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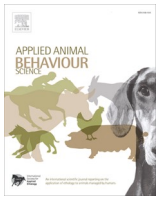

The impact of gastrointestinal parasitism on the behaviour and welfare of weaned housed lambs

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ABSTRACT

Gastrointestinal (GI) parasitism is a health and production concern in sheep, yet its impact on animal welfare remains unclear. The impact of subclinical infections is especially ambiguous as GI parasitism often remains undiagnosed until clinical signs such as diarrhoea are evident. This study applied quantitative and qualitative methods to examine the effects of subclinical *Teladorsagia circumcincta* infection on the behaviour and welfare of 96 Suffolk-cross lambs (24 pens of 4 lambs) weaned at 10 weeks old. The hypothesis that parasitism causes negative affective states was tested. Lambs were divided into three groups at the pen level: ad-lib fed control (AC), restricted-fed control (RC), and ad-lib fed parasitised (AP). Parasitised lambs (AP) were dosed three times weekly with 7000 third stage *T. circumcincta* larvae (L_3) from 16 weeks of age. Lambs in the RC group were pair fed to match AP feed intake to separate the effects of infection-induced anorexia from the potential direct impacts of infection. From 7 days pre-infection to 23 days post-infection, scan and behaviour samples were taken from video recordings to quantitatively monitor behaviour, and animal-based measures such as faecal soiling score (FSS) were recorded as welfare indicators. Lying, standing, eating, play and social behaviour were monitored. Qualitative behaviour assessment (QBA) was conducted weekly using the AWIN (2015) protocol to gain insight into the lambs' affective states over the onset of infection. Parasitised lambs were more likely to stand inactive than AC lambs as the infection progressed ($P=0.006$). They were also less likely to display eating behaviour in the third daily scan sample than RC lambs ($P<0.001$). Principal Component Analysis of the QBA data revealed that the first dimension (PC1) described arousal levels, the second (PC2) described the valence of the animals' affective states, and the third (PC3) described fearfulness and aggression levels. Parasitised lambs (est=10.64, SE=0.33) scored higher than RC lambs (est=9.42, SE=0.33) on PC3, the fearfulness dimension ($P=0.030$). There were no differences between fearfulness scores of AC and AP lambs or RC lambs and treatment group had no significant impact on the distribution of scores on PC1 or PC2. These findings demonstrate that subclinical GI parasitism negatively impacts lamb welfare not only in the health domain but in the behaviour and mental domains as well. This has implications for welfare assessments and early disease detection in lambs. Future research could explore remote monitoring of the indicators of parasitism identified in this study.

1. Introduction

Gastrointestinal (GI) parasitism is a health and production concern in sheep, costing the United Kingdom's sheep industry €47 million annually (Charlier et al., 2020; Coop et al., 1985). This cost may rise in the future as the UK's grazing season is predicted to lengthen due to climate change, increasing the time during which sheep are exposed to GI parasites (Phelan et al., 2016). However, this condition's effects on behaviour can be difficult to monitor on farm and evidence of its impact on animal welfare is sparse. If lambs adjust their behaviour in early infection it may be possible to use these adjustments as early indicators of parasitism. Early indicators are by definition present and identifiable before the symptoms of clinical disease are visible. Subclinical infection is defined as the presence of parasites in the gastrointestinal tract

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without the presence of clinical signs such as diarrhoea (Gunn and Irvine, 2003). Without prompt treatment, subclinical GI parasitism leads to morbidity rather than mortality (Kenyon and Jackson, 2012), extending the duration of its welfare impacts. Behavioural effects include anorexia, and changes in diet selection, grazing and social behaviour (Hutchings et al., 1999, 2000b; Morris et al., 2022). Ewe lambs infected with *Teladorsagia circumcincta* spend less time grazing each day and have a lower feed intake than non-parasitised sheep due to their shorter grazing bouts (Hutchings et al., 2000a). Parasitised lambs have lower activity levels and fewer social interactions (Morris et al., 2022). Lying behaviour increased in parasitised lambs in one indoor study (Hempstead et al., 2023), but decreased in a study on pasture (Högberg et al., 2021). Animal-based indicators are the most appropriate tools to provide insight into the welfare state of animals (EFSA, 2012; Smulders and Algers, 2009). Since behavioural symptoms are often visible before clinical signs, studies of sheep behaviour can provide insight into the animals' experiences of welfare challenges (Gougoulis et al., 2010). Qualitative methods like Qualitative Behaviour Assessment (QBA) can complement these approaches by directly assessing animals' affective states.

The experience of subclinically infected lambs remains unclear despite that when asked to rank sheep welfare concerns, UK stakeholders consistently name parasitism as a top issue (Dwyer et al., 2021; Rioja-Lang et al., 2020). There are few studies using an animal welfare science approach to assess the impacts of GI parasitism. The term "affective state" is used here to describe the subjective experience of an animal caused by bodily events and external stimuli (Panksepp, 2005). This study grounds itself in the Five Domains framework, which uses affective states as a measure of the experiment's overall impact on welfare (Mellor, 2016). The five domains are nutrition, environment, health, behaviour and mental state, and the interaction between them provides a systematic assessment of animal welfare (Mellor et al., 2020). By collecting data on the health, behaviour and mental domains of parasitised lambs, we aim to gather information on lambs' affective states during the early stages of infection. Understanding the welfare costs of GI infection by identifying which domains are impacted could help centre it as a welfare issue, as well as a production issue. There is some evidence of altered affective state in ewes infected with Strongylids: they were scored as more "depressed/suspicious" and "unsettled/apprehensive" than non-parasitised ewes using QBA (Grant et al., 2020). Reliable tools to measure the effect of parasitism on welfare are needed to manage it effectively and address welfare concerns. Infection is ubiquitous, and treatment relies on regular anthelmintic treatments (Morgan and van Dijk, 2012). As resistance to anthelmintic drugs increases, the risk of clinical disease rises (Barger, 1999). By treating only infected sheep, in refugia parasite populations parasites are preserved and the anthelmintics' efficacy is prolonged (Kenyon et al., 2009). Targeted selective treatment is a method of identifying individual infected animals based on production factors such as live weight gain (Kenyon et al., 2009). More tools for early identification of infected sheep are needed to avoid blanket treatments of entire flocks.

The aims of this study were to identify early behavioural indicators of *T.circumcincta* infection through scan and focal sampling, and to explore its impact on lamb welfare through QBA, behavioural change and welfare indicators like faecal soiling score (FSS) and gut fill score (Phythian et al., 2013, 2019). We hypothesised that infected lambs would reduce their activity levels, feeding and social behaviour compared to non-infected lambs. They would have higher FSS and lower gut fill scores. We hypothesised that QBA would capture infected lambs' negative affective states through higher scores on terms like `listless` and `apathetic.`

2. Methods

2.1. Ethical approval

Ethical approval for this study was granted by SRUC's Animal Experiment Committee, as a subset of a larger experimental trial (AE Number: SHE AE 03–2021). Humane end points for parasite infection were set in the ethical approval documentation. These outlined that any lamb showing profuse diarrhoea for more than 24 hours will be given veterinary treatment, including anthelmintic drugs. However, the parasite dose administered was not expected to result in severe clinical disease and no animals reached this endpoint throughout the trial. All work is reported to be fully compliant with the ARRIVE2.0 guidance.

