The dynamics of ovine gastrointestinal nematode infections within ewe and lamb cohorts on three Scottish sheep farms

Kim Hamer, Jennifer McIntyre, Alison G. Morrison, Amy Jennings, Robert F. Kelly, Sarah Leeson, David J. Bartley, Umer Chaudhry, Valentina Busi, Neil Sargison

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**A B S T R A C T**

Gastrointestinal nematodes (GIN) are a serious concern for sheep producers worldwide. However, there is a paucity of evidence describing the epidemiology of GIN on modern UK sheep farms. The aim of this paper was to understand whether expected seasonal variations of infection are still found in ewes and lambs under varying management strategies in temperate climates. Faecal egg counts (FEC) were conducted on freshly voided samples collected from groups of ewes and lambs every third week for twelve months on three farms in southeast Scotland. The patterns of egg output have been described here in relation to management practices undertaken on the farms. Despite changes in farming practice and climatic conditions, the findings complement historical studies detailing the epidemiology of GIN. Findings include a periparturient rise in ewe FEC on two of the farms, while lambing time treatment appeared to suppress this on the third farm. On the same two farms lamb FEC increased during the summer, reaching a peak in the autumn. The work also highlights how the ad hoc use of anthelmintics does little to impact these patterns.

**1. Introduction**

Current planned management of ovine gastrointestinal nematodes (GIN) in temperate climates is largely based on epidemiological studies that are becoming increasingly dated (Morgan et al., 1951; Farnell, 1954; Brunsdon, 1970; Coop et al., 1990). These refer to farming systems prior to the introduction of the most recent broad-spectrum anthelmintic drug classes, the recognition of climate change and the widespread development of anthelmintic resistance (Sargison et al., 2007; Mitchell et al., 2010; Glover et al., 2017; Hamer et al., 2018). The impact of GIN could be significantly reduced by improved control methods (Nieuwhof and Bishop, 2005). However, meta-analysis assessing their impact on small ruminants indicated that they continue to be a major factor associated with loss of productivity and profitability on sheep farms (Mavrot et al., 2015). Therefore, current dogma concerning the epidemiology of GIN at farm level needs to be re-examined to challenge the understanding of the principles of sustainable control and how these are applied.

The accepted pattern of ovine GIN infections in temperate climates includes a spring periparturient rise (PPR) in ewe faecal worm egg output (Coop et al., 1990), which contaminates pasture for naïve lambs (Brunsdon, 1966) initiating the cycle of infection. This is followed by the spring/early summer appearance of *Nematodirus battus* in lambs. *Teladorsagia circumcincta* and *Trichostrongylus* species egg contamination on pasture increases as the naïve lambs multiply the infection throughout the summer, while they develop a protective immune response. The increase of pasture contamination and lamb nematode burden leads to increased risk of clinical parasitic gastroenteritis (PGE). Pasture contamination is thought to decline in autumn and winter in temperate climates, when lamb immunity increases, stocking densities decrease and larval development reduces due to decreased ambient temperatures (Armour, 1986; Smith and Grenfell, 1994).
2. Materials and methods

2.1. Study site and farms

An observational study was carried out on three spring (March to May) lambing farms in the south east of Scotland. The three farms were selected partly because of convenience and the existence of strong working relationships (essential for long-term studies), and partly because of their differing management practices that represent common sheep farming practices in temperate climates. The study farms included two lowland farms (Farms 1 and 2) and a hill farm (Farm 3). There was no movement of sheep between the farms within the study period. A summary of sheep management on the three study farms is given here and more details can be found in supplementary Appendices 1 and 2.

