

Original Article

Effects of lambing season on nematode faecal egg output in ewes

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ARTICLE INFO

Keywords:

Nematode
 Haemonchus
 Sampling
 Diagnostics
 ddPCR
 McMaster
 Faecal egg count
 Spring rise
 Periparturient rise
 Parasite control

ABSTRACT

In this study, we assessed the occurrence of a periparturient rise (PPR) in winter and spring lambing ewes in Sweden and where nematode egg excretion patterns were investigated mainly for diagnostic purposes. Gastro-intestinal nematodes and *Haemonchus contortus* presence were monitored in parallel in all animals in each experimental group on four farms in samples that were collected mainly when the animals were stabled. Faecal examinations of the same animals were conducted on four sampling occasions between January and June 2018. Each group consisted of 12 crossbreed ewes of similar genotype. One group's peak lambing was in January or February (early), and the other in March or early May (late). The first (S1) and third samples (S3) were from approximately one to two weeks before parturition in the early (winter lambing) and late (spring lambing) group respectively, whereas the second (S2) and fourth samples (S4) were collected approximately three to five weeks post-parturition in the same groups. During the course of the study, there was a significant rise in faecal egg counts (FEC) in both groups on all farms. On three farms with a substantial amount of *Haemonchus*, we observed a difference in the egg excretion patterns between the two groups of ewes as revealed by a significant interaction between the sampling point and lambing period. Also, when samples corresponding to each other in relation to the number of weeks that had elapsed post-parturition (three to five weeks post-parturition, S2 for early and S4 for late) were compared, FEC were significantly lower in the early group lambing in winter than the late group lambing in spring. This indicates that besides lambing, the rise in nematode egg count is also influenced by other factors unrelated to the lambing period, such as the increased daylight in spring. Due to study limitations, we cannot provide a more detailed explanation for this, but only state that the rise appeared to be more closely linked to season than physiology as measured by day relative to parturition. Still, our results suggests that when turned out, winter lambing ewes contribute to pasture contamination to a lesser extent than those lambing in spring. These results will be used in stipulating evidence-based advice to farmers in their flock management to reduce use of anthelmintics, and at the same time efficiently produce prime lambs.

1. Introduction

Grazing sheep for prime lamb production is currently an expanding way of producing livestock in Sweden. Apart from providing meat and pelts, grazing sheep contributes to ecological functions, that maintain open landscape's biodiversity and aesthetic values of grassland (Ben-thien et al., 2018; Metera et al., 2010). In the meantime, grazing animals are constantly exposed to pasture borne nematode parasite infections that can contribute to ill-thrift and associated welfare and health issues unless the infection levels are controlled (Sutherland and Scott, 2010). Although effective parasite control can usually be achieved through informed use of anthelmintics usually in combination with grazing

management strategies, there is increasing evidence of anthelmintic resistance (AR) in Europe which in some regions threatens the sheep sector (Vineer et al., 2020). By determining the faecal eggs counts before drug use, treatment frequency can be reduced and it will thereby slow down the selection for AR (Charlier et al., 2014).

Farm and Animal Health (www.gardochedjurhalsan.se) is a Swedish veterinary organisation offering advice on the use of anthelmintics in sheep farms for decades. This, by providing diagnostic services related with parasitic nematode eggs count. Accordingly, sheep should be dewormed to reduce the risk for build-up of high infection levels of larvae on pasture when the welfare and health of the animals are at risk. This is in accordance with the principle of a targeted anthelmintic

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Received 4 March 2021; Received in revised form 31 August 2021; Accepted 5 September 2021

Available online 8 September 2021

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treatment strategy, whereby the animals are only dewormed if it is to the long-term benefit of the animals (Kenyon et al., 2009). Thereby the use of anthelmintics is reduced and hence slows selection for AR. In a previous study we investigated an enhanced sampling strategy for larger commercial sheep flocks specialised in pasture-based lamb production. We demonstrated that the likelihood of finding the most pathogenic parasite *Haemonchus contortus* increases with the sampling intensity (Höglund et al., 2019). Together with *Teladorsagia circumcincta*, these are the two dominant species in Swedish sheep, while for example *Trichostrongylus colubriformis* which is common in other parts of the world is missing (Halvarsson and Höglund, 2021). However, in order to apply an effective targeted deworming strategy, it is essential to find out how the sampling time affects the outcome of the faecal examination. Under Swedish conditions, this applies not least to lambing ewes before they are turned-out on pasture. Due to the phenomenon of periparturient egg rise (PPR), the sampling time of the ewes was assumed to influence the investigation's results.