2.2. Animals

Ninety-six Suffolk cross male (48) and female (48) lambs were studied in this experiment. Eighty-four were Suffolk X Texel and the remaining twelve were Suffolk X Blueface Leicester lambs balanced across the three treatment groups described below. All but five of the lambs were twins so the singletons were balanced across treatment groups. They were born within 10 days of each other on the experimental farm and remained with their dams until weaning at 10 weeks of age. All lambs had tails docked and males were castrated. They were housed indoors until the experiment began when they were 4 months of age to ensure they were naïve to GI parasites. Prior to the start of the study, lambs were fed commercial pelleted feed (Tarff Valley Ltd., Castle Douglas, UK). During the study, they were housed in a naturally ventilated shed where 24 pens were made of metal railing in blocks of four, each block being separated by a walkway. Lambs were kept in groups of four according to their treatment in the pens with a space allowance of 1.96 m^2 per lamb. Each pen contained at least four feeders and one drinker, with saw dust bedding. Pens were bedded with fine wood shaving and completely cleaned out every 8 days, with daily fresh bedding added as necessary.

2.3. Experimental design

2.3.1. Treatment groups

There were three experimental treatments with 8 replicates, each consisting of a pen of 4 lambs balanced for live weight. Computer programming (RStudio) was used to allocate lambs to each treatment group. Lambs were initially ranked according to starting trial weight, then grouped together to minimise weight difference between each replicate of 4 lambs per treatment. The treatment groups were ad-libitum fed control (AC), restricted-fed control (RC), and ad-libitum fed parasitised (AP). The latter were orally trickle dosed three times per week (with an interval of 2 or 3 days) with approximately 7000 *T. circumcincta* L_3, a dose known to lead to subclinical infection (Coop et al., 1982; Fox et al., 2018). The AC and RC groups were sham infected with 4 mL of water, following the same protocol as the AP group. The first doses of larvae and sham doses were administered to lambs, pen by pen, on a rolling basis over 6 days. Infection was monitored through faecal egg counts every 10 days from the various days of first infection for each pen using the modified flotation method with a sensitivity of one egg per gram (epg) of faeces (Christie and Jackson, 1982). Feed intake for the ad-lib fed parasitised lambs and ad-lib fed control lambs was measured daily. Feed intake per pen was recorded based on systematic weighing of feed given and leftover feed. The lambs' diet was made up of grass pellets (For Farmers UK Ltd., Bury St Edmunds, UK) consisting of 939 g/kg of dry matter and 122 g/kg DM of crude protein. They were fed once a day between 9 and 10 am. The RC group was restricted-fed to match the feed intake of the parasitised group, on a 3-day rolling average basis, as they developed parasite-induced anorexia. This meant that after the onset of infection, RC lambs were given a restricted diet. This was to control for the

confounding effect of anorexia and allow for the assessment of the true impact of parasitism on behaviour and welfare. The mean daily feed intake of the parasitised group over the previous 3 days was calculated to smooth-out natural fluctuations in daily feed intake and this calculated amount of feed was given to the RC group. Mean feed intake was recalculated for the RC group on a daily basis since both the growth of the lambs and the degree of parasite-induced anorexia impacted the daily feed intake of the parasitised lambs. Once restrictions were in place, RC pens had 5 feeders to minimise fighting. Before the beginning of the trial, the mean body weight of AC lambs was 29.6 kg, while RC lambs weighed 30 kg and AP lambs weighed 29.9 kg on average.

2.3.2. Parasitology

Lambs on-farm (but outwith the present trial) were inoculated with *T. circumcincta* to maintain a supply of fresh parasite larvae for the trial. Faeces were collected daily throughout the week using collection bags, then incubated in stable conditions for at least 10 days before the hatched L_3 larvae were collected using the Baermann technique (Walker and Wilson, 1960). The quality and quantity of larvae collected was visually assessed using microscopy, then the larvae were stored in water at 5 °C until they were about to be used. Prior to use, the concentration of viable larvae was assessed using microscopy and either concentrated or diluted to ensure that 7000 viable L_3 would be given within a 3–5 mL volume of the suspension. The consistency of the larval concentration was checked prior to dosing the trial lambs. Anthelmintic treatment was given to all lambs in the days immediately prior to them being moved into the trial location for a settling-in period, and infected lamb were treated again at the end of the trial.

2.4. Data collection

2.4.1. Video recordings

Data collection occurred over 4 weeks, from day of infection (DOI) −7 pre-infection to 23 post-infection. Twelve cameras were placed on posts above 4 pens of each treatment (16 lambs/ treatment) and connected to a computer running GeoVision surveillance software (GeoVision Inc., Taipei, Taiwan). Each camera clearly captured the entirety of one pen. Video was recorded every day for one hour between 13:00 h and 14:00 h for 28 days. This time slot was selected through observing 48 hours of continuous video footage captured one week prior to the beginning of the experiment and selecting the time of day where video quality was highest and disturbances were minimal. Management and experimental procedures were complete by 1 pm, meaning the lambs were mostly undisturbed, and the natural light in the barn led to good image quality. Video data were downloaded onto a hard drive every other day and uploaded to an institutional server at the end of the experiment. The functioning and placement of the cameras were checked every morning and they were adjusted as needed. The four individual lambs in each pen were identified by a livestock marker paint (Ritchey Livestock ID, Brighton, USA) dot on their shoulders, mid-back, or rump and the fourth lamb was identified by the lack of a marking.

Behavioural sampling from the videos was conducted by a trained observer blind to the lambs' treatment groups using The Observer XT 15 (Tracksys Ltd., Nottingham, UK). The observer had seven years of animal behaviour and welfare research experience and data collection protocols were approved by senior researchers. Three scan samples at 30-minute intervals (minutes 0, 30 and 60 of each video recording) and one 30–minute pen-level continuous focal sample was taken from each daily recording to record social behaviour and play, using the ethogram shown in Table 1. Scan samples were carried out at the individual lamb level while focal samples were conducted at the pen-level.

2.4.2. Qualitative behaviour assessment (QBA)

QBA was carried out on each pen weekly between 11:00 h and 13:00 h, a time chosen to avoid disturbances in the barn. The same observer, blind to the lambs' treatment groups, performed QBA every week. The observation protocol was reviewed and approved by senior researchers. After entering or changing positions in the barn, the observer allowed sufficient time for the animals to settle before beginning the observations. For example, if vigilance behaviour began when the observer took their place, observations did not begin until vigilance behaviour disappeared. Once the animals were judged to have resumed their ongoing behaviour, each pen was observed for 2 minutes, starting with the farthest pen and ending with the nearest. The protocol and list of terms presented in the EU Animal Welfare Indicators (AWIN) project Protocol for Welfare Assessment in Sheep (AWIN, 2015) was used to score the lambs' demeanour using a visual analog scale for every term on a tablet (Xperia S, Sony Europe Ltd., Weybridge, UK). Ninety-six pen--level assessments were carried out over four weeks, with each of the 24 pens being observed 4 times.

Table 1
Ethogram of lamb behaviours collected by scan and focal sampling for penned lambs kept in groups of 4 to determine the effects of parasitism on behaviour, where behaviours without an asterisk (*) were only used in scan sampling and behaviour marked with an asterisk (*) were used in scan and focal sampling.