Farm 1 was a lowland sheep-only farm of 370 Mule, Cheviot cross Texel and Texel ewes, lambed indoors and turned out to grass within 48 h of lambing. This farm used set stocked grazing until weaning, then rotation of silage aftermath for weaned lambs. This farm used a suppressive anthelmintic regime, including oral moxidectin (Cydectin Oral Drench; Zoetis UK limited) treatment of periparturient ewes and routine parasitological monitoring of individual animals, which would have resulted in diminishing numbers of samples as the year progressed. Significant attempts were made to avoid repeat sampling of individual animals, by covering large areas of the field during collection. Samples were collected opportunistically from ten ewes and ten lambs on each farm. Ten samples per group were examined individually to provide a representative sample of the overall group mean FEC, given the over-dispersed nature of FEC in sheep (Morgan et al., 2005).

Details of the groups of ewes and lambs included in the study are provided in Fig. 1. Consistent groups were sampled consecutively where possible, however groups were changed and mixed at key management points during the study, for example lambing, weaning and breeding. In mid-pregnancy ewes underwent transabdominal ultrasound scanning to determine the number of foetuses present, this was carried out by a non-veterinary technician, as is common practice on UK sheep enterprises. The ewes were then divided into groups according to foetus number. After lambing, the ewes that had lambed were kept at grass with their lambs, in carefully managed groups. These groups contained only ewes with similar numbers of lambs, except for Farm 3 where ewes with single and twin lambs were mixed (Fig. 1).

Lamb sampling started when the lambs were approximately six weeks old. After weaning, the populations of lambs were ever changing as animals moved off the farms to slaughter (Fig. 1).

2.2. Parasitological methods

Faecal sample transportation to the laboratory was a maximum of 10 min’ by car, they were stored in air-tight bags (with air squeezed out before closure), inside air-tight containers at room temperature until processing was possible. The majority of samples were processed within 48 h of collection, less than 10% of samples were processed a maximum of 120 h post collection and were stored at 4 °C when this was the case. FECs were performed using a salt flotation cuvette method with a potential sensitivity of one egg per gram (Christie and Jackson, 1982). Strongylid, Nematodirus and Strongyloides eggs were distinguished morphologically. The strongylid and Nematodirus eggs were counted, while only the presence or absence of Strongyloides was noted. For the analysis here only the counts of the strongylid and Nematodirus eggs are included. The excess faeces were pooled and cultured to generate third stage larvae (MAFF, 1986). DNA lysates were prepared from pools of recovered L3 and used as templates for metabarcoded deep amplicon sequencing as described by Avramenko et al. (2015) to quantify their species composition.
2.5. Interaction between ewe and lamb FEC

Lamb FEC patterns were compared with ewe FEC from the same farm using graphical representation. For each farm a time delay was estimated between the spring peak in ewe FEC and when mean lamb FEC first increased above 200 epg on the same farm.

All graphical figures were produced with the package ggplot2 (Wickham, 2009), using R (R Core Team, 2019) in RStudio, version 1.2.1335 (RStudio Team, 2018).
3. Results

During 19 visits each to Farms 1 and 2 and 18 visits to Farm 3, a total of 1100 faecal samples were collected (558 from ewes and 542 from lambs). At occasional sampling points nine or eleven samples were collected and analysed rather than ten, and when the lambs were approximately six weeks old fewer samples were collected.

3.1. Ewes

Fig. 2 shows the ewe FEC results for the study period. There were distinct overall patterns in ewe FEC through the study period, with a PPR observed on all three farms between April and July 2016. A similar rise was also observed on Farms 1 and 2 at the end of the observation period, in March and April of 2017. The PPR appeared to be shortened on Farm 1 in association with the lambing-time moxidectin treatment (Cydectin Oral Drench; Zoetis UK limited). On this farm, in 2016, the peak mean FEC was 912 eggs per gram (epg) (median = 675 epg) in peri-parturient ewes immediately prior to treatment. On Farm 2 the FEC increased as lambing progressed, but the median remained below 375 epg throughout the PPR. These ewes received no treatment. The ewes on Farm 3 received a pre-lambing mebendazole treatment (Mebadown Super; Downland), the FEC of these ewes was low in late March and early April and began to rise in the second half of lambing to a peak mean of 592 epg (median = 508 epg) and then decreased again in June and July. Towards the end of the PPR of 2016 there was a small, short-lived elevation in FEC approximately two weeks after the cessation of concentrate feeding on all three farms (Fig. 2).