The PPR during gastrointestinal nematode infection in ewes is reported to be characterized by an increase or rise in faecal egg counts (FEC) around parturition which remains elevated for some time. To prevent pasture contamination with infective larvae, knowledge about the parasite egg excretion patterns in relation to the arrested larvae resumption connected with lambing is of practical importance (Gibbs, 1986a). Larval arrestment is an important feature in the epidemiology of GIN ruminants (Eysker, 1997), and especially for *H. contortus* that under Swedish farming conditions has evolved to survive the winters almost entirely within the host as hypobiotic larvae (Waller et al., 2004). Although several studies suggest a massive increase in FEC around parturition, others give contradictory results (Falzon et al., 2013). In regions where larval arrestment occurs it has been suggested that PPR is at large due to the reactivation of inhibited mucosal stages (Gibbs, 1986b). Although the precise mechanism behind PPR is still unclear (Beasley et al., 2012; Falzon et al., 2013), a favoured explanation is that the sudden rise in nematode eggs is because of temporary immunity loss in the ewe around parturition (Beasley et al., 2010a). PPR in sheep is affected by the host animals genotype, where breeds showing nematode resistance sometimes show a reduced PPR compared to susceptible sheep (Courtney et al., 1984; Rocha et al., 2004; Williams et al., 2010), but also to litter size (Notter et al., 2017; Romjali et al., 1997), as well as to stage of lactation and nutrient supply (Beasley et al., 2010b; Houdijk, 2008). Furthermore, it has been speculated that PPR may be due to hormonal changes related to pregnancy (Mahieu and Aumont, 2007), even though there was no clear evidence of a hormonal (progesterone, oestradiol, cortisol, prolactin and leptin) initiator or contributor to the maintenance of the PPR during late pregnancy and lactation in *T. colubriformis* infected Merino ewes (Beasley et al., 2012; Beasley et al., 2010b).

PPR in sheep has never before been systematically investigated in Sweden. It has been discussed which is the most suitable time point for screening periparturient ewes for a long time. To the best of our knowledge, few studies have focused on the influence of the time of year in relation to their lambing period that can occur from winter to late spring or early summer. To refine and improve the faecal diagnosis, we investigated how the lambing period affects nematode egg output in two groups of naturally infected winter and spring lambing ewes on four commercial sheep farms. Both groups were studied in parallel on four sampling occasions when the animals were stabled without access to pasture, apart from the last sample on one farm that was collected soon after turn-out. Besides that, knowledge about the PPR phenomenon is of fundamental interest, it is also of practical importance for the timing of faecal sample investigations upon which treatment decisions often are made before ewes are turned out to pasture in late spring.

2. Material and methods

2.1. Farms and animals

The study was conducted between December 2017 and June 2018 on four commercial Swedish sheep farms located in the nemoral agro-climatic zone of Europe. This region is characterized by a cool temperate climate where sheep are usually stabled from early October to May. The farms under investigation were of varying size and had between 130 and 980 naturally infected breeding ewes (A = 170, B = 600, C = 130, D = 980), each with two groups of multiple bearing crossbred ewes of similar genotypes (farm A = Texel and Swedish Finewool, B = Dorset and Swedish Finewool; C = Gotland Pelt, and D = Dorset, Texel and Suffolk), where one group was lambing during the winter period with a peak in January or February (early), and a second group (late) was lambing in spring with a peak in March or early May (farm A). At the start of the experiment, 12 periparturient (early) and 12 early gestation (late) ewes were randomly selected on each farm (A-D). On each farm, all of these had grazed together the previous year and had not been treated with any anthelmintic during or for at least 9 months before the study.