Behaviour	Definition
Feeding	Lamb has head within 10 cm of the feed or water trough, may be seen biting, chewing or obtaining feed.
Drinking	Lamb has head within 10 cm of the water trough, may be seen to be licking, mouthing the trough or obtaining water from trough.
Locomotion	Lamb moves feet, leading to motion in any direction for more than 2 seconds.
Lying	Lamb's body is touching the ground from shoulder to back end, neck and head touching the ground or upright.
Standing	Lamb remains still in a posture where head is raised above the level of the back, up on all four legs.
Pen Exploration	Lamb nudges, noses or chews any object or structure, other than feed, water, bedding or the brush head.
Locomotor play *	Lamb moves rapidly in any direction for more than 2 seconds with no obvious destination to reach, jumping or pivoting for no obvious reason
Social play *	Lamb puts its head down and runs to butt heads with another lamb, or jumps up onto back legs and rests its front half on the back of another lamb
Social behaviour *	Lamb is in any kind of active physical contact with another lamb, including nudging, nuzzling, or nosing. Excludes passively lying close to another lamb and touching it.
Object play *	Lamb's face is within 5 cm of the brush head, or it interacts with the brush head by sniffing, butting, pawing or jumping on it.
Unclear	Lamb's behaviour is concealed by a visual barrier e.g. feeder or another lamb.

2.4.3. Live weight, faecal sampling and visual scores

All lambs were weighed on day −7, 2, 12 and 21 of parasite infection. Before being moved to the weighing area, faecal samples (approx. 6 g per animal) were collected in the pen following natural expulsion of faecal matter. If a sufficient faecal sample could not be obtained naturally, a direct faecal sample was collected. Lambs were then moved to a holding pen linked to a weigh crate. While in the holding pen, FSS and gut fill scores were assigned to every lamb based on visual inspection. Faecal soiling was scored on the scale from 0 to 4 developed by AWIN (AWIN, 2015), where:

- 0: No faecal soiling, the wool around the breech area and under the tail is clean
- 1: A small quantity of faecal matter in the wool around the anus
- 2: Some soiling around the anus and dags (matted areas of faecal matter adhering to the wool) in this area only
- 3: Soiling and dags extending beyond the anus to the tail and onto the upper part of the legs
- 4: Wider area of soiling with dags extending down the legs as far as the hocks.

To record gut fill, lambs were scored as 2 for bloated, 1 for full or

0 for emaciated, as previously described (Phythian et al., 2013). Lambs were then individually weighed and returned to their home pens.

2.5. Statistical analysis

For all analyses, data were separated into pre-infection (DOI −7 to −1) and post-infection (DOI 0–23). The pre-infection dataset was used to determine the baselines of feed intake, behaviour and mental state, while the post-infection dataset showed the effect of infection on these variables. Unless stated otherwise in the model descriptions, pen number was included as the random effect in the models. Generalised Linear Mixed Models (GLMM) and cumulative linear mixed models (CLMM) were used to analyse feed intake, behaviour, and welfare indicators because of their ability to process repeated measures taken over time from the same individuals and to handle unbalanced designs, as well as the possibility of include random effects. Fixed and random effects were chosen to answer the research questions and account for possible confounding factors. Missing data were included in the data set as blank cells.

Scan and behaviour samples were exported from The Observer XT 15 into Microsoft Excel. All statistical analysis was conducted in R 4.2.2 (R Core Team, 2022) via R Studio (version 3.0). To determine if changes in feed intake took place, a GLMM [glmmTMB package (Brooks et al., 2017)] was utilised using pen as the experimental unit with negative binomial distribution with a quadratic parameterization (nbinom2) link function. Fixed effects included treatment (AC, RC and AP) and day of infection (DOI) as a covariate, as well as the interaction between the two.

Behaviours performed more than 5 % of the time during scan sampling were analysed. To determine the relationships between the binary behaviours (presence/absence (0,1)) performed during scan sampling and the treatment groups, GLMMs [glmmTMB package (Brooks et al., 2017)] were performed with a binomial probability distribution (binomial) where each lamb acted as the experimental unit. Fixed effects included treatment (AC, RC and AP), scan sample (0, 30 or 60 mins) and day of infection (DOI) as a covariate. Interaction terms included 2-way interactions between *DOI * Treatment*, *DOI * scan*, and *scan * Treatment*. Lamb ID nested within pen number was included as a random effect.

Behavioural analysis during focal sampling included comparisons of total durations and frequencies across treatment groups at pen level (4 lambs combined within pen) for each 30-minute focal sample via GLMMs [glmmTMB package (Brooks et al., 2017)]. Social play, locomotory play and object play were combined to form a single play behaviour response variable. The family link function was set to negative binomial distribution with a quadratic parameterization (nbinom2). Fixed effects were DOI and treatment (AC, RC and AP), as well as an interaction (*DOI * Treatment)*. Pen was included as the random effect. Differences in social behaviour and play were compared between the pre-infection and the post-infection period. Negative binomial GLMMs were also used for this analysis where fixed effects included a factor describing the timing of each observation (pre-infection, post-infection) and treatment group, and an interaction term *timing*treatment* was included.

Principal Component Analysis (PCA) (Wold et al., 1987) was used to explore differences in lamb affective state across treatment groups as assessed by QBA. A PCA was run on the scores for the descriptive terms (21 total) across observations and pens using the R package *stats*. A scree plot was produced using the package *factoextra* (Kassambra and Mundt, 2020) and the three dimensions that accounted for the highest levels of variance (more than 10 %) were retained for graphical representation and modelling. The base R function *print* was applied to the resulting PCA to produce a covariance matrix for the 21 terms and the PCA dimensions. This allowed for interpretation of each dimension. The R package *factoextra* (Kassambra and Mundt, 2020) was used to create graphs of the distribution of pens along the dimensions. It was also used to extract the coordinates of each observation along the first three dimensions. This new dataset contained variables called Arousal, Valence and Aggression, which described the placement of each observation along the respective dimensions. For these three variables, GLMMs were used to evaluate whether the lambs' loadings were related to treatment group or day of infection, with Y+10 to account for negative values in the response variable without disrupting variance. The family link function was set to either negative binomial distribution with a quadratic parameterization (nbinom2) or Gaussian distribution, dependent on model fit and overdispersion parameters (Hardin and Hilbe, 2007). Fixed effects included treatment (AC, RC and RP) and DOI as a covariate, as well as the interaction between the two (*DOI * Treatment)*.

A CLMM [ordinal package (Christensen, 2022) and RVAideMemoire (Hervé, 2023)] with the threshold set to flexible was used to determine the relationships between FSS and treatment. Model fitness was verified by log-likelihood test in the *ordinal* package (Christensen, 2022). Fixed effects included treatment (AC, RC and RP) and DOI as a covariate, as well as an interaction between the two (*DOI * Treatment)*. Lamb ID nested within pen number was included as the random effect.

For all GLMMs, model fitness, normality of residuals and homogeneity of variance was graphically confirmed using the DHARMa package (Hartig, 2022). The ANOVA function in the *car* package (Fox and Weisberg, 2018) was used to determine the significance of explanatory variables based on a $p < 0.05$ threshold and to examine differences between fixed effects and interactions. Pairwise comparisons of estimated marginal means (i.e. adjusted or least-squares means) and associated standard errors were derived with the *emmeans* function of the *emmeans* package (Lenth, 2023) with mode set to "mean.class" to obtain the average probability distributions as probabilities of the visual scores and "response" to obtain estimates of the probability distribution in the response scale for each treatment group, with Tukey adjustment of p-values accounting for multiplicity. *Emmeans* (Lenth, 2023) was also used to examine linear trends between fixed effects and covariates. Graphical representations of results were produced using *ggplot2* (Wickham, 2016) with corrected pairwise comparisons with standard error (SE) and 95 % confidence intervals (CIs) reported.