Between July and September ewe FEC were relatively low on all three farms: on Farm 1 they remained close to zero; on Farm 2 most were between zero and 125 epg; and on Farm 3 the median ewe FEC remained below 125 epg, but at most sample time points some samples had over 500 epg. On Farm 1, ewe FEC increased slightly in the autumn, however there was a more obvious autumn rise on Farm 3 (peak mean = 486 epg; median = 419 epg). Ewes received a routine pre-breeding treatment with ivermectin (Noromectin oral drench; Norbrook) on Farm 3, which appeared to disrupt this rise. On farms 1 and 2, the median FEC remained below 130 epg at all samplings outside of the PPR.

3.2. Lambs

Fig. 3 shows the lamb FEC results for the study period. On Farm 1, median lamb FEC remained below 250 epg throughout most of the grazing season, there was a slight increase in the autumn (peak mean = 174 epg; median = 116 epg) but still within these limits. On Farms 2 and 3 there was a gradual increase in FEC through the summer, followed by a rapid rise in the autumn, to a peak mean FEC of 1014 epg (median 860 epg) and 1823 epg (median = 1638 epg), respectively. Throughout this time there was an overall increase in FEC, however counts were undulating, apparently in response to anthelmintic treatments. From July until late October, each post-anthelmintic drop in FEC appeared to last less than six weeks. In contrast, anthelmintic treatments given to either ewes or lambs after mid-October were followed by lowered mean FEC for a longer period (until the end of sampling, 2 months in lambs on Farms 1 and 2, and 5 months in all ewes and the Farm 3 lambs).

There were two main peaks of Nematodirus species egg output in the
lambs on each farm (Fig. 4), the first between May and July and the second peak in September or October. On Farm 1 the spring peak occurred in mid-May, with a median of 189 epg and mean 180 epg, from only four lambs sampled as the lambs were still less than 2 months old. The autumn peak occurred in late September, mean 49.4 epg and median 15 epg, (30 lambs sampled for a FECRT). Farm 2 had a small peak Nematodirus species FEC in late-May (mean 32.5 epg, median 42 epg), a second small peak in late July (mean 41.6 epg, median 27 epg) and larger autumn peak in early September (mean 195 epg, median 148.5 epg). On Farm 3 by comparison, the peaks were delayed, with one in mid-July (mean 118.8 epg, median 121.5 epg) and the other in late October (mean 305.1 epg, median 171 epg).

Each farm gave four anthelmintic treatments to the lambs in the study groups. On Farm 1 the first anthelmintic treatment was given in late May, nine weeks after the start of lambing, followed by two treatments at three-week intervals, then one treatment in September, which included a faecal egg count reduction test (FECRT). Treatments on Farm 3 followed a similar predefined pattern but were started in late June, ten weeks after the first lambs were born, followed by two treatments at four- to five-week intervals, then one treatment in late October. Farm 2 used reactive treatments based on FEC results, the first treatment occurred at the start of June, nine weeks after the start of lambing, the next was eight weeks later in August (this treatment included a FECRT with 10 untreated control lambs), the third treatment was six weeks later in September and the fourth in October after another four weeks.

