Effects of nematode parasitism on activity patterns in first-season grazing cattle

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ABSTRACT

We investigated the effects of gastrointestinal nematode (GIN) challenge on activity patterns in first season grazing (FSG) steers exposed to two different levels of Ostertagia ostertagi and Cooperia oncophora. At turn-out, experimental animals were allocated to one of two treatment groups grazing in different enclosures each with 32 animals. The first group (High) received 5000 third stage (L3) O. ostertagi (50%) and C. oncophora (50%) larvae; whereas the second group (Low) were dewormed monthly with 0.5 mg ivermectin (Noromectin®) per kg bodyweight. Activity patterns were monitored by fitting some animals in each group (High, n = 10; Low, n = 8) with leg mounted sensors (IceTag® 3D-accelerometers) during three two-week periods. In animals fitted with sensors body weight gain (BWG) was recorded every fortnight, whereas faecal and blood samples were collected every four weeks for nematode faecal egg count (FEC) and serum pepsinogen concentrations (SPC). Differences between the periods in daily (P = 0.046) and diurnal (P = 0.0502) activities were recorded between groups during the course of the study. A significant (P = 0.038) increase in the number of lying bouts was recorded in group High during the second period (days 74–86), which was correlated (r = 0.55, P = 0.018) to an increase in SPC = 85 days after turn-out. BWG was reduced (P = 0.037) in group High compared to group Low, deviating from day 45. Strongyle nematode eggs were observed in both groups 29 days after turn-out, however the mean EPG remained low in group Low throughout the experiment. An increase in SPC was observed (P < 0.0038) in group High with levels peaking on day 58. In conclusion, our data supports that changes in activity patterns monitored with sensors could contribute to the identification of animals challenged with GIN, but also improve our understanding in the potential welfare impairments caused by such infections.

1. Introduction

Pasture borne gastrointestinal nematode (GIN) parasites are very common in grazing cattle and thereby represent a significant economic and welfare burden to the global ruminant livestock industry (Sutherland and Leathwick, 2011). Faecal egg counts (FEC) are commonly used as an indicator of patent GIN infections but are often poorly correlated to infection levels and impact on animal performance (Clarebout and Vercruysse, 2000). Thus, alternatives to FEC have been suggested to enable targeted selective treatments (TST) of grazing livestock, such as individual treatment in the herd based on reduced weight gains (Höglund et al., 2013; Jackson et al., 2017) and/or the level of diarrhoea or body condition score (Kenyon and Jackson, 2012). In theory, it is possible to maintain effective parasite control of nematode-induced impact on animals by the use of TST strategies while decreasing the risk of selection for anthelmintic resistance (Charlier et al., 2014). However, this should not occur at the expense of reduced animal health, animal performance and impaired animal welfare.

An alternative to the above mentioned TST indicators would be to focus on host sickness behaviour (Weary et al., 2009). Research into host behaviour, which can underpin novel parasite control methods, remains understudied.

To date assessment of activity patterns as an indicator of health and welfare impairment has mainly been investigated in housed animals (Caja et al., 2016). For example, in dairy cows a decrease in food intake or reduction in number of meals has been shown to be able to predict the onset of ketosis (González et al., 2008), mastitis (Sepúlveda-Varas et al., 2016) and metritis (Neave et al., 2018). Furthermore,
transition cows a change in lying time and number of lying bouts could predict ketosis (Itle et al., 2015) and subclinical metritis (Neave et al., 2018). In addition, a recent study on fattening cattle showed a decrease in daily activity before onset of clinical signs of bovine respiratory disease (Marchesini et al., 2018).

In contrast, there is only a handful of studies investigating responses in host activity in relation to parasite infections. Heifer calves with naturally acquired GIN showed a significant decrease in grazing time 70 days after turnout (Forbes et al., 2000). Also the eating time, mean meal duration, total bites and idle time were all significantly decreased in GIN infected dairy cows (Forbes et al., 2004). Recently, studies on housed Holstein-Friesian bull calves experimentally challenged with trickle doses of Ostertagia ostertagi showed a significant decrease in number of steps taken and decrease in number of standing and lying bouts 21 days post inoculation with infective larvae (Szyszka et al., 2013). Furthermore, there are indications that alteration of behavioural response to GIN challenges acts in a threshold level response with intermediate infective doses having similar effects on behaviour and only higher infective doses showing a dose dependent relationship (Szyszka and Kyriazakis, 2013). In line with studies in cattle, sheep challenged with GIN showed a decrease in number of steps taken (Hutchings et al., 2000). In addition, goats with naturally acquired haemonchosis show lower activity levels (Babayani, 2016).