3. Results

3.1. Pre-infection results

There was a significant effect of treatment group on feed intake between DOI −7 and −1 (mean feed intakes: RC=7931±89.2 g, AC=8580 ±96.5 g, AP=8674±97.8 g, $X^2_{(2,238)}$=37.66, P<0.001), when all animals were being fed ad libitum. There were no significant differences in the likelihood of performing lying behaviour (odds ratios: AC=0.478±0.04, RC=0.516±0.04, AP=0.500±0.04, $X^2_{(2,655)}$=0.33, P=0.850), standing behaviour (odds ratios: AC=0.242±0.004, RC=0.208±0.04, AP=0.161 ±0.04, $X^2_{(2,655)}$=1.81, P=0.404), or eating behaviour (odds ratios: AC=0.204±0.03, RC=0.164±0.03, AP=0.208±0.03, $X^2_{(2,655)}$=1.65, P=0.438) across treatment groups. Analysis of focal samples revealed no significant differences in total durations of play (AC=0.5±0.6 s, RC=0.4 ±0.9 s, AP=3.5±3.6 s, $X^2_{(2,54)}$=2.58, P=0.276) or social behaviour (total durations: AC=42.1±15.7 s, RC=30.6±11.4 s, AP=37.4±15.1 s, $X^2_{(2,56)}$=0.36, P=0.836), nor in number of bouts of play (mean bout counts: AC=0.05±0.05, RC=0.25±0.18, AP=0.27±0.19, $X^2_{(2,56)}$=4.18, P=0.124) or social behaviour (mean bout counts: AC=3.10±0.85, RC=2.81±0.78, AP=3.30±0.95, $X^2_{(2,56)}$=0.13, P=0.932) between treatment groups. QBA loadings along the arousal dimension increased for all treatments across the pre-infection period, although there was a significant difference in the rate of that increase between AP and RC lambs (slopes: AC=1.300±0.36, RC=1.847±0.33, AP=0.316± 0.32, $X^2_{(2,29)}$=11.61, P=0.007). There was a significant effect of treatment on FSS in the pre-infection period (mean scores: AC=1.86±0.15, RC=1.99 ±0.16, AP=1.70±0.16, $X^2_{(2,29)}$= 40.24, P<0.001).

3.2. Post infection results

3.2.1. Faecal Egg Counts (FEC)

The parasitised treatment group (AP) was the only group whose FEC rose above zero for the entire study period, and only from DOI 11. That day, AP lambs began showing low FECs of 1.4±0.6epg (mean±SE). On DOI 12, AP lambs had a mean FEC of 3.2±0.7epg. Ten days later, on DOI 21, all 32 AP lambs were shedding eggs, with a mean FEC of 77.2 ±14.7epg, and AC and RC lambs' FEC remained at 0. A Kruskal-Wallis test of FEC on DOI 21, the first day of the patent period of infection when lambs are expected to start shedding parasite eggs, found a significant difference between APlambs and RC and AC lambs ($X^2_{(2)}$=90, P<0.001).

3.2.2. Feed intake

Feed intake increased over time for all three treatment groups as the lambs grew. Mean feed intake during the infection period for AC lambs was 10213±72.9 g, 9585 g±54.0 g for RC lambs and 10059±70.3 g for AP lambs. There was a significant effect of the interaction between DOI and treatment group on feed intake ($X^2_{(2,491)}$=11.53, P=0.003). The increase in feed intake over time for AC lambs was significantly greater than for AP lambs (slopes: AC=0.006±0.001, RC= 0.003±0.001, AP=0.001±0.001, Z_{ratio}=3.39, P=0.002). There was no significant difference in feed intake over DOI between RC and AC (Z_{ratio}=1.85, P=0.155) or RC and AP lambs (Z_{ratio}= −1.52, P=0.281).

3.2.3. Scan samples

Across all treatment groups, the most frequently recorded behaviour was lying (61.6 % of observations), and the least frequently observed was object play (0.01 % of observations). For AC lambs, lying was recorded in 60.6 % of observations while standing and eating accounted for 15.2 % and 16.2 % of observations, respectively. Lambs in the RC group were recorded as lying, standing and eating during 60.8 %, 14.6 % and 17.2 % of observations, respectively. In AP lambs, lying, standing and eating made up 63.6 %, 15.5 % and 15.7 % of observations, respectively. The other behaviours in the ethogram (Table 1) were seen less than 5 % of the time across treatment groups, and therefore were not analysed.

3.2.3.1. Lying behaviour. Scan number had a significant effect on lying behaviour (probabilities: Scan 1=0.48±0.02, Scan 2=0.68±0.02, Scan 3=0.70±0.02, $X^2_{(2,2307)}$=95.92, P<0.001). Lying was less likely to occur during scan 1 than scan 2 (OR=0.42±0.04, Z_{ratio}= −8.11, P<0.001) and scan 3 (OR=0.40±0.04, Z_{ratio}= −8.64, P<0.001) for all treatment groups. There was no significant effect of treatment group on lying behaviour ($X^2_{(2,2307)}$=1.37, P=0.504) and no significant interaction between DOI and treatment group (($X^2_{(2,2307)}$=0.86, P=0.649).

3.2.3.2. Standing behaviour. When modelling standing behaviour, there was a significant interaction between DOI and treatment group (slopes: AC=0.02±0.02, RC=0.06±0.02, AP=0.10±0.02, $X^2_{(2,2307)}$=9.55, P=0.008). As shown in Fig. 1, AP lambs were more likely to be standing as DOI increased than AC lambs (est= −0.08±0.03, Z_{ratio}= −3.06, P=0.006), especially from DOI 14 onwards. The RC lambs' likelihood of standing behaviour did not differ from AC (est= −0.05±0.03, Z_{ratio}= −1.73, P=0.193) or AP lambs (est=0.04±0.04, Z_{ratio}=1.26, P=0.416) (Fig. 1). This means that AP lambs may have reduced their activity levels as infection progressed, if standing is considered an inactive behaviour.

There was a significant interaction between treatment group and scan number for standing behaviour ($X^2_{(4,2307)}$=23.47, P<0.001). Lambs in the AC group showed a significant decrease in likelihood of standing behaviour between scans 1 and 3 (probabilities: Scan 1=0.21±0.03, Scan 3=0.10±0.02, OR=2.26±0.57, Z_{ratio}=3.25, P=0.003), while RC's decreased between scans 1 and 2 (probabilities: Scan 1=0.27±0.03, Scan 2=0.08±0.02, OR=4.21±1.13, Z_{ratio}=5.35, P<0.001) and scans 1 and 3 (probabilities: Scan 1=0.27±0.03, Scan 3=0.07±0.02, OR=4.90 ±1.42, Z_{ratio}=5.48, P<0.001). Lambs in the AP group (probabilities: Scan 1=0.16±0.03, Scan 2=0.10±0.02, Scan 3=0.16±0.03) showed no significant difference in standing behaviour likelihood between scan 1 and scan 2 (OR=1.78±0.49, Z_{ratio}=2.10, P=0.089), scan 1 and scan 3 (OR=1.00±0.25, Z_{ratio}=0.01, P=0.999), or scans 2 and 3 (OR=0.57 ±0.16, Z_{ratio}= −2.08, P=0.09), meaning they were equally likely to be standing across the entire scan sampling period.

