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Early signals of parasitism expressed through behaviour but modulated by social context

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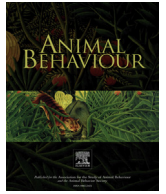
Early signals of parasitism expressed through behaviour but modulated by social context

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Sickness behaviours are believed to be an adaptive response to infection. However, the degree to which these behaviours can be expressed may be impacted by an individual's social environment. Here we tested, first, whether parasitism reduces the activity behaviour of lambs, *Ovis aries*, second, whether this occurs prior to other observed costs of parasitism and, third, whether the infection status of other individuals affects the degree to which these behaviours are expressed. Sixty lambs were separated into replicate groups within three treatments: (1) parasitized: all lambs were infected with the parasitic nematode *Teladorsagia circumcincta*; (2) nonparasitized; all lambs were dosed with water; (3) mixed: some of the group were infected and some were dosed with water. Activity behaviour was monitored before, during and after parasite infection. Parasitized groups had reduced activity levels following infection, and this occurred before any other impact or measure of parasitism was detected. Infected animals in the mixed groups had reduced activity levels following infection, but the level of change was less than that in animals in the fully parasitized groups. Activity levels remained low until lambs were treated with anthelmintic when activity levels of the groups that had been parasitized returned to the same level as nonparasitized groups. These findings show that parasite-induced behavioural changes occur earlier than other more commonly observed signals of infection, but the infection profile of an individual's group can shape these behavioural responses to infection.

Parasites are ubiquitous in the environment and can have a major impact on the health of both wild and domesticated animal populations (Charlier et al., 2014; Hudson et al., 2006; Lafferty et al., 2006; Marcogliese, 2004; Poulin, 1999). Infection can induce inflammatory immune responses which in turn can lead to sickness behaviours such as reduced feed intake, reduced activity levels and changes in social behaviour (Ayres & Schneider, 2009; Bilbo et al., 2002; Dantzer, 2004; Hart, 1988; Kelley et al., 2003; Lopes et al., 2012; Moore, 2002). It has been hypothesized that these sickness behaviours may be an adaptive response by the host to reallocate energy resources to fight off infection (Hart, 1988; Hutchings et al., 1998). However, focusing resources to fight infection could remove resources away from other important activities, such as reproductive success (Bilbo et al., 2002; Owen-Ashley & Wingfield, 2006), protection of offspring (Aubert et al., 1997; Weil et al., 2006), territorial defence (Friedman et al., 1996) and maintenance of social status (Cohn & de Sá-Rocha, 2006; Lopes et al., 2012). Therefore, animals may be expected to adjust the expression of sickness behaviours across different environments (Lopes et al., 2012). This includes an animal's social environment where the consequences of sickness behaviours may affect competition with their conspecifics for resources (Hamilton and Zuk, 1982; Huzzey et al., 2006), or social cohesion, as healthy animals might actively avoid sick individuals (Behringer et al., 2006; Kiesecker et al., 1999; Tobler & Schlupp, 2008). For this reason, it is expected that social animals that benefit from being part of a group may alter the extent to which they demonstrate any signs of vulnerability by masking sickness behaviours under certain social conditions (Weary et al., 2009).

With the development of recent technology that enables the continuous and simultaneous remote monitoring of animal behaviour, it is now possible to identify these subtle differences in the behaviour changes of infected animals. As such, there has been a rise in studies that

have used remote monitoring technology to identify behaviour change in animals that can be associated with parasite infection. For example, proximity loggers have shown interaction rates between Tasmanian devils, *Sarcophilus harrisii*, with facial tumours decreased as tumour load increased (Hamilton et al., 2020) and that TB test-positive badgers, *Meles meles*, were socially isolated from their own groups (Weber et al., 2013). Accelerometers and activity loggers have shown sheep, *Ovis aries*, treated with anthelmintics to remove any naturally occurring parasites had higher activity levels than their untreated counterparts (Burgunder et al., 2018; Högberg et al., 2021; Ikurior et al., 2020). Randomized experimental trials of infection have also detected similar patterns confirming such changes in activity levels may be related directly to parasitism. For example, video image analysis could detect altered movements of pigs, *Sus scrofa*, experimentally infected with African swine fever virus (Martínez-Avilés et al., 2017), and the use of accelerometers demonstrated cows, *Bos taurus*, experimentally infected with the roundworm *Ostertagia* had reduced step rate and increased frequency of lying bouts (Högberg et al., 2019). Using experimental infections in the same range as subclinical natural infections removes the possibility that naturally infected individuals may be a biased subset of the population. For example, individuals that are naturally more active could be exposed to higher levels of infection while feeding. Experimental infection minimizes these potential confounding factors that could be explaining changes in behaviour. Furthermore, experimental infection allows the study of the parasitism from the moment that individuals are dosed and can follow the development of the infection, allowing the exact timing of any behavioural changes to be established.

Experimental studies also show there is potential to use behaviour change to identify early signs of parasitism in animal populations. However, in both natural and agricultural systems, groups are made up of individual members whose behaviour can impact the dynamics of the whole group. Furthermore, parasitism is also often overdispersed within groups, meaning not all individuals will be of the same infection status within a socially interacting group (Woolhouse et al., 1997). While there is evidence that parasitism can affect activity, it is unknown how an individual's group can affect their behavioural response to parasitism, and how an individual within a group can be affected by the parasitic status of its group members. These effects of parasitism have the potential to impact both parasitized and nonparasitized members in positive and negative ways (Granroth-Wilding et al., 2015). This in turn may affect the ability to use remote sensing to provide early identification of parasitized animals.

Understanding how animals balance the costs and benefits of sickness behaviours across different social environments will aid in our understanding of both the evolutionary and ecological impact of disease on animal populations and the impact of social structure and demography on infection and disease. There are also direct applications in using behaviour as a noninvasive tool to identify and treat only infected individuals in domesticated systems (Kenyon et al., 2009). Such methods may be beneficial in slowing resistance by reducing the use of drugs to control parasitism (Van Wyk, 2001; Vercruysse & Claerebout, 2001). Identification of infected individuals is usually based on a biological indicator of infection, such as faecal egg counts, body condition score and reduced weight gains (Kenyon et al., 2009; Stafford et al., 2009; Van Wyk, et al., 2002). However, these occur late in infections when there has already been a loss in production and a reduction in welfare of the animals (Leathwick et al., 2006). Moreover, as behavioural changes are thought to be one of the most valuable ways to detect disease at the earliest stages (Weary et al., 2009), using behaviour change as an early signal of infection would be a useful tool across different areas of research, monitoring and practical application.

Here we investigated the effect of parasite infection on the behaviour of a highly gregarious social species and the effect an individual's social group can have on their behavioural response to infection. We used a group of domesticated sheep, experimentally infected with the gastrointestinal nematode *Teladorsagia circumcincta*, a common parasite of both economic and welfare importance (Papadopoulos et al., 2012). Specifically, we asked: (1) does experimental infection lead to a change in activity levels; (2) are these effects detectable prior to detectable physiological costs or observable measures of parasitism; and (3) are these behaviours affected by the infection status of group members through social modulation?

METHODS

Ethical Note

The experiment was carried out in accordance with the U.K.'s regulation of animal use in science and approved by SRUC'S Animal Ethics Committee (approval number SHE AE 12-2019).

Lambs were given an experimental trickle dose infection of the parasite *T. circumcincta* which represented a subclinical natural infection of domestic sheep. This allowed the investigation of behavioural change in sheep in relation to the stage of the parasite infection, which has not been accomplishable by previous studies. Throughout the study the lambs were checked daily by experienced animal technicians or the named animal care and welfare officer to ensure effects of the infection remained subclinical and to confirm the health and welfare of the lambs were not compromised. All experimental work and procedures (blood sampling, faecal sampling, parasite dosing) were carried out under Home Office licence with the approval of the SRUC's animal ethics committee. Experimental procedures were carried out quickly with minimal handling to reduce stress and discomfort to the lambs. Animals recovered from blood sampling very quickly, displaying normal sheep behaviour immediately after release. They were monitored for at least 30 min after blood sampling and showed no signs of discomfort or stress afterward. Daily monitoring of the lambs ensured there were no postprocedure impacts. All remote monitoring devices were validated prior to the study (Morris, 2022) for accuracy of the behaviour data and to ensure the attachment method did not cause any skin abrasions or impact the lamb's behaviour.

Animals and Experimental Design

Sixty 12-week-old Texel x Bluefaced Leicester lambs were selected randomly from a commercial flock of sheep that had been reared indoors since birth, under conditions that excluded nematode infection and so were considered parasite naïve. The lambs were divided into one of three treatment groups with four replicate groups of five lambs within each treatment. These were: (1) parasitized: all lambs were infected with the parasitic nematode *T. circumcincta* and were of the same parasitic status; (2) nonparasitized: all lambs were dosed with water, remained parasite naïve and were of the same parasitic status; and (3) mixed: a group containing animals of mixed parasitic status, three animals being dosed with water and two with *T. circumcincta* larvae. Each replicate group was standardized for sex (three females and two males per group) and weight (mean liveweight ± SD 27.6 ± 0.13 kg). Given the small number of replicate groups, it was decided not to randomize the animals that were infected in the mixed group, but to have a structured approach and infect the smallest female and largest male in all groups. This approach was chosen to account for any potential effect of sex and weight, and so reduce the residual variation and thus increasing the power to detect the effect of parasitism in these groups. To ensure all animals within each group had similar social experiences with conspecifics no siblings were allocated to the same group. One week before the experiment start

date groups were put onto set stock pasture in individual plots laid out in a six by two grid, with each plot measuring 30 × 30 m and separated by sheep netting. All plots had been free from grazing ruminants for the previous 3 years and animals were given ad libitum access to water. To control for any effect the plot could have on the behaviour of the lambs, groups were rotated clockwise to a new grazing plot twice weekly, so each plot had animals from each treatment group for the same amount of time.

The experiment was conducted in summer 2019. The experimental timetable (a total of 9 weeks) was divided into four phases: preparasite (week 1), a period when all lambs would be kept parasite naïve; prepatent (weeks 2−4), a period when lambs identified for infection would be parasitized but not yet showing any pathological physiological effects of parasitism and not yet shedding eggs; patent-parasite (weeks 5−7), a period when lambs would show physiological responses to infection and shed eggs in their faeces; postparasite (weeks 8−9), a period when all lambs would be dosed with anthelmintic and considered parasite free. On the first day of week 2, lambs identified for infection, which included all lambs in the parasitized groups and two out of five lambs in the mixed groups, received an oral dose of 5000 L3 stage *T. circumcincta* larvae; lambs identified to remain noninfected were handled in the same way and received a dose of water. All lambs were then trickle dosed with either water or *T. circumcincta* larvae three times per week for 6 weeks. The trickle infection chosen (5000 L3/day) would ensure a subclinical infection would be established in the lambs and also represented a level similar to that encountered by sheep naturally when grazing on contaminated pastures (Coop et al., 1982; Wood et al., 1995). On the first day of week 8 all lambs were treated with anthelmintic (Albendazole, 1 ml/10 kg) and infections were cleared. The experiment was designed to operate within the life cycle of the parasite so that natural parasite exposure that could arise from eggs shedding from our experimentally infected individuals was not an issue. At the end of the study all lambs were returned to a commercial flock.

Activity Behaviour

Activity behaviour of lambs in all groups was continuously and simultaneously recorded 24 h per day, using IceRobotics IceQube activity monitors (IceRobotics Ltd, Edinburgh, U.K.). One week prior to the start date of the experiment, an activity monitor was fitted to the rear ankle of each lamb; this was activated on day 1 of the experiment. The IceQubes use a three-axis accelerometer to continuously capture highly detailed information on the animal's movement behaviour and store the data in 15 min increments of time. The IceQubes recorded four activity behaviours including step count (the number of times the lamb lifts its leg), motion index (a broader measurement of the animal's activity which is related to the total amount of energy used by the lamb), lying time (the period when the sensor is horizontal) and lying bouts (the number of times the sensor changes from vertical to horizontal and back to vertical).

Data from each IceQube were downloaded twice weekly. During this time IceQubes were rotated between social groups to reduce the effect of interlogger variation. Activity data recorded while lambs were being handled during the experiment were excluded from any analysis.

Animal Measurements

On the first day of each week rectal faecal samples were taken from all 60 lambs within their plots to estimate the number of

nematode eggs per gram of faeces using a modified salt-flotation method (see below; Jackson, 1974). Lambs were weighed to measure weekly weight gain. Blood samples were taken by jugular venepuncture at the start of weeks 1, 7 and 9 (one measurement during preparasite, patent-parasite and postparasite phases) to measure serum pepsinogen level (an indication of parasite-induced gut damage) using a sheep pepsinogen ELISA assay kit (BlueGene Biotech, Shanghai, China). The blood samples were spun within 2 h of collection at 3660 rpm at 4 °C for 15 min; the serum was removed and stored at −20 °C. At the end of the experiment, a faecal sample and weight measurement were taken from every animal, to assess the final weights and parasite load of the lambs.

Faecal Egg Counts

One day after sample collection, 1 g of faeces was weighed out and placed in a fresh bag with 10 ml of water and emulsified. The sample was taken and dispensed through a 1 mm sieve into a beaker, with the retentate washed into the beaker with an additional 5 ml of water. The retentate was transferred to a 15 ml cellulose acetate tube and centrifuged at 1000 rpm for 2 min. The supernatant was removed using a vacuum line, and the faecal pellet was suspended in 10 ml saturated sodium chloride solution and centrifuged at 1000 rpm for 2 min. Artery forceps were used to clamp off the tube just below the meniscus and the fluid in the upper chamber was poured into a cuvette. One millilitre of NaCl solution was used to rinse the upper chamber of the tube and added to the cuvette. The cuvette was inverted to homogenize the eggs, filled to the top with NaCl and sealed with a cuvette cap. The cuvette was filled with NaCl solution and nematode eggs were counted to a precision of 1 egg/g.