In the future, technology development of data transfer from sensors will enable remote real-time monitoring of animals also on pasture.

The aim of this study was to investigate activity patterns (i.e. lying time, number of steps, number of lying bouts and total activity) and standard diagnostic indicators (i.e., body weight gain (BWG), FEC and serum pepsinogen (SPC)) in first season grazing cattle (FGG) when exposed to two different levels of O. ostertagi and Cooperia oncophora under natural conditions. We predicted that BWG would be reduced and FEC and SPC increased in FSG exposed to a higher level of GIN. For the activity recordings, it was hypothesized that FSG exposed to a higher dose of GIN would be lying more, taking fewer steps, having a lower activity levels (Babayani, 2016).

In the future, technology development of data transfer from sensors will enable remote real-time monitoring of animals also on pasture.

2. Material and methods

The study took place at SLU Götala Beef and Lamb Research Centre, Sweden (58° 42′ N, 13° 21′ E; elevation 150 m a.s.l.) from May 2nd until September 20th 2016. The study was approved by the Committee on Animal Experiments in Gothenburg (registration number 187–2014).

2.1. Pasture

The pasture consisted of 28 ha of permanent semi-natural pastures, which previous year had been used by beef suckler cows and their calves. For the present experiment it was split up into two similar enclosures, both consisting of approximately 20% dry, 60% mesic and 20% wet areas. The pasture was mainly open, but included small areas of mixed deciduous trees. The dominant plant species was Deschampsia cespitosa (tufted hairgrass), but Festuca rubra (red fescue) was also prominently present.

Sward height and chemical composition of the pasture herbage were measured every four weeks from turn-out to housing, to ensure similar conditions in the two enclosures. In each enclosure, sward height measurement followed a W-shaped route according to Frame (1993), with 120–150 recordings performed with a rising plate meter (0.3 x 0.3 m, weight 430 g). To estimate chemical composition, 25–30 herbage samples were cut with a handheld machine in 3-mm diameter circles along the route. The samples were analysed for concentrations of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and in vitro organic matter digestibility. The DM concentration was determined at 130 °C for 24 h, CP was determined according to Dumas (1831) and NDF was determined according to Chai and Udén (1998). Metabolisable energy (ME) concentration was calculated from in vitro disappearance of rumen organic matter according to Lindgren (1979).

2.2. Animals

FSG steers of two different genotypes purchased at 2–3 months of age from the same commercial farm of which some were monitored (see 2.5.). There were in total 31 purebred dairy calves (Swedish Red and Swedish Holstein) and 32 crossbreeds between dairy and beef breed (Charolais). The birth date of the steers ranged from April 18th to November 1st 2015. Before turn-out, animals were housed in an un-insulated building on deep straw and fed a forage based total mixed ration at ad libitum intake. Average age at turn-out was 283 ± 58 days. The animals had unlimited access to fresh water, salt and vitaminized minerals at pasture.

2.3. Experimental design

The experiment involved two grazing groups exposed to two different level of GIN, where the two genotypes were randomized according to birthdate and evenly allocated between the two groups. The high level parasite exposure group (High) was primed at turn-out with about 5000 infective third stage larvae (L3) of O. ostertagi and C. oncophora (1:1). In contrast, the calves in the low parasite exposure group (Low) was treated with an ivermectin pour-on solution (Noromectin® Pour-on, 0.5 mg/kg body weight) at four-week intervals from turn-out until end of trial (i.e. after 142 days on pasture). Both groups of animals were turned out on May 2nd into one out of two similar pasture enclosures of 14 ha, naturally contaminated with nematodes the previous year exposing both groups to overwintering strongyle larvae in the grass.