2.2. Sampling

Sampling was done in parallel in both groups of ewes on four occasions: i) S1 = approximately 10 to 17 days before the expected parturition in the early group, ii) S2 = about 22 to 29 days after parturition in the early group, iii) S3 = about 8 to 15 days before the start of parturition in the late lambing group, and iv) S4 = about 18 to 37 days post-parturition in the late group (Fig. 1). At all samplings (S1-S4), the animals on all farms were indoors and fed with hay and silage supplemented with concentrate, except for the last sample on farm A (S4) where the ewes at this time point had been grazing for approximately three weeks.

The farmer or a field technician were instructed to collect approximately two tablespoons of fresh faeces from each animal either from a fresh dropping or the rectum. The samples were placed immediately in marked zip-lock plastic bags whereafter the air was pressed out before sealing and then being sent over night on the same day by national post to the diagnostic laboratory (Vidilab AB).

2.3. Parasitological investigation

Upon arrival of the samples at the laboratory the following day, the number of GIN eggs were first counted using a modified McMaster technology based on 3 g and where each egg counted represented 50 nematode eggs per gram faeces (EPG) as has been previously described (Ljungström et al., 2018). Then subsamples of approximately 2 g from all animals in each group were pooled in separate plastic containers. After blending the faeces with vermiculite, the eggs were cultured for between 7 and 10 days in a moist chamber at ≈ 20 °C. Infective third-stage larvae (L3) were harvested using the Petri-dish method, concentrated in a Falcon tube and collected into an Eppendorf-tube before storing in a freezer at approximately -18 °C (Elmahalawy et al., 2018). After thawing, DNA was extracted using the Nucleospin® tissue kit according to the manufacturers' instructions. The pooled larval DNA was finally screened in a duplex reaction using two different primer probe sets targeting different positions in the internal transcribed spacer region 2 (ITS2) situated in the ribosomal RNA gene for absolute DNA copy number quantification of: i) universal strongyle egg DNA and ii) *Haemonchus* specific DNA. For this we used the BioRad® droplet digital PCR assay platform as described before (Elmahalawy et al., 2018). The proportion of *Haemonchus* ITS2 copies in the sample was calculated in relation to the universal copy numbers in the same sample measuring total strongyle DNA.

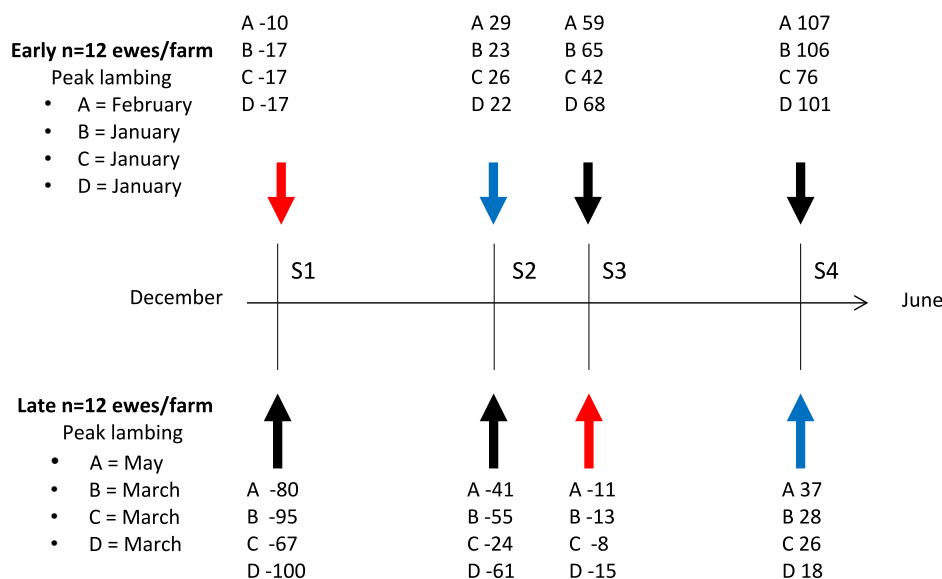


Fig. 1. Graphical display of the sampling schedule. Two groups of ewes on four farms (A-D) lambing at in winter (early) or in spring (late) were sampled at different points (S1-S4). With exception of S4 on farm A, each group consisted of 12 stabled multiple-bearing ewes that were sampled on every occasion. Approximate time points from midpoint lambing are indicated (in days) near the arrows. Note that the lambing periods were not synchronized between farms. The arrows indicate sampling occasions when the lambing status was similar for the two groups, before (red) and after lambing (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.4. Statistical analyses