Statistical Analysis

All analyses were performed in R version 4.0.3 (RStudio Team, 2020). Activity models were fitted using the package 'glmmTMB' (Brooks et al., 2017) and animal measurement models (weight and pepsinogen) were fitted with the packages 'lme4' and 'lmerTest' (Bates et al., 2014). Final model formulae and definitions of fixed and random effects are listed in Appendix Tables A1 and A2.

All activity data were aggregated on an hourly level. Using generalized linear mixed models with the REML algorithm, the impact of parasitism on activity (motion index, step count, frequency of lying bouts, lying time) throughout the trial, was assessed by analysing a phase effect (preparasite, prepatent, patent-parasite and postparasite phases) on the activity levels of the three treatment groups (nonparasitized, parasitized and mixed), and between animals in the mixed and single-state groups. Data were also analysed for an effect of week to account for differences in time periods between the phases and to give greater resolution within phase periods. We fitted Animal ID nested within Group ID, IceQube ID and Plot as random effects in all models for motion index, lying bouts and lying time. IceQube ID was initially fitted as a random effect for step count models, but we found one IceQube tag was more sensitive at recording step count than all others throughout the experiment (Appendix Fig. A1); thus, IceQube ID was included as a fixed effect in all step count models rather than a random effect to explain the variance caused by this tag rather than control for it. Other fixed effects considered for the models were: Treatment group, Phase (preparasite, prepatent, postpatent, postparasite), Week, Parasitic status (infected or noninfected), Group type (mixed-parasitic state groups or single-parasitic state groups) and Sex. To avoid confounding, Phase and Week were not fitted in the same model. The best fit model was selected using a backward elimination process using Akaike's

information criterion (AIC; Akaike, 1974) as the comparison criterion between models. Where two models had an AIC within 2 of each other we chose the simplest model. AIC does not equate directly to a *P* value; however, this approach results in a model that is most parsimonious. Statistical significance was calculated for coefficients by the software once the optimum model had been selected by AIC. Coefficients described as being significant are statistically significant, where the calculated *P* value was less than 0.05 throughout.

Before models were run, the mean−variance relationship was assessed to verify the model structure and to ensure the appropriate distribution was used for each response variable. For step count and motion index we used mixed models fitted with negative binomial-distributed errors (Appendix Fig. A2a, b) and for lying bouts we used mixed models fitted with Poisson-distributed errors (Appendix Fig. A2c). As lying data had a U-shape distribution, they were converted to fit a binomial distribution (1 = lambs were lying ≥ 1800 s/h and 0 = lambs were lying < 1800 s/h) and analysed using mixed models with a binomial-distributed error. We found abnormally large data spikes at precisely 15, 30 and 45 min during each hour within the lying time data, owing to a technical malfunction of the equipment, so these data points were not included in the analysis. As lambs were likely to spend more time lying during the night, models for lying time were run separately for day and night.

Mixed-effect models were used to assess the impact of parasitism on the weight of the lambs fitted with a Gaussian-distributed error (Appendix Fig. A2d) and compared the liveweights between lambs in the mixed and single-state groups by analysing data containing animals that were exposed to the same treatment. We also used mixed-effects models with a Poisson-distributed error (Appendix Fig. A2e) to assess the impact of parasitism on blood serum pepsinogen levels as a measure of parasite-induced physiological gut damage.

In all models the referent treatment group was the non-parasitized treatment group, and the referent time point was the preparasite phase (week 1). The main effect of treatment reported for the models is therefore the difference in treatment groups in week 1, i.e. prior to being infected with parasites. We therefore did not expect a significant effect of treatment as a main effect. Similarly, the main effect of time is to describe the trajectory of non-parasitized animals over the course of the experiment. Conversely, we would expect this to be significant as it describes changes as the animals mature. These results are not discussed but are available in the Appendix. The effect of interest in these models is therefore the interaction between treatment group and time, and parasitic status, group type and time, as this describes how differences between treatment groups and between infected individuals in mixed and single-state groups develop over time. We restrict the results below to a discussion of these interactions.

RESULTS

Measures of Infection and Associated Physiological Costs

When lambs were put onto pasture, all faecal egg counts were zero and they remained zero for all noninfected animals throughout the experiment (Fig. 1). Faecal egg counts of all infected lambs increased to 603.6 ± 137.6 (mean ± SE) eggs/g by week 5 of the patent period, 3 weeks after they were first dosed with larvae (Appendix Fig. A3). Within the treatment groups faecal egg counts of infected animals in the parasitized groups increased to 631.2 ± 177.4 (mean ± SE) and in the mixed groups to 534.6 ± 202.8 (Fig. 1). Faecal egg counts of all infected lambs remained high until lambs were dosed with anthelmintic at the start of week 8 when they returned to zero by week 9. Serum pepsinogen concentrations
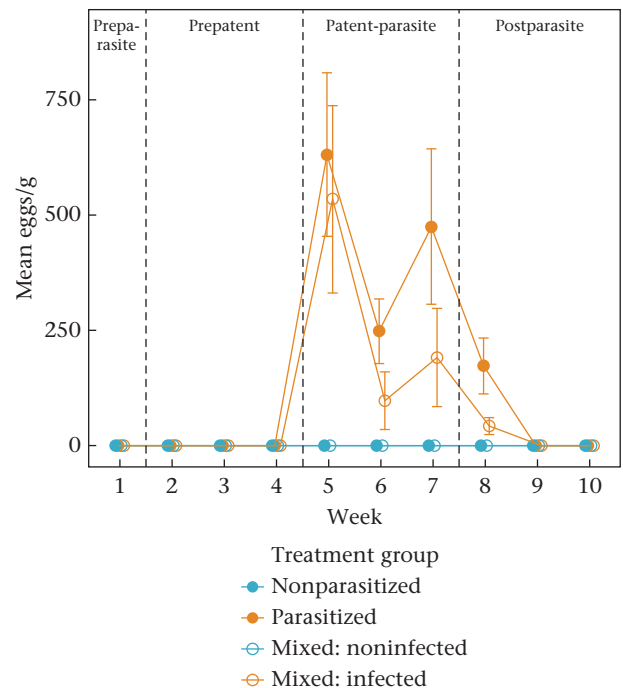
Figure 1. Mean ± SE faecal egg counts (eggs/g) of infected and noninfected lambs in each treatment group, nonparasitized (*N* = 4), parasitized (*N* = 4) and mixed (noninfected and infected; *N* = 4), during each week of the experiment, including the final sampling day at the beginning of week 10. The dashed lines separate the experiment into the four phases (preparasite, prepatent, patent-parasite and post-parasite). Lambs were dosed with *T. circumcincta* larvae at the start of week 2 and infections were cleared at the start of week 8 after faecal samples were collected.
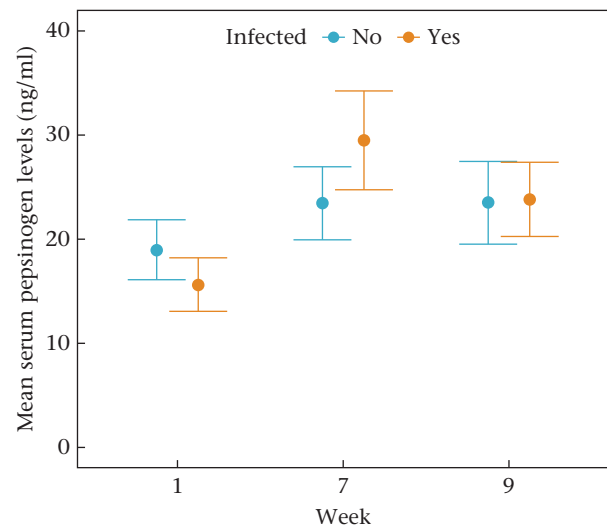
Figure 2. Mean ± SE serum pepsinogen levels (ng/ml) of infected (*N* = 14) and noninfected (*N* = 14) lambs during the three blood sampling weeks. Blood samples were taken during the preparasite (week 1), patent-parasite (week 7) and postparasite phase (week 9).

of infected lambs were significantly higher by the patent-parasite sampling day (Week 7; estimate = 0.42, *P* = 0.02; Fig. 2), whereas noninfected lambs' concentrations showed no significant change. Before parasitism and following treatment with anthelmintic there was no significant difference in the serum pepsinogen levels between infected and noninfected lambs (Appendix Table A3).

The average weight of infected and noninfected lambs in each treatment group during each week of the experiment is shown in

Figure 3. Mean ± SE liveweight of infected and noninfected lambs in each treatment group, nonparasitized ($N = 4$), parasitized ($N = 4$) and mixed (noninfected and infected; $N = 4$), during each week of the experiment, including the final weighing day at the beginning of week 10. The dashed lines separate the experiment into the four phases (preparasite, prepatent, patent-parasite and postparasite).
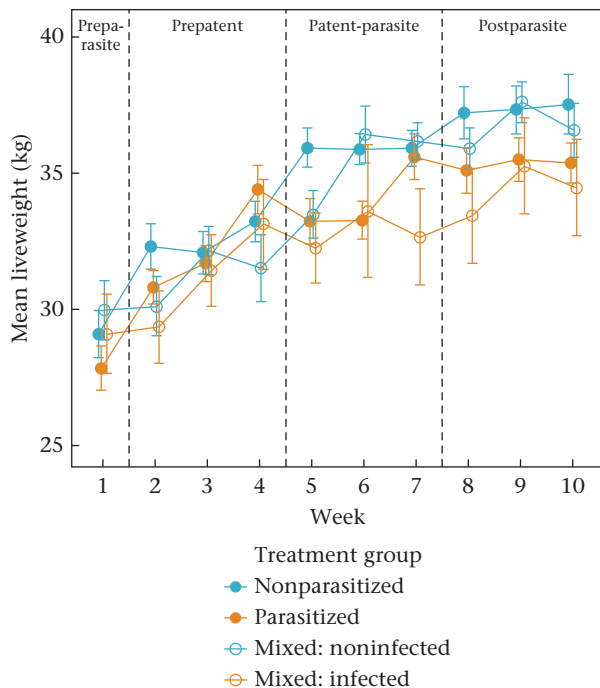
Fig. 3. Although there was no significant interaction between treatment group and week on liveweight (Appendix Table A4) there was a significant interaction between week and parasitic status on the liveweight of the lambs ($F_9 = 3.62$, $P < 0.001$). Overall, the mean weight of infected lambs was significantly lower than that of noninfected lambs on the final day of the experiment (estimate = −1.74, $P = 0.04$; Appendix Table A5). We also found infected lambs in mixed-state groups had lower liveweights than infected lambs in single-state groups during week 7 of the patent-parasite phase but this was not significant at the 5% level (estimate = −4.19, $P = 0.053$; Fig. 3, Appendix Table A6).

All dosed animals had faecal egg counts above zero from week 5 to week 8 that decreased following treatment with anthelmintic and were zero by week 9. In comparison the faecal egg counts of noninfected animals were zero throughout, demonstrating the expected/predicted difference between infected and noninfected animals and thus creating the required framework to investigate the questions being addressed.

We next investigated whether changes in activity could be detected in both single-state and mixed-state groups and whether these effects were observable prior to the patent period when the physiological costs of parasitism could be measured.

Impact of Parasitism in Relation to Treatment Group

Motion index

There was a significant interaction between treatment group and phase on motion index (Wald test: $W_6 = 33.08$, $P < 0.001$; Appendix Table A7). Parasitized groups had significantly lower motion index than the nonparasitized groups during the prepatent (estimate = −0.09, $P < 0.001$) and patent-parasite (estimate = −0.07, $P = 0.015$) phases of infection compared to nonparasitized groups (Fig. 4a). The mixed groups also had reduced motion index during the prepatent phase of infection but this was not significant at the 5% level (estimate = −0.05, $P = 0.059$). There was no significant difference in the motion index between the three treatment groups during the preparasite phase when all lambs were parasite naïve and following treatment with anthelmintic during the postparasite phase (Fig. 4a). Analysis on a finer scale (e.g. weekly) demonstrated that the drop in motion index in the parasitized groups was consistent throughout all weeks of the prepatent and patent-parasite phases (see Appendix Table A8).

Step count

There was a significant interaction between treatment group and phase on step count (Wald test: $W_6 = 45.60$, $P < 0.001$; Appendix Table A7). Parasitized groups had significantly lower step counts during the prepatent (estimate = −0.11, $P < 0.001$) and patent-parasite (estimate = −0.11, $P < 0.001$) phases of infection compared to the nonparasitized groups (Fig. 4b). The step count of the mixed groups was also significantly lower than that of the nonparasitized groups during the prepatent phase of the study (estimate = −0.07, $P = 0.033$; Fig. 4b). There was no significant difference in step count between the three treatment groups during the preparasite phase when all lambs were parasite naïve and following treatment with anthelmintic during the postparasite phase (Fig. 4b). Analysis on a finer scale demonstrated that the decrease in step count in the parasitized groups was consistent throughout all weeks of the prepatent and patent-parasite phases (see Appendix Table A8).

Frequency of lying bouts

There was a significant interaction effect between treatment group and phase on frequency of lying bouts (Wald test: $W_6 = 15.37$, $P = 0.018$; Appendix Table A7). The frequency of lying bouts of the parasitized groups was significantly reduced during the prepatent (estimate = −0.06, $P = 0.043$), patent-parasite (estimate = −0.09, $P = 0.004$) and postparasite (estimate = −0.07, $P = 0.036$) phases compared to the nonparasitized groups (Fig. 4c). There was no significant difference in the frequency of lying bouts between the mixed and nonparasitized groups during each phase of the experiment (Fig. 4c). However, the frequency of lying bouts of the mixed groups was significantly lower than that of the nonparasitized groups in week 4 (estimate = −0.08, $P = 0.028$) and week 7 (estimate = −0.08, $P = 0.036$; Appendix Table A8).

Lying time

There was no significant interaction between phase and treatment group on lying time (night data: Wald test: $W_6 = 6.15$, $P = 0.406$; day data: Wald test: $W_6 = 4.41$, $P = 0.621$; Appendix Table A7). However, there was an interaction effect between treatment group and week as well as a diurnal effect on lying time (night data: Wald test: $W_{16} = 26.01$, $P = 0.054$; Appendix Table A8), as the parasitized groups were more likely to spend time lying down during the night in week 4 (estimate = 0.50, $P = 0.021$; Fig. 4d) than the nonparasitized groups.

