Veterinary Parasitology 296 (2021) 109500

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Nematode parasitism affects lying time and overall activity patterns in lambs following pasture exposure around weaning

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ARTICLE INFO

Keywords:
Accelerometers
Activity
Behaviour
Gastrointestinal nematodes
Health monitoring
Sheep

ABSTRACT

We investigated the effects of gastrointestinal nematode (GIN) challenge on activity in first season grazing lambs naturally exposed to two different levels of multispecies GIN infections. Ewes and their twin-born lambs were turned-out to graze in two permanent pasture enclosures naturally contaminated with GIN the previous year, thereby exposing them to overwintering strongyle larvae. Animals in the low parasite exposure group (LP) were dewormed monthly with 0.2 mg ivermectin (Ivomec® vet, oral suspension) per kg body weight, whereas those in high parasite exposure group (HP) were left untreated. At weaning, lambs were allocated to one out of four groups based on weight and sex (HPE, n = 15; HPR, n = 15; LPE, n = 14; LPR, n = 14), in four nearby non-contaminated ley enclosures of similar size. Activity patterns were monitored from day −7, i.e. 7 days pre-weaning, until day 49, i.e. 49 days post-weaning, by fitting all lambs with IceQube sensors (IceRobotics). Body weight was monitored weekly from day −21, whereas faecal samples were investigated at days −21, 7, 35 and 49 for nematode faecal egg counts (EPG) using McMaster-technology and a validated Droplet Digital PCR protocol to determine nematode composition. All statistical analyses were performed in R studio, using mixed models with repeated measures. In the data analyses, weekly recordings was treated as a period, generating a total of eight periods. Average daily lying time had a significant interaction between parasite exposure and period ($P = 0.0013$), with animals in HP having a 101 ± 31 min shorter daily lying time compared to LP. Motion Index (MI; absolute value of the 3-D acceleration) had a significant interaction between parasite exposure and period ($P = 0.0001$) with lambs in group HP having a lower average daily MI compared with LP. Both body weight gain and EPG levels were significantly different ($P < 0.0001$) between HP and LP groups during the course of the study. The molecular investigation showed that animals were predominantly infected with *Teladorsagia* spp., combined with low proportions of *Haemonchus* spp. In conclusion, this study shows that lying time and Motion Index of lambs around weaning was affected by moderate nematode infections. This indicates that there is a potential use of automated behaviour recordings as a diagnostic tool for detection of nematode parasites in lambs even at moderate infection levels.

1. Introduction

Infections with gastro-intestinal nematode (GIN) parasites is a global problem in pasture-based sheep herds and it is associated with reduced animal health and welfare which affect farm productivity and profitability (Charlier et al., 2020). Current control practices of GIN infections depend largely on use of anthelmintic drugs, often in conjunction with grazing management strategies (Sutherland and Scott, 2010). However, misuse of these drugs has led to a widespread development of drug-resistant worm populations (Rose Vineer et al., 2020). This underlines the need for sustainable management approaches that minimize overuse of anthelmintic drugs and thereby the selection for anthelmintic resistance (AR) (Vande Velde et al., 2018).

Targeted selective treatment (TST), where only individual animals within a group are treated, has been proposed as a sustainable long-term strategy to yield individual benefits to animal health and at the same time decrease the risk for AR (as reviewed by Charlier et al., 2014). Several indicators have been proposed, i.e. faecal egg counts (FEC), weight gains and other production traits, as well as pathophysiological indicators such as the FAMACHA© system. However, the

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https://doi.org/10.1016/j.vetpar.2021.109500
Received 3 March 2021; Received in revised form 2 June 2021; Accepted 9 June 2021
Available online 11 June 2021

implementation of TST approaches is today limited on commercial farms and the future integration is dependent of user-friendly, reliable and affordable indicators. Sickness behaviour has been suggested as an applicable indicator for monitoring various diseases in animals. Furthermore, deviating feeding behaviour and general activity can provide specific information about an animals health and welfare (Weary et al., 2009). The advancement of Precision Livestock Farming (PLF) enable real-time monitoring of such behaviours (Berckmans, 2017) and could potentially be utilized to monitor GIN challenge. However, the knowledge of responses in host activity in relation to GIN infections in ruminants is today limited and needs to be developed before it can be integrated in parasite management (Vercruysse et al., 2018).