2.4. Weighting, sampling and parasitological examinations

BWG of the animals equipped with sensors (N = 18, see 2.5.) was recorded manually at turn-out (start of experiment) and after 142 days at housing (end of experiment), as well as every fortnight in between. Average weight of group High (n = 10) at experimental start was 317 ± 62 kg and of group Low (n = 8) 374 ± 89 kg. Similarly, rectal faecal samples were collected at turn-out, housing and at four-week intervals in between. FEC was determined according to a modified McMaster technique based on 5 g of faeces with a minimum detection level of 20 nematode eggs per gram (EPG). Every four weeks, 2 × 5 ml blood samples were taken from the coccygeal vein (Vacutainer®, Becton Dickinson). The SPC was determined according to a micro-method (Charlier et al., 2011).

2.5. Activity measurements

The ten and eight heaviest animals in group High and Low, respectively, were fitted with IceTag® 3D-accelerometers (IceRobotics Ltd, Edinburgh, UK; Validated by Ungar et al., 2017) on the left hind leg above the fetlock during three two-week periods (1: 14–29 June, 2: 12–26 July and 3: 9–23 August). Sensor dimensions were 65 × 60 × 30 mm and 197 g. The tri-axial accelerometer operates using a sample rate of 16 Hz with a time resolution of 1 s. It continuously recorded standing (indicates whether the animal is upright or not), lying (indicates whether the animal is lying down or not), steps (number of steps taken), lying bouts (indicates frequency of lying bouts) and Motion Index (MI) (the measured net acceleration, indicates total activity). Behavioural recordings from IceTags, expressed as minutes per hour and 24 h and numbers of steps and lying bouts per hour and 24 h, were downloaded at the end of the study using the download station IceReader.
2.6. Statistical analysis

Data retrieved from the IceTags was assorted in Microsoft® Excel® (16.0.4591.1000). Data from nine days during period 3 was missing from one sensor and was treated as missing data in the analysis. All statistical analyses were performed using R (v. 3.4.3). Assumptions of variance homogeneity and normal distribution of residuals were checked by inspection of residual plots. For lying bouts registrations, log-transformation of the data was used to meet assumptions of normal distribution. Daily and diurnal activity measures were analysed in mixed models, respectively, with repeated measures using the LME function in the NLME package (Pinheiro et al., 2011). Experimental group (High, Low) reflecting the level of GIN exposure and period (1, 2, 3) were selected as fixed effects with the individual as random effect. Start weight and genotype were selected as covariates. To account for time autocorrelation, a continuous autoregressive structure for a continuous time covariate (corCar1) was fitted. Final model selection was based on AIC (Appendix 1 and 2). Pairwise differences in behaviour were compared with ANOVA in the NLME package. Tukey’s pairwise comparisons was performed with the emmeans package (Lenth et al., 2018). Behavioural recordings of time spent standing were not analysed as it is a direct mirroring to time spent lying. EPG counts and SPC were compared in a repeated measures mixed model with experimental group (High, Low) and day as fixed effects and the individual animal as a random effect. A continuous time covariate (corCar1) was also fitted to account for autocorrelation. In addition, the mean values for lying time, number of lying bouts and steps were calculated for each period and correlated to corresponding SPC, sampled in the end of each period, through a Pearson correlation using the cor.test() function. All graphical illustrations were made in the ggplot2 package (Wickham, 2016).

3. Results

3.1. Pasture characteristics

Pasture sward height was similar in the two enclosures throughout the grazing period with 4.7 ± 2.5 cm in the enclosure used by the High group, and 4.4 ± 2.5 cm in the enclosure for Low group. Likewise, chemical composition of the grass was similar in the two enclosures with 288 ± 26 and 300 ± 21 g DM/kg herbage, 157 ± 29 and 161 ± 24 g CP/kg DM, 475 ± 40 and 451 ± 49 g NDF/kg DM and 10.3 ± 1.1 and 10.2 ± 0.9 MJ/kg DM for the High and Low exposure groups, respectively.