Impact of Parasitism in Relation to Infection Status of Groupmates

Motion index

There was no significant interaction between parasitic status, group type and phase (Wald test: $W_3 = 3.48$, $P = 0.32$; Appendix Table A9) or between parasitic status, group type and week on motion index (Wald test: $W_8 = 9.67$, $P = 0.299$;
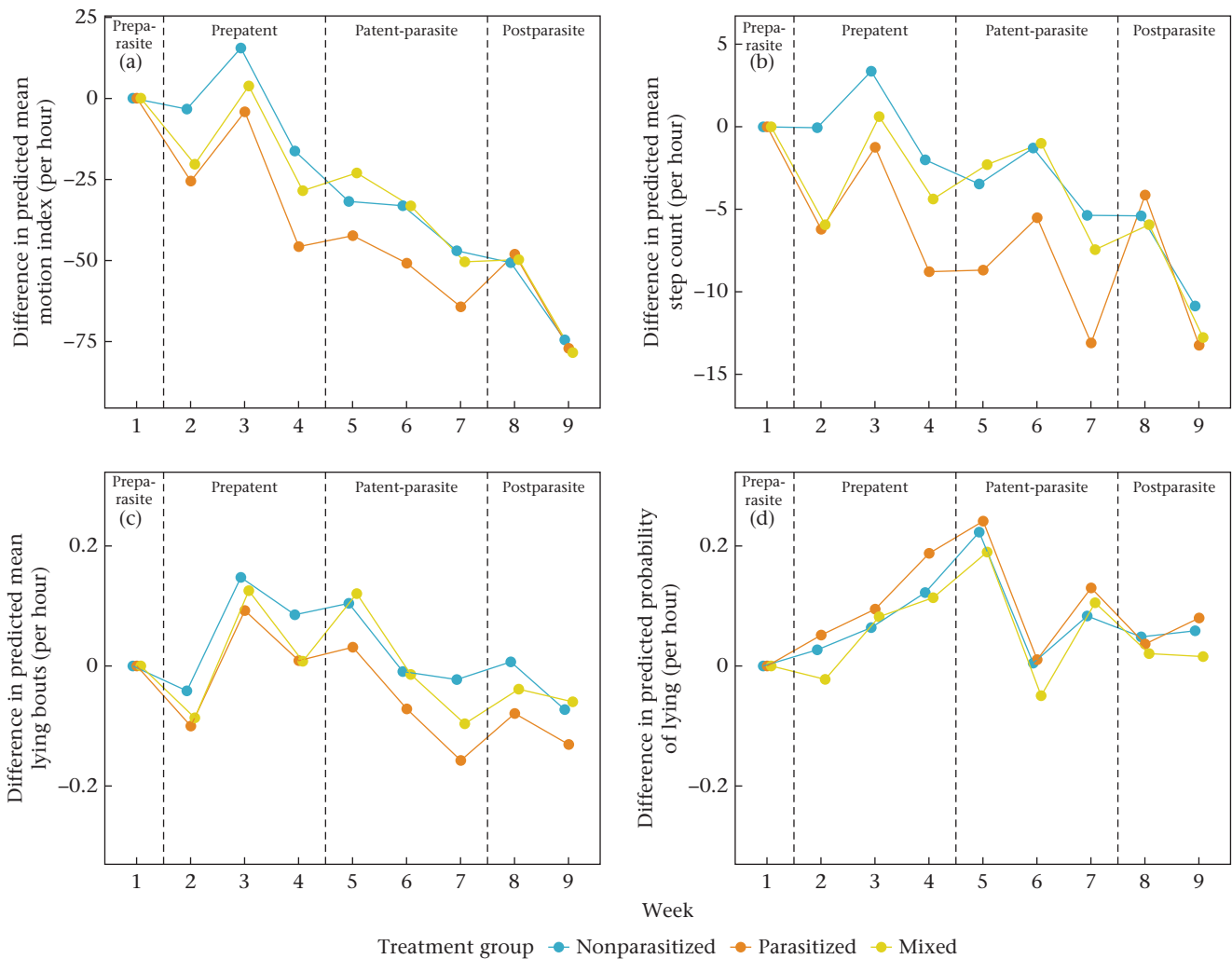
Figure 4. Difference in activity behaviour of individuals in each treatment group, nonparasitized ($N = 4$), parasitized ($N = 4$) and mixed ($N = 4$), during each week of the study compared to the preparasite phase (week 1). The dashed lines separate the experiment into the four phases (preparasite, prepatent, patent-parasite and postparasite). (a) Difference in model-predicted mean motion index per hour. (b) Difference in model-predicted mean step count per hour. (c) Difference in model-predicted mean frequency lying bouts per hour. (d) Difference in model-predicted probability of lying down (night data).

Appendix Table A10). Thus, the pattern of behaviour found between infected lambs in mixed- and single-state groups (Fig. 5a) and between noninfected lambs in mixed- and single-state groups did not differ.

Step count

There was no interaction between parasitic status, group type and phase on step count (Appendix Table A9); however, when this was investigated on a finer scale of week, there was an interaction between parasitic status, group type and week on step count (Wald test: $W_8 = 32.82$, $P = 0.001$; Fig. 5b, Appendix Table A10). In week 2, the step count of noninfected lambs in the mixed-state groups was significantly lower than that of noninfected lambs in the single-state groups (estimate $= -0.13$, $P = 0.004$; Fig. 5b) and the step count of infected lambs in the mixed-state groups was significantly higher than that of infected lambs in the single-state groups (estimate $= 0.17$, $P = 0.01$; Fig. 5b). There was also a difference in week 8 following treatment with anthelmintic where the step count of infected lambs in the mixed-state groups was significantly lower than that of infected lambs in the single-state groups (estimate $= -0.15$, $P = 0.023$; Fig. 5b). Individuals in the single-state groups returned to the level of noninfected individuals

following anthelmintic treatment but previously infected animals in the mixed-state groups did not.

Frequency of lying bouts

There was no interaction between parasitic status, group type and phase on frequency of lying bouts (Wald test: $W_3 = 4.65$, $P = 0.19$; Appendix Table A9). However, again there was an interaction between parasitic status, group type and week on frequency of lying bouts (Wald test: $W_8 = 14.51$, $P = 0.06$; Appendix Table A10). The frequency of lying bouts of infected lambs in the mixed-state groups was higher than that of infected lambs in the single-state groups in week 6 (estimate $= 0.13$, $P = 0.057$) and week 7 (estimate $= 0.18$, $P = 0.007$; Fig. 5c) and the frequency of lying bouts of noninfected lambs in the mixed-state groups was significantly lower than that of noninfected lambs in the single-state groups in week 7 (estimate $= -0.09$, $P = 0.036$).

Lying time

There was no significant interaction between parasitic status, group type and phase on lying time (night data: Wald test: $W_3 = 4.66$, $P = 0.19$; day data: Wald test: $W_3 = 1.99$, $P = 0.58$; Appendix Table A9), and no significant interaction between parasitic
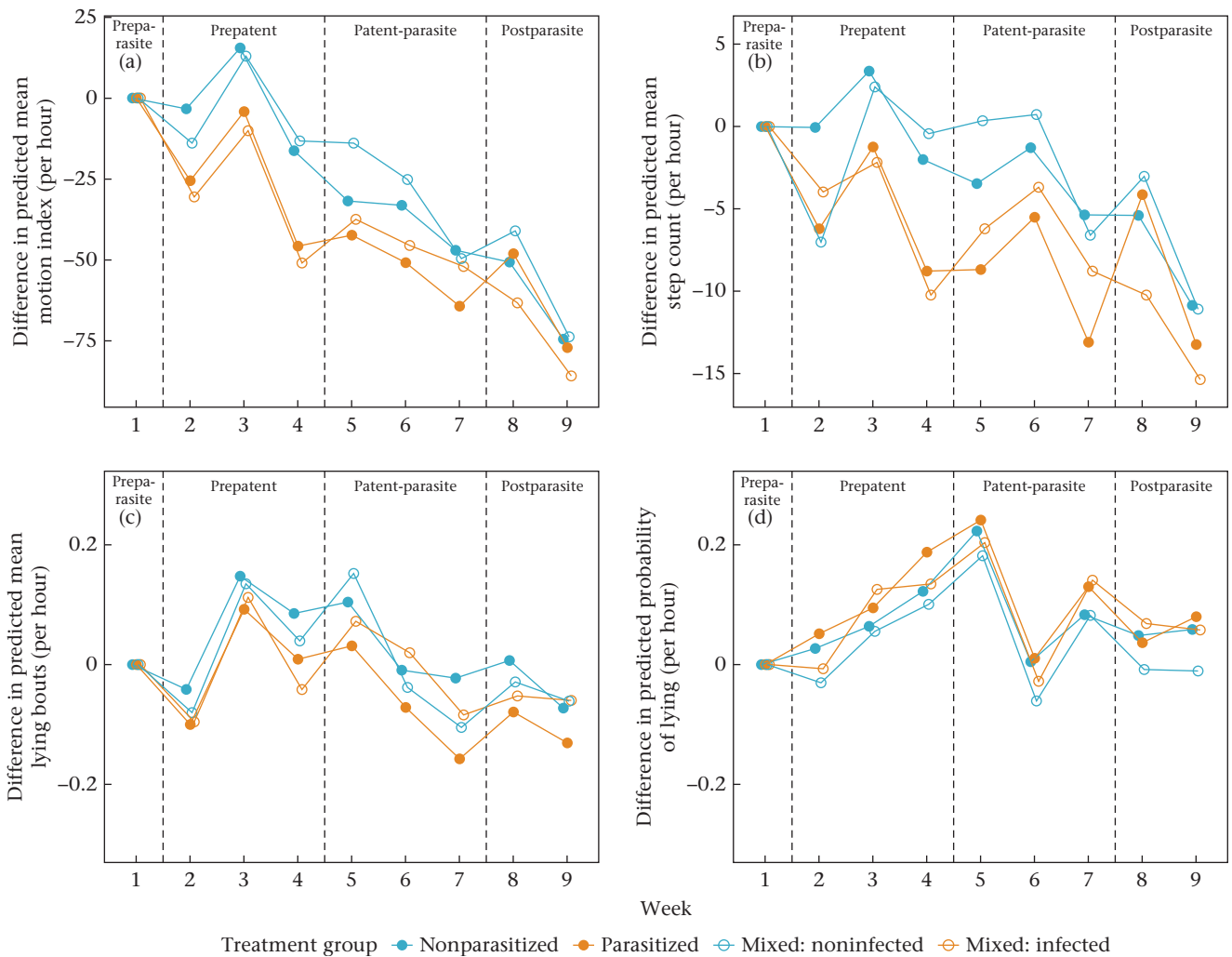
Figure 5. Difference in activity behaviour between infected and noninfected individuals in each treatment group, nonparasitized ($N = 4$), parasitized ($N = 4$) and mixed (noninfected and infected; $N = 4$), during each week of the study compared to the preparasite phase (week 1). The dashed lines separate the experiment into the four phases (preparasite, prepatent, patent-parasite and postparasite). (a) Difference in model-predicted mean motion index per hour. (b) Difference in model-predicted mean step count per hour. (c) Difference in model-predicted mean frequency lying bouts per hour. (d) Difference in model-predicted probability of lying down (night data).

status, group type and week on lying time (night data: Wald test: $W_8 = 7.23$, $P = 0.51$; day data: Wald test: $W_8 = 5.57$, $P = 0.70$; Appendix Table A10, Fig. 5d).

DISCUSSION

Here we have shown that parasitism induced detectable changes in behaviour early in the prepatent period of infection and that these changes could be detected in both single-parasitic state and mixed-parasitic state groups. However, in mixed groups, social modulation of behaviour altered the activity behaviour of all group members, indicating that the cost of disease may impact both infected and uninfected members of the group.

In this study we successfully established experimental treatment groups, detected parasitism and induced measurable costs of infection. We found faecal egg counts of infected lambs were detectable 3 weeks after the initial infection dose with *T. circumcincta* larvae consistent with other studies suggesting a prepatent period of 17–21 days (Wood et al., 1995). Faecal egg counts remained high until infections were cleared by treating with an anthelmintic. The faecal egg counts of lambs dosed with water remained at zero throughout the experiment. There was an

increase in serum pepsinogen levels of infected animals during week 7, which arises from mucosal damage of the abomasum surface by late larval and adult stages of *T. circumcincta*, resulting in secretion of pepsinogen into the blood (Scott et al., 2000). We also found infected lambs had lower liveweights than noninfected animals from week 5 through to the end of the study. The parasite infection model therefore successfully established clear preparasitized, prepatent, patent-parasite and postparasite phases across the treatment groups.

We were able to identify behavioural changes during both the prepatent and patent-parasite phases of infection. During the prepatent phase, infected lambs in both the single-state (parasitized groups) and mixed-state groups (mixed groups) had reduced motion index and step count, which first occurred in week 2 before any noticeable impact of parasitism or measure of parasitism was observable. We also found that parasitized groups spent less time transitioning between standing and lying during the prepatent, patent-parasite and postparasite phases and spent more time lying down at night in week 4, during the prepatent phase of infection. These prepatent observations are in line with classic sickness behaviours exhibited by parasitized animals during the patent stage of infection

across both domestic and wild systems (Besier et al., 2016; Ghai et al., 2015; Högberg et al., 2021; Hutchings et al., 2000; Szyszka et al., 2013). As smaller social groups of sheep show more vigilant behaviours and forage less (Dumont & Boissy, 2000; Penning et al., 1993; Sevi et al., 1999), infected animals spending more time lying down would reduce the total number of animals within a social group grazing together, which may have implications for the foraging efficiency of the entire social group. Following treatment with the anthelmintic there was no difference in activity between the three treatment groups.

