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# Early signals of parasitism expressed through changes in host activity and social behaviour

Alexandra Morris

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## Authorship Declaration

I declare that I am the sole author of this thesis. All writing and analyses are my own work, conducted with the help of my supervisors. Lesley Smith, Emma Cunningham, Giles Innocent and Mike Hutchings provided comments on writing, analyses and methodology for the data chapters.

Data for Chapter 2 is comprised of multiple field experiments. I carried out the trials with the assistance of student helpers and technicians. All statistical analyses in this chapter were completed by me.

Data for Chapter 3 and Chapter 4 is comprised of a field experiment and lab work. I carried out the fieldwork with the assistance of student helpers and technicians. I collected all activity and contact behaviour data. I performed all of the faecal egg counts and ELISA assays. All statistical analyses in Chapter 3 and Chapter 4 were completed by me.

Many students and technicians played a role in field data collection and sample processing for fieldwork represented in this thesis. I use the “we” throughout the data chapters because they were written as papers.

Alexandra Morris, 30/09/2021

## Thesis Abstract

Parasites are ubiquitous in the environment and can profoundly impact the health and welfare of their hosts. Infected animals will often exhibit an array of behavioural responses that are termed sickness behaviours. By exhibiting these behaviours, animals can potentially reallocate energetic resources to reduce the severity of infection. However, focusing energetic resources to fight infection could remove resources from other activities that are more beneficial to host fitness. Infected animals may therefore, modulate their behavioural response to infection across different environments including their social environment.

This thesis comprises a series of experimental work in a domestic sheep (*Ovis aries*) system. I first validated two remote monitoring systems (activity monitors and proximity loggers) (Chapter 2) that would be used to record the activity and social behaviour of lambs. The validation work aimed to compare the level of agreement between the behaviours recorded using remote monitoring systems and live focal observations during a series of experiments and evaluate the capabilities of the proximity system to be used in future hypothesis testing. In Chapter 2, I found a positive correlation between live behavioural observations and the data collected by the remote sensors. However, proximity loggers provided a more detailed representation of animal behaviour and could detect subtle changes in behaviour earlier than what could be detected using focal observations.

I then carried out a large-scale field trial to investigate how parasite infection affects the activity behaviour (Chapter 3) and social behaviour (Chapter 4) of groups of lambs of different parasitic status, to understand what stages of infection these behavioural changes occur, and what affect the infection status an individual's social group can have on their behavioural response to infection.

I monitored the activity and social contact behaviour of lambs during four phases of infection (pre-parasite, pre-patent; patent-parasite, post-parasite). Lambs were part of one of three treatments: Parasitised; all lambs were experimentally infected with the gastrointestinal nematode *Teladorsagia circumcincta*, Non-parasitised; all lambs were given a sham infection and dosed with water, Mixed; part of the group were infected with *T. circumcincta,* and part of the group were dosed with water. Faecal samples were taken each week to measure the number of nematode eggs per gram of faeces, blood samples were taken at three time points to measure serum pepsinogen levels to give an indication of gut wall damage and lambs were weighed weekly to measure liveweight gain. Analysis of the animals' measurements (faecal egg counts, pepsinogen levels and weight) demonstrated experimental infection was successful in all cases and lambs to remain parasite free remained clear of parasites throughout the study (Chapter 3 and 4).

In Chapter 3, I found that parasitism affects the activity behaviour of lambs in both single-parasitic state and mixed-parasitic state groups immediately after exposure to parasitism, during the pre-patent phase, three weeks before parasitism could be detected through standardised assessment measures of parasitism and before any noticeable impact of parasitism on physiological measures or condition/weight. However, the extent of this behaviour change was affected by the infection status of an individual's social group. I also show that following treatment with anthelmintic, the behaviour of infected animal's returns to pre-parasite levels, providing further evidence these effects are a direct consequence of parasitism.

In Chapter 4, I found that all individuals in the parasitised groups had reduced contact frequency during the pre-patent, patent-parasite and post-parasite phases, but increased duration of contacts during the pre-patent phase. There was also a reduction in the frequency of contacts in the mixed groups relative to the non-

parasitised groups; however this was driven by a reduction in contacts between infected individuals only, as there was no change in the social contact behaviour between infected and non-infected animals. I also found that although infected animals in mixed-state groups had reduced contact frequency, there was no change in the network architecture of the group as non-infected animals maintained pre-infection levels of social interactions.

These results show that parasitism can affect the activity and social behaviour of infected individuals. However, in mixed-parasitic state groups the parasitic status of other group members can socially modulate the behaviour of both infected and non-infected individuals. Moreover, given the social effects of parasitism and the impact on traits associated with host fitness as well as on behaviour, this research highlights that parasite-mediated behavioural changes can vary due to an individual's social environment. This may have implications for our understanding of how sociality impacts infection across different populations.

## Lay Summary

Parasites are present in all wild and domesticated systems and represent a major problem for the health and welfare of animals. Most individuals will become infected at some stage of their life, and infection can often lead to changes in animal behaviour. These behaviour changes may be a direct consequence of infection or an adaptive response to reduce the costs of infection. The most common behavioural changes include reduced activity levels and feed intake and changes in an individual's social interactions. However, groups of animals often contain individuals of different parasitic status, and parasitism may have indirect effects on the behaviour of non-infected individuals. Understanding the behavioural response of groups of animals that contain individuals of different parasitic status is therefore important to predict the consequences of infection throughout a population.