3.2.3.3. Eating behaviour. There was a significant interaction between treatment group and scan number for eating behaviour ($X^2_{(4,2307)}$=18.54, P<0.001). As illustrated in Fig. 2, during scan 1 (probabilities: AC=0.22±0.03, RC=0.20±0.03, AP=0.27±0.03) there were no significant differences between AP and AC lambs (OR=0.78 ±0.17, Z_{ratio}= −1.10, P=0.512), nor between AP and RC (OR=1.44 ±0.33, Z_{ratio}=1.62, P=0.239) or AC and RC (OR=1.13±0.26, Z_{ratio}=0.53, P=0.855). During scan 2 (probabilities: AC=0.14±0.02,

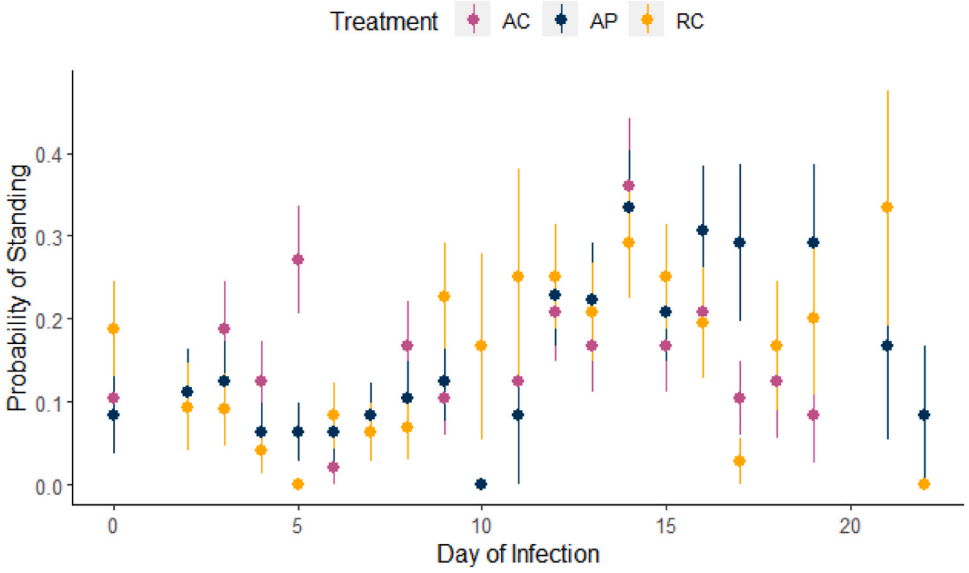

Fig. 1. Mean probability with standard error of lamb standing behaviour by treatment group from day 0 of infection to day 23 of infection, where AC=ad-lib fed control, RC=restricted-fed control and AP=ad-lib fed parasitised.

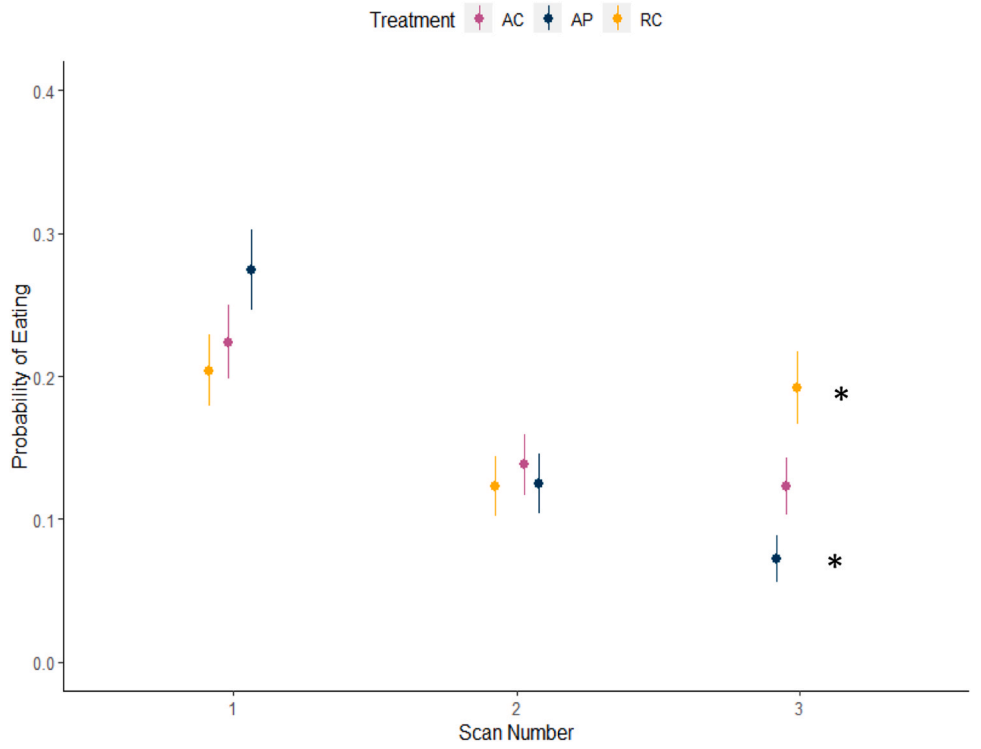

Fig. 2. Mean probability and standard error of lamb eating behaviour across the three daily scan samples by treatment group, where AC=ad-lib fed control, RC=restricted-fed control and AP=ad-lib fed parasitised. Dots with differing star symbols are significantly different from each other.

RC=0.12±0.02, AP=0.12±0.02) there were again no significant differences between AP and AC lambs (OR=1.14±0.31, Z_{ratio}= 0.47, P=0.887), nor between AP and RC (OR=1.01±0.28, Z_{ratio}=0.02, P=0.874) or AC and RC (OR=1.14±0.31, Z_{ratio}=0.50, P=0.874) (Fig. 2). However, during scan 3, AP lambs were significantly less likely than RC lambs to be performing eating behaviour (probabilities: AC=0.12±0.02, RC=0.19±0.03, AP=0.07±0.02, OR=0.32±0.10, Z_{ratio}= −3.74, P<0.001), which is visible in Fig. 2. This result may reflect the expected parasite-induced anorexia in AP lambs.

3.2.4. Focal samples

As a whole, lambs performed social behaviour and play behaviour 295 and 45 times respectively (Table 2). As expected and shown in Table 2, play behaviour occurred less often than social behaviour.

There was a significant interaction between DOI and treatment group when modelling total duration of play ($X^2_{(2,24)}$=6.13, P=0.047). As seen in Fig. 3, play bout duration decreased over time for AC and AP lambs but increased for RC lambs (slopes: AC=-0.13±0.07, RC=0.12±0.08, AP= −0.06±0.08), especially from DOI 14. The difference in play bout duration trend over time between AC and RC lambs was significant (estimate= −0.25±0.10, Z_{ratio}= −2.43, P=0.040) though the differences between AC and AP (estimate= −0.07±0.10, Z_{ratio}= −0.63, P=0.802) and AP and RC were not significant (estimate= −0.19±0.11, Z_{ratio}= −1.70, P=0.207) (Fig. 3).