Behaviour changes following parasite infection usually comprise lower activity levels, reduced feed intake and changes to sociality (Gauly et al., 2007; Ghai et al., 2015; Hart, 1988; Kazlauskas et al., 2016; Kyriazakis et al., 1998; Moore, 2002; Poulin, 1995; Szyszka & Kyriazakis, 2013). Treatment with anthelmintics to remove naturally occurring parasites has been demonstrated to lead to an increase in activity of lambs with natural parasite infections (Grant et al., 2020; Ikurior et al., 2020) suggesting parasitism to be a direct cause of this change. Owing to our experimental design, we believe that these behaviour changes can be directly attributed to parasitism and occur during the first week of infection, 3 weeks before any measure of parasitism (faecal egg count) or noticeable impact of parasitism (weight loss) was observed. The motion index gives an indication of the total amount of energy used; therefore, a decrease in motion index could be associated with a reduction in other behaviours such as grazing rates, as we know reduced feed intake and anorexia are commonly associated with parasite infections (Adamo et al., 2010; Hart, 1988; Hite et al., 2020; Hutchings et al., 2000; Kyriazakis et al., 1996; Murray & Murray, 1979). While we did not measure forage intake during this study, we did find that, overall, infected lambs had consistently lower weights than noninfected animals during the patent-parasite and postparasite phases.

There are several potential explanations for the expression of sickness behaviours by infected animals. For example, sickness behaviours are thought to reflect the early conservation of energy by the host to mount an immune response to fight infection (Kyriazakis et al., 1998). This link between behaviour and the immune response has been reported in many systems (Adelman et al., 2009; Dantzer, 2004; Lopes, 2017; Lopes et al., 2012; Stockmaier et al., 2018), and studies have shown that antibody levels in lambs infected with *T. circumcincta* start to increase within the first week of infection (Halliday et al., 2007; Henderson & Stear, 2006; Houdijk et al., 2005). Alternatively, changes in host behaviour may also be a side-effect of the pathology associated with infection (Holland & Cox, 2001; Klein, 2003), a result of the physical presence of the parasite (Jolles et al., 2020; Lafferty & Shaw, 2013) or a response to pathogen—host signalling through molecular mechanisms (Claycomb et al., 2017).

Behavioural responses were also affected by the parasitic status of other individuals in a group. Both infected and noninfected individuals altered their behaviour in different ways depending on group composition. For example, during the early stages of infection at week 2, we found the step count of noninfected lambs in the mixed-state groups was lower than that of noninfected lambs in the single-state groups suggesting that noninfected animals decreased their activity in the presence of the less active infected individuals. We also found that infected lambs in the mixed groups had reduced step count and motion index during the prepatent and patent-parasite phase; however, the change in activity was to a lesser degree during the patent-parasite phase compared to infected lambs in the single-state groups (Fig. 4).

These findings indicate that parasitism affected the behaviour of lambs in both single-state and mixed-state groups. However, in the mixed-state groups this effect was modulated by the noninfected lambs, as infected individuals increased their activity in the presence of more active noninfected individuals, thus suggesting that social group and social facilitation may have affected the activity behaviour in response to parasitism of lambs in the mixed-state groups.

The extent to which animals engage in different sickness behaviours can often vary depending on their environment (Cohn & de Sá-Rocha, 2006; Lopes et al., 2012; Lopes et al., 2021), and in certain circumstances infected animals could adjust the expression of sickness behaviours in favour of other behaviours that may be more beneficial at the time (Cohn & de Sá-Rocha, 2006; Lopes et al., 2012). Like most grazing herbivores, lambs are highly social prey animals that will benefit from being part of a large social group (Hamilton, 1971; Krause and Ruxton, 2002; Lima, 1995). Studies have shown that sheep will choose to graze with members of their social group rather than graze alone in more favourable areas, and when part of a larger group they will reduce vigilant behaviours and spend more time foraging (Dumont & Boissy, 2000; Penning et al., 1993; Sevi et al., 1999). It has also recently been suggested that animals may benefit from group living by using social behaviour to increase parasite tolerance (Almberg et al., 2015; Ezenwa & Worsley-Tonks, 2018). As reduced activity levels can lead to an individual having reduced sociality (Hart, 1988; Lopes et al., 2016; Hawley et al., 2021), parasitized individuals could also lose the associated benefits of group living (Behringer et al., 2006; Kiesecker et al., 1999; Krause and Ruxton, 2002; Tobler & Schlupp, 2008). Thus, the higher activity levels of infected lambs and lower activity levels of noninfected animals in the mixed groups could indicate social facilitation, with infected animals modulating their activity to maintain group cohesion. However, as sickness behaviours are believed to have evolved as an adaptive response to fight infection, nonexpression of these behaviours may have damaging effects on the health of the animal (Lopes, 2014). Interestingly, we found the liveweight of infected lambs tended to be lower in the mixed-state groups than in the single-state groups towards the end of the patent-parasite phase. This suggests not expressing these sickness behaviours may have led to more severe consequences for the health of the animals in the mixed-state than in the single-state groups. In theory, reduced activity levels could reduce foraging efficiency or antipredator behaviours in healthy animals, meaning activity modulation in response to parasitism in noninfected animals may also come at a cost to the individual.

The return of behaviour of infected animals to normal levels after treatment with an anthelmintic was consistent with parasite removal experiments that have shown rapid changes in behaviour (Gauly et al., 2007; Hutchings et al., 2002; Sharma et al., 2016; Szyszka & Kyriazakis, 2013). Furthermore, by removing the experimental treatment, we lost the behavioural signal of infection, which further shows that the behaviour change exhibited by infected lambs was driven by the effect of parasitism on the animals. Unlike other behaviours, frequency of lying bouts of parasitized groups did not return to normal levels until week 9. This lag in behaviour reversal could reflect infected animals overcompensating for the reduced food intake in the previous weeks. As parasitized lambs have been reported to have increased bodyweight gain following treatment with an anthelmintic (Sharma et al., 2016), animals could be spending more time grazing and less time transitioning between standing and lying after anthelmintic treatment.

We have shown that parasitism can impact behaviour at the very early stages of infection. These changes in behaviour occurred immediately after exposure to parasites, at an earlier stage than any classical indicators of parasitism such as faecal egg counts, indicators of gut wall damage and changes in liveweight. Although livestock producers already have an indicator of infection by measuring weight loss, they are detecting this after experiencing a loss in production. By identifying a change in behaviour associated with early subclinical parasitism there is potential to target individuals within a group to reduce the amount of drugs in agriculture systems, slowing the rate of anthelmintic resistance while keeping parasite numbers low. Thus, there is the potential to use these parasite-induced changes in behaviour for early infection detection to inform targeted parasite control strategies, and to improve the welfare of the animals. We have also shown that the behavioural response of an individual can be modulated by its social environment, as both infected and uninfected animals in the mixed-state groups altered their behaviour to a different degree during the patent-parasite phase of the study than those with similar burdens in single-state groups. These findings demonstrate the importance of taking the parasitic status of all animals within a social group into account as certain social contexts may limit the expression of behaviours that are optimal for fitness in both infected and uninfected members of the group.

Author Contributions

Alex Morris: Conceptualization, Methodology, Formal analysis, Investigation, Writing—Original Draft**,** Writing—Review & Editing, Visualization. **Giles Innocent**: Conceptualization, Methodology, Resources, Writing—Review & Editing, Supervision. **Emma Cunningham**: Conceptualization, Methodology, Writing—Review & Editing, Supervision. **Spiridoula Athanasiadou**: Investigation, Resources, Writing—Review & Editing. **Michael Hutchings**: Conceptualization, Methodology, Writing—Review & Editing, Supervision, Funding acquisition. **Lesley Smith**: Conceptualization, Methodology, Investigation, Writing—Review & Editing, Supervision, Project administration, Funding acquisition.

Data Availability

Data will be made available on request.

Acknowledgments

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Appendix

Motion Index

There was a statistically significant interaction between treatment group and week on motion index (Wald test: $W_{16} = 30.38$, $P = 0.001$). The parasitized groups had a statistically significant lower motion index between weeks 2 and 7 compared to nonparasitized groups (Fig. 3a, Appendix Table A4). There was no statistically significant difference in motion index between mixed and nonparasitized groups during each week of the study (Appendix Table A4).

Step Count

There was a statistically significant interaction between treatment group and week on step count (Wald test: $W_{16} = 63.31$, $P < 0.001$). Parasitized groups had a statistically significant lower step count than the nonparasitized groups between weeks 2 and week 7 (Fig. 3b, Appendix Table A4), and the step count of the mixed groups was statistically significantly lower than the nonparasitized groups during week 2 (estimate $= -0.11$, $P = 0.005$).

Frequency of Lying Bouts

There was also a statistically significant interaction effect between treatment group and week on frequency of lying bouts (Wald test: $W_{16} = 29.23$, $P = 0.02$). Parasitized groups had a statistically significant lower frequency of lying bouts during week 4 (estimate $= -0.08$, $P = 0.031$), week 7 (estimate $= -0.15$, $P < 0.001$) and week 8 (estimate $= -0.09$, $P = 0.021$; Fig. 3c) compared to the nonparasitized groups. Mixed groups also had a statistically significant lower frequency of lying bouts during week 4 (estimate $= -0.08$, $P = 0.028$) and week 7 (estimate $= -0.08$, $P = 0.036$) compared to the nonparasitized groups. There was no statistically significant difference in the frequency of hourly lying bouts between the three treatment groups following treatment with anthelmintic at week 9 (Fig. 3c, Appendix Table A4).

Table A1
Model formulae for analyses of activity behaviour (step count, motion index, frequency of lying bouts and lying time) and measurements of lambs during the experiment

Model group	Response	Model class	Model family	Fixed effects	Interactions	Random effects
Activity behaviour	Step count	GLMM	Negative binomial	Sex + **Ice.Qube**	**Treatment Group*Phase**	**Group ID/Lamb ID + Plot**
Activity behaviour	Step count	GLMM	Negative binomial	Sex + **Ice.Qube**	**Treatment Group*Week**	**Group ID/Lamb ID + Plot**
Activity behaviour	Step count	GLMM	Negative binomial	Sex + **Ice.Qube**	**Parasitic status*Group type*Phase**	**Group ID/Lamb ID + Plot**
Activity behaviour	Step count	GLMM	Negative binomial	Sex + **Ice.Qube**	**Parasitic status*Group type*Week**	**Group ID/Lamb ID + Plot**
Activity behaviour	Motion index	GLMM	Negative binomial	Sex	**Treatment Group*Phase**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Motion index	GLMM	Negative binomial	Sex	**Treatment Group*Week**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Motion index	GLMM	Negative binomial	Sex	**Parasitic status*Group type*Phase**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Motion index	GLMM	Negative binomial	Sex	**Parasitic status*Group type*Week**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Frequency of lying bouts	GLMM	Poisson	Sex	**Treatment Group*Phase**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Frequency of lying bouts	GLMM	Poisson	Sex	**Treatment Group*Week**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Frequency of lying bouts	GLMM	Poisson	Sex	**Parasitic status*Group type*Phase**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Frequency of lying bouts	GLMM	Poisson	Sex	**Parasitic status*Group type*Week**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Lying time	GLMM	Binomial	Sex	**Treatment Group*Phase**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Lying time	GLMM	Binomial	Sex	**Treatment Group*Week**	**Group ID/Lamb ID + IceQube ID + Plot**
Activity behaviour	Lying time	GLMM	Binomial	Sex	**Parasitic status*Group type*Phase**	**Parasitic status*Group type*Week**
Activity behaviour	Lying time	GLMM	Binomial	Sex	**Parasitic status*Group type*Week**	**Group ID/Lamb ID + IceQube ID + Plot**
Animal measurements	Weight	GLMM	Gaussian	Sex	**Parasitic status*Week**	**Group ID/Lamb ID + Plot**
Animal measurements	Weight	GLMM	Gaussian	Sex	**Treatment Group*Week**	**Group ID/Lamb ID + Plot**
Animal measurements	Weight	GLMM	Gaussian	Sex	**Infected Group*Week**	**Group ID/Lamb ID + Plot**
Animal measurements	Pepsinogen	GLMM	Poisson	Sex	**Parasitic status*Week**	**Group ID/Lamb ID + Plot**
Animal measurements	Pepsinogen	GLMM	Poisson	Sex	**Treatment Group*Week**	**Group ID/Lamb ID + Plot**

Bold indicates terms that were included in the minimal model.

Table A2
Description of fixed and random effects

Term	Class	Description
Parasitic status	Factor (2 levels)	Infected (dosed with parasites); noninfected (dosed with water)
Treatment Group	Factor (3 levels)	Nonparasitized (social groups of noninfected lambs); parasitizsed (social groups of infected lambs); mixed (social groups of a mixture of infected and noninfected lambs)
Phase	Factor (4 levels)	Preparasite (first week of experiment when all lambs were parasite naïve); prepatent (weeks 2–4 when lambs are infected but not shedding eggs); patent-parasite (weeks 5–7 when infected lambs are shedding eggs); postparasite (weeks 8–9 after lambs were treated with anthelmintic)
Week	Factor (9 levels)	Week of experiment (week 1–9)
Group type	Factor (2 levels)	Mixed-parasitic state (individual is in the mixed group containing infected and noninfected lambs), single-parasitic state (individual is in the parasitized or nonparasitized group)
Sex	Factor (2 levels)	Male and female
Lamb ID	Factor (60 levels)	ID of lamb
Group ID	Factor (12 levels)	ID of the social group
Plot	Factor (12 levels)	ID of plot
IceQube ID	Factor (65 levels)	ID of IceQube

Table A3
Model estimates for fixed effects of final generalized linear mixed models on mean serum pepsinogen levels of infected and noninfected lambs during the three blood sampling weeks

Fixed effect	Estimate	SE	z	P
Parasitic status, Infected	−0.19	0.21	−0.92	0.360
Phase, Patent-parasite	0.21	0.11	1.92	0.055
Phase, Postparasite	0.21	0.16	1.35	0.176
Parasitic status, Infected*Phase, Patent-parasite	**0.42**	**0.18**	**2.32**	**0.020**
Parasitic status, Infected*Phase, Postparasite	0.21	0.23	0.92	0.359
(Intercept)	2.94	0.15	19.51	<0.001
				AIC = 614.8

Blood samples were taken during the preparasite (week 1), patent-parasite (week 7) and postparasite phase (week 9). AIC value is presented from the final model. Bold indicates significant result.