3.2. Weighing, sampling and parasitological examinations

3.2.1. Body weight gain

The change in body weight for all animals fitted with sensors throughout the experiment is shown in Fig. 1. At turn-out the mean weight in group High was 372 ± 51 kg and in group Low 406 ± 40 kg. There was a significant effect of parasite exposure group (F1, 16 = 5.19, P = 0.037) and time (F1, 78 = 5.19, P < 0.0001) with a reduced weight gain in group High. During the first two weeks on pasture, the animals in group Low lost 55 ± 15 kg whereas animals in group High lost 51 ± 13 kg. From the third week and onwards all animals increased in weight (F1, 142 = 1776.8, P < 0.0001), with animals in group High diverging and gaining weight at a slower rate (F1, 142 = 5.89, P = 0.027) than group Low animals. The growth rate from turn-out until housing was 636 ± 23 g/day for group Low and 542 ± 29 g/day for group High.

3.2.2. Parasitology

Nematode eggs appeared in both groups after the animals had been on pasture for 29 days (Fig. 1). Throughout the study, FEC in group High was significantly higher than in group Low (F1, 16 = 11.42, P < 0.0038) which peaked at day 30 and then declined. Likewise, SPC levels were significantly higher in group High (F1, 16 = 37.01, P < 0.001). Arithmetic SPC levels in group High ranged from 3.25 ± 1.00 IU tyrosin whereas group Low ranged from 0.55 ± 0.23 to 1.08 ± 0.18 IU tyrosin.

3.3. Activity measurements

3.3.1. Lying duration

No exposure effect on average daily lying time (Fig. 2) was observed (F1, 48 = 0.44, P = 0.52). However, there was an effect of period (F2, 671 = 22.99, P < 0.0001) and exposure by period interaction (F2, 671 = 20.52, P < 0.0001) on lying time. Group High showed an increase in average daily lying time from period 1–2 (P < 0.0001) and a decrease from period 2–3 (P < 0.0001). Variation of diurnal lying
time (Fig. 3) was not affected by the level of GIN exposure ($F_{1, 14} = 0.209, P = 0.65$) but varied for both groups over the periods ($F_{2, 15671} = 3.67, P = 0.025$). A trend of a diurnal parasite exposure by period interaction was found ($F_{2, 15671} = 2.68, P = 0.069$), with group High showing a decrease in hourly lying time between period 2 and 3 ($P = 0.0084$).

### 3.3.2. Lying bouts

The average number of daily lying bouts (Fig. 2) were not affected by the level of parasite exposure ($F_{1, 14} = 0.10, P = 0.75$), but there was an effect of experimental period ($F_{1, 671} = 49.80, P < 0.0001$) and an interaction between parasite exposure and experimental period ($F_{1, 671} = 79.44, P < 0.0001$). The pairwise comparison showed a difference between experimental groups during period 2 ($P = 0.038$), with group High performing more lying bouts. Group High showed an increase in number of lying bouts from period 1–2 ($P < 0.0001$) and a decrease from period 2–3 ($P < 0.0001$), whereas group Low, in contrast, showed a decrease from period 1–2 ($P = 0.025$). The number of diurnal lying bouts (Fig. 3) was not affected by level of parasite exposure ($F_{1, 14} = 2.58, P = 0.13$) but varied over period ($F_{2, 15671} = 128.60, P < 0.0001$) and showed a parasite exposure by period interaction ($F_{1, 15671} = 148.43, P < 0.0001$). Group High showed a higher number of lying bouts on a diurnal level during period 2 ($P = 0.0035$) compared to group Low. Furthermore, group High showed an increase in number of diurnal lying bouts from period 1 to 2 ($P < 0.0001$) and decrease from period 2–3 ($P < 0.0001$), whereas group Low, in contrast, showed a decrease from period 1–2 ($P = 0.0075$).

### 3.3.3. Step counts

No effect of parasite exposure was found on the average number of steps taken per day ($F_{1, 14} = 2.44, P = 0.14$) (Fig. 2). However, there was an effect of period ($F_{2, 671} = 51.48, P < 0.0001$) and level of parasite exposure by period interaction ($F_{2, 671} = 13.99, P < 0.0001$). There was no difference between groups within the three different periods. (Fig. 2). However group Low showed a decrease in steps taken from period 2–3 ($P < 0.0001$) and group High from period 1–2 ($P = 0.0014$). A trend to a difference between the two experimental groups.