Data summaries were completed in Microsoft Excel® for mac version 16.16.6 (Microsoft Corporation®). Then data were imported into the GraphPad Prism® mac version 8.4.2, for graph production, statistical analyses and temporal FEC pattern comparisons between the two groups of ewes (early or late lambing period) on each farm. For this we used the repeated measures two-way ANOVA platform with the mixed-effect analysis option to evaluate responses across measurement times. In the model, FEC + 1 log-transformed values were the dependent variable whereas sampling time (S1–4) and lambing period (early and late) were independent factors. Also, the interaction term between the sampling point and lambing period was calculated. Model assumptions were checked visually using the built-in QQ and homoscedasticity scatter plot

functions matching the actual and absolute values of the residuals versus the predicted residuals, respectively. Differences in the abundance of *H. contortus* between groups of ewes and farms were compared using contingency analysis by testing each sample occasion separately for each sample and by combining the data from S1 with S2 and S3 with S4. Also, S2 and S4 FEC results for the early and late groups on each farm were compared with Mann-Whitney non-parametric test. All tests were considered statistically significant at $P < 0.05$.

3. Results

3.1. Faecal egg counts

As shown in the violin plots, some farms were more heavily infected

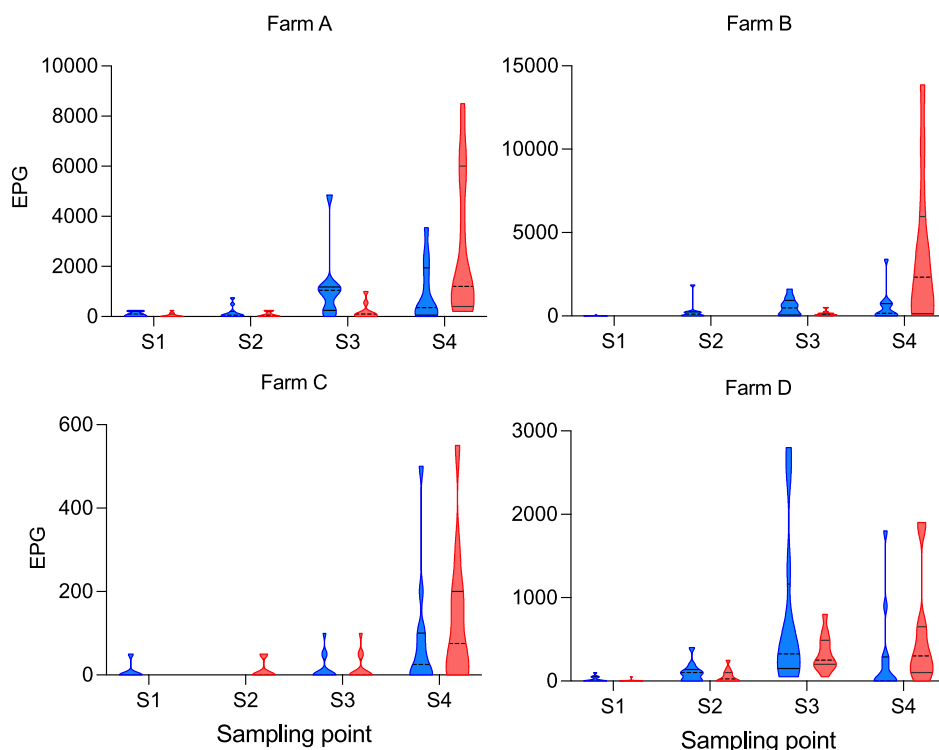


Fig. 2. Truncated violin plots showing the strongyle egg numbers in both groups on the four sampling occasions S1 to S4. Plots in blue refer to the early lambing ewes in winter ($n = 12$) and plots in red indicate late lambing in spring ($n = 12$). S1 and S3 were collected one to two weeks before lambing, whereas S2 and S4 were collected three to five weeks post-parturition in the early and late group respectively. Note that all ewes were stabled apart from the S4 on farm A and that scales in the graphs are different. The lines within the plots represent the median (dotted) and quartiles (straight). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