Table A4
Model estimates for fixed effects of final generalized linear mixed models on mean weight of the three treatment groups (nonparasitized, parasitized and mixed) during each week of the experiment

Fixed effect	Estimate	SE	z	P
Group, Parasitized	−1.29	1.41	−0.92	0.364
Group, Mixed	0.49	1.41	0.35	0.732
Week, 2	**3.17**	**1.08**	**2.93**	**<0.001**
Week, 3	**2.94**	**0.70**	**4.17**	**<0.001**
Week, 4	**4.08**	**1.08**	**3.77**	**<0.001**
Week, 5	**6.79**	**1.08**	**6.27**	**<0.001**
Week, 6	**6.73**	**0.70**	**9.56**	**<0.001**
Week, 7	**6.77**	**1.08**	**6.26**	**<0.001**
Week, 8	**8.06**	**1.08**	**7.45**	**<0.001**
Week, 9	**8.18**	**0.70**	**11.62**	**<0.001**
Week, 10	**8.38**	**1.08**	**7.75**	**<0.001**
Group, Parasitized*Week, 2	−0.21	1.73	−0.12	0.903
Group, Parasitized*Week, 3	0.89	0.99	0.91	0.366
Group, Parasitized*Week, 4	2.46	1.73	1.42	0.175
Group, Parasitized*Week, 5	−1.42	1.73	−0.82	0.424
Group, Parasitized*Week, 6	−1.32	0.99	−1.33	0.184
Group, Parasitized*Week, 7	0.97	1.73	0.56	0.582
Group, Parasitized*Week, 8	−0.83	1.73	−0.48	0.639
Group, Parasitized*Week, 9	−0.53	0.99	−0.54	0.592
Group, Parasitized*Week, 10	−0.85	1.73	−0.49	0.629
Group, Mixed*Week, 2	−2.98	1.73	−1.72	0.104
Group, Mixed*Week, 3	−0.70	0.99	−0.71	0.479
Group, Mixed*Week, 4	−1.55	1.73	−0.89	0.384
Group, Mixed*Week, 5	−3.43	1.73	−1.98	0.064
Group, Mixed*Week, 6	−1.07	0.99	−1.08	0.281
Group, Mixed*Week, 7	−1.64	1.73	−0.95	0.358
Group, Mixed*Week, 8	−2.77	1.73	−1.60	0.128
Group, Mixed*Week, 9	−1.15	0.99	−1.16	0.245
Group, Mixed*Week, 10	−2.29	1.73	−1.32	0.204
(Intercept)	29.16	1.00	29.17	<0.001
				AIC = 2852.044

AIC value is presented from the final model. Bold indicates significant results.

Table A5
Model estimates for fixed effects of final generalized linear mixed models on mean weight of infected and noninfected lambs during each week of the experiment

Fixed effect	Estimate	SE	z	P
Parasitic status, Infected	−1.07	0.94	−1.14	0.258
Week, 2	**1.84**	**0.56**	**3.27**	**<0.001**
Week, 3	**2.64**	**0.54**	**4.85**	**<0.001**
Week, 4	**3.56**	**0.56**	**6.33**	**<0.001**
Week, 5	**5.36**	**0.56**	**9.54**	**<0.001**
Week, 6	**6.61**	**0.54**	**12.13**	**<0.001**
Week, 7	**6.99**	**0.56**	**12.42**	**<0.001**
Week, 8	**7.06**	**0.56**	**12.57**	**<0.001**
Week, 9	**7.95**	**0.54**	**14.60**	**<0.001**
Week, 10	**8.14**	**0.56**	**14.47**	**<0.001**
Parasitic status, Infected*Week, 2	0.55	0.85	0.65	0.515
Parasitic status, Infected*Week, 3	0.76	0.79	0.96	0.340
Parasitic status, Infected*Week, 4	**1.75**	**0.85**	**2.06**	**0.040**
Parasitic status, Infected*Week, 5	−0.43	0.85	−0.50	0.615
Parasitic status, Infected*Week, 6	−1.46	0.79	−1.84	0.067
Parasitic status, Infected*Week, 7	−0.95	0.85	−1.12	0.262
Parasitic status, Infected*Week, 8	−0.45	0.85	−0.54	0.592
Parasitic status, Infected*Week, 9	−0.74	0.79	−0.93	0.354
Parasitic status, Infected*Week, 10	**−1.74**	**0.85**	**−2.06**	**0.040**
(Intercept)	29.40	0.72	40.64	<0.001
				AIC = 2845.068

AIC value is presented from the final model. Bold indicates significant results.

Table A6
Model estimates for fixed effects of final generalized linear mixed models on mean weight of infected lambs in the mixed and single state groups during each week of the experiment

Fixed effect	Estimate	SE	z	P
Group, Mixed	1.03	1.76	0.59	0.562
Week, 2	**2.95**	**1.14**	**2.60**	**0.019**
Week, 3	**3.83**	**0.73**	**5.23**	**<0.001**
Week, 4	**6.54**	**1.14**	**5.75**	**<0.001**
Week, 5	**5.37**	**1.14**	**4.72**	**<0.001**
Week, 6	**5.41**	**0.73**	**7.38**	**<0.001**
Week, 7	**7.74**	**1.14**	**6.81**	**<0.001**
Week, 8	**7.23**	**1.14**	**6.36**	**<0.001**
Week, 9	**7.65**	**0.73**	**10.43**	**<0.001**
Week, 10	**7.53**	**1.14**	**6.62**	**<0.001**
Sex, Male	2.22	1.12	1.98	0.059
Group, Mixed*Week, 2	−2.69	2.04	−1.32	0.201
Group, Mixed*Week, 3	−1.51	1.37	−1.10	0.274
Group, Mixed*Week, 4	−2.51	2.04	−1.23	0.232
Group, Mixed*Week, 5	−2.23	2.04	−1.10	0.286
Group, Mixed*Week, 6	−0.91	1.37	−0.66	0.508
Group, Mixed*Week, 7	**−4.19**	**2.04**	**−2.06**	**0.053**
Group, Mixed*Week, 8	−2.89	2.04	−1.42	0.171
Group, Mixed*Week, 9	−1.50	1.37	−1.09	0.277
Group, Mixed*Week, 10	−2.17	2.04	−1.06	0.300
(Intercept)	26.99	1.12	24.18	24.18
				AIC = 1359.69

AIC value is presented from the final model. Bold indicates significant results.

Table A7
Model estimates for fixed effects of final generalized linear mixed models on activity behaviour of the three treatment groups during each experiment phase

Fixed effect	Estimate	SE	z	P
Motion index				
Group, Parasitized	0.01	0.04	0.16	0.873
Group, Mixed	0.03	0.04	0.63	0.531
Prepatent	**−0.14**	**0.02**	**−7.18**	**<0.001**
Patent-parasite	0.00	0.02	−0.14	0.889
Postparasite	**−0.25**	**0.02**	**−12.04**	**<0.001**
Group, Parasitized*Phase, Prepatent	**−0.09**	**0.03**	**−3.40**	**<0.001**
Group, Parasitized*Phase, Patent-parasite	**−0.07**	**0.03**	**−2.42**	**0.015**
Group, Parasitized*Phase, Postparasite	−0.01	0.03	−0.19	0.849
Group, Mixed*Phase, Prepatent	**−0.05**	**0.03**	**−1.89**	**0.059**
Group, Mixed*Phase, Patent-parasite	0.01	0.03	0.23	0.821
Group, Mixed*Phase, Postparasite	−0.01	0.03	−0.18	0.855
(Intercept)	5.65	0.03	174.75	<0.001
				AIC = 1087531.2
Step count				
Group, Parasitized	0.04	0.06	0.64	0.524
Group, Mixed	0.02	0.06	0.39	0.698
Prepatent	0.01	0.02	0.49	0.625
Patent-parasite	**−0.06**	**0.02**	**−2.73**	**0.006**
Postparasite	**−0.15**	**0.02**	**−6.54**	**<0.001**
Group, Parasitized*Phase, Prepatent	**−0.11**	**0.03**	**−3.49**	**<0.001**
Group, Parasitized*Phase, Patent-parasite	**−0.11**	**0.03**	**−3.59**	**<0.001**
Group, Parasitized*Phase, Postparasite	−0.01	0.03	−0.38	0.701
Group, Mixed*Phase, Prepatent	**−0.07**	**0.03**	**−2.14**	**0.033**
Group, Mixed*Phase, Patent-parasite	−0.07	0.03	−2.23	0.026
Group, Mixed*Phase, Postparasite	−0.01	0.03	−0.28	0.783
(Intercept)	4.10	0.05	78.55	<0.001
				AIC = 818275.0
Frequency of lying bouts				
Group, Parasitized	**0.07**	**0.03**	**2.04**	**0.041**
Group, Mixed	0.05	0.03	1.36	0.174
Prepatent	**0.07**	**0.02**	**3.09**	**0.002**
Patent-parasite	0.03	0.02	1.14	0.254
Postparasite	−0.04	0.02	−1.64	0.101
Group, Parasitized*Phase, Prepatent	**−0.06**	**0.03**	**−2.02**	**0.043**
Group, Parasitized*Phase, Patent-parasite	**−0.09**	**0.03**	**−2.90**	**0.004**
Group, Parasitized*Phase, Postparasite	**−0.07**	**0.03**	**−2.10**	**0.036**
Group, Mixed*Phase, Prepatent	**−0.05**	**0.03**	**−1.73**	**0.085**
Group, Mixed*Phase, Patent-parasite	−0.02	0.03	−0.76	0.447
Group, Mixed*Phase, Postparasite	−0.02	0.03	−0.53	0.597
(Intercept)	−0.10	0.03	−3.84	<0.001
				AIC = 239872.5
Lying time (night data)				
Group, Parasitized	−0.12	0.15	−0.81	0.419
Group, Mixed	0.07	0.15	0.49	0.623
Prepatent	**0.43**	**0.11**	**3.87**	**<0.001**
Patent-parasite	**0.63**	**0.11**	**5.63**	**<0.001**
Postparasite	**0.30**	**0.11**	**2.71**	**0.007**
Group, Parasitized*Phase, Prepatent	0.23	0.16	1.43	0.154
Group, Parasitized*Phase, Patent-parasite	0.12	0.16	0.73	0.466
Group, Parasitized*Phase, Postparasite	0.01	0.16	0.07	0.943
Group, Mixed*Phase, Prepatent	−0.05	0.16	−0.30	0.768
Group, Mixed*Phase, Patent-parasite	−0.11	0.16	−0.66	0.507
Group, Mixed*Phase, Postparasite	−0.19	0.16	−1.22	0.224
(Intercept)	1.01	0.11	9.61	<0.001
				AIC = 239872.5
Lying time (day data)				
Group, Parasitized	−0.03	0.10	−0.29	0.776
Group, Mixed	−0.07	0.10	−0.67	0.501
Prepatent	**−0.33**	**0.07**	**−4.79**	**<0.001**
Patent-parasite	**−0.28**	**0.07**	**−3.98**	**<0.001**
Postparasite	**−0.62**	**0.08**	**−7.92**	**<0.001**
Group, Parasitized*Phase, Prepatent	−0.05	0.10	−0.49	0.626
Group, Parasitized*Phase, Patent-parasite	−0.02	0.10	−0.21	0.836
Group, Parasitized*Phase, Postparasite	−0.01	0.11	−0.11	0.911
Group, Mixed*Phase, Prepatent	−0.13	0.10	−1.31	0.191
Group, Mixed*Phase, Patent-parasite	−0.01	0.10	−0.13	0.898
Group, Mixed*Phase, Postparasite	0.01	0.11	0.08	0.938
(Intercept)	−0.30	0.07	−4.16	<0.001
				AIC = 38668.2

AIC values are presented from final models. Bold indicates significant results.

Table A8
Model estimates for fixed effects of final generalized linear mixed models on the activity behaviour of the three treatment groups during each week of the experiment. AIC values are presented from final models. Bold indicates significant results.