In this thesis, I investigated the effect infection with a well-known gastrointestinal parasitic worm of domesticated sheep had on the activity and contact behaviour of lambs that were part of groups that differed in proportion of individuals that were infected. Before conducting any experimental work, I first validated two remote monitoring systems (activity monitors and proximity loggers) that would be used to remotely monitor the activity and social behaviour of groups of lambs in future studies. I found a positive correlation between live behavioural observations and the data collected by the remote sensors. However, proximity loggers enabled a more detailed representation of animal behaviour to be collected simultaneously across multiple animals than would be feasible by focal observation and therefore enabled subtle changes to be detected that would not otherwise be possible. I then used standardised experimental infections to test the effect of parasitism within a group on their activity and social contact behaviour (with the previously validated systems) before, during and post-parasite infection. I first tested the effect of infection on activity

behaviour. I found that parasitism reduces the activity behaviour of infected lambs before any other observable effect of parasitism (e.g., weight loss or measures of infection). However, the extent of this change can be affected by the infection status of other animals in their group, showing that an animal's social environment is important in how they deal with infection. I next tested the effect of infection on social contact behaviour of lambs in single and mixed parasitic-state groups. I found that parasitism reduces the frequency of contacts between infected lambs in both mixed and single-parasitic state groups at the earliest stages of infection, but the degree of behaviour change exhibited by infected animals is influenced by the parasitic status of other individuals within the group. I also found that although infected animals in mixed groups had reduced contact frequency, non-infected animals maintained pre-infection levels of social interactions with their infected groupmates. Overall, my thesis demonstrates that parasitism can affect the behaviour of infected individuals and that these behavioural changes can occur at the earliest stages of infection, but the level of behaviour change may be modulated by the infection status of an individual's social group.

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# Chapter 1

## 1. General Introduction

Parasites defined broadly here to include both macroparasites (i.e. helminths, ectoparasites) and microparasites (i.e. bacteria, viruses, protozoans) are ubiquitous in the environment and are a major problem for the health and welfare of wild and domesticated animals (Poulin, 1999; Marcogliese, 2004; Hudson et al. 2006; Lafferty et al. 2006; Charlier et al. 2014). As 40% of species on the planet are parasites (Dobson et al. 2008), almost all animals will inevitably become infected at some stage in their life. As a result, many hosts will alter their behaviour to reduce parasite exposure, or limit the severity of infection (Moore, 2002). These behavioural alterations in response to infection play an important role in mediating the effects of infection and are considered the first line of defence against parasitism (Hart, 1990).

Behaviour strategies in response to parasitism are used to avoid, control, or remove parasites from the host. Grooming and preening behaviours are used to remove ectoparasites from the hosts body (Hart, 1990). Head tossing, ear twitching and tail switching are fly-repelling behaviours used to reduce the number of bites from blood feeding ectoparasites (e.g. biting flies) and changing feed intake or exploiting new habitats and social groups, is an effective way to reduce parasite exposure (Hart,

2011). However, these behaviours do not serve to remove the risk of infection completely, and animals do succumb to infection at some stage in their life.

When animals become infected, they will often exhibit an array of behaviour changes during illness. There behaviour changes can occur as a result of parasite manipulation of host behaviour in ways that favour parasite transmission (Klein, 2003; Doherty, 2020). In contrast, infections can induce sickness behaviours by the host, such as, reduced activity, reduced feed intake, increased somnolence and changes in sociality (Hart, 1988; Kyriazakis et al. 1998; Ghai et al. 2015; Lopes et al. 2021). These sickness behaviours are believed to divert energy to immune responses or promote host tolerance to infection (Hart, 1988; Kelley et al. 2003; Medzhitov et al. 2012). Benjamin Hart (1988) was the first to suggest that sickness behaviours are potentially adaptive, enabling animals to reallocate energetic resources to fight infection. However, redirecting resources to fight infection, could remove resources away from other important activities (Lopes et al. 2012) such as foraging, territorial defence and mating. Expressing behavioural changes may be particularly important for social species where the expression of sickness symptoms may remove them from their social group, and thus the associated benefits of group living (Loehle, 1995). Therefore, under certain environmental conditions infected hosts may modulate the degree to which sickness behaviours are expressed.

This introductory chapter begins with an overview of the ways in which parasitism affects the behaviour of animals. I then describe the potential adaptive benefits of these behaviours, the ways in which behavioural changes may have evolved to reduce the severity of infection, and also the impact an individual's environment can have on their behavioural response to infection. I then discuss the costs and benefits of social group living, the impact of parasitism on social behaviour and the costs and benefits of exhibiting changes in sociality in response to infection when part of a social

group. Lastly, I introduce my study system, domesticated sheep *Ovis aries and* the gastrointestinal nematode *Teladorsagia circumcincta,* discuss the direct benefits of using behaviour change as an indicator of infection in domestic systems and outline the aims of this thesis.

## 1.1 Parasitism and the behaviour of animals

### 1.1.1 Parasite manipulation of host behaviour

Parasitism modifying the behaviour of animals is well documented across almost all animal taxa (Poulin, 1994; Moore, 2002), but also see Doherty, (2020). Behaviour change as a response to parasitism can occur for a variety of reasons. Parasites may manipulate host behaviour to enhance their own fitness (Moore, 2002). Host manipulation of parasite transmission is defined as any alteration in host phenotype that has fitness benefits to the parasite (Poulin, 2010). Although, there are many examples of host manipulation, in general infected hosts behave in a manner that facilitate the transmission or dispersal of the parasite, and thus the completion of the parasite's life cycle. For example, the brain worm, *Dicrocoelium dendriticum*, is a species of trematode that must be transmitted by ingestion from an ant to a sheep. The trematode causes infected ants to climb to the tip of grass blades, bite hard and wait for a grazing sheep (Carney, 1969). *Leucochloridium* is another species of trematode that alters the size, shape and colour of the tentacles of its snail intermediate host, causing them to pulsate violently in response to light, attracting the attention of birds to which the parasite must be transmitted to next (Wesołowska and Wesołowski, 2014). Many arthropod parasite vectors have also been shown to suffer from parasite interference. Bacot and Martin first noticed that fleas harbouring the bacterium responsible for the plague exhibited blocked proventriculi that limited their success in blood feeding (Bacot and Martin, 1914). This blockage led to increased

plague transmission. Since then, many arthropod vectors have been shown to suffer from parasite interference in hematophagy that could well increase parasite transmission to vertebrate hosts (Moore, 2002; Rogers and Bates, 2007).