than others (Fig. 2). Overall, the highest mean values for all data combined was observed on farm B (648 EPG), followed by farm A (644 EPG), D (264 EPG) and C (34 EPG). Although the strongyle egg counts varied a lot between different animals even within the same sample groups (Fig. 2), there was a significant ($p < 0.001$) increase in FEC in both lambing groups on all farms during the course of the study. Of the 192 samples, 34 (18%) had egg counts exceeding 1000 EPG. Most of the samples with high egg counts were observed on the last two sampling occasions (S3 and S4), whereas few or no nematode eggs were counted at S1 and S2 (Fig. 2). In the late lambing ewe group, the highest EPG values were nearly always observed in S4. The increase in FEC over the four sampling occasions was not as pronounced in the early group, and especially on farms A and D, where the highest values were noticed in S3. In the multiple pairwise FEC value comparisons between both groups on the different sampling occasions, a significant difference was only seen on farm D at S3 with a slightly higher value in the early group than the late group (Fig. 2). However, we observed a significant interaction between the sampling point and lambing interval on all farms with the exception of farm C (A, $p = 0.01$; B, $p < 0.001$; C, $p = 0.32$; D, $p = 0.003$, Table 1), showing that the FEC evolution patterns were different in the two lambing groups on most farms. Also, when comparing the FEC from the two groups in samples collected at the same time in relation to the number of weeks that had elapsed after lambing on the same farms, S2 for early and S4 for late, significantly higher values were always observed in the late group (A, $p = 0.0001$; B, $p = 0.0041$; C, $p = 0.0013$; D, $p = 0.0103$), (Fig. 2).

3.2. Molecular findings

According to the ddPCR investigation, *Haemonchus* was present in S4 on all farms (Fig. 3). However, it is evident that farms A, B and D had a higher proportion of *Haemonchus* than C, as shown in the graph. Overall, the highest mean values were observed on farm A, with an average of 40%, followed by B with 23% and D with 17%, whereas farm C had less than 1%. Furthermore, *Haemonchus* was present in all samples on farm A, whereas on B and D its occurrence increased first in the early lambing group and then in the late group. Also, when comparing differences on these three farms, significantly different proportion *Haemonchus* patterns between the two ewe groups were observed on farm B and D, but not on farm A.

4. Discussion

To identify GIN egg excretion patterns in peri-parturient ewes before they are turned out to pasture is important to identify how they contribute to parasite pasture contamination. The purpose of the current study was to assess how the nematode egg output in ewes is affected by their lambing period. By using a replicated pairwise sequential study design, we investigated the evolution of strongyle FEC and how it is influenced by the presence of the abomasal parasite *Haemonchus*. The study was conducted in two groups of 12 ewes on four farms, each characterized by a different lambing period. Accordingly, two cross-breed groups of a similar genotype were monitored in parallel on four sampling occasions from winter to spring on each farm mainly when the ewes were stabled. We found that the FEC increased gradually post lambing in both groups irrespective of their lambing period. However,

Table 1
Main effects of time (S1 to S4), lambing (early and late) and the interaction term between time and lambing on the four farms.

Fixed effects	Time	Lambing	Interaction
Farm A	$F_{(2.43, 45.4)} = 11.3^{***}$	$F_{(1, 20)} = 1.27$	$F_{(3, 56)} = 4.13^{**}$
Farm B	$F_{(2.15, 63.0)} = 38.1^{***}$	$F_{(1, 88)} = 2.09$	$F_{(3, 88)} = 9.38^{***}$
Farm C	$F_{(2.09, 61.2)} = 10.1^{***}$	$F_{(1, 88)} = 0.775$	$F_{(3, 88)} = 1.18$
Farm D	$F_{(2.26, 49.6)} = 27.3^{***}$	$F_{(1, 22)} = 0.0674$	$F_{(3, 66)} = 5.21^{**}$