Contrary to what was hypothesised, total duration of social behaviour was not significantly affected by DOI ($X^2_{(1,80)}$=1.39, P=0.239) or treatment group ($X^2_{(2,80)}$=1.04, P=0.0594). The number of bouts of social behaviour was similarly unaffected by DOI ($X^2_{(1,192)}$=0.28, P=0.600) or treatment group ($X^2_{(2,192)}$=0.54, P=0.762). No statistically significant relationships existed between the number of bouts of play performed by each pen and DOI ($X^2_{(1,192)}$=0.003, P=0.956) or treatment group ($X^2_{(2,192)}$=1.39, P=0.500). When comparing before and after infection, there was a significant decrease in the number of social behaviour bouts after infection for all treatment groups (OR=0.45 ±0.11, Z_{ratio}= −3.41, P<0.001).

Table 2
Total number of bouts, total duration of bouts, and mean duration of bouts for social behaviour and play at the pen level for the infection period across treatment groups.

	Treatment Group	Play	Social Behaviour
Total number of bouts	AC	21	94
	RC	10	81
	AP	14	120
	Total	**45**	**295**
Total duration of bouts (s)	AC	830.6	856.8
	RC	1279.4	1057.6
	AP	328.3	1244.6
	Total	**2438.3**	**3159.0**
Mean ± SE duration of bouts (s)	AC	2.5 ± 1.3	3.0 ± 0.6
	RC	12.5 ± 6.5	6.8 ± 2.0
	AP	3.2 ± 1.1	4.4 ± 0.8
	Mean	**6.0 ± 1.8**	**4.7 ± 1.8**

3.2.5. QBA

The PCA revealed that principal component 1 (PC1) accounted for 36.7 % of the variance, PC2 accounted for 15.1 % of the variance, and PC3 accounted for 12.8 % of the variance. Cumulatively, PC1, PC2 and PC3 accounted for 64.6 % of the variance in the QBA data.

Table 3 was used to interpret the meaning of the PCA dimensions. PC1 seemed to described arousal levels, with terms such as `Calm`, `Relaxed`, and `Subdued` on one end and `Active`, `Vigorous` and `Assertive` on the other (Table 3). PC2 may have described the valence of the animals' affective states, running from `Agitated`, `Apathetic` and `Physically Uncomfortable` to `Content` and `Bright` (Table 3). PC3 suggested it may reflect the spectrum of fear and aggression, running from `Sociable` and `Aggressive` to `Alert`, `Fearful`, and `Tense` (Table 3).

Treatment group had no significant impact on the distribution of pens along PC1 ($X^2_{(2,65)}$=0.09, P=0.956) or PC2 ($X^2_{(2,65)}$=1.13, P=0.569)

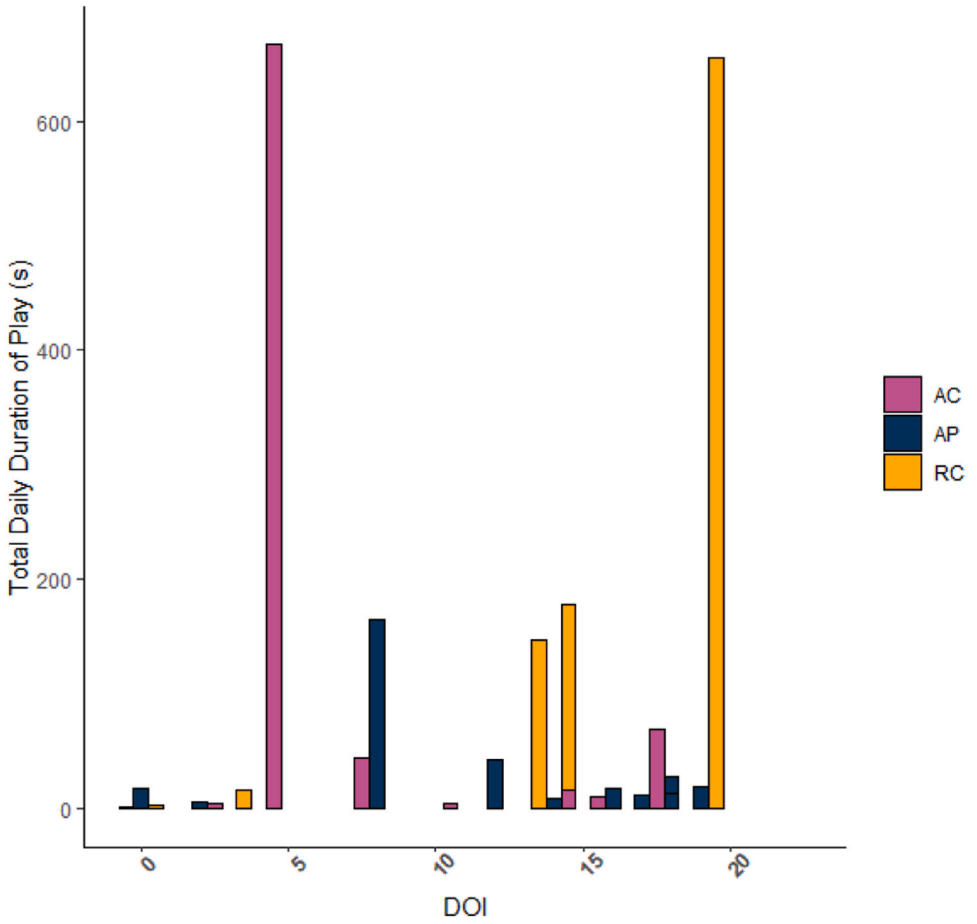

Fig. 3. Total daily duration of play behaviour in seconds every day of infection (DOI) for the three treatment groups, where AC=ad-lib fed control, AP=parasitised and RC=restricted-fed control lambs.

Table 3
Matrix of the 21 QBA terms for pen-level observations. Cells with a single border show the two terms with the highest positive values and cells with a double border show the two lowest negative values.

Term	PC1	PC2	PC3
Alert	-0.1207	-0.2635	-0.2807
Active	-0.2933	-0.0123	0.2037
Relaxed	0.2728	-0.2555	-0.0638
Fearful	-0.1132	0.1860	-0.4681
Content	0.1555	-0.4150	-0.0330
Agitated	-0.1220	0.3182	-0.0968
Sociable	-0.2061	-0.0754	0.2383
Aggressive	-0.1940	0.1129	0.2099
Vigorous	-0.3153	-0.0598	0.1383
Subdued	0.2837	0.2012	0.1028
Physically uncomfortable	0.0742	0.2876	-0.0218
Defensive	-0.1671	0.0629	0.1963
Calm	0.3206	-0.1458	-0.1049
Frustrated	-0.1108	0.2482	-0.0460
Apathetic	0.2560	0.2836	0.1723
Wary	-0.0963	0.0887	-0.4704
Tense	-0.1427	0.2567	-0.3883
Bright	-0.2525	-0.3055	-0.0996
Inquisitive	-0.2703	0.0189	0.0482
Assertive	-0.3010	0.0106	0.1375
Listless	0.2038	0.2766	0.1735

over the infection period of the experiment, contrary to the hypothesis that parasitism would impact AP lambs' PC2 or valence loadings. However, loadings along PC3, the dimension describing aggression and fear, were different across treatment groups (mean loadings: AC=9.92 ±0.33, RC=10.62±0.33, AP=9.42±0.33, $X^2_{(2,65)}$=6.89, P=0.0.32) (Fig. 4a). Lambs in the AP group had significantly lower PC3 loadings than RC lambs (estimate= −1.20±0.46, Z_{ratio}= −2.59, P=0.032), meaning they were behaving more fearfully than RC lambs (Fig. 4b). This is highlighted in Fig. 4b by the difference along the Y axis between the placement of the blue (AP) and yellow (RC) ellipses. The AC's PC3 loadings were not significantly different from either RC (estimate= −0.70±0.47, Z_{ratio}= −1.50, P=0.297) or AP (estimate=0.50±0.46, Z_{ratio}=1.09, P=525) (Fig. 4b).