The potential for parasite manipulation is not limited to arthropod hosts. The protozoan parasite *Toxoplasma gondii*, which must be transmitted from a rat intermediate host to a cat definitive host, causes infected rats to be attracted to cat odour (Berdoy et al. 2000). Humans can also become infected with *T. gondii*. Although this is a dead end for parasite transmission, *T. gondii* nevertheless induces neurochemical changes in humans, and people with *T. gondii* infections often show different personality traits and reaction times than those that are uninfected (Flegr et al. 2008; Sugden et al. 2016).

### 1.1.2 Behavioural avoidance of parasite infection

Almost all aspects of an animal's behaviour is associated with exposure to some type of parasite (Ezenwa et al. 2016b). For instance, mating behaviours increase the transmission of sexually transmitted diseases between animals (Thrall et al. 2000), foraging is a major route of infection for environmental transmitted parasites and social behaviour contributes to the transmission of various directly transmitted parasites (Altizer et al. 2003). However, behaviour can also play a role in how hosts defend themselves against parasitism, and alterations such as avoidance behaviours are thought to be the first line of defence against parasite infection (Hart, 1990; Hart, 1994).

Parasite avoidance can occur through mate selection (Hamilton and Zuk, 1982), habitat movement (Poulin and Fitzgerald, 1989), the formation of selfish herds (animals in larger groups receive fewer fly bites (Cote and Poulin, 1995; Moore, 2002)), changing postures (animals can minimise body surface area exposure to reduce

ectoparasite infestation (van Riper et al. 1986)) and by exhibiting behaviours such as fly swatting and grooming (to reduce bites from ectoparasites and parasite vectors (Dudley and Milton, 1990; Clayton, 1991)). Parasite avoidance behaviour may not always be exhibited by potential susceptible hosts. In some eusocial insect species, there are examples of infected hosts isolating themselves away from their social group to avoid infecting other members of the group. For example, cold seeking behaviour by conopoid fly infected bees slows down parasite development, so each night infected individuals move outside the nest into lower temperatures (Müller and Schmid-Hempel, 1993). It is thought these behaviours not only retard parasite growth but ensures the larvae living inside the bees cannot infect the siblings of the bee they are infecting.

Susceptible hosts may also avoid parasitism by recognising and avoiding infected conspecifics. Targeted avoidance of diseased individuals is an effective method in minimising the exposure to directly transmitted parasites (Loehle, 1995). Evidence of avoidance behaviours have been documented across many taxa, including frogs (Kiesecker et al. 1999), rats (Kavaliers et al. 2004), lobsters (Behringer et al. 2006), fish (Croft et al. 2011) and birds (Zylberberg et al. 2013) that use either chemical or visual cues to avoid contact with infected individuals. However, avoidance of diseased conspecifics does not always occur. Banded mongooses with clinical sings of tuberculosis were not avoided by healthy conspecifics (Fairbanks et al. 2015), and house finches demonstrated a preference for feeding near individuals that were visibly infected with transmissible conjunctivitis (Bouwman and Hawley, 2010). Bottlenose dolphins had a higher association with conspecifics that were visibly infected with tattoo skin disease than those that were not (Powell et al. 2020). What determines whether healthy animals avoid their infected conspecifics is currently unknown, but it is thought to be associated with parasite virulence, mode of transmission, and the

benefits of expressing that behavioural alteration to host fitness. For instance, if parasitism affects competitive ability, then the benefits of lower competition for resources might outweigh any risks of infection.

## 1.2 Sickness behaviours as an adaptive response to infection

Changes in host behaviour may also result from immunological or pathological consequences of infection (Ezenwa et al. 2016b). Such behaviour changes do not benefit the parasite, but rather benefit the host by minimizing the severity of infection. These behavioural changes are termed 'sickness behaviours', a term used to describe the collective suite of behaviours exhibited by infected animals in response to infection (Hart, 1988; Bilbo et al. 2002; Moore, 2002; Kelley et al. 2003; Ayres and Schneider, 2009). Stereotypical behaviours include reduced feed and water intake, reduced activity levels, changes to grooming and exploratory behaviours, increased somnolence, decreased libido and changes to an individual's sociality (Ayres and Schneider, 2009; Lopes et al. 2012; Hawley et al. 2021).

Sickness behaviours have been reported in almost all animal taxa (e.g., mammals (Murray and Murray, 1979; Bilbo et al. 2002; Stockmaier et al. 2018), birds (Owen-Ashley and Wingfield, 2006; Lopes et al. 2012), reptiles (Garrido and Pérez-Mellado, 2014), amphibians (Rollins-Smith and Woodhams, 2012; Rakus et al. 2017), fish (Kirsten et al. 2018) and insects (Ayres and Schneider, 2009)). The behavioural response of most species falls into one of the classic stereotypical behaviours such as anorexia, reduced activity levels and social isolation, however, there are also some species-specific behaviour changes, such as increased sociality, as shown in rhesus monkeys following a lipopolysaccharide (LPS) challenge (Willette et al. 2007). This change in behaviour of rhesus monkeys occurred when animals received a low dose of LPS and is thought to be motivated by young animals seeking out comfort from a

familiar companion. Animals overcoming sickness behaviours in response to their social environment is something that has been previously reported (Aubert 1997; Cohn and de Sá-Rocha, 2006). However, it is also something that is believed may be overcome by higher doses of infection, as shown in the rhesus monkey system where individuals that received a higher dose of LPS had reduced social interactions with their groupmates.