To date there are only a handful of studies assessing activity patterns with a sensor approach as an indicator of GIN infections in sheep on pasture. Burgunder et al. (2018) observed with 3D-accelerometers that GIN infected sheep exhibited a smaller behavioural complexity compared with dewormed animals, suggesting that organizational patterns of their behaviour changes with GIN infections. Ikurior et al. (2020), showed that sheep naturally infected with strongyles had a lower activity level compared with dewormed animals after 42–46 days on pasture. Moreover, first season grazing (FSG) steers infected with GIN showed a lower activity level compared with dewormed animals as well as a higher number of conducted lying bouts 74–86 days after turn-out (Högberg et al., 2019). In addition, FSG steers inoculated with *Ostertagia* and *Cooperia* at turn-out showed a longer lying time the 40 first days on pasture as well as a higher number of steps day 62–69, compared with dewormed animals (Högberg et al., 2021).

The aim of this study was to investigate how activity patterns; i.e. lying time, number of lying bouts and total activity along with standard diagnostic indicators; i.e., body weight gain (BWG) and FEC in naive grazing lambs, are influenced when exposed to contrasting levels of GIN under natural grazing conditions. We predicted that total activity and number of conducted lying bouts would decrease whereas lying time would increase in lambs exposed to a higher level of GIN.

2. Material and methods

The study took place at Götala Beef and Lamb Research Centre, Sweden (58° 42′N, 13° 21′E; elevation 150 m MSL) from April 25th until August 13th 2019.

2.1. Animals

A total of 34 multiparous ewes with two lambs of each sex from the same commercial herd were included. The ewes consisted of 23 pure Dorset breed and 11 Dorset and Swedish Finewool cross-breeds. Two ewes from HP and three ewes from LP were treated for mastitis six to seven weeks prior to weaning and were excluded from the study. One ram lamb in group HR was treated with benzylpenicillin (Penovet® vet) and meloxicam (Metacam® 20 mg/mL) for lameness, six weeks post weaning. Altogether 58 lambs were used in the experiment (for details see experimental design).

2.2. Pasture

The pasture used pre weaning consisted of permanent semi-natural pastures with minor areas of former arable land. For the present experiment, it was split up into two enclosures with similar stocking rates. The grass-dominated pasture was mainly open including small areas of deciduous trees. The ley grazed by the lambs post-weaning contained a grass-clover mixture and was stocked three weeks after a first harvest cut. To ensure similar conditions sward height and chemical composition of the herbage was measured at weaning and at days 28 and 49 post weaning. In each enclosure, sward height measurements was made according to Frame (1993), with 120–150 recordings performed with a rising plate meter. To estimate chemical composition, 25–30 herbage mass samples were cut in 3-m diameter circles along the route. The composed herbage samples from each enclosure were analysed for concentration of crude protein (CP), neutral detergent fibre (NDF) and *in vitro* organic matter digestibility. The CP was determined according to Dumas (1831) and NDF was determined according to Chai and Udén (1998). Metabolisable energy (ME) concentration was calculated *in vitro* disappearance of rumen organic matter according to Lindgren (1979). In addition, the animals had *ad libitum* access to fresh water, salt and vitaminized minerals.

2.3. Experimental design

The study consisted of two periods, one pre-experimental period of five weeks from turn-out until three weeks pre-weaning followed by an experimental period with data collection lasting to seven weeks post-weaning. At turn-out, ewes with their twin-born lambs were allocated to one out of two pre-experimental groups (HP, high, and LP, low parasite exposure). The ewe groups were balanced for breed, body weight (HP: 68.9 ± 9.5 kg; LP: 72.9 ± 13.2 kg), body condition score (HP: 2.8 ± 0.5; LP: 3.0 ± 0.4) and age (HP: 3.8 years; LP: 3.5 years). Ewes in group LP were dewormed prior to turn-out with 0.2 mg ivermectin (Ivomec® vet, oral suspension) per kg body weight and thereafter at four-week intervals, whereas their lambs were dewormed four weeks after turn-out and thereafter at four-week intervals until the end of the experiment. Each group was released into one of two similar permanent pasture enclosures, naturally contaminated by sheep with strongyle nematode larvae the previous year. At weaning, lambs were allocated to one out of four groups based on sex (E = ewe; R = ram) and experimental group (HPE, n = 15; HPR, n = 15; LPE, n = 14; LPR, n = 14). Each experimental group was allowed to graze in one of four non-contaminated 1.0 ha ley enclosures, whereas ewes were removed.

