or by mixed grazing with older cattle (Nansen et al., 1990; Šarkūnas et al., 2000). However, it must be emphasized that although rotational grazing with SSG cattle was successful in this study, caution should always be used when the same turn-out pasture is provided to FSG cattle for consecutive years (Larsson et al., 2006).

Although tracer worm counts varied and generally were higher in late season (September), compared to early in the season (May), tracers grazing paddock RT overall harboured less worms, in particular Ostertagia spp., compared to tracers on the FD and UT paddocks. On no occasion during the 3 years of this study were tracers grazing the RT paddock found to harbour >20,000 worms of Ostertagia spp. and the pasture larval counts never exceeded 1200 L3 per kg DM in this paddock at the time for spring turn-out. However, it should be remembered that pasture larval counts in general produce results that are difficult to compare between laboratories (Couvillion, 1993; Eysker and Ploeger, 2000). Still, the relatively low tracer worm counts and pasture larval counts in the present study correspond with data presented in the companion paper on animal performance (Larsson et al., 2006).

At turn-out in 2003, however, numbers of nematode larvae recorded from the paddock grazed by the supplementary fed calves (FD) were similar to those observed in the samples from the paddock grazed by untreated calves (UT), with numbers exceeding levels critical for clinical ostertagiosis according to pepsinogen values and clinical symptoms of FSG animals.
grazing these paddocks (Larsson et al., 2006). The low numbers of infective larvae in the herbage samples from paddock FD during the second part of the grazing season was confirmed by moderate worm burden in September—tracers of that paddock and was certainly a result from the previous doramectin salvage treatment.

The ecology of the pre-parasitic stages of GI nematodes is largely influenced by prevailing climate and weather conditions (for review, see Stromberg, 1997). Taken the RT, FD, and UT treatments together, the overall low pasture infectivity and tracer worm counts observed in late 2002, contrasted by the relatively high numbers of overwintered larvae in 2003, may be explained by dry conditions in late 2002 that delayed the breakdown of dung pats. Previously it has been observed that massive numbers of larvae are released when the dung pat is thoroughly moisturised by rain, which facilitates a synchronous translation of infective larvae (Dimander et al., 2003a,b). The non-disintegrated dung pat provides a shield for developing larval stages and accumulated infective third stage larvae which facilitates successful overwintering (Dimander et al., 2003b). Not surprisingly in this study, pasture samples and tracer tests performed at the time of spring turn-out in 2003 showed comparably high levels of overwintered larvae. Large numbers of overwintered infective larvae often appear on a contaminated pasture following extended periods of dry weather and have been observed before both in Sweden (Dimander et al., 2003a) and Denmark (Nansen et al., 1989) as well as in Australia (Barger et al., 1984). Understandably, for inert SSG cattle to effectively act as “vacuum cleaners”, a dry summer may obliterate the intended role of SSG cattle in this type of parasite control strategy. Consequently, even if the RT treatment strategy showed promising results in this experiment, caution should be used if contamination in early season is followed by an extended period of dry weather. Based on findings in this and previous studies, a guarded definition of a clean or parasite free pasture under Swedish conditions may be an aftermath pasture spelled in early summer and where a silage cut has been taken in early summer. Furthermore, such aftermath pastures seem to be relatively clean in mid-summer regardless of the level of contamination the previous year.

Even though this study was not primarily designed to make methodological comparisons between techniques for pasture infectivity estimations, results from pasture larval counts and tracer calves overall agreed well. An obvious advantage with herbage sampling is that no animals destined to estimate pasture infectivity levels are necessary in these studies, thus making it a favourable technique from an animal welfare perspective. The disadvantage is that pasture sampling, processing and finally differential larval counts are laborious and results may be highly variable (Eysker and Ploeger, 2000). Pasture infectivity will vary over time and since larval counts from herbage samples are spot estimates, sampling has to be performed on repeated occasions to obtain accurate estimates of exposure levels. However, in longitudinal studies where preferably experienced staff performs sampling, processing and analysis, the herbage larval count technique is useful to study the population dynamics of larvae on a particular pasture.

While pasture sampling is a random estimate of larvae on pasture, the tracer technique provides an integrated expression of the total acquisition of L3 over an extended period of time, and the subsequent establishment of these nematodes in the host (Waller et al., 1981). Thus, the use of tracer calves would give a better estimate of the exposure of the animals to parasites than do pasture larval counts. In addition, the tracer technique makes it possible to determine inhibition patterns of the nematodes. One drawback with tracer animals may be the general variation in intake and establishment of nematodes between individuals, which requires a fairly large number of tracer calves to obtain representative worm counts (Eysker and Ploeger, 2000). Still, in the present trial the worm burdens of the paired tracer calves showed good agreement except at housing in 2003 when huge variations were observed between individuals from all paddocks except DO. There are several possible explanations related to individual tracer calf variation. Examples may be differences in grazing behaviour and immunological responses, e.g. it has been observed that expulsion of C. oncophora may occur in calves following huge exposure (Kanobana et al., 2002). However, any of these explanations will remain speculative in the present study.

 Arrested development in early L4 stage was almost exclusively found in tracer animals grazing in September, and to a slightly higher extent for Cooperia spp. compared to Ostertagia spp. Many factors like climate, parasite strain, management as well as host age and immunity may influence the induction of hypobiosis in nematodes (Armour and Duncan, 1987; Eysker, 1997). Important factors observed from studies in the northern hemisphere seem to be seasonal (Michel et al., 1974, 1975) with a primary stimulus being low temperatures (Armour and Bruce, 1974). In a Danish tracer test study, almost a 10-fold increase in the
proportion of inhibited EL4 *O. ostertagi* was observed in September/October compared to in August the same year (Satrija and Nansen, 1993). Seasonal inhibition has been shown in different nematode species of both cattle and sheep in the cool temperate regions of the Northern hemisphere (Almeria et al., 1996; Claerebout et al., 1997; Waller et al., 2004). Therefore, by using parasite naïve calves of approximately the same age on all occasions the clear difference between the percentages of arrested EL4 found in late season compared to early season was expected in the present trial.

In conclusion, the pasture rotation technique evaluated in this study shows that it is possible to maintain pasture infectivity at acceptable levels in Sweden by one mid-summer move of FSG cattle to aftermath in combination with grazing of the contaminated spring turn-out pasture by SSG cattle. This was observed for consecutive years and without the use of anthelmintics.

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### References


Kanobana, K., Ploeger, H., Vervelde, L., 2002. Immune expulsion of the trichostrongylid *Cooperia oncophora* is associated with increased eosinophilia and mucosal IgA. Int. J. Parasitol. 32, 1389.