Sickness behaviours were once assumed a result of debilitation of the host and were simply a consequence of infection serving no major function. However, in 1988, Hart first proposed the idea that sickness behaviours may be an adaptive response by the host to fight infection and increase host survival. Fever typically accompanies sickness behaviours in endotherms. Whereas, in species that cannot produce a fever physiologically (i.e., ectotherms), sickness behaviours can involve a behavioural fever whereby animals move to warmer environments to elevate core body temperature (Rakus et al. 2017). According to Hart's theory, sickness behaviours contribute to self-preservation through reallocation of energy from activities such as foraging and grooming, into components of the immune response. This can eventually lead to animals becoming anorexic and having reduced ingestion of nutrients that are considered essential for pathogen growth. Since then, many studies in different vertebrate and invertebrate have shown that hosts have increased survival or reduced pathogen growth when they exhibit a behavioural response to parasite infection (Murray and Murray, 1979; Müller and Schmid-Hempel 1993; Boltaña et al. 2013; Sauer et al. 2019). Research of sickness behaviours in livestock has also been of interest given the economic implications of disease outbreaks on farms. However, this research has highlighted the difficulty that animals may have in displaying sickness behaviours, such as self-isolation or reduced activity when maintained in high densities (Proudfoot et al. 2012).

Sickness behaviours have also been hypothesized to serve as a signalling function to other group members (Tiokhin, 2016) or to have evolved to protect kin (Shakhar and Shakhar, 2015). These hypotheses were proposed because host survival theory does not explain such behaviours as anorexia. If activating an immune response is energetically costly, it seems counterproductive to simultaneously reduce caloric intake during illness. However, it is argued that the induction of anorexia occurs at a time when the body needs an increase in energy to support the demand from the immune system (Kyriazakis et al. 1998; Kelley et al. 2003; Dantzer, 2004). It has been proposed that this reduction in appetite is to help the host fight off infection to optimize the immune response (Kyriazakis et al. 1998). Allowing the individual to overcome its sickness by adjusting interactions between the immune system and other physiological processes (Murray and Murray, 1979; Hart, 1988; Kyriazakis et al. 1998; Ayres and Schneider, 2009; Adamo et al. 2010). This was demonstrated by Murray and Murray (1979), who force fed a proportion of mice infected with the bacteria *Listeria monocytogenes* to the same level of uninfected controls, whilst allowing the other infected mice to feed ad libitum. The infected mice that were allowed to regulate their own feed intake consumed 58% the amount of food as uninfected mice and were more likely to survive than the force-fed individuals.

It has also been stated that the onset of sickness behaviours such as anorexia could allow animals to be more selective in their diet reducing the risk of further infection (Kyriazakis et al. 1998). This was demonstrated by Hutchings et al. (1998) who found sheep infected with helminths selected a higher proportion of their diet from clean sward patches instead of heavily contaminated sward patches. Such diet selection also has the benefit of allowing infected animals to select for certain foods that contain specific anti-parasitic compounds (Githiori et al. 2006; Tzamaloukas et al. 2006; Athanasiadou et al. 2007). Self-medicating behaviour has been well documented in

chimpanzees (Huffman, 1997), but has also been reported in some species of caterpillar (Karbant and English-loeb, 1997). In relation to the kin protection hypotheses, withdrawal from social environments and reduced feed and water intake, could be favored for kin selection, as these behaviours would reduce social interaction and the likelihood of transmission. However, there has been limited research around this area, and one study testing this hypothesis in a wild mouse system (*Mus domesticus*) found no support for it (Lopes et al. 2016).

### 1.2.1 Environmental context modulates sickness behaviours

During an infection, most animals will react by having reduced activity levels, reduced feed intake and show changes in their social interactions (Hart, 1988; Kelley, 2003). Although these sickness behaviours could enhance the chance of recovery, life-history theory predicts that under certain environmental circumstances individuals may supress the expression of such sickness behaviours, even if it is detrimental to an individual's own health (Friedman et al. 1996; Aubert et al. 1999; Bilbo et al. 2002; Cohn and de Sá-Rocha, 2006; Owen-Ashley and Wingfield, 2006; Weil et al. 2006). For example, when lactating mice were exposed to life-threatening conditions (low ambient temperatures), dams that had received LPS injection to induce sickness behaviours, were able to suppress sickness symptoms and maintain nest building behaviour (Aubert, 1999), therefore investing in offspring over their own health. In song sparrows, the effect of LPS injections was less noticeable during the breeding season than during other parts of the year (Owen-Ashley and Wingfield, 2006). In male Siberian hamsters (*Phodopus sungorus*), the duration of sickness behaviours were reduced during short day lengths compared to long day lengths (Bilbo et al. 2002), increasing survival rate by reducing energy expenditure during times of energy shortage (winter). There has also been research into how an individual's social environment may influence the extent to which sickness behaviours are expressed.

For gregarious species, the expression of sickness behaviours could lead to a loss of social position, mating opportunities and associated fitness benefits of group living (Lopes et al. 2012). This behaviour was reported by Lopes et al (2012), who found Zebra finches (*Taeniopygia guttata*) challenged with an LPS injection kept in isolation had markedly reduced activity compared to individuals kept in a colony setting (Lopes et al. 2012). Demonstrating that in a social setting, sickness behaviours might be reduced, or masked, to allow for the participation in behaviours that are more beneficial to host fitness. As not expressing sickness symptoms can have severe costs to host health (Murray and Murray, 1979), the extent to which a given host exhibits a particular behaviour will depend on parasite virulence/load (Stephenson, 2019; Powell et al. 2020), mode of transmission and the benefit of that behaviour to host fitness (Ezenwa et al. 2016b).

## 1.3 Social group living and parasite infection

Living in social groups is considered one of the major transitions in evolution (Szathmáry and Smith, 1995) that resulted in the formation of permanent groups of variable size, composition and stability (Krause and Ruxton, 2002). However, living in social groups comes with both costs and benefits. Advantages include reduced predation risk and increased opportunities for cooperation, such as defence of shared resources including food, territory and offspring (Bertram, 1978; Mosser and Packer, 2009; Cornwallis et al. 2010). Whereas the costs of social group living are increased susceptibility to disease (Capitanio et al. 1998; Sapolsky et al. 2000), and increased risk of parasite transmission (Freeland 1976; Anderson and May 1979; Loehle, 1995).