3.2.6. *Visual scores*

3.2.6.1. Gut fill. All lambs scored a gut fill of 1 (normal fill) at every sampling day throughout the study, so no analysis of the score's relationship with parasitism could be conducted and the gut fill hypothesis could not be tested.

3.2.6.2. Faecal soiling scores (FSS). For all treatment groups during the infection period, FSS 1 was most often recorded, and FSS 4 was only recorded 5 times. The AC group had a median FSS of 3 (IQR=2), RC lambs' median FSS was 2 (IQR=1) and AP lambs' median FSS was 2 (IQR=1). FSS increased over time across all treatment groups ($X^2_{(3,90)}$=36.34, P<0.001) but there was no significant effect of treatment group on FSS ($X^2_{(2,90)}$=3.84, P=0.147), leading us to reject the hypothesis that parasitised lambs would have higher scores.

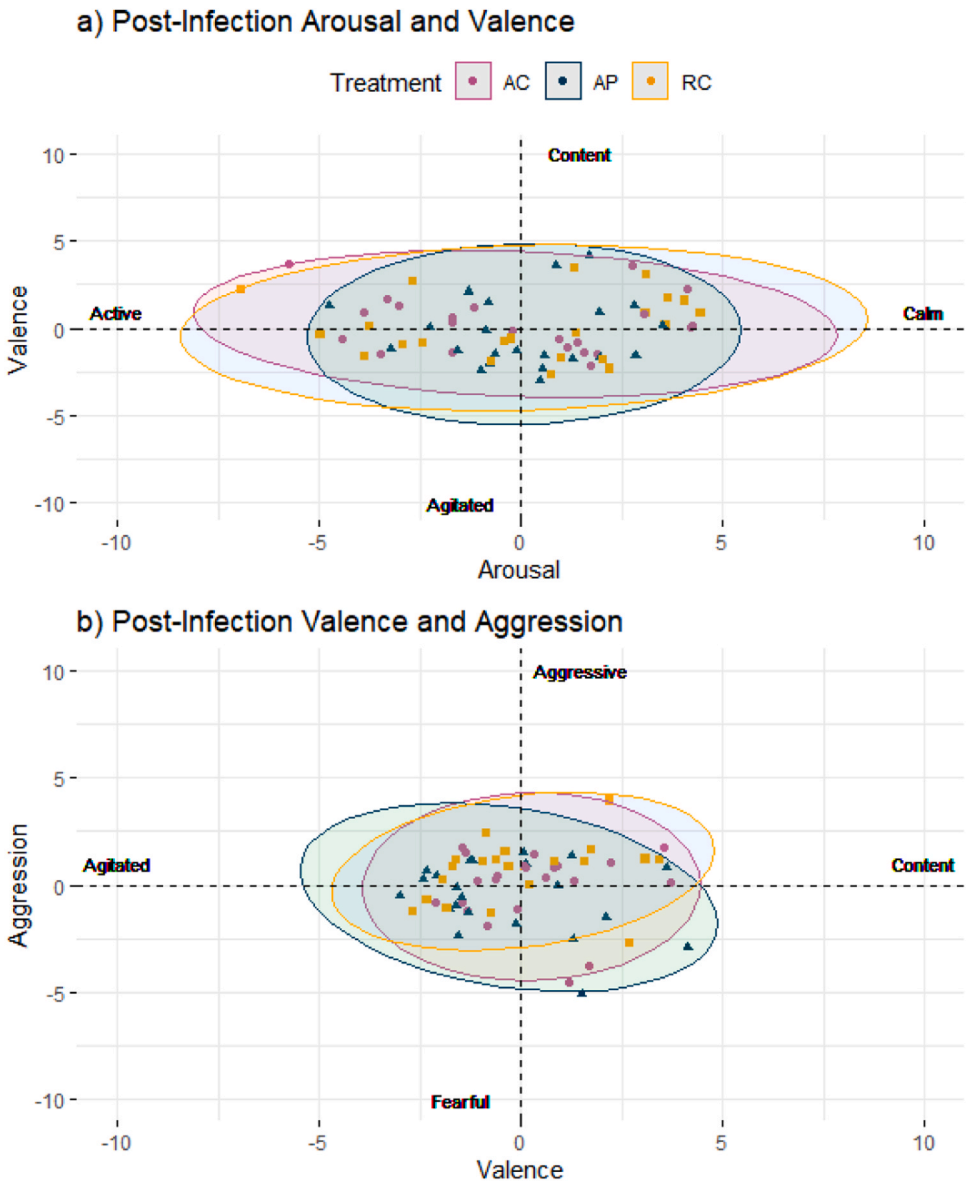

Fig. 4. Plots of pens over the infection period with a) PC1 (Arousal) on the x axis and PC2 (Valence) on the y axis and b) PC2 (Valence) on the x axis and PC3 (Aggression) on the y axis. Terms at both ends of the axes are anchors for the principal components. AC=ad-lib fed control, AP=parasitised and RC=restricted-fed control lambs.

4. Discussion

This study aimed to identify early indicators of GI parasitism and to understand its welfare impact on lambs. Subclinically parasitised lambs were more likely to stand and less likely to display eating behaviour than unparasitised lambs. QBA found that they scored lower on the dimension describing aggressivity than non-parasitised lambs.

T.circumcincta egg counts were low, as the study period extended 23 days after infection, capturing the prepatent phase of infection and beginning of the patent phase. Egg shedding begins in the patent phase, 15–21 days after infection (Roeber et al., 2013; Wood et al., 1995). As this study intended to identify early indicators of parasitism, this focus on early infection was justified. However, further longitudinal research on the patent phase is necessary to complete the understanding of behaviour changes throughout infection. Lambs infected with *T. circumcincta* in a previous study had a lower motion index, step count and fewer lying bouts than control lambs in the prepatent phase (Morris et al., 2022). This finding is similar to the present study's behavioural findings, despite the fact that Morris et al. (2022) studied lambs outdoors on pasture and used accelerometers to collect behavioural data.

AP lambs had a smaller increase in feed intake over time than AC lambs. The RC lambs had a lower mean intake than AP lambs, especially pre-infection and in the first 5 days of infection. The reason behind this lower intake is unknown. The purpose of the RC group was to separate behavioural and welfare impacts of hunger from those of parasite infection. This separation was rendered impossible by the RC lambs seemingly eating to satiation despite their restriction. The change in feed intake over time in AP lambs was significantly different from the pattern in AC lambs, and likely reflects the onset of parasite-induced anorexia. This reduction in feed intake has been reported in modelled subclinical *T.circumcincta* infection of lambs of the same age as the ones studied here (Laurenson et al., 2011). Some differences in lying, standing and eating behaviour across the three scan samples likely reflect the lambs' daily routine; they were fed between 9:00 h and 11:00 h every morning, and scans 1, 2 and 3 occurred at 13:00 h, 13:30 h and 14:00 h, respectively. Scan 1 was closest to feed time and more pellets likely remained in feed boxes than during later scans. The decreased likelihood of lying during

scan 1 may reflect the increased likelihood that lambs were standing and eating.