Fixed effect	Estimate	SE	z	P
Motion index				
Group, Parasitized	0.002	0.04	0.06	0.954
Group, Mixed	0.02	0.04	0.55	0.579
Week, 2	−0.01	0.02	−0.48	0.632
Week, 3	0.05	0.02	2.35	0.019
Week, 4	−0.06	0.02	−2.53	0.012
Week, 5	**−0.12**	**0.02**	**−4.88**	**<0.001**
Week, 6	**−0.12**	**0.02**	**−5.10**	**<0.001**
Week, 7	**−0.18**	**0.02**	**−7.60**	**<0.001**
Week, 8	**−0.20**	**0.02**	**−8.05**	**<0.001**
Week, 9	**−0.30**	**0.02**	**−12.83**	**<0.001**
Group, Parasitized*Week, 2	**−0.08**	**0.04**	**−2.29**	**0.022**
Group, Parasitized*Week, 3	**−0.07**	**0.03**	**−2.08**	**0.038**
Group, Parasitized*Week, 4	**−0.12**	**0.03**	**−3.46**	**<0.001**
Group, Parasitized*Week, 5	−0.04	0.04	−1.19	0.235
Group, Parasitized*Week, 6	**−0.07**	**0.03**	**−2.09**	**0.037**
Group, Parasitized*Week, 7	**−0.08**	**0.03**	**−2.17**	**0.030**
Group, Parasitized*Week, 8	0.01	0.04	0.30	0.761
Group, Parasitized*Week, 9	−0.01	0.03	−0.37	0.713
Group, Mixed*Week, 2	−0.06	0.04	−1.73	0.083
Group, Mixed*Week, 3	−0.04	0.03	−1.23	0.220
Group, Mixed*Week, 4	−0.04	0.03	−1.32	0.187
Group, Mixed*Week, 5	0.04	0.03	1.02	0.308
Group, Mixed*Week, 6	0.00	0.03	0.09	0.927
Group, Mixed*Week, 7	−0.01	0.03	−0.29	0.772
Group, Mixed*Week, 8	0.01	0.04	0.23	0.818
Group, Mixed*Week, 9	−0.01	0.03	−0.34	0.737
(Intercept)	5.65	0.03	175.26	<0.001
				AIC = 10873.27
Step count				
Group, Parasitized	0.03	0.06	0.58	0.564
Group, Mixed	0.02	0.06	0.37	0.714
Week, 2	0.01	0.03	0.01	0.989
Week, 3	0.06	0.02	2.23	0.026
Week, 4	−0.03	0.03	−1.32	0.188
Week, 5	**−0.06**	**0.03**	**−2.2**	**0.028**
Week, 6	−0.02	0.03	−0.83	0.404
Week, 7	**−0.09**	**0.03**	**−3.62**	**<0.001**
Week, 8	**−0.09**	**0.03**	**−3.57**	**<0.001**
Week, 9	**−0.2**	**0.03**	**−7.83**	**<0.001**
Group, Parasitized*Week, 2	**−0.11**	**0.04**	**−2.70**	**0.007**
Group, Parasitized*Week, 3	**−0.08**	**0.04**	**−2.08**	**0.037**
Group, Parasitized*Week, 4	**−0.12**	**0.04**	**−3.26**	**0.001**
Group, Parasitized*Week, 5	**−0.09**	**0.04**	**−2.37**	**0.018**
Group, Parasitized*Week, 6	**−0.07**	**0.04**	**−1.91**	**0.056**
Group, Parasitized*Week, 7	**−0.15**	**0.04**	**−3.81**	**<0.001**
Group, Parasitized*Week, 8	0.03	0.04	0.66	0.511
Group, Parasitized*Week, 9	−0.04	0.04	−1.10	0.269
Group, Mixed*Week, 2	**−0.10**	**0.04**	**−2.65**	**0.008**
Group, Mixed*Week, 3	−0.05	0.04	−1.27	0.204
Group, Mixed*Week, 4	−0.04	0.04	−1.10	0.270
Group, Mixed*Week, 5	0.02	0.04	0.56	0.573
Group, Mixed*Week, 6	0.01	0.04	0.14	0.888
Group, Mixed*Week, 7	−0.04	0.04	−0.99	0.324
Group, Mixed*Week, 8	−0.01	0.04	−0.20	0.842
Group, Mixed*Week, 9	−0.04	0.04	−0.95	0.341
(Intercept)	4.10	0.05	78.43	<0.001
				AIC = 818081.0
Frequency of lying bouts				
Group, Parasitized	**0.08**	**0.03**	**2.20**	**0.028**
Group, Mixed	0.05	0.03	1.34	0.180
Week, 2	−0.05	0.03	−1.63	0.104
Week, 3	**0.15**	**0.03**	**5.84**	**<0.001**
Week, 4	**0.09**	**0.03**	**3.42**	**<0.001**
Week, 5	**0.11**	**0.03**	**4.01**	**<0.001**
Week, 6	−0.01	0.03	−0.37	0.710
Week, 7	−0.03	0.03	−0.95	0.341
Week, 8	0.01	0.03	0.26	0.793
Week, 9	**−0.08**	**0.03**	**−3.09**	**0.002**
Group, Parasitized*Week, 2	−0.06	0.04	−1.52	0.128
Group, Parasitized*Week, 3	−0.06	0.04	−1.66	0.097
Group, Parasitized*Week, 4	**−0.08**	**0.04**	**−2.15**	**0.031**

Table A8 (continued)

Fixed effect	Estimate	SE	z	P
Group, Parasitized*Week, 5	**−0.08**	**0.04**	**−1.96**	**0.050**
Group, Parasitized*Week, 6	−0.07	0.04	−1.68	0.093
Group, Parasitized*Week, 7	**−0.15**	**0.04**	**−3.85**	**<0.001**
Group, Parasitized*Week, 8	**−0.09**	**0.04**	**−2.32**	**0.021**
Group, Parasitized*Week, 9	−0.06	0.04	−1.57	0.116
Group, Mixed*Week, 2	−0.05	0.04	−1.24	0.215
Group, Mixed*Week, 3	−0.03	0.04	−0.71	0.475
Group, Mixed*Week, 4	**−0.08**	**0.04**	**−2.19**	**0.028**
Group, Mixed*Week, 5	0.01	0.04	0.28	0.782
Group, Mixed*Week, 6	−0.01	0.04	−0.13	0.896
Group, Mixed*Week, 7	**−0.08**	**0.04**	**−2.10**	**0.036**
Group, Mixed*Week, 8	−0.05	0.04	−1.23	0.218
Group, Mixed*Week, 9	0.02	0.04	0.48	0.628
(Intercept)	−0.10	0.03	−3.88	<0.001
				AIC = 239516.2
Lying time (night data)				
Group, Parasitized	−0.12	0.15	−0.81	0.416
Group, Mixed	0.08	0.15	0.54	0.590
Week, 2	0.14	0.14	1.01	0.311
Week, 3	**0.36**	**0.14**	**2.60**	**0.009**
Week, 4	**0.77**	**0.14**	**5.41**	**<0.001**
Week, 5	**2.07**	**0.21**	**9.80**	**<0.001**
Week, 6	0.03	0.13	0.22	0.825
Week, 7	**0.49**	**0.13**	**3.67**	**<0.001**
Week, 8	**0.27**	**0.13**	**2.11**	**0.035**
Week, 9	**0.33**	**0.12**	**2.71**	**0.007**
Group, Parasitized*Week, 2	0.12	0.21	0.59	0.555
Group, Parasitized*Week, 3	0.16	0.20	0.83	0.410
Group, Parasitized*Week, 4	**0.50**	**0.21**	**2.32**	**0.021**
Group, Parasitized*Week, 5	−0.02	0.30	−0.05	0.959
Group, Parasitized*Week, 6	0.03	0.18	0.15	0.881
Group, Parasitized*Week, 7	0.27	0.19	1.40	0.163
Group, Parasitized*Week, 8	−0.08	0.19	−0.44	0.660
Group, Parasitized*Week, 9	0.10	0.17	0.56	0.573
Group, Mixed*Week, 2	−0.25	0.20	−1.24	0.214
Group, Mixed*Week, 3	0.14	0.20	0.71	0.477
Group, Mixed*Week, 4	−0.02	0.21	−0.11	0.909
Group, Mixed*Week, 5	−0.44	0.28	−1.54	0.123
Group, Mixed*Week, 6	−0.27	0.18	−1.48	0.139
Group, Mixed*Week, 7	0.19	0.19	0.99	0.322
Group, Mixed*Week, 8	−0.15	0.18	−0.84	0.404
Group, Mixed*Week, 9	−0.25	0.17	−1.41	0.158
(Intercept)	1.01	0.11	9.63	<0.001
				AIC = 18618.4
Lying time (day data)				
Group, Parasitized	−0.02	0.10	−0.16	0.873
Group, Mixed	−0.08	0.10	−0.78	0.436
Week, 2	−0.14	0.09	−1.57	0.115
Week, 3	**−0.45**	**0.08**	**−5.38**	**<0.001**
Week, 4	**−0.38**	**0.08**	**−4.55**	**<0.001**
Week, 5	**−0.40**	**0.09**	**−4.61**	**<0.001**
Week, 6	**−0.32**	**0.09**	**−3.55**	**<0.001**
Week, 7	−0.10	0.09	−1.16	0.248
Week, 8	**−0.47**	**0.09**	**−4.95**	**<0.001**
Week, 9	**−0.78**	**0.09**	**−8.34**	**<0.001**
Group, Parasitized*Week, 2	−0.14	0.13	−1.12	0.262
Group, Parasitized*Week, 3	0.01	0.12	0.08	0.938
Group, Parasitized*Week, 4	−0.04	0.12	−0.37	0.708
Group, Parasitized*Week, 5	−0.02	0.13	−0.19	0.852
Group, Parasitized*Week, 6	0.04	0.13	0.30	0.765
Group, Parasitized*Week, 7	−0.11	0.12	−0.92	0.358
Group, Parasitized*Week, 8	−0.08	0.14	−0.61	0.544
Group, Parasitized*Week, 9	0.05	0.13	0.39	0.698
Group, Mixed*Week, 2	0.00	0.13	0.02	0.981
Group, Mixed*Week, 3	−0.10	0.12	−0.82	0.415
Group, Mixed*Week, 4	**−0.24**	**0.12**	**−1.99**	**0.047**
Group, Mixed*Week, 5	0.04	0.13	0.29	0.770
Group, Mixed*Week, 6	0.02	0.13	0.14	0.891
Group, Mixed*Week, 7	−0.05	0.12	−0.41	0.683
Group, Mixed*Week, 8	0.05	0.14	0.36	0.721
Group, Mixed*Week, 9	0.02	0.13	0.13	0.896
(Intercept)	−0.30	0.07	−4.09	<0.001
				AIC = 38505.0

Table A9
Model estimates for fixed effects of final generalized linear mixed models on the activity behaviour of infected and noninfected lambs in the mixed and single parasitic state groups

Fixed effect	Estimate	SE	z	P
Motion index				
Parasitic status, Infected	0.01	0.04	0.16	0.875
Group type, Mixed	0.00	0.05	0.07	0.944
Phase, Prepatent	**−0.14**	**0.02**	**−7.18**	**<0.001**
Phase, Patent-parasite	0.00	0.02	−0.14	0.886
Phase, Postparasite	**−0.25**	**0.02**	**−12.05**	**<0.001**
Parasitic status, Infected*Group type, Mixed	0.05	0.07	0.71	0.477
Parasitic status, Infected*Phase, Prepatent	**−0.07**	**0.03**	**−2.42**	**0.016**
Parasitic status, Infected*Phase, Patent-parasite	**−0.09**	**0.03**	**−3.40**	**0.001**
Parasitic status, Infected*Phase, Postparasite	−0.01	0.03	−0.19	0.853
Group type, Mixed*Phase, Prepatent	0.03	0.03	0.81	0.419
Group type, Mixed*Phase, Patent-parasite	−0.02	0.03	−0.49	0.622
Group type, Mixed*Phase, Postparasite	0.02	0.03	0.49	0.621
Parasitic status, Infected*Group type, Mixed*Phase, Prepatent	0.02	0.05	0.41	0.683
Parasitic status, Infected*Group type, Mixed*Phase, Patent-parasite	0.00	0.05	0.09	0.932
Parasitic status, Infected*Group type, Mixed*Phase, Postparasite	−0.05	0.05	−0.98	0.327
(Intercept)	5.65	0.03	174.90	<0.001
				AIC =1087533.0
Step count				
Parasitic status, Infected	0.04	0.06	0.64	0.523
Group type, Mixed	−0.01	0.06	−0.17	0.866
Phase, Prepatent	0.01	0.02	0.49	0.626
Phase, Patent-parasite	**−0.06**	**0.02**	**−2.73**	**0.006**
Phase, Postparasite	**−0.15**	**0.02**	**−6.54**	**<0.001**
Parasitic status, Infected*Group type, Mixed	0.04	0.09	0.51	0.612
Parasitic status, Infected*Phase, Prepatent	**−0.11**	**0.03**	**−3.50**	**<0.001**
Parasitic status, Infected*Phase, Patent-parasite	**−0.11**	**0.03**	**−3.59**	**<0.001**
Parasitic status, Infected*Phase, Postparasite	−0.01	0.03	−0.38	0.700
Group type, Mixed*Phase, Prepatent	−0.04	0.03	−1.22	0.223
Group type, Mixed*Phase, Patent-parasite	0.02	0.04	0.58	0.559
Group type, Mixed*Phase, Postparasite	0.01	0.04	0.35	0.723
Parasitic status, Infected*Group type, Mixed*Phase, Prepatent	0.05	0.05	0.88	0.378
Parasitic status, Infected*Group type, Mixed*Phase, Patent-parasite	0.04	0.05	0.75	0.453
Parasitic status, Infected*Group type, Mixed*Phase, Postparasite	−0.08	0.06	−1.52	0.130
(Intercept)	4.11	0.05	78.54	<0.001
				AIC =818278.3
Frequency of lying bouts	0.07	0.03	2.04	0.041
Parasitic status, Infected	0.03	0.04	0.72	0.470
Group type, Mixed	**0.07**	**0.02**	**3.09**	**0.002**
Phase, Prepatent	0.03	0.02	1.14	0.254
Phase, Patent-parasite	−0.04	0.02	−1.64	0.101
Phase, Postparasite	−0.02	0.06	−0.43	0.669
Parasitic status, Infected*Group type, Mixed	**−0.06**	**0.03**	**−2.02**	**0.043**
Parasitic status, Infected*Phase, Prepatent	**−0.09**	**0.03**	**−2.90**	**0.004**
Parasitic status, Infected*Phase, Patent-parasite	**−0.07**	**0.03**	**−2.10**	**0.036**
Parasitic status, Infected*Phase, Postparasite	−0.04	0.04	−1.03	0.302
Group type, Mixed*Phase, Prepatent	−0.02	0.04	−0.63	0.526
Group type, Mixed*Phase, Patent-parasite	−0.01	0.04	−0.35	0.724
Group type, Mixed*Phase, Postparasite	0.02	0.05	0.41	0.683
Parasitic status, Infected*Group type, Mixed*Phase, Prepatent	0.09	0.05	1.67	0.096
Parasitic status, Infected*Group type, Mixed*Phase, Patent-parasite	0.06	0.06	1.07	0.286
Parasitic status, Infected*Group type, Mixed*Phase, Postparasite	**0.07**	**0.03**	**2.04**	**0.041**
(Intercept)	−0.10	0.03	−3.85	<0.001
				AIC = 239877.7
Lying time (night data)				
Parasitic status, Infected	−0.12	0.15	−0.80	0.424
Group type, Mixed	0.14	0.17	0.81	0.419
Phase, Prepatent	**0.43**	**0.11**	**3.87**	**<0.001**
Phase, Patent-parasite	**0.63**	**0.11**	**5.63**	**<0.001**
Phase, Postparasite	**0.30**	**0.11**	**2.71**	**0.007**
Parasitic status, Infected*Group type, Mixed	−0.04	0.25	−0.17	0.862
Parasitic status, Infected*Phase, Prepatent	0.23	0.16	1.42	0.157
Parasitic status, Infected*Phase, Patent-parasite	0.12	0.16	0.72	0.469
Parasitic status, Infected*Phase, Postparasite	0.01	0.16	0.07	0.948
Group type, Mixed*Phase, Prepatent	−0.13	0.18	−0.72	0.470
Group type, Mixed*Phase, Patent-parasite	−0.18	0.18	−0.98	0.326
Group type, Mixed*Phase, Postparasite	−0.35	0.18	−1.90	0.058
Parasitic status, Infected*Group type, Mixed*Phase, Prepatent	0.00	0.28	−0.02	0.988
Parasitic status, Infected*Group type, Mixed*Phase, Patent-parasite	0.08	0.28	0.27	0.786
Parasitic status, Infected*Group type, Mixed*Phase, Postparasite	0.39	0.28	1.41	0.159
(Intercept)	1.01	0.11	9.62	<0.001
				AIC = 19119.3