Increased susceptibility to disease is believed to be a consequence of stress induced by social living. As sociality is often associated with hierarchal systems, when resources are low, food intake may be reduced for individuals of lower rank in the

social group (Ceacero et al. 2012). For some group members, continuous low social ranking status often coincides with social conflict which can result in chronic stress (Goymann and Wingfield, 2004; Rubenstein, 2007). Thus, physiological consequence of social stress in combination with poor resources can affect individual susceptibility to disease.

The second and biggest cost associated with social group living is increased risk of parasite transmission (Freeland, 1976; Anderson and May, 1979; Loehle, 1995). Group living mammals, birds and insects are known to have higher infection levels than solitary species (Tella 2002; Ezenwa, 2004). While the transmission route of infection is often parasite-specific and can depend on social contact, the risk of social transmission is increased in group living animals compared to solitary species because of the spatio-temporal concentration of potential hosts. However, as there is variation in group size, frequency and type of social contact there are variabilities in the probability of parasite transmission.

Group size is the most widely used metric to capture the effects of social behaviour on parasite transmission as it provides an intuitive proxy for the number of social interactions an individual experiences. Although the strength of association often varies depending on the measure of parasitism (e.g., abundance, prevalence) and mode of transmission (e.g., direct, faecal-oral), there is a large body of work on the relationship between group size and parasitism, that shows group size is an important predictor of parasite transmission (Cote and Poulin, 1995; Altizer et al. 2003; Patterson and Ruckstuhl, 2013). However, this work also reveals that group size alone does not take into account many factors about animal social organization that are important for parasite transmission. For instance, some individuals may change groups frequently which can affect parasite transmission (Ezenwa 2004; Griffin and

Nunn, 2012), and recently it was reported that group size may only explain some of the variance associated with parasite transmission (Briard and Ezenwa, 2021).

As not all aspects of social group living increases the risk of infection, parasite transmission can depend on the type of social interaction between hosts and transmission mode of the parasite (Craft, 2015). The limitations of group size as a metric of true social interactions and the recent advances in computer power, have added to the rise of social network analysis (SNA) as a tool for understanding the associations between social behaviour and parasite transmission (Craft, 2015; Briard and Ezenwa, 2021). SNA provides a powerful tool to understand patterns of social interactions and has been applied to a number of systems to understand the patterns of parasitism across a population (Godfrey, 2013; White et al. 2017). For example, the frequency of contacts among Tasmanian devils (*Sarcophilus harrisii*) predicted their probability of contracting facial cancer through biting (Hamede et al. 2009). Whereas the type and direction of social contact was important for understanding the transmission of tuberculosis *Mycobacterium bovis* in Meercats (*Suricata suricatta*)*, as* transmission increased during aggressive interactions and not during grooming bouts between conspecifics (Drewe, 2010). Thus, it is now thought that characteristics of a group's social network and how individuals within the group are interacting with each other might be more important for controlling and predicting parasite transmission (Briard and Ezenwa, 2021).

### 1.3.1 Parasitism influences the social behaviour of infected hosts

Social behaviour has long been recognized as a factor that can aide in parasite transmission (Loehle, 1995), and host species that exhibit social behaviours are hypothesized as being at a greater risk of contracting parasite infections (Krause and

Ruxton, 2002). However, there is another aspect of this relationship that has received relatively less attention, and that is how parasitism can alter the social behaviour of infected hosts (Hawley et al. 2021).

Changes in host behaviour during infection can reduce the degree of interactions with other conspecifics and can potentially reduce the spread of socially transmitted parasites, especially if the change in sociality occurs during the infectious period of infection (Hawley et al. 2021). For example, Lopes et al. (2016), injected wild house mice *Mus musculus domesticus* with bacterial endotoxin to stimulate sickness behaviours, and found immune activation reduced activity levels resulting in fewer social interactions between group members. Tasmanian devils with facial tumours decreased social interactions as tumour load increased (Hamilton et al. 2020), and TB test-positive badgers were more socially isolated from their own groups (Weber et al. 2013b).

As with other sickness behaviours, the extent to which infected hosts alter their social behaviour may depend on the energetic costs of infection and the importance of that social behaviour for maintaining host fitness (Ezenwa et al. 2016b). As a result, infected hosts may not always reduce their social interactions with other members of the group. In some systems hosts social behaviours may be maintained during infection (Powell et al. 2020) which is thought to be associated with low parasite virulence. There are also systems where infected animals may maintain social interactions with a subset of the group (Poirotte et al. 2017), for example, vampire bats injected with an endotoxin to induce sickness behaviours continued to groom close kin to remove ectoparasite infestations but reduced the extent to which they groomed non-kin (Stockmaier et al. 2020). Given the importance of host social behaviours for parasite transmission understanding the ways in which parasites and

host social behaviours interact is therefore critical for predicting parasite evolution (Schmid-Hempel, 2017), and disease dynamics (Ezenwa et al. 2016a).

### 1.3.2 Parasitism influences the social behaviour of uninfected hosts

Parasitism can also alter social interactions by changing the behaviour of uninfected hosts towards their infected conspecifics. In some systems, infected or immune activated hosts may display visual or release chemical cues that infected hosts use to avoid them (Anderson and Behringer, 2013; Zylberberg et al. 2013), or to remove them from the colony (honeybees (*Apis mellifera*) (Baracchi et al. 2012). However, avoidance of infected conspecifics does not always occur, more so when there is a high degree of relatedness between groupmates. For example, some social insects are known to care for infected conspecifics (Cremer et al. 2018). Similarly, Mandrills (*Mandrillus sphinx*), reduced frequency of grooming bouts (to remove ectoparasites) towards infected partners that were not related but maintained grooming contagious partners if they were offspring or close maternal kin (Poirotte and Charpentier, 2020). However, even in systems where groupmates are not closely related, uninfected individuals often maintain social interactions with their infected conspecifics. For example, non-infected vampire bats continued to share food with their infected conspecifics that were visibly exhibiting sickness behaviours (Stockmaier et al. 2020). Mongoose (*Mungos mungo*) and vampire bats (*Desmodus rotundus*) continued to groom visibly diseased conspecifics even when allogrooming reciprocity from these infected individuals was reduced (Fairbanks et al. 2015; Stockmaier et al. 2018). There are also systems were uninfected individuals have been reported to increase social interactions after their conspecifics became infected. Uninfected male house finches (*Haemorhous mexicanus*) preferentially chose to feed near groupmates that were visibly infected with conjunctivitis (Bouwman and Hawley, 2010), and uninfected

mice had increased social contact with their infected groupmates (Edwards, 1988). Bouwman and Hawley, (2010) suggested male house finches used the behavioural changes in their conspecifics as an opportunity to assert dominance over their infected groupmates. Whereas Edward's (1988) suggested the increase in sociality of uninfected mice occurred simply through social exploration following a change in behaviour of their infected conspecifics.