3.3. Nemabiomes

The nematode species proportions are only summarised here, as their analysis is beyond the scope of this paper. On Farm 1, similar proportions of T. circumcincta, Trichostrongylus vitrinus, Trichostrongylus axei and Cooperia curticei were present in ewes throughout the year, with small proportions of Oesophagostomum venulosum also present, whereas similar proportions of T. circumcincta and T. vitrinus were present in the lambs at the beginning and end of the sampling period, with T. circumcincta predominating for the remainder of the time. On Farm 2, similar proportions of T. circumcincta, T. axei and O. venulosum were present in both the ewes and lambs for most of the sampling period, with C. curticei appearing later in the year. On Farm 3 mixed infections with similar proportions of T. circumcincta, T. vitrinus, T. axei, C. curticei and O. venulosum were seen throughout the year in the ewes, whereas only T. circumcincta and T. vitrinus were present in the lambs, with a predominance of T. circumcincta.

3.4. Interaction between ewe and lamb FEC

The mean lamb FEC first increased above 200 epg on Farms 2 and 3 approximately two months after the spring peak in ewe FEC (Figs. 5 and 6). The interactions between ewe and lamb FEC on Farm 1 were more difficult to assess because testing appeared to start part way through the PPR, and the PPR was subsequently suppressed by the lambing-time anthelmintic treatment of the ewes before they were turned out to grass with their lambs. Nevertheless, Fig. 7 shows that mean lamb FEC went above 200 epg approximately six months after the spring peak in ewe FEC for Farm 1.

3.5. Weather

The weather for 2016 and early 2017 was typically mild and wet for the south east of Scotland. The mean fortnightly temperature remained over 5 °C and below 17 °C for nearly ten months of the twelve-month period. The minimum temperature did not fall below −7 °C for the whole year. The rainfall was reliable throughout the year and relative
humidity remained above 70% (Fig. 8).

4. Discussion

This study provides updated evidence for the well-established epidemiological patterns of ovine GIN in temperate climates, whilst taking into account the complex influence of modern farming practices and climatic conditions. Previously identified patterns, which were also seen on two of the study farms here, include the PPR in ewe FEC, which results in pasture contamination for lambs, then subsequent multiplication of GIN in the lambs through from May to peak in October. Here anthelmintic treatments had little impact on these patterns unless used in a suppressive regime, as on Farm 1, the study farm without the expected seasonal FEC variations.

The purpose of this study was to describe the epidemiology of ovine GIN over a year in standard field conditions in the context of modern farming practices in northern Europe. Field studies are important because they reflect on-farm scenarios, which controlled experimental studies struggle to do, however all study designs have limitations. In the case of observational field studies, there is a lack of control over conditions, therefore it can be difficult to precisely assess the impact of climatic conditions and husbandry practices on ovine GIN epidemiology. This study was undertaken on three farms that were geographically close, therefore no difference in climatic conditions could be assessed.

On these farms there was a lack of control over the mixing of groups, which reduced the consistency of animals included in the study groups. Therefore, the described study design was chosen in order to maintain the required number of samples throughout the year. Attempting to monitor the same individuals would have resulted in animals being lost from the study, reducing the sample number and increasing the impact of the over-dispersed nature of FEC. The impact of group changes should have been minimised by the fact that all classes of animals on each farm underwent similar management and grazed similar or the same pasture throughout the study period. Furthermore, this particular study is useful in describing the differences in GIN epidemiology between different husbandry systems. The use of multi-level mixed models was considered to explore the data from this study. However the repeat measures, significant changes in FEC (for example after anthelmintic treatment) and mutually exclusive variables (such as for lambs, co-grazing with ewes and the stress of weaning) were difficult to incorporate into the models. Therefore rigorous description of the observed FEC changes was considered most appropriate, and useful for current farming practice.