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Fig. 2. a) Duration of average lying time (min), b) mean number of lying bouts, c) mean number of steps and d) mean Motion Index in two groups of first season grazing steers. One group was infected at turn-out and thereby exposed to a high parasite challenge (High, $n = 10$, in blue), whereas the other group was dewormed with Ivermectin® (0.5 mg kg$^{-1}$) monthly, thus being exposed to a low parasite challenge (Low, $n = 8$ in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
was found on the diurnal number of steps (Fig. 3) where the number of diurnal steps varied over periods ($F_{2, 15671} = 19.75, P < 0.0001$). Diurnal period differences within the groups were found, with group High decreasing diurnal steps from period 1–2 ($P = 0.0014$) and group Low showing a decrease from period 2–3 ($P < 0.0001$).

### 3.3.4. Motion index

There was a significant effect of parasite exposure on average daily MI ($F_{1, 14} = 4.80, P = 0.046$) with group High showing a lower overall activity (Fig. 2), as well as effects of period ($F_{1, 671} = 54.4, P < 0.0001$) and parasite exposure by period interaction ($F_{1, 671} = 11.62, P < 0.0001$). Both groups reduced their MI from period 2–3 ($P < 0.0001$). A trend in parasite exposure effect on diurnal MI was found ($F_{1, 14} = 4.59, P = 0.0502$), with group High showing a lower diurnal activity (Fig. 3). The diurnal MI was also affected by period ($F_{1, 15671} = 29.29, P < 0.0001$) and by interaction between these factors ($F_{1, 15671} = 7.40, P = 0.006$). Both groups showed a decrease in diurnal MI from period 2–3 ($P < 0.0001$).

### 3.3.5. Correlations

A significant positive correlation ($r = 0.55, P = 0.018$) was found between the number of lying bouts and SPC during period 2 (Table 1). In addition, significant negative correlations were found between lying time and SPC during period 2 and 3 ($r = -0.58, P = 0.011; r = -0.53, P = 0.028$) and between the amount of steps and SPC during period 2 ($r = -0.50, P = 0.011$).

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**Table 1**

Pearson’s correlation coefficients ($r$) between mean activity measures and associated serum pepsinogen during three periods in two groups of first season grazing calves ($N = 18$).

| Period | 1   | 2       | 3   |
|--------|-----|---------|-----|
| Lie    | -0.58* | 0.35 | -0.53* |
| Lying bouts | 0.067 | 0.55* | 0.075 |
| Steps  | -0.14 | -0.50* | 0.031 |

Notes: *$P < .05$.

$(F_{1, 14} = 4.59, P = 0.057)$ was found on the diurnal number of steps (Fig. 3) where the number of diurnal steps varied over periods ($F_{2, 15671} = 19.75, P < 0.0001$). Diurnal period differences within the groups were found, with group High decreasing diurnal steps from period 1–2 ($P = 0.0014$) and group Low showing a decrease from period 2–3 ($P < 0.0001$).
= -0.50, P = 0.034).

4. Discussion

We detected effects on the standard diagnostic indicators (BWG, FEC, SPC) in animals exposed to higher exposure levels of GIN parasites. Interestingly, the differences in BWG occurred already from the third week until housing 17 weeks later, whereas significant responses in FEC and SPC were observed not until the fourth week. SPC levels higher than > 3.5 IU tyrosin, indicating clinical ostertagiosis (Charlier et al., 2011), were only observed at three occasions in different animals in group High. This implies a subclinical course of disease in most animals in this group. These parasitological findings and effects on BWG in this study are basically in agreement with previous studies with FSG in Sweden (Dimander et al., 2003; Larsson et al., 2007; Höglund et al., 2013).