The mechanisms underlying these patterns of behaviour between infected and uninfected hosts remain unknown, but it has been suggested that the maintenance of some social interactions during infection may be a form of tolerance, allowing hosts to reduce the impact of infection (Ezenwa et al. 2016b). For example, Ezenwa and Worsely-Tonks (2018) suggested that association with larger groups benefited grazing Grants gazelles (*Nanger granti)*, allowing them to reduce the cost of infection-induced anorexia. Moreover, given that infected hosts experience higher predation risk and anorexia, gregariousness may be a common form of tolerance in many taxa (Alzaga et al. 2008; Stephenson et al. 2016). Understanding how animals balance the costs and benefits of sickness behaviours across different social environments, will help understand the conditions in which parasitism induces changes in behaviour, which in turn will enhance the understanding of how infections are likely to spread across populations.

## 1.4 Study system (*Ovis aries* and *Teladorsagia circumcincta*)

In this thesis, I investigate the effect of parasitism on the behaviour of social groups of lambs and the impact an individual's social environment may have on their behavioural response to infection. Domestic sheep (*Ovis aries*) are an ideal model organism to answer the aims and proposed questions in this thesis, as they are naturally gregarious animals that form stable social bonds with members of their group

(Keller et al. 2011). Previous work has also shown that although dominant behaviour can occur in some breeds of domestic sheep (Lynch et al. 1992), dominant hierarchies are not commonly formed amongst groups of ewes and weathered animals of similar ages (Fischer and Mathews, 2001), and so they are easy to manipulate into replicated social groups for hypotheses testing in an experimental framework.

The species of nematode used to experimentally infect the lambs was *Teladorsagia circumcincta*. *T. circumcincta* is an abomasal gastrointestinal nematode that represents a major parasitic infection of sheep (Coop et al. 1982; Burgess et al. 2012). *T. circumcincta* is a predominantly resistant species of gastrointestinal nematode, with some strains found to be resistant to all classes of anthelmintic (Bartley et al. 2004; Sargison et al. 2007), making *T. circumcincta* one of the most economically important gastrointestinal nematode species of sheep worldwide (Papadopoulos et al. 2012; Venturina et al. 2013; Charlier et al. 2014).

The pathology of *T. circumcincta* infections is associated with the larvae developing in the abomasal gastric glands damaging the gut's ability to digest food. Infected animals suffer with dehydration, diarrhoea, loss of body condition and anaemia (Coop et al. 1982), and as infections develop lambs suffer with anorexia (Kyriazakis et al. 1998). Anorexic sheep have reduced production efficiency due to reductions in voluntary feed intake and reduced utilisation of feed efficiency (Coop, 1996; Mavrot et al. 2015). This means they often remain on the farm for longer periods of time, and as a result have increased greenhouse gas emissions (Thornton, 2010; Houdijk et al. 2017).

*T. circumcincta* has a direct life cycle, meaning the parasite lives the majority of its life and reproduces within one host. The life cycle of *T. circumcincta* comprises of two main phases – an adult stage of males and females who reproduce within the host and a free-living egg and three larval stage phase which occurs in the environment

(Wood et al. 1995). Adult worms live in the abomasum of the gut. Worms reside, mate and females shed eggs into the environment via faeces (between 0-350 per day) (Stear et al. 1999). Under optimal temperature and humidity the eggs develop on pasture through first (L1), second (L2) and third (L3) stage larvae. Third stage larvae are unable to feed and are the infective stage. L3 stage larvae can survive on pasture without feeding for up to 12 months. Under optimal conditions, of high humidity and high temperature the developmental process from eggs to L3 stage larvae requires 7-10 days (Stear et al. 1999). Sheep then encounter the L3 infectious stage larvae on pasture whilst grazing and infection occurs upon ingestion. Once in the host, L3 stage develop within 48 hours into fourth stage larvae (L4) in the gastric glands, which then develop into young adults, mature and breed (Stear et al. 1995). *T. circumcincta* goes from egg to egg (full life cycle) within 25-31 days, although this can vary dependent on environmental conditions.

Transmission of *T. circumcincta* is driven by an infected host shedding eggs produced by adult female worms within the environment via faeces following ingestion of infectious larval stages. Following ingestion, there is a pre-patent period that usually lasts between 17-21 days (Wood et al. 1995) during which hosts are not showing any physiological or pathological symptoms of infection and are not shedding any eggs. Infections are usually diagnosed through changes in live weight, dag scoring (Dagginess is a subjectively, visually assessed trait using a 6-point scale: zero (no dagginess) to five (complete coverage of the breech and down the legs by faecal material)) (Morris et al. 1995) and confirmed through faecal egg counts. Lambs with high levels of infections are currently treated with anthelmintics.