Table A9 (*continued*)

Fixed effect	Estimate	SE	z	P
Lying time (day data)				
Parasitic status, Infected	−0.03	0.10	−0.25	0.803
Group type, Mixed	−0.02	0.12	−0.21	0.836
Phase, Prepatent	**−0.33**	**0.07**	**−4.77**	**<0.001**
Phase, Patent-parasite	**−0.27**	**0.07**	**−3.88**	**<0.001**
Phase, Postparasite	**−0.62**	**0.08**	**−7.93**	**<0.001**
Parasitic status, Infected*Group type, Mixed	−0.08	0.18	−0.44	0.664
Parasitic status, Infected*Phase, Prepatent	−0.05	0.10	−0.48	0.633
Parasitic status, Infected*Phase, Patent-parasite	−0.03	0.10	−0.26	0.794
Parasitic status, Infected*Phase, Postparasite	−0.01	0.11	−0.11	0.915
Group type, Mixed*Phase, Prepatent	−0.15	0.11	−1.33	0.183
Group type, Mixed*Phase, Patent-parasite	−0.10	0.11	−0.86	0.389
Group type, Mixed*Phase, Postparasite	−0.04	0.13	−0.34	0.735
Parasitic status, Infected*Group type, Mixed*Phase, Prepatent	0.09	0.17	0.56	0.578
Parasitic status, Infected*Group type, Mixed*Phase, Patent-parasite	0.22	0.17	1.29	0.196
Parasitic status, Infected*Group type, Mixed*Phase, Postparasite	0.15	0.19	0.77	0.440
(Intercept)	−0.30	0.07	−4.21	<0.001
				AIC = 38580.2

AIC values are presented from final models. Bold indicates significant results.

Table A10
Model estimates for fixed effects of final generalized linear mixed models on the activity behaviour of infected and noninfected lambs in the mixed and single parasitic state groups

Fixed effect	Estimate	SE	z	P
Motion index				
Parasitic status, Infected	0.001	0.04	0.05	0.957
Group type, Mixed	0.001	0.05	0.00	1.000
Week, 2	−0.01	0.02	−0.49	0.627
Week, 3	**0.05**	**0.02**	**2.34**	**0.019**
Week, 4	**−0.06**	**0.02**	**−2.53**	**0.011**
Week, 5	**−0.12**	**0.02**	**−4.88**	**<0.001**
Week, 6	**−0.12**	**0.02**	**−5.10**	**<0.001**
Week, 7	**−0.18**	**0.02**	**−7.61**	**<0.001**
Week, 8	**−0.20**	**0.02**	**−8.06**	**<0.001**
Week, 9	**−0.30**	**0.02**	**−12.84**	**<0.001**
Parasitic status, Infected*Group type, Mixed	0.06	0.07	0.78	0.437
Parasitic status, Infected*Week, 2	**−0.08**	**0.04**	**−2.29**	**0.022**
Parasitic status, Infected*Week, 3	**−0.07**	**0.03**	**−2.07**	**0.038**
Parasitic status, Infected*Week, 4	**−0.12**	**0.03**	**−3.46**	**<0.001**
Parasitic status, Infected*Week, 5	**−0.04**	**0.04**	**−1.18**	**0.236**
Parasitic status, Infected*Week, 6	**−0.07**	**0.03**	**−2.08**	**0.037**
Parasitic status, Infected*Week, 7	**−0.07**	**0.03**	**−2.17**	**0.030**
Parasitic status, Infected*Week, 8	0.01	0.04	0.31	0.757
Parasitic status, Infected*Week, 9	−0.01	0.03	−0.36	0.717
Group type, Mixed*Week, 2	−0.04	0.04	−0.95	0.341
Group type, Mixed*Week, 3	−0.01	0.04	−0.23	0.820
Group type, Mixed*Week, 4	0.01	0.04	0.29	0.771
Group type, Mixed*Week, 5	0.07	0.04	1.72	0.085
Group type, Mixed*Week, 6	0.03	0.04	0.79	0.428
Group type, Mixed*Week, 7	−0.01	0.04	−0.27	0.787
Group type, Mixed*Week, 8	0.04	0.04	1.00	0.316
Group type, Mixed*Week, 9	0.00	0.04	0.06	0.949
Parasitic status, Infected*Group type, Mixed*Week, 2	0.02	0.06	0.42	0.674
Parasitic status, Infected*Group type, Mixed*Week, 3	−0.01	0.06	−0.18	0.854
Parasitic status, Infected*Group type, Mixed*Week, 4	−0.02	0.06	−0.35	0.727
Parasitic status, Infected*Group type, Mixed*Week, 5	−0.04	0.06	−0.67	0.504
Parasitic status, Infected*Group type, Mixed*Week, 6	0.01	0.06	0.03	0.980
Parasitic status, Infected*Group type, Mixed*Week, 7	0.08	0.06	1.32	0.188
Parasitic status, Infected*Group type, Mixed*Week, 8	−0.09	0.06	−1.53	0.125
Parasitic status, Infected*Group type, Mixed*Week, 9	−0.02	0.06	−0.38	0.707
(Intercept)	5.65	0.03	175.23	<0.001
				AIC =1087331.2
Step count				
Parasitic status, Infected	0.03	0.06	0.55	0.579
Group type, Mixed	−0.01	0.06	−0.22	0.825
Week, 2	0.00	0.03	−0.04	0.970
Week, 3	**0.05**	**0.02**	**2.20**	**0.028**
Week, 4	−0.03	0.03	−1.37	0.172
Week, 5	**−0.06**	**0.03**	**−2.27**	**0.023**
Week, 6	−0.02	0.03	−0.84	0.400
Week, 7	**−0.10**	**0.03**	**−3.68**	**<0.001**
Week, 8	**−0.10**	**0.03**	**−3.63**	**<0.001**

Table A10 (*continued*)

Fixed effect	Estimate	SE	z	P
Week, 9	**−0.20**	**0.03**	**−7.87**	**<0.001**
Parasitic status, Infected*Group type, Mixed	0.05	0.09	0.54	0.587
Parasitic status, Infected*Week, 2	**−0.11**	**0.04**	**−2.71**	**0.007**
Parasitic status, Infected*Week, 3	**−0.08**	**0.04**	**−2.08**	**0.037**
Parasitic status, Infected*Week, 4	**−0.12**	**0.04**	**−3.27**	**0.001**
Parasitic status, Infected*Week, 5	**−0.09**	**0.04**	**−2.36**	**0.018**
Parasitic status, Infected*Week, 6	−0.07	0.04	−1.92	0.055
Parasitic status, Infected*Week, 7	**−0.15**	**0.04**	**−3.82**	**<0.001**
Parasitic status, Infected*Week, 8	0.03	0.04	0.66	0.511
Parasitic status, Infected*Week, 9	−0.04	0.04	−1.11	0.267
Group type, Mixed*Week, 2	**−0.13**	**0.04**	**−2.85**	**0.004**
Group type, Mixed*Week, 3	−0.01	0.04	−0.36	0.720
Group type, Mixed*Week, 4	0.03	0.04	0.66	0.509
Group type, Mixed*Week, 5	0.07	0.04	1.51	0.130
Group type, Mixed*Week, 6	0.03	0.04	0.79	0.428
Group type, Mixed*Week, 7	−0.02	0.04	−0.56	0.574
Group type, Mixed*Week, 8	0.04	0.04	0.96	0.336
Group type, Mixed*Week, 9	−0.01	0.04	−0.18	0.860
Parasitic status, Infected*Group type, Mixed*Week, 2	**0.17**	**0.07**	**2.59**	**0.010**
Parasitic status, Infected*Group type, Mixed*Week, 3	0.001	0.06	0.00	1.000
Parasitic status, Infected*Group type, Mixed*Week, 4	−0.05	0.06	−0.75	0.454
Parasitic status, Infected*Group type, Mixed*Week, 5	−0.02	0.07	−0.26	0.798
Parasitic status, Infected*Group type, Mixed*Week, 6	0.00	0.07	0.01	0.993
Parasitic status, Infected*Group type, Mixed*Week, 7	0.12	0.06	1.80	0.072
Parasitic status, Infected*Group type, Mixed*Week, 8	**−0.15**	**0.06**	**−2.28**	**0.023**
Parasitic status, Infected*Group type, Mixed*Week, 9	−0.03	0.06	−0.41	0.679
(Intercept)	4.11	0.05	78.46	<0.001
				AIC = 818071.6
Frequency of lying bouts				
Parasitic status, Infected	**0.08**	**0.03**	**2.20**	**0.028**
Group type, Mixed	0.03	0.04	0.71	0.476
Week, 2	−0.05	0.03	−1.63	0.104
Week, 3	**0.15**	**0.03**	**5.84**	**<0.001**
Week, 4	**0.09**	**0.03**	**3.42**	**<0.001**
Week, 5	**0.11**	**0.03**	**4.01**	**<0.001**
Week, 6	−0.01	0.03	−0.37	0.709
Week, 7	−0.03	0.03	−0.95	0.341
Week, 8	0.01	0.03	0.26	0.793
Week, 9	**−0.08**	**0.03**	**−3.09**	**0.002**
Parasitic status, Infected*Group type, Mixed	−0.03	0.06	−0.53	0.596
Parasitic status, Infected*Week, 2	−0.06	0.04	−1.52	0.128
Parasitic status, Infected*Week, 3	−0.06	0.04	−1.66	0.097
Parasitic status, Infected*Week, 4	**−0.08**	**0.04**	**−2.15**	**0.031**
Parasitic status, Infected*Week, 5	**−0.08**	**0.04**	**−1.96**	**0.050**
Parasitic status, Infected*Week, 6	−0.07	0.04	−1.68	0.093
Parasitic status, Infected*Week, 7	**−0.15**	**0.04**	**−3.85**	**<0.001**
Parasitic status, Infected*Week, 8	**−0.09**	**0.04**	**−2.32**	**0.020**
Parasitic status, Infected*Week, 9	−0.06	0.04	−1.57	0.116
Group type, Mixed*Week, 2	−0.04	0.05	−0.97	0.330
Group type, Mixed*Week, 3	−0.02	0.04	−0.36	0.718
Group type, Mixed*Week, 4	−0.05	0.04	−1.12	0.263
Group type, Mixed*Week, 5	0.04	0.04	0.96	0.336
Group type, Mixed*Week, 6	−0.03	0.05	−0.68	0.494
Group type, Mixed*Week, 7	**−0.09**	**0.04**	**−2.09**	**0.036**
Group type, Mixed*Week, 8	−0.04	0.04	−0.86	0.388
Group type, Mixed*Week, 9	0.02	0.04	0.38	0.703
Parasitic status, Infected*Group type, Mixed*Week, 2	0.05	0.07	0.73	0.468
Parasitic status, Infected*Group type, Mixed*Week, 3	0.03	0.06	0.55	0.586
Parasitic status, Infected*Group type, Mixed*Week, 4	0.01	0.06	−0.07	0.946
Parasitic status, Infected*Group type, Mixed*Week, 5	0.01	0.06	−0.02	0.981
Parasitic status, Infected*Group type, Mixed*Week, 6	**0.13**	**0.07**	**1.90**	**0.057**
Parasitic status, Infected*Group type, Mixed*Week, 7	**0.18**	**0.07**	**2.70**	**0.007**
Parasitic status, Infected*Group type, Mixed*Week, 8	0.07	0.07	1.03	0.303
Parasitic status, Infected*Group type, Mixed*Week, 9	0.06	0.07	0.98	0.325
(Intercept)	−0.10	0.03	−3.89	<0.001
				AIC = 239521.0
Lying time (night data)				
Parasitic status, Infected	−0.12	0.15	−0.81	0.417
Group type, Mixed	0.14	0.17	0.85	0.394
Week, 2	0.14	0.14	1.01	0.312
Week, 3	**0.36**	**0.14**	**2.60**	**0.009**
Week, 4	**0.77**	**0.14**	**5.41**	**<0.001**
Week, 5	**2.07**	**0.21**	**9.79**	**<0.001**
Week, 6	0.03	0.13	0.22	0.825