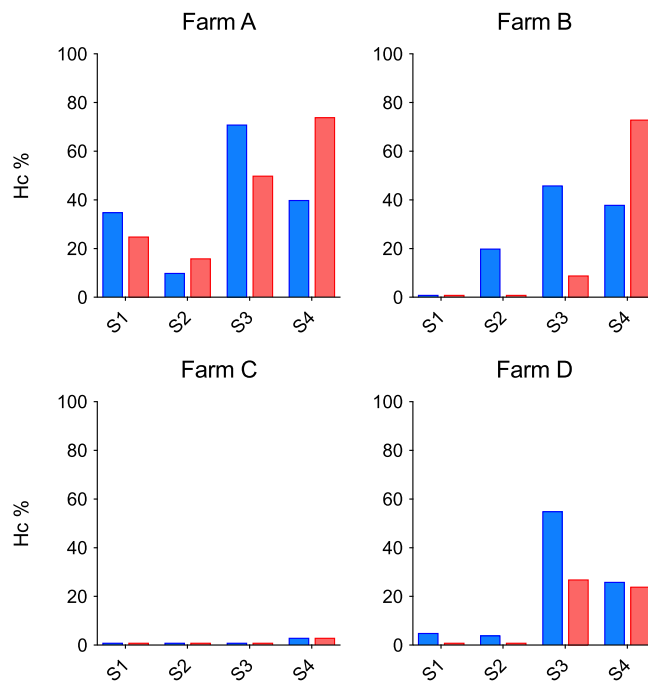


Fig. 3. Bar charts showing the proportions of *Haemonchus* ITS-2 copies in pooled larval cultures in both lambing groups on the four sampling occasions from winter to spring/early summer. S1 and S3 were collected one to two weeks before lambing, whereas S2 and S4 were collected three to six weeks post-parturition in the early and late group respectively. Blue refers to group of ewes lambing in winter (early) and in red to the group lambing in spring (late). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

when the two groups on each farm were compared with respect to the number of weeks that had elapsed in relation to lambing (i.e., S2 for early and S4 for late), we noticed significant differences. However, the evolution of the FEC patterns differed only on three out of four farms (A, B and D in Fig. 2). All of these were characterized by relatively high FEC levels and had a large proportion of *Haemonchus* than farm C (Fig. 3). Since the rise in egg counts started somewhat later in relation to the lambing and was less intense in the early winter lambing group, it suggests that other factors than the lambing period per se contributed to the PPR.

Several studies have demonstrated a seasonal effect, where changes in temperature and day length during the grazing season triggers an arrested or inhibited *H. contortus* development to survive cold winter temperatures (Eysker, 1997; O'Connor et al., 2006). This is in line with previous studies establishing that the *H. contortus* majority in Swedish sheep overwinter inside the host as inhibited larvae (Troell et al., 2005; Waller et al., 2004). Adult *H. contortus* only survive for some months in the abomasum and stabled animals are not likely to be exposed to infective larvae (Besier et al., 2016). Thus, we can assume that the observed rise in FEC within the studied farms, where a majority of animals sampled while stabled, was due to a more or less simultaneous maturation of arrested stages. Since the last sample (S4) on farm A was collected when the ewes had grazed for approximately three weeks, it can be argued that these were exposed to overwintering infective larvae on the pasture. However, at the same time, it has been demonstrated by tracer tests that the pasture contamination of *H. contortus* is low soon after turn-out in Sweden (Waller et al., 2004). Nevertheless, when we compared FEC levels between the two groups, three to five weeks post lambing (S2 for early and S4 for late), they were consistently higher in the late group on all farms. A significant interaction between lambing interval and sampling point was also observed on farms A, B and D, but not on farm C, with fewer *Haemonchus* (Fig. 2). This indicates that the

FEC patterns differed between winter and spring lambing ewes where *Haemonchus* is abundant (Fig. 3). From this, it follows that *Haemonchus* contributed to the PPR, which also seemed to be more intense in the spring lambing ewes.