## 1.5 Gastrointestinal nematodes in domestic sheep

There are direct applications in using domestic sheep as a model organism to answer the proposed questions. Early detection of behaviour change that can be associated with gastrointestinal parasitism has potential to be used as a non-invasive tool to identify and treat only infected individuals in domesticated systems, reducing the amount of anthelmintic going into domestic systems (Kenyon, et al. 2009). Targeted control strategies have proven to reduce the impact of parasitism and slow down the rate of anthelmintic resistance in domestic systems (van Wyk, 2001; Soulsby, 2007). This method aims to treat only individuals within a group based on a biological indicator of infection (Vercruysse and Claerebout, 2001; Kenyon et al. 2009; Kenyon and Jackson, 2012). A number of indicators have been used to identify infected animals, including faecal egg counts, anaemia, dag scores, body condition and reduced weight gains (van Wyk and Bath, 2002; Kenyon, et al. 2009; Stafford et al. 2009). Although it is possible to identify infection using these methods, the time taken between identifying the infected sheep and retrieving and testing the sample means there has already been a loss in production and a reduction in the welfare of the animals (Leathwick et al. 2006). Thus, there is an interest within the sheep industry in using behaviour change as a non-invasive early indicator of infection that would be used to identify and treat only animals exhibiting behavioural signals of parasitism. Furthermore, as most animals may not show clinical signs of disease during the early stages of gastrointestinal nematode infection (Miller et al. 2012) there is potential for behavioural indicators to identify infected animals before the animal progresses into a clinical stage of infection, improving the welfare and success of parasite management programs.

### 1.5.1 Parasite infection model

Lambs in the study were experimentally infected with L3 stage *T. circumcincta* larvae that were cultured using a parasite infection model. The culture process involved four 12-week-old male donor lambs that were experimentally infected with 15,000 L3 stage *T. circumcincta* larvae (Wood et al. 1995). The donor lambs were housed indoors from birth and were considered parasite naïve. Lambs were kept indoors throughout their infection in two 8x4m pen (two lambs in each pen), were given ad lib access to water and fed silage and concentrate twice per day.

After 21 days, lambs were fitted with a body harness to catch faecal materials in an attached bag. Faeces were collected and incubated for a 10-day period at 20°C. Larvae were then collected from the faeces using a standard Baerman procedure (Cabaret et al. 1980) and stored in tap water (1000 L3/ml) at 4°C until administered to experiment lambs orally. The strain used to initially infect the donor lambs was the Moredun ovine anthelmintic susceptible strain. This infection model has repeatedly been used to induce sub-clinical parasitism in sheep (Houdijk et al. 2003; Houdijk et al. 2006; Zaralis et al. 2009; Kidane et al. 2010; Houdijk et al. 2017).

## 1.6 Aims of this thesis

The primary research question of this thesis was: What is the effect of parasitism on the behaviour of infected lambs? My specific aims were:

1. To validate the use of two types of remote monitoring systems (activity monitors and proximity loggers) in recording the activity behaviour and social contact behaviour of groups of lambs (Chapter 2).

2. To examine the effect of parasitism on the activity behaviour of lambs infected with the gastrointestinal nematode *T. circumcincta*, determining whether these behaviour changes are detectable before any physiological costs or observable measures of parasitism (Chapter 3).

3. To determine the effect an individual's social environment can have on their behavioural response to parasite infection (Chapter 3).

4. To examine the impact of parasitism on the social contact behaviour of lambs housed in single-parasitic state and mixed-parasitic state groups (Chapter 4).

5. To determine the effect of parasitism on the network architecture of groups of lambs of mixed-parasitic state (Chapter 4).

In Chapter 5 (Discussion), I synthesise the results of my thesis in the context of current sociobiology and behavioural ecology research and discuss their use in agricultural systems. Specifically, I link the results of Chapter 2 to the findings in Chapter 3 and 4

and discuss the use and practicality of different types of remote monitoring sensors in monitoring the health and welfare of animals in domesticated systems. I link the results of Chapter 3 and 4 to explore the effects of parasitism on the activity and social behaviour of lambs and discuss how the impact of parasitism on one behaviour may have influenced other behaviours. Finally, I discuss limitations of this study and address areas of ongoing and future work to complement the results of the thesis. Discussing how greater understanding of the process underlying the behavioural response of animals to parasitism could be used to predict disease spread across populations.

# Chapter 2

# Assessing the capabilities of two remote monitoring systems in measuring lamb behaviour

## 2.1 Lay summary

Remote monitoring of behaviour offers great potential for expanding the scope of questions that can be examined in animal behaviour. However, these systems require validation to confirm they accurately convey the information required to answer specific questions. Two types of remote sensors (activity monitors and proximity loggers) were validated to determine their capabilities of monitoring the activity and social behaviour of lambs in future studies. Live focal observation data of lamb activity behaviour and social contact behaviour were compared to behaviour data recorded using activity monitors and proximity loggers, to assess the level of agreement between the two behaviour-monitoring techniques. We also determined if the longer monitoring periods of the loggers provided different information from the shorter focal periods. Although overall activity measures differed slightly between focal and remote

methods, we found no systematic bias across different activity levels suggesting they provide a reliable index of behaviour. We also found that the proximity loggers could detect subtle changes in lamb behaviour earlier than could be detected using focal observations.

## 2.2 Abstract

Monitoring of animal behaviour is a valuable indicator of health and welfare of animals. Animal behaviour is often monitored using live focal observations or video monitoring. However, such methods can be labour intensive and be analytically time consuming. Where appropriate, remote sensors (activity monitors and proximity loggers) enable the continuous and simultaneous monitoring of animal behaviour over long periods of time. However, sensors are often used without first assessing their capability in monitoring the behaviour of the system they are to be deployed into. The objective of this study was to (i) determine the capabilities of IceQube activity monitors in recording lamb activity behaviour (experiment 1 and experiment 2), (ii) determine the optimal signal strength (UHF) of Sirtrack proximity loggers to detect social contacts between lambs that come within 1-1.5m (experiment 3), (iii) determine the capabilities of proximity loggers in accurately documenting lamb social behaviour (experiment 4), (iv) determine the capabilities of the proximity system at detecting the changes in patterns of social behaviour and (v) to determine if the longer monitoring periods of the loggers provide different information from the shorter focal periods (experiment 5).