2.4. Activity measurements

One week prior to weaning all lambs were fitted with IceQube® 3D-accelerometers (IceRobotics Ltd, Edinburgh, UK; Validated for use in lambs by: Högberg et al., 2020) on the left hind leg above the fetlock. Sensor dimensions were 55 × 55 × 27 mm and 75 g. The tri-axial accelerometer operates using a sample rate of 4 Hz with a time resolution of 15 min, with a 9-day internal memory. The accelerometers continuously recorded **Standing** (indicates whether the animal is upright or not), **Lying** (indicates whether the animal is lying down or not), **Lying bouts** (indicates the start of a lying bout) and **Motion Index** (the measured net acceleration, indicates total activity). Recordings from IceQubes, expressed as minutes per 24 h and lying bouts per 24 h, were downloaded at weekly intervals from one week before weaning and then for the seven consecutive weeks, using the download station IceReader, when the animals were handled. In the data analyses, recordings from the same lambs (i.e. 7 days of data) was treated as a period, generating a total of eight periods. Recordings from the first day of period 1 and last day of period 8 was not included so that each analysed day contained 24 h of data.

2.5. Weighing, sampling and parasitological examination

Body weights of lambs were registered on a digital scale three weeks from prior to weaning and thereafter every week for ten weeks. In connection with weighing, rectal faecal samples were collected i) three weeks prior to weaning, and ii) one week iii) five weeks, and iv) seven weeks post weaning. FEC was determined using a modified McMaster method based on 3 g faeces dispersed in 42 mL saturated NaCl, providing a minimum diagnostic sensitivity of 50 strongyle eggs per gram (EPG) faeces. In addition, remaining faecal slurry was transferred into 15 ml sterile tubes (Sarstedt, Nümbrecht, Germany) and stored at −18 °C. Total DNA was then extracted using the NucleoSpin DNA Stool

kit®, following the guidelines issued by the manufacturer (Macherey Nagel, Germany). The proportions of *Haemonchus* spp. and *Teladorsagia* spp. DNA copies situated in the internal transcribed spacer region 2 (ITS2) of the ribosomal RNA gene array were then determined in relation to the universal strongyle egg DNA copies in duplex reactions using a droplet digital (dd)PCR assay (BioRad), as described earlier by Elmahalawy et al. (2018).