The PPR of the ewes on these farms followed a traditional pattern (Coop et al., 1990), starting in the two weeks before lambing and persisting for approximately six weeks after the end of lambing. However, there was a slight increase in FEC in the two weeks after the...
cessation of concentrate feeding. Feeding was discontinued three to four weeks after the end of lambing, when many of the ewes would have been near peak lactation. The decision to discontinue concentrate feeding is normally based on date and grass growth, when grass supply is considered to be sufficient for lactating ewes (Wright and Genever, 2016). On Farm 1, this rise in FEC could also have been associated with the end of the persistence period of oral moxidectin, as described by Sargison et al. (2012a). Lambing-time anthelmintics were not used on Farms 2 and 3. The scientific basis for a nutritional effect on PPR has been explored in a review by Houdijk (2008). It is suggested that increasing the nutritional supply for periparturient and lactating ewes removes the need for ewes to partition suboptimal dietary protein between milk production and maintenance, including immune function, particularly for multiparous ewes (Houdijk et al., 2006). There was an autumn rise in the ewe FEC in the hill ewes on Farm 3, but the reason for this is unclear.

Significant increases in lamb FEC occurred in the autumn after weaning (September and October) on Farms 2 and 3. This pattern of autumn GI nematode burdens has been noted in other work (Parnell, 1954; Boag and Thomas, 1977). The peak in lamb FEC appears to occur in the autumn as a result of the build-up of pasture contamination and lamb infection throughout the summer, however there are often changes in nematode species dominance through the grazing season, due to environmental factors and lamb immunity acquisition (Brunsdon, 1970; Vlasso et al., 2001). With this seasonal variation in mind, there is concern that the period of high pasture contamination will be prolonged in response to climate change (van Dijk et al., 2008; McMahon et al., 2012). Environmental shifts in temperature and rainfall may promote the hatching of nematode eggs on pasture for longer periods (Morgan and van Dijk, 2012). This extension of the risk period for GIN could provide an explanation for the magnitude of the October lamb FEC on Farms 2 and 3 in this study, which had means between 1000 and 2000 epg. According to the nemabiome analyses, *Haemonchus contortus*, a GIN expected to give high FEC (Coyne et al., 1991), was not present on these farms. Therefore, FEC results of over 1000 suggest serious infection levels with other nematode species, such as *T. circumcincta* and *Trichostrongylus* species, and an associated risk of clinical PGE. The extended risk for PGE could be expected to impact production through reduced growth rates in store lambs and replacement breeding animals. Further research is needed to determine the full impact of these alterations in GIN epidemiology.

*Nematodirus* species eggs were seen in faeces of lambs in May to July and September to October, according to the spring and autumn patterns of infection we have come to expect (van Dijk and Morgan, 2010). Despite the May peak on Farm 2 being small (mean 32.5 epg, median 42 epg), clinical signs consistent with nematodirosis, including severe diarrhoea (Kingsbury, 1953), were reported by the stockman at that time. By contrast no obvious clinical signs were reported in September,
when the peak was larger (mean 195 epg, median 148.5 epg). This does not prove that there were no clinical signs in September, but inconsistencies between *Nematodirus* species FEC and clinical presentation (Herbeuval, 2003), or even worm burden (Thomas, 1991), are well recognised.

Comparison with historical data for untreated lambs (Parnell, 1954; Boag and Thomas, 1977) would suggest that the use of anthelmintic treatments between May and July on Farms 2 and 3 did not interfere with the natural accumulation of infective nematode burdens in the lambs, which still rose to a significant peak in the autumn. These treatments may however have influenced the relationship between ewe faecal egg output and the build-up of lamb FEC seen previously (Brunsdon, 1966; Wilson et al., 2008). On Farm 1, lamb FEC remained low and the lag between peak ewe FEC and mean lamb FEC reaching 200 epg was three-times longer than on Farms 2 and 3, despite the use of set stocking on Farm 1. This may have been in response to the low ewe FEC after lambing-time anthelmintic treatment with oral moxidectin, or it could have resulted from the suppressive anthelmintic treatment of lambs in the early part of their grazing period (three-weekly from late May to early July). This pattern of anthelmintic use may not be sustainable, due to the lack of a refugia nematode population on pasture and potential selection for anthelmintic resistance (Sargison et al., 2010; Kenyon et al., 2013). Albeit the same total number of group treatments (four) were given to the lambs on each farm, but the treatments were more spread out on Farms 2 and 3 than on Farm 1. On Farm 2, no anthelmintic treatments were used in the ewes and the August lamb treatment with levamisole did not produce an equivalent drop in FEC to those seen after the other anthelmintic treatment events, possibly indicating the presence of GIN resistant to levamisole. Therefore larval contamination of pasture could be expected to be high on this farm and may provide an explanation for the high lamb FEC in the autumn.