In experiment one, activity data (step count, lying duration and frequency of lying bouts) was collected from 18 lambs over 13 days using IceQube activity monitors and live focal observations. The two datasets were compared to determine the level of agreement between the two behaviour monitoring methods. In experiment two, the date/time of transition times were recorded using video recordings and compared to

the date/time of transitions recorded using IceQubes. The data was used to assess the rate of false positives and false negatives recorded by the IceQubes. In experiment three, the optimal signal strength of the proximity loggers was determined to detect social contacts between lambs that come within 1-1.5m. In experiment four, contact data (frequency, duration and total duration) were collected from 18 lambs that came within 1-1.5m of each other over 13 days using Sirtrack proximity loggers and live focal observations to compare the level of agreement between the two behaviour monitoring methods. In experiment five, we carried out a mini social disturbance experiment to test whether the proximity system could detect changes in patterns of social behaviour, and to determine if the longer monitoring periods of the loggers provide different information from the shorter focal periods. Social contact behaviour of two social groups of lambs (Control; lambs were kept in a stable social group ($n$ = 6), Removal; lambs were exposed to a social disturbance whereby a proportion of the group were trickle removed ($n$ = 12)) was recorded using proximity loggers and focal observations over 13 days. We compared the social behaviour of individuals in each group before and after the social disturbance, to determine the effect the social disturbance had on the remaining group behaviour and ensure similar findings were synthesized from the data collected from both monitoring techniques.

We found a positive relationship between activity behaviour (step count, lying duration and frequency of lying bouts) recorded using IceQubes loggers and live focal observations and between contact duration recorded by the proximity loggers and focal observations. Both sensors underestimated absolute levels of activity and contact behaviour, but this was consistent across a range of different behaviours. Data recorded by the sensors therefore provide a good index, rather than absolute measure of behaviour. As we were looking to determine if sensors could be used to monitor patterns in behaviour and not absolute values, we believe the sensors can be

used in future hypothesis testing of lamb behaviour. We also found that removing lambs from a social group increased the duration of contacts between remaining individuals. This behavioural alteration was detected through both focal observations and proximity system. However, the proximity loggers could detect changes in behaviour earlier than using live focal observations. The result of this chapter demonstrates that both systems are capable at monitoring lamb behaviour and show they can be used in an experimental framework in future studies.

## 2.3 Introduction

Monitoring of animal behaviour is often used to assess the health and welfare of animals (Weary et al. 2009). Early detection of disease through behaviour observations in domestic systems can reduce the associated welfare implications and improve overall productivity of the animal (Kenyon et al. 2009). In wild systems monitoring the impact of disease on the behaviour of individuals can aide our understanding of the impact of disease in a population (Weber et al. 2013a; Ezenwa et al. 2016b; Lopes et al. 2016). Behaviour observations in both wild and domestic systems are often reliant on live focal observations or video monitoring. However, both methods can be labour intensive, take a long time to analyse (Trénel et al. 2009; Weary et al. 2009; Richeson et al. 2018), and it has long been known that the presence of a human observer can influence animal behaviour (Carpenter, 1934; Schneirla, 1950). Furthermore, the number of animals that can be observed at any one time or the duration of an observation may be limited, which could mean your sample size may not be appropriate to pick up subtle but consistent changes in behaviour patterns. Live focal observations are also unlikely to record behaviours during unsociable hours, and video recordings are limited by camera angles, clarity of the picture, and often if the video recording is of a group of animals the focal animal

can sometimes be obstructed by other animals in the pen. Therefore, depending on the research question, remote monitoring can overcome these limitations.

Recent development in remote sensor technology has enabled the continuous and simultaneous monitoring of animal behaviour over longer periods than an observer can often manage (Krause et al. 2007, Krause et al. 2013). Remote sensors offer high precision and resolution for data collection and have the potential to objectively quantify subtle and otherwise undetectable changes in animal behavioural patterns. Accelerometers and proximity loggers are two of the most commonly used remote sensor systems for monitoring animal behaviour. As diseased animals often show changes in their activity and social behaviour (Hart, 1988), both systems provide a useful tool to monitor changes in behaviour following infection (Bohm et al. 2009; Weber et al. 2013a; Doyle et al. 2016; Högberg et al. 2019, Hamilton et al. 2020; Högberg et al. 2021). Furthermore, as animals in social groups may alter the extent to which they demonstrate signs of sickness (Lopes et al. 2016), there is potential to use remote monitoring techniques to detect subtle changes in behaviour that are associated with illness and stress in animals when there are otherwise no clinical symptoms exhibited (Weary et al. 2009; Mathews et al. 2016; Neethirajan & Kemp, 2021).

The use of accelerometers originated to assess changes in human activity levels in relation to health (Inman and Eberhard, 1953). As technology progressed and the size and weight of the sensors reduced, there was an interest from researchers in using remote sensors to monitor animal behaviour. Accelerometers have since been used in a wide range of species (e.g., penguins, Yoda et al. 2004; brown bears, Gervasi et al. 2006; reef sharks, Whitney et al. 2007; goats, Moreau et al. 2009; cattle, Szyszka and Kyriazakis, 2013; dogs, Jones et al. 2014), and have proven useful for connecting behavioural changes to various health impairments in animals where the activity

behaviour is expected to be affected. For instance, accelerometers have linked changes in lying duration to mastitis infections in dairy cattle (Fogsgaard et al. 2015), and reduced activity levels with gastrointestinal nematode infections in cattle (Högberg et al. 2019) and sheep (Burgunder et al. 2018; Ikurior et al. 2020; Högberg et al. 2021).

Proximity loggers are a tool designed to monitor the contact behaviour of individuals to understand the social cohesion and social interactions between individuals, and what factors can affect contact behaviour (Prange et al. 2006; Bohm et al. 2009; Broster et al. 2010; Marsh et al. 2011; Drewe et al. 2012; Freire et al. 2012; Boyland et al. 2013; Doyle et al. 2016; Silk et al. 2017). Proximity loggers have previously been deployed in domestic and wild systems. For instance, they have been used to study the social behaviour of wild racoons (*Procyon lotor*) (Prange et al. 2006), the rate of interactions between cows and their calves (Swain and Bishop-Hurley, 2007) and ewes with their