It has been shown that informative periparturient FEC in ewes can be obtained from one week before until approximately five weeks after the start of lambing (Notter et al., 2017). According to older studies, the rise of nematode eggs in breeding ewes last for more than a month with a peak approximately from six weeks after parturition, although variation exists in the magnitude, duration and pattern of increase (Brunsdon, 1964; Procter and Gibbs, 1968). It cannot be excluded that we have missed the peak in egg production. However, if so, then most likely only in the spring lambing ewes (late group). Although the sampling points varied somewhat between the four farms, we aimed for a uniform strategy where samples from both groups correspond to each other in relation to the pre- and post-lambing period for the early and late ewes respectively. When comparing the FEC in relation to lambing (S2 for early and S4 for late), it was less pronounced in the early group where the highest levels were observed somewhat later (i.e., at S3). Although the causes for this remain obscure, our results suggest that changes leading to larval development within the host in relation to lambing were also affected by the increased daylight. In Sweden, the day length increases show considerable fluctuations during the year. In fact, it increases with ≈ 8 h from February to May (<https://www.worlddata.info/europe/sweden/sunset.php>). We therefore propose that the term spring rise is a more appropriate term than PPR under Swedish conditions. This observation is in agreement with Falzon et al. (2013), who studied PPR in ewes on Canadian sheep farms practicing out-of-season lambing. According to these authors the magnitude and timing of maximum faecal egg shedding for each production stage varied between seasons, suggesting that PPR in ewes depends on both environmental and animal physiological factors.

PPR in strongyle nematode egg excretion and its role in the GIN epidemiology of ruminants is well known (Sutherland and Scott, 2010). Still, despite much work on the mechanism leading to a more or less simultaneous maturation of adult worms, it is yet not fully understood (Beasley et al., 2012; Beasley et al., 2010a; Falzon et al., 2013). Although PPR has been linked to the ewes' breeding and nutritional status (Coop and Holmes, 1996; Houdijk, 2008), it has been shown that its intensity and magnitude is variable (Agyei et al., 1991; Brunsdon, 1964). In a detailed study of the immune response against the intestinal nematode *T. colubriformis*, infected pregnant and lactating Merino ewes exhibited a classic PPR which was preceded by changes in circulating cell counts and antibody titres (Beasley et al., 2010a). Also in breed comparisons, changes in PPR may have been attributed to differences in the periparturient relaxation of immunity rather than in the ability to acquire immunity to worm infections (Courtney et al., 1985; Courtney et al., 1984). However, it has also been suggested that the PPR intensity is linked to endocrine hormonal changes such as in *H. contortus* infected ovariectomized ewes receiving prolactin (Fleming and Conrad, 1989), although there was little evidence to support a hormonal basis for initiation and maintenance of the PPR in *T. colubriformis* according to Beasley et al. (2010b).

Most of the above-mentioned confounding factors could be ruled out in the pairwise FEC pattern farm comparisons in winter (early) and spring (late) lambing ewes when studied at S1 and S3 as well as at S2 and S4 for the early and late group, respectively. Although we investigated somewhat different crossbreed genotypes on the four farms, the two groups of ewes only differed with respect to their lambing interval, whereas the feeding regime was the same for both groups on each farm during housing. From our data, it appears that the rise in nematode egg counts in the early winter lambing ewes on farms with a substantial amount of *Haemonchus* occurred later and was less pronounced than the spring lambing ewes. This was irrespective of the genotype studied. At the same time, we realize that the significance of each individual factor is impossible to determine from these data and thus requires further

attention.

5. Conclusion

This study indicates that the rise in FEC patterns differed between winter and spring lambing ewes on farms where *Haemonchus* was prevalent. From our results, it seems like the rise in nematode egg count was closely associated with season. Regardless of the mechanism involved, the likelihood of finding *Haemonchus* increases if the ewes are sampled late in spring or early summer, irrespective of their lambing period. From a practical point of view it seems less risky to turn out untreated ewes lambing in winter than those giving birth later in spring. However, also winter lambing ewes contribute to pasture contamination and is therefore important not to ignore this group around turn out. This information is of value for sustainable on-farm control strategies.

Ethical statement

Treatments and sample collection conform with the national animal ethics guidelines.

Declaration of Competing Interest

There is no conflict of interest.

Acknowledgement

This work was supported by the Swedish Foundation of Agricultural Research grant O-16-20-742 and Formas 2016-1744. The authors would like to gratefully acknowledge the willingness and assistance of our farmer collaborators during the course of this study and to thank Safaa T. Elmahalawy for helping with the ddPCR analyses.

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