The majority of anthelmintic treatments used on these farms during the study period did result in significant reductions in FEC in ewes and lambs in the weeks immediately following treatment; hence they appear to have been effective. However, re-infection of the lambs was rapid, with FEC returning to pre-treatment levels (or higher) within six weeks of treatment. Rapid re-infection from contaminated pasture, as seen here, would have significantly impacted lamb growth rates (Coop et al., 1982). On Farm 3, a short acting anthelmintic, which reduced the lamb FEC to nearly zero within two weeks, failed to prevent significant contamination of pasture. The anthelmintic was given a week after these lambs were weaned and moved to new pasture, which had not been grazed between May and mid-August. That one week appeared to be long enough to allow high contamination of the pasture, resulting in subsequent rapid increase in FEC. In addition, on Farm 3, the treatment of ewes with mebendazole in late March, two weeks prior to lambing, did not prevent a PPR. This is likely to have been due to lack of efficacy.
of mebendazole against inhibited nematode larvae, due to poor aqueous solubility (Chen et al., 2012). Also, the mebendazole would not have prevented the ewes from acquiring new infection from overwintered larvae on pasture. In contrast to these situations, short-acting anthelmintics did appear to have a more prolonged impact on ewe and lamb FEC in late October and early November when the ambient temperatures decreased, possibly because larvae ingested after these treatments became hypobiotic rather than developing into egg-laying adults (Gaba and Gourbière, 2008).

Another potential impact of the anthelmintic treatments used during this study could have been a change in the species population composition of the GIN present. This can occur due to variation in the levels of anthelmintic resistance in different GIN species (Wilson et al., 2008). This will be discussed in more detail in a subsequent publication.

This study has shown that in a diversity of field situations, with rotation grazing and summer lamb treatments, the connection between pasture contamination by ewes and subsequent lamb infections is not simple and further work is needed to find sustainable ways of breaking this transmission link in different years and on different farms. A number of areas for further research and improvements for future study designs have been highlighted. For example, the monitoring of individual animals in these complex systems would make it easier to establish the effect of the various management practices.

5. Conclusions

This study brings the epidemiology of ovine GIN up-to-date in various commercial farming systems in Scotland. The results support the stable, but complex, nature of overall patterns of infection in temperate regions, despite changes in climate, farming practices and the widespread use of modern anthelmintics. The results from three farms, with different management practices, show trends of GIN in ewes and lambs in temperate climates and provide insight into their epidemiology. Ewes in early lactation appeared to have high FEC, which was dependent on anthelmintic treatment and possibly ewe nutrition. Lamb FEC built up gradually through the year regardless of short-acting anthelmintic treatments, although these did reduce lamb FEC immediately after treatment. This study will help to inform the sustainable control of ovine GIN, by providing insight into on-farm epidemiology.

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Authors’ contributions

KH, NS: Study design, sample collection and analysis, data analysis, manuscript preparation.
AJ, RK, VB: Data interpretation and manuscript revision for intellectual content.
JM, DB, AM, UC: Sample collection and analysis, manuscript preparation.
SL: Weather data.
All authors had sight of and contributed to the final manuscript.

Ethical approval

Ethical approval was acquired through Veterinary Ethics Review Committee (VERC) at the University of Edinburgh, reference number VERC 10 16. Verbal consent was given by the relevant management teams to carry out this work on the farms.

Declaration of Competing Interest

None.
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Appendix A. Supplementary data

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References