Behaviour categories were mutually exclusive in this study's ethogram, therefore standing can be considered inactive behaviour. These results reflect previous findings where activity in many species was reduced during a health challenge (Gauly et al., 2007; Ghai et al., 2015; Hart, 1988; Morris et al., 2022). Although the exact reason for increased standing of AP lambs cannot be confirmed, it is possibly due to abdominal discomfort caused by abomasal damage inflicted by parasite larvae. *T.circumcincta* larval stages cause most pathogenic effects, as opposed to its adult stages (Roeber et al., 2013). Larvae development creates nodules in the abomasal mucosa and causes considerable damage to parietal cells, which increases the abomasum pH (Anderson et al., 1985; McKellar, 1993). Standing immobile has been reported as a reaction to castration pain in lambs since it avoids or reduces stimulation of the hyperalgesic tissue (Molony et al., 1993; Molony and Kent, 1997). It is possible that parasitised lambs were more likely to stand immobile to avoid stimulating their damaged abomasal tissue. This result leads us to accept our hypothesis that parasitised lambs reduced their activity levels compared to uninfected lambs.

Probably due to parasite-induced anorexia, the likelihood of observing eating behaviour remained low after scan 1 for AP lambs, whereas control lambs were just as likely to be eating during other scans. This reflects previous studies' findings where reduced feeding bouts in parasitised ruminants in varied experimental environments with varying levels and types of infection were reported (Fox et al., 2013; Hutchings et al., 2000b, 2002). Sheep have the ability to make complex grazing decisions to reduce parasite ingestion on pasture (Bricarello et al., 2023; Hutchings et al., 1999) but the experimental environment of this study did not allow for changes in feeding strategy. Based on the results, we accepted the hypothesis that parasitised lambs reduced their feeding activity.

Reduced play and socialising are components of sickness behaviour in many mammalian species (Dantzer and Kelley, 2007; Hart and Hart, 2019; Johnson, 2002; Weary et al., 2009). One study reported that when parasitised with *T.circumcincta* on pasture, social contacts between parasitised lambs were reduced compared to those between non-infected lambs (Morris, 2022). Contrary to these previous findings, the reduction in social behaviour after infection was seen across all treatment groups in this study. We rejected our hypothesis that parasitised lambs would reduce their social behaviour. Interactions between lambs could have decreased over time as the lambs aged and became accustomed to their surroundings. Social interactions are subject to breed differences, with English lowland breeds and Scottish hill breeds such as the ones in this trial being some of the least gregarious in outdoor settings (Dwyer and Lawrence, 1999). Further research in different breeds with focal observations of young lambs could shed more light on the dynamics of play and social behaviour during parasite infection. Play is influenced by the environment (Berger, 1979) but the pens used here were relatively bare, so space for play and social interaction may have acted as a limiting factor (Berger, 1979). That RC lambs' play bout duration increased over time post-infection could be because they were a particularly playful or aggressive group of lambs, as shown through their non-significantly higher aggression loadings in QBA pre-infection. It was not possible to differentiate between antagonistic and playful bouts of head-butting and jumping during observations, so it is unclear if the RC lambs were truly more aggressive, or if they were simply more playful.

The PCA's PC3 described a spectrum of behaviour from freezing alert to antagonistic social interactions. Post-infection, AP lambs' behaviour was characterised by this alert freezing response, differing from RC lambs who had higher loadings on the aggression side of the axis. This reflects non-significant results in the pre-infection period where RC lambs had higher aggression loadings than AP lambs. It is possible that sick prey animals would increase their vigilance behaviour, as they are more vulnerable to predators. Lambs experiencing pain showed more vigilant behaviour in the presence of predators (Young, 2006). On pasture, observers scored inappetent sheep as more `reluctant`, `tense` and `wary` than control sheep, although the reason for their inappetence was not reported (Grant et al., 2018). These findings suggest that qualitative assessments of behavioural expression could contribute to identifying GI parasitism in sheep. This leads us to accept the hypothesis that parasitised lambs experienced a negative mental state.

The gut fill score may have been too crude to account for minor differences between lambs, and could only detect significant welfare impacts. This score had been useful as part of a wider welfare assessment index due to its good inter-observer agreement (Phythian et al., 2013). Rumen fill is often used in cattle studies but rarely appears in sheep trials (Zufferey et al., 2021). Its use did not lead to any analysis or conclusions in this study, therefore we must reject the hypothesis that parasitism causes lower gut fill scores. In this experiment, FSS was not associated with FEC. In one study, FSS had a low positive phenotypic correlation with FEC, although the FSS scale used was not described in detail (Bisset et al., 1992). Contrarily, Morris et al. (2000), (2005) found an increased FSS in their low FEC line of Romney sheep. Other studies found low genetic correlations between FEC and FSS in Merino sheep. FSS was an indicator of scouring, but it was different from FEC as an indicator of infection (Pollott et al., 2004). This reflects our FSS findings, leading us to reject our hypothesis that parasitised lambs would have higher FSS.

The GLMMs used to analyse behavioural data met the assumption of linear residuals, but the dispersion of the residuals was not entirely homogenous. This is likely due to sources of variation that were unaccounted for during data collection. This limitation was considered when interpreting the results of the models. Further work using models that account for nonlinear patterns of behaviour over time could help address this.

These findings could be applied to on-farm monitoring early behavioural indicators of parasitism, such as lambs standing immobile. Digital technologies like accelerometers could monitor this type of behavioural change remotely, while video cameras and machine-learning algorithms have the potential to detect immobile lambs in a barn. These tools could support farmers in early identification of infected animals and encourage prompt, individual treatment. The finding that parasitism may lead to negative mental states through increased fear is important if lamb welfare is to be improved. As parasitism is ubiquitous in grazing sheep, the implications of poor welfare in infected animals are wide-reaching.

5. Conclusion

Early indicators of disease are crucial to encouraging prompt treatment of health issues in extensively farmed sheep and lessening their impact on animal welfare. We demonstrate that subclinically parasitised lambs increased standing behaviour and decreased eating behaviour over time compared to non-parasitised lambs. These changes have the potential to act as early indicators of GI parasite infection. If behaviour can be monitored remotely by digital technology in extensively farmed sheep, infection could be detected early and at the individual level without gathering the flock. The QBA results suggest that parasitised lambs experienced more negative affective states linked to fear and anxiety compared to non-parasitised lambs. This finding contributes to the small body of evidence that GI parasitism, even at a subclinical level, negatively impacts lamb welfare not only in the health domain but in the behaviour and mental domains as well. Future research into tools to monitor early behavioural indicators such as accelerometers could help improve lamb welfare and encourage prompt and individual treatment, which could contribute to fighting anthelmintic resistant. Repeating similar studies in extensive conditions and with different sheep breeds could help apply the findings to the variety of commercial sheep farming conditions.

CRediT authorship contribution statement

Fiona Kenyon: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Jessica E Martin:** Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing. **Emma M Baxter:** Conceptualization, Methodology, Visualization, Writing – review & editing. **Cathy M Dwyer:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **Michelle C Reeves:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Naomi Booth:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Naomi J Fox:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **Jo Donbavand:** Investigation, Methodology, Writing – review & editing. **Mhairi Jack:** Conceptualization, Investigation, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2024.106323.

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