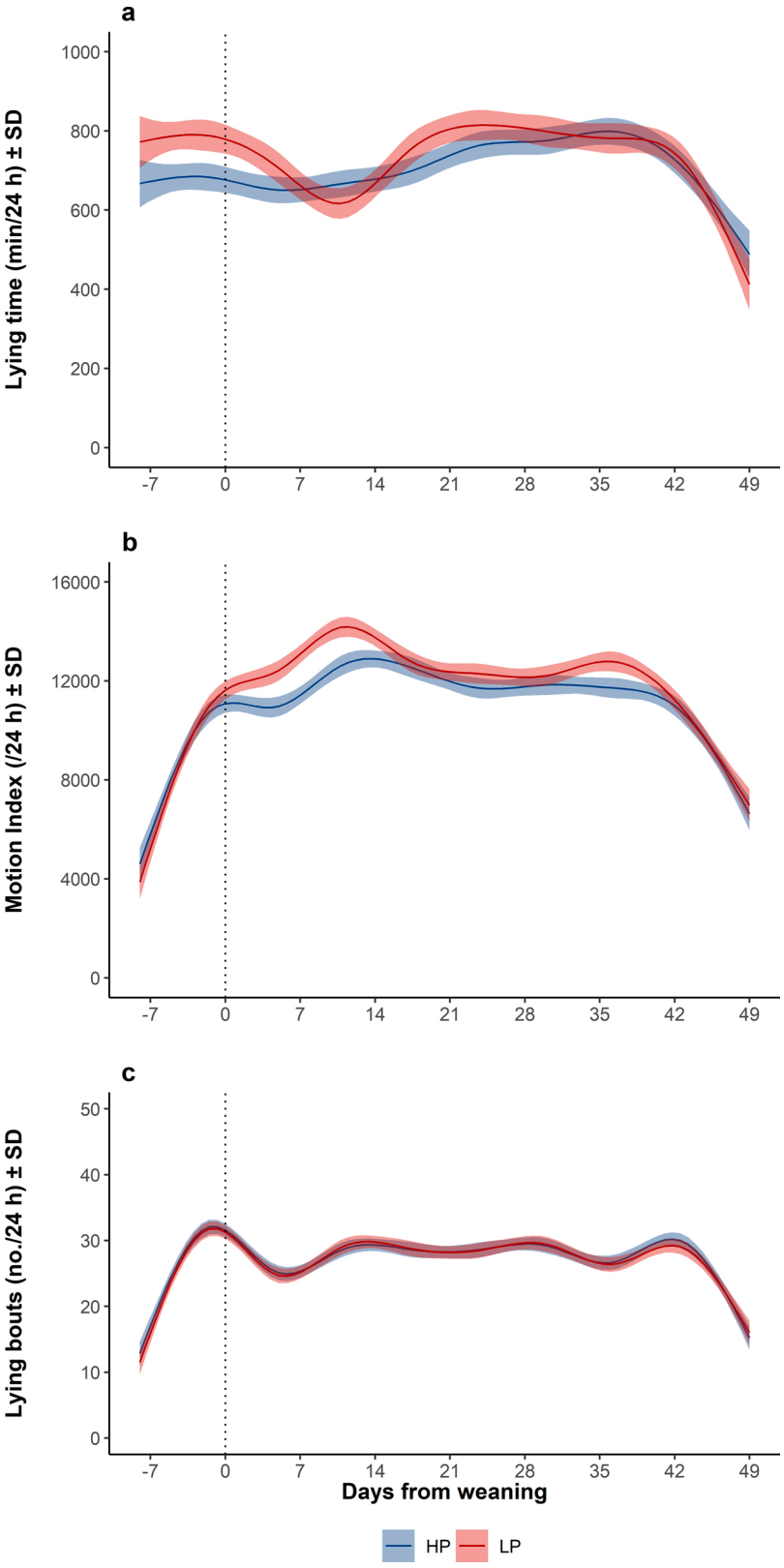

Fig. 1. a) Duration of mean lying time ± SD (min/24 h), b) mean Motion Index (/24 h), and c) mean number of lying bouts (no./24 h) in four groups, based on sex (E = ewe; R = ram) and experimental group (HP = high parasite; LP = low parasite), of first season grazing lambs exposed to overwintering strongyle larvae at pasture. One experimental group (LP) (red) was dewormed with Ivomec® (0.2 mg kg^{-1}) monthly (LPE, n = 14; LPR, n = 14), exposing them to a lower parasite challenge compared with HP (blue) (HPE, n = 15; HPR, n = 15). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2.6. Statistical analysis

The statistical analyses were performed using R studio (v. 1.2.5033). Assumptions of variance homogeneity and normal distribution of residuals of the data were checked by inspection of residual plots. Differences in activity were analysed using mixed models, with repeated measures using the LME function in the NLME package (Pinheiro et al., 2020). For IceQube data (Lying time, Lying bouts and Motion Index), GIN exposure level (HP, LP), and experimental period (1–8) were treated as fixed factors with the experimental group and individual as nested random effects. Start weights were treated as a covariate in the model. Data from one animal in group HPR, treated for lameness, was excluded from period 6 until the end of the study (and was treated as missing data). To account for time autocorrelation, a continuous autoregressive structure for a continuous time covariate (CorAr1) was fitted for the model. Final model selection was based on AIC. Pairwise differences were compared with ANOVA in the NLME package. Differences between experimental groups during the different periods were compared using Tukey's pairwise comparisons with the emmeans package (Lenth et al., 2018). Activity recordings of time spent standing was not analysed, as it is a direct mirroring of time spent lying. BWG and EPG were analysed in a repeated measure mixed model with GIN exposure level (HP, LP) and day as fixed factors and the experimental group and individual as nested random effects. A continuous time covariate (corAR1) was also fitted to account for autocorrelation. Results are reported as mean values and SD. Significance was set at $P < 0.05$. All graphical illustrations were made using the ggplot2 package (Wickham, 2016).

3. Results

3.1. Activity measurements

Average daily lying time had a significant interaction between GIN exposure and period ($P = 0.0013$), that was most pronounced from day -7 until day 7, with mean lying times being 667 ± 244 in HP and 760 ± 257 in LP, during these days, respectively (Fig. 1a). The pairwise comparisons for each period did not show any significant differences for specific periods.

Motion Index had a significant interaction between GIN exposure and period ($P = 0.0001$), that was most pronounced from day 0 until day 14, with mean Motion Index being 11,836 ± 250 in HP and 13,156 ± 278 in LP, during these days, respectively (Fig. 1b). The pairwise comparisons for each period did not show any differences.

The number of lying bouts recorded across the study period was not affected by GIN exposure ($P = 0.51$) or exposure by period interaction ($P = 0.82$) (Fig. 1c).

3.2. Host performance, nematode egg counts and larval specification

The mean body weight (Fig. 2) seven days prior to weaning did not differ between exposure groups ($P = 0.58$) and was 28.1 ± 4.68 kg in HP and 28.7 ± 3.7 kg in LP, respectively. The average body weight gain (BWG) was on average 19.1 % higher ($P < 0.0001$) in LP (362 ± 5 g) compared with HP (304 ± 6 g) from three weeks prior to weaning until seven weeks post weaning.

Fluctuations in FEC are shown in Fig. 3. Strongyle eggs were present in both experimental groups at day – 21 from weaning. The FEC was higher ($P < 0.0001$) in HP than in LP throughout the study, decreasing from day – 21 to day 7, to then increase again at day 35. The highest FEC in LP was observed at day – 21 and then declined throughout the study. Animals shedding *Haemonchus* spp. and *Teladorsagia* spp. eggs in each group are shown in Table 1. *Haemonchus* spp. was found in both experimental groups, with 7 % of animals in LP and 100 % of animals in HP being positive at least on one occasion. *Teladorsagia* spp. was found in all animals on at least one occasion.

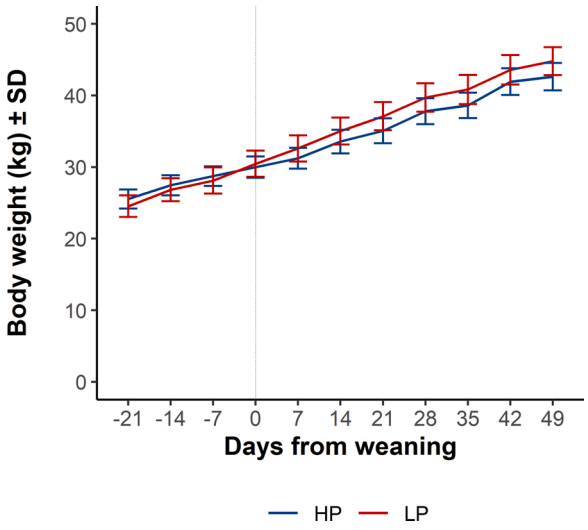

Fig. 2. Mean body weight ± SD in four groups, based on sex (E = ewe; R = ram) and experimental group (HP = high parasite; LP = low parasite), of first season grazing lambs exposed to overwintering strongyle larvae at pasture. One experimental group (LP) (red) was dewormed with Ivomec® (0.2 mg kg^{-1}) monthly (LPE, n = 14; LPR, n = 14), exposing them to a lower parasite challenge compared with HP (blue) (HPE, n = 15; HPR, n = 15). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

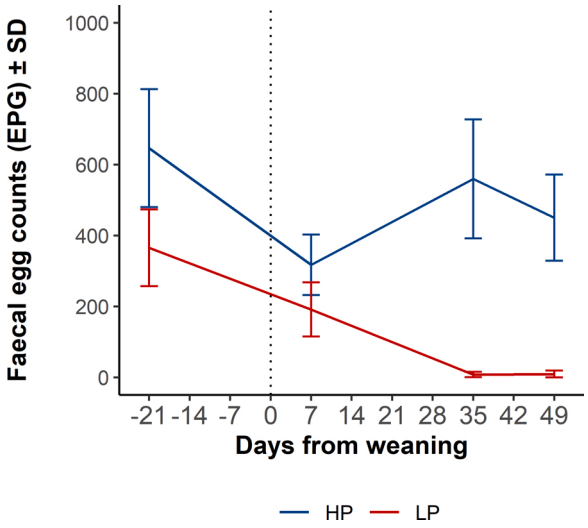

Fig. 3. Mean gastrointestinal nematode faecal egg counts (EPG) ± SD in four groups, based on sex (E = ewe; R = ram) and experimental group (HP = high parasite; LP = low parasite), of first season grazing lambs exposed to overwintering strongyle larvae at pasture. One experimental group (LP) (red) was dewormed with Ivomec® (0.2 mg kg^{-1}) monthly (LPE, n = 14; LPR, n = 14), exposing them to a lower parasite challenge compared with HP (blue) (HPE, n = 15; HPR, n = 15). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.3. Pasture

All weaned lamb groups had similar nutritional conditions with abundant pasture herbage of high nutritional quality. Across the post-weaning period, the average sward height was 7.8–8.5 (SD 1.7–2.5) cm in the four enclosures. The ranges of chemical composition across the enclosures were 224−234 g CP, 336−358 g NDF, and 11.5–11.8 MJ ME per kg of dry matter herbage.

Table 1
Prevalence (%) of *Haemonchus* spp. and *Teladorsagia* spp. and proportion (% ± SD) *Haemonchus* spp. and *Teladorsagia* spp. of FEC in four groups, based on sex (E = ewe; R = ram) and experimental group (HP = high parasite; LP = low parasite), of first season grazing lambs exposed to overwintering strongyle larvae at pasture. One experimental group (LP) (red) was dewormed with Ivomec® (0.2 mg kg^{-1}) monthly (LPE, n = 14; LPR, n = 14), exposing them to a lower parasite challenge compared with HP (blue) (HPE, n = 15; HPR, n = 15).

	HP		LP	
Days from weaning	Prevalence of *Haemonchus* spp. (%)	Proportion *Haemonchus* spp. of FEC (%)	Prevalence of *Haemonchus* spp. (%)	Proportion *Haemonchus* spp. of FEC (%)
−21	0.0	0.0	4.0	0.2 ± 0.8
7	60.0	0.9 ± 2.8	0.0	0.0
35	80.7	12.8 ± 12.4	3.7	1.7 ± 8.7
49	73.9	14.9 ± 19.3	0.0	0.0
	HP		LP	
Days from weaning	Prevalence of *Teladorsagia* spp. (%)	Proportion *Teladorsagia* spp. of FEC (%)	Prevalence of *Teladorsagia* spp. (%)	Proportion *Teladorsagia* spp. of FEC (%)
−21	100.0	78.7 ± 11.5	100.0	79.6 ± 14.4
7	100.0	54.3 ± 25.2	100.0	79.7 ± 18.6
35	92.3	13.9 ± 16.7	42.3	26.1 ± 37.0
49	87.0	5.6 ± 8.0	32.1	21.2 ± 35.0

4. Discussion

In this study strongyle nematode eggs were observed in both groups three weeks prior to weaning, but FEC was significantly higher in HP compared with LP throughout the study. *T. circumcincta* and *H. contortus* were identified as the two most abundant pathogenic species. This is in line with a recent study of the nemabiome composition in Swedish sheep (Halvarsson and Höglund, 2021), where these were among the most prevalent GIN species, along with *Trichostrongylus vitrinus, Oesophagostomum venulosum* and *Chabertia ovina*. Thus, the unidentified ratio of the species composition is likely composed of these, of which the two latter are considered as apathogenic (Sutherland and Scott, 2010). *T. circumcincta* was more abundant in both experimental groups, appearing at day -21, and initially contributing to a high proportion of eggs shed. *H. contortus* was present in both groups, but at a higher rate in HP compared with LP animals. The pattern with *H. contortus* eggs appearing first at day seven in HP suggests that the main source of infection originated from eggs shed by the ewes rather than from overwintering pasture contamination, in agreement with Troell et al. (2005). The relatively low EPG levels in combination with predominantly low proportions of *H. contortus*, indicates moderate infections with this parasite also in HP animals. Still, we observed a difference in BWG throughout the study period with the daily weight gain in LP being 75 ± 18 g higher than HP. No differences in pasture composition and availability was observed. Furthermore, no clinical signs of parasitism, such as diarrhoea and anaemia, were observed. Together, this implies a moderate subclinical course of disease also in HP.

Despite all of this, our results indicate that the differences in GIN infections generated herein had effects on the activity patterns in lambs and that could be registered with commercially available on-animal sensors. Animals in HP had a lowered MI compared with LP throughout the study. This finding is in agreement with those of Ikurior et al. (2020) that also detected a reduced activity in sheep challenged with subclinical levels of GIN. Similarly, FSG steers inoculated with *O. ostertagi* and *C. oncophora* at turn-out and further exposed to larvae on pasture, had a reduced activity compared with dewormed animals (Högberg et al., 2019). Previously, a reduction in activity in grazing steers have been suggested to be linked with a dose dependent mucosal damage mainly associated with *O. ostertagi* infection (Högberg et al., 2019). The lambs in the present study were primarily exposed to *Teladorsagia* spp. that inhabit the same niche in sheep as *O. ostertagi* in cattle, with similar pathological effects on the host (Sutherland and Scott, 2010). This further underlines the possibility of a connection between nematode induced mucosal damage in the abomasum and deviation in host activity patterns. This contrasts the results of Ikurior et al. (2020), where sheep were predominantly infected with *Cooperia* spp. This suggest that the effect of GIN parasitism on host activity is not limited to mucosal damage solely. Therefore, there is a need for future studies in grazing livestock where different host-parasite relationships, including mixed infections and different levels of exposure, are investigated.

There was also an exposure by period interaction on lying time with HP animals having a shorter daily lying time compared with LP throughout the study. This is in direct contrast with the findings of Högberg et al. (2021), where FSG steers challenged with a subclinical infection of GIN had a longer daily lying time during the 40 first days after infection, compared with dewormed animals. Again, this emphasises the need for species-specific interpretation of associated sickness behaviour for different host-parasite relationships. In addition, the differences in lying time patterns observed in the lambs studied herein was present already at the start of recording. This indicates that the influence of GIN infection on the activity patterns in sheep may arise early and could be explained by the difference in FEC levels observed three weeks prior to weaning, reflecting a manifest GIN infection. This underlines the potential use of behavioural variation as an early indicator of GIN infection, as suggested by Szyszka et al. (2013). In contrast, long term differences in activity may have a more limited value for the implementation of a TST-system. However, more studies are clearly needed to determine the relationship between infection levels and individual behavioural responses, under varying exposure conditions.

Nevertheless, in our study the variation of FEC within HP was moderate reflecting minor variations in GIN infection levels between the animals in this group. Also individual traits, such as body weight, has been shown to affect the behavioural response to GIN infections in sheep (Ikurior et al., 2020). This underlines that individual variation needs to be taken into account when developing PLF-systems evaluating treatment thresholds that can be used in parasite control programs.

5. Conclusion

This study constitutes an attempt to evaluate the effects of multi-species nematode parasitism on the activity level in grazing lambs in connection to weaning, using commercially available on-animal sensors. The results show that the activity measurements lying time and Motion Index differed between HP and LP, despite that infection levels were considered to be low also in HP. Although additional research regarding individual variation in connection to GIN infections is needed, we have demonstrated the potential use of automated behaviour recordings as a diagnostic tool for detection of major nematode infections of sheep to be integrated into future parasite control programs.

Ethics statement

The study was approved by the Committee on Animal Experiments in Gothenburg (registration number 824-2017).

Data and model availability statement

None of the data were deposited in an official repository.

Funding

The study was funded by the Swedish research council FORMAS (grant number 22304000), Västra Götalandsregionen (grant number RUN-2018-00137), Agroväst and Swedish farmers' foundation for agricultural research.

CRediT authorship contribution statement

Niclas Högberg: Investigation, Conceptualization, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Anna Hessle:** Conceptualization, Supervision, Project administration. **Lena Lidfors:** Conceptualization, Supervision, Project administration. **Nizar Enweji:** Investigation. **Johan Höglund:** Conceptualization, Supervision, Project administration.

Declaration of Competing Interest

Boehringer Ingelheim supplied the Ivomec® used in the study.

Acknowledgements

We thank Jonas Dahl, David Johansson, Annelie Carlsson, Frida Dahlström, Josefine Norrback and Andreas Bladh for the practical work at SLU Götala Beef and Lamb Research Centre. Claudia von Brömsen at SLU is thanked for assistance on statistical questions and Boehringer Ingelheim for sponsoring of anthelmintics.

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