The pathology of GIN and especially for O. ostertagi is known to be caused by mucosal damage due to the presence of late larval and adult stages (Fox, 1997). Thus, damage due to GIN is likely induced already within a few days after exposure to infective larvae (Murray et al., 1970; Simpson, 2000). In contrast, FEC are only informative following the prepatent period and do not reflect the infection level of the animal after development of immunity (Eysker and Ploeger, 2000). Similarly, SPC start to increase during the prepatent period (Jennings et al., 1966), but it has been concluded that SPC should preferably be used on a herd level at housing (Charlier et al., 2011; Forbes et al., 2009). Also clinical performance-based methods to indicate GIN infection has been suggested as an indicator for TST in FSG cattle in Sweden (Höglund et al., 2009), but is yet impractical since it is difficult to identify universal treatment thresholds (Höglund et al., 2013).

Thus, there is an urgent need to evaluate alternative novel methods for the determination of GIN infection levels in FSG cattle such as those based on activity measurements. Earlier studies looking at animals challenged with trickle doses of Ostertagia ostertagi showed a deviation of activity from day 22, four days after parasite eggs appeared in the faeces (Szyszka et al., 2013). In addition to these findings indicating early activity alterations, our results showed modest changes in long-term activity of animals challenged with GIN, with animals in the highly exposed group showing a significantly lower MI over all three periods. As MI measures the total movement of the sensor this finding indicates that the highly exposed animals have a generally lower activity compared to dewormed animals. The effects are underlined by the significant parasite exposure by time interaction resulting in group High taking fewer steps and showing a lower MI over time. We also observed a parasite exposure by time interaction resulting in a shorter lying time in group High over time.

The reason for a decline in activity in animals challenged with different health impairments is not fully understood, but it has been suggested to be connected to lethargy associated with sickness behaviour, enabling energy conservation (Hart, 1988; Weary et al., 2009). Furthermore, Forbes et al. (2000) showed that grazing time in FSG is negatively affected by GIN which in turn result in a reduced dry matter intake. Possibly this can explain the differences in BWG and activity seen in this study. Further studies combining assessment of activity and feeding behaviour in FSG cattle is needed to fully comprehend the interaction.

A key finding was that animals in group High showed an increase in number of lying bouts during period 2. The behavioural response recorded is not in line with classical sickness behaviour with a decrease in behavioural change. This could be reasoned to be due to pain or a general feel of discomfort. Similar discrepancies from classical sickness behaviour in housed dairy cattle with mastitis has been interpreted as a response to soreness and pain and could therefore be important from a welfare point of view (Fogsgaard et al., 2015). The high number of recorded lying bouts in both groups probably reflects what can be seen as false lying bouts (i.e. leg movements) rather than an actual behavioural change (Ungar et al., 2017) and could similarly be interpreted as an indication of discomfort. In addition, a moderate positive correlation between SPC and the amount of lying bouts was found during this period, coinciding with the highest SPC levels observed.

This may indicate that the behavioural response only occurs when the mucosal damage reaches a certain threshold level. This idea is supported by earlier findings that behaviour response to GIN challenges are not dose dependent but rather acts in a threshold level response (Szyszka and Kyriazakis, 2013). It has also been described that these patterns viewed over time, seen as the complexity of behaviour, may be considered as an indicator of stress as a result of health and welfare challenges (Alados et al., 1996). Recently it was shown that sheep naturally infected with Strongylids exhibited a smaller behavioural complexity (Burgunder et al., 2018), providing evidence for the possibility to use behavioural observations as a method for welfare monitoring of GIN infected animals. However, this lies beyond the scope of the present study.

The study provides a basis for further development into a Precision Livestock Farming-system where animals are continuously monitored for activity and that changes in behavioural pattern would be an indicator of parasitic infestation. Thereby, affected animals can be treated with anthelmintics and thus reduce the amount of substance, suggesting less risks of development of resistance among parasites.

In conclusion, this study constitutes a first attempt to evaluate the effects of multispecies nematode parasitism on activity patterns in FSG cattle using 3D-accelerometers. The results show that several activity measurements are affected by ongoing GIN infection and demonstrate the potential use of automated behavioural observations as a diagnostic tool. It has also given more insight into the potential welfare impairments caused by GIN infections. The ongoing development and use of sensors on pasture opens up for further understanding of the links between behaviour and disease. Thus, further studies are required to evaluate if such methods could be implemented in the monitoring of grazing livestock.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vpoa.2019.100011.

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