Table A10 (*continued*)

| Fixed effect	Estimate	SE	z	P
Week, 7	**0.49**	**0.13**	**3.67**	**<0.001**
Week, 8	**0.27**	**0.13**	**2.11**	**0.035**
Week, 9	**0.33**	**0.12**	**2.71**	**0.007**
Parasitic status, Infected*Group type, Mixed	−0.04	0.25	−0.17	0.863
Parasitic status, Infected*Week, 2	0.12	0.21	0.59	0.556
Parasitic status, Infected*Week, 3	0.16	0.20	0.82	0.410
Parasitic status, Infected*Week, 4	**0.50**	**0.21**	**2.32**	**0.021**
Parasitic status, Infected*Week, 5	−0.02	0.30	−0.05	0.959
Parasitic status, Infected*Week, 6	0.03	0.18	0.15	0.882
Parasitic status, Infected*Week, 7	0.27	0.19	1.39	0.163
Parasitic status, Infected*Week, 8	−0.08	0.19	−0.44	0.660
Parasitic status, Infected*Week, 9	0.10	0.17	0.56	0.575
Group type, Mixed*Week, 2	−0.30	0.23	−1.29	0.197
Group type, Mixed*Week, 3	−0.02	0.23	−0.10	0.922
Group type, Mixed*Week, 4	−0.10	0.23	−0.42	0.675
Group type, Mixed*Week, 5	−0.45	0.32	−1.39	0.163
Group type, Mixed*Week, 6	−0.34	0.21	−1.61	0.107
Group type, Mixed*Week, 7	0.04	0.22	0.17	0.863
Group type, Mixed*Week, 8	−0.32	0.21	−1.51	0.131
Group type, Mixed*Week, 9	−0.39	0.20	−1.96	0.050
Parasitic status, Infected*Group type, Mixed*Week, 2	0.00	0.35	0.00	0.999
Parasitic status, Infected*Group type, Mixed*Week, 3	0.29	0.36	0.79	0.428
Parasitic status, Infected*Group type, Mixed*Week, 4	−0.30	0.37	−0.81	0.418
Parasitic status, Infected*Group type, Mixed*Week, 5	0.03	0.48	0.07	0.944
Parasitic status, Infected*Group type, Mixed*Week, 6	0.15	0.32	0.46	0.646
Parasitic status, Infected*Group type, Mixed*Week, 7	0.12	0.34	0.35	0.726
Parasitic status, Infected*Group type, Mixed*Week, 8	0.51	0.32	1.62	0.105
Parasitic status, Infected*Group type, Mixed*Week, 9	0.28	0.30	0.92	0.360
(Intercept)	1.01	0.10	9.65	<0.001
				AIC =18630.2
Lying time (day data)				
Parasitic status, Infected	−0.02	0.10	−0.16	0.872
Group type, Mixed	−0.04	0.12	−0.35	0.729
Week, 2	−0.14	0.09	−1.57	0.115
Week, 3	**−0.45**	**0.08**	**−5.38**	**<0.001**
Week, 4	**−0.38**	**0.08**	**−4.55**	**<0.001**
Week, 5	**−0.40**	**0.09**	**−4.61**	**<0.001**
Week, 6	**−0.32**	**0.09**	**−3.55**	**<0.001**
Week, 7	−0.10	0.09	−1.16	0.247
Week, 8	**−0.47**	**0.09**	**−4.95**	**<0.001**
Week, 9	**−0.78**	**0.09**	**−8.34**	**<0.001**
Parasitic status, Infected*Group type, Mixed	−0.09	0.18	−0.48	0.630
Parasitic status, Infected*Week, 2	−0.14	0.13	−1.12	0.262
Parasitic status, Infected*Week, 3	0.01	0.12	0.08	0.938
Parasitic status, Infected*Week, 4	−0.04	0.12	−0.37	0.709
Parasitic status, Infected*Week, 5	−0.02	0.13	−0.19	0.853
Parasitic status, Infected*Week, 6	0.04	0.13	0.30	0.765
Parasitic status, Infected*Week, 7	−0.11	0.12	−0.92	0.359
Parasitic status, Infected*Week, 8	−0.08	0.14	−0.61	0.545
Parasitic status, Infected*Week, 9	0.05	0.13	0.39	0.698
Group type, Mixed*Week, 2	−0.02	0.14	−0.15	0.884
Group type, Mixed*Week, 3	−0.09	0.14	−0.67	0.505
Group type, Mixed*Week, 4	−0.27	0.14	−1.96	0.050
Group type, Mixed*Week, 5	0.01	0.14	0.06	0.951
Group type, Mixed*Week, 6	−0.12	0.15	−0.79	0.430
Group type, Mixed*Week, 7	−0.14	0.14	−0.98	0.325
Group type, Mixed*Week, 8	−0.03	0.16	−0.20	0.846
Group type, Mixed*Week, 9	−0.01	0.15	−0.07	0.944
Parasitic status, Infected*Group type, Mixed*Week, 2	0.20	0.21	0.95	0.342
Parasitic status, Infected*Group type, Mixed*Week, 3	−0.02	0.21	−0.11	0.915
Parasitic status, Infected*Group type, Mixed*Week, 4	0.12	0.21	0.59	0.556
Parasitic status, Infected*Group type, Mixed*Week, 5	0.10	0.21	0.45	0.651
Parasitic status, Infected*Group type, Mixed*Week, 6	0.28	0.22	1.28	0.201
Parasitic status, Infected*Group type, Mixed*Week, 7	0.33	0.21	1.57	0.116
Parasitic status, Infected*Group type, Mixed*Week, 8	0.28	0.23	1.21	0.227
Parasitic status, Infected*Group type, Mixed*Week, 9	0.02	0.23	0.09	0.927
(Intercept)	0.30	0.07	−4.09	<0.001
				AIC = 38517.4

AIC values are presented from final models. Bold indicates significant results.
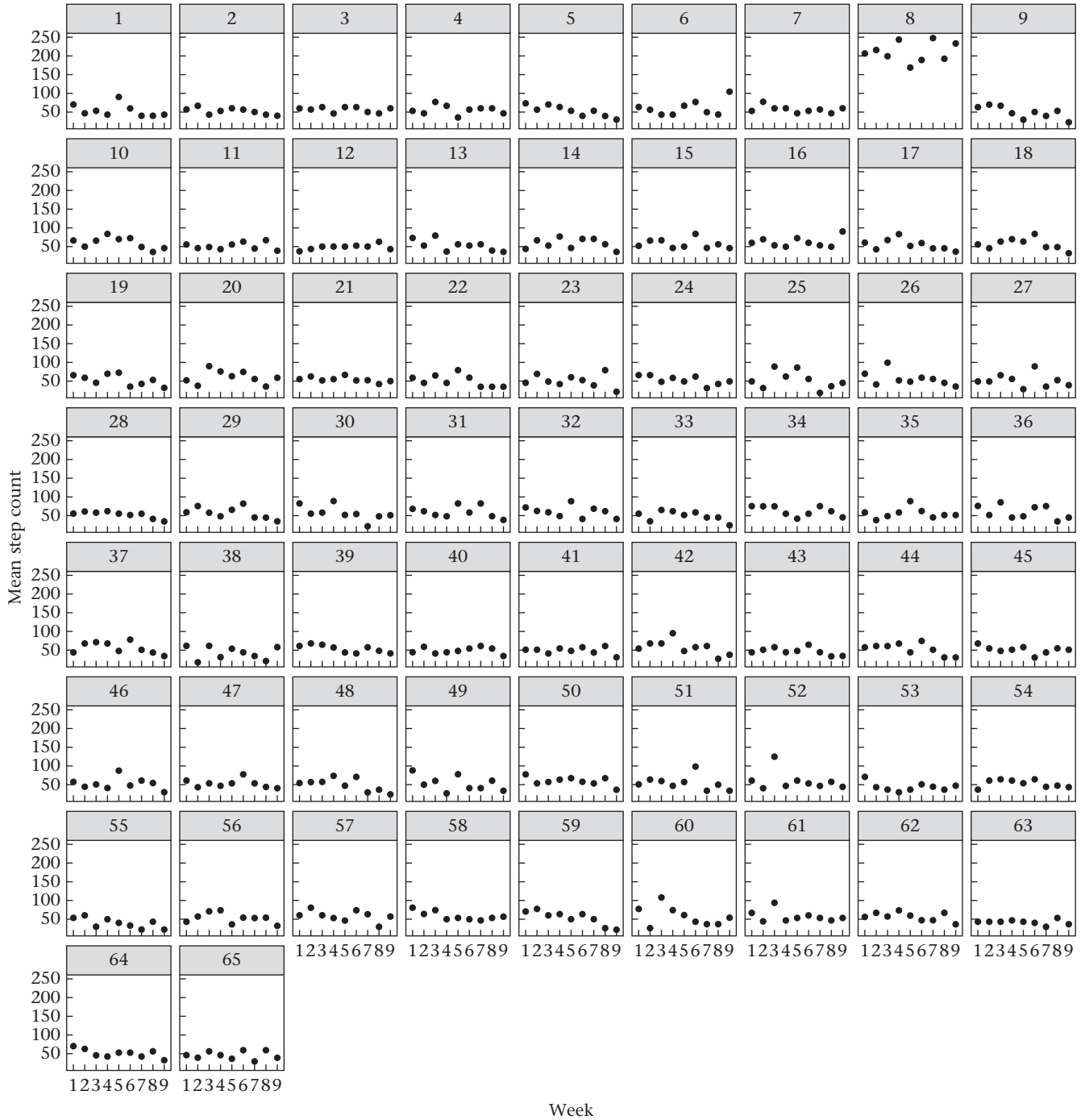
Figure A1. Mean step count recorded by each IceQube (*N* = 65) during each week of the experiment. As one IceQube (IceQube 8) was more sensitive at recording step count than all others and consistently recorded a higher step count each week, IceQube ID was included in the GLMMs for step count as a fixed effect to explain the variance rather than control for it.
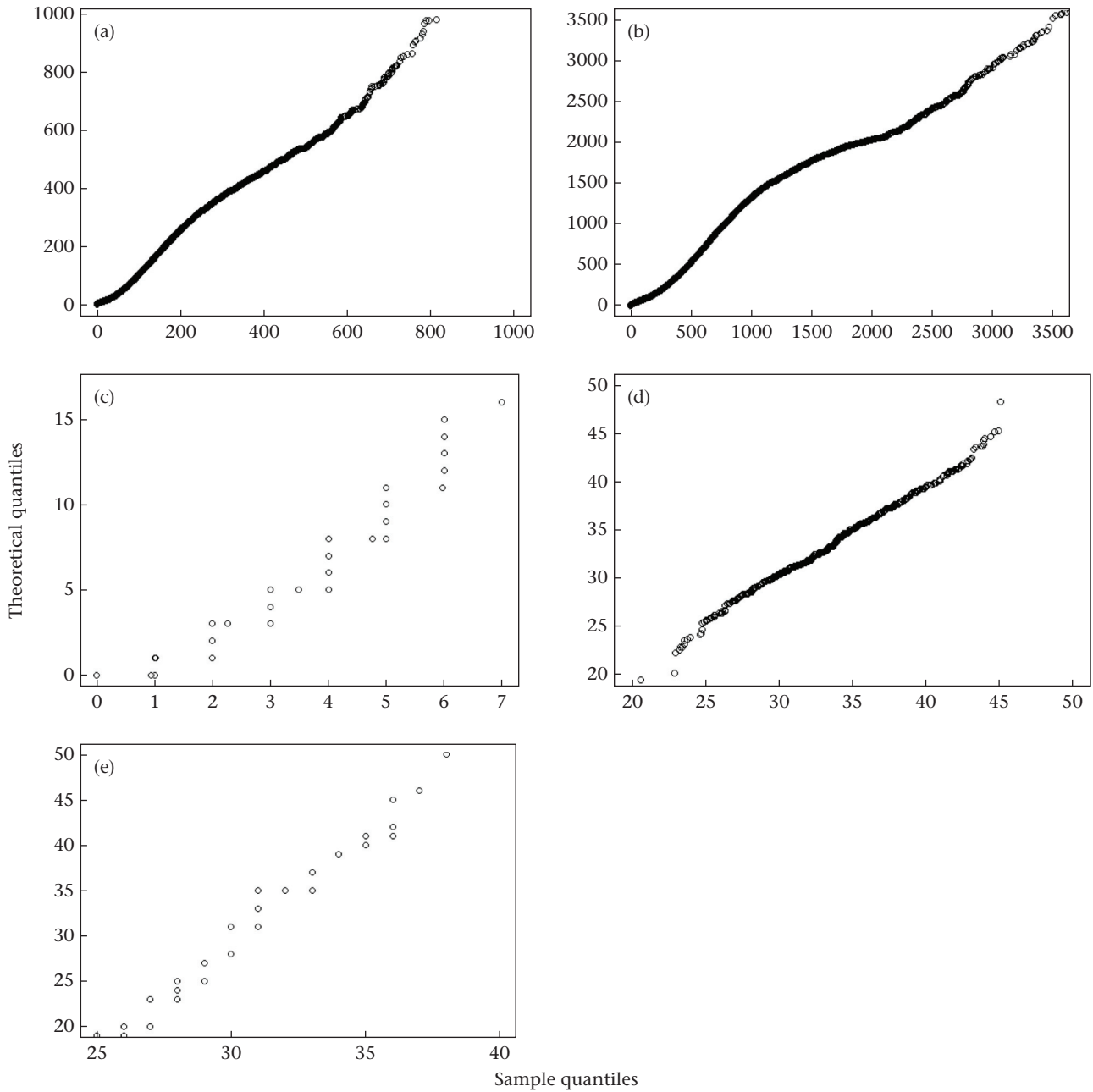
Figure A2. Quantile−quantile plots showing the empirically observed quantiles of (a) step count and (b) motion index as a function of quantiles expected from a negative binomial distribution, (c) lying bouts as a function of quantiles from a Poisson distribution, (d) weight as a function of quantiles from a Gaussian distribution and (e) serum pepsinogen as a function of quantiles from a Poisson distribution.
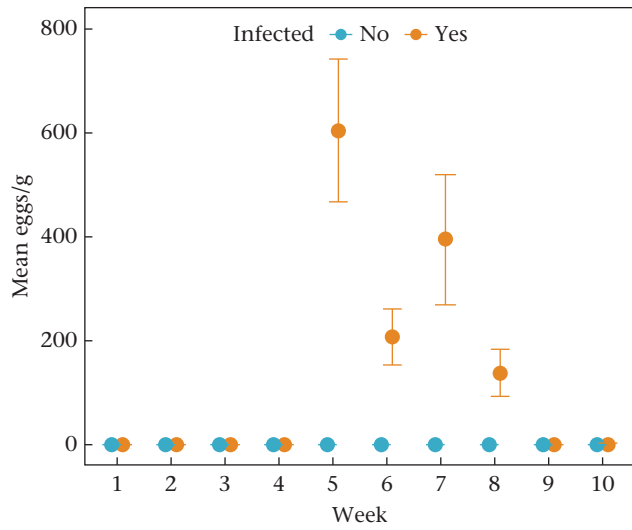
Figure A3. Mean ± SE faecal egg counts (eggs/g) of infected (*N* = 28) and noninfected (*N* = 32) lambs each week of the experiment, including the final sampling day at the beginning of week 10. Lambs were dosed with *T. circumcincta* larvae at the start of week 2 and infections were cleared at the start of week 8 after faecal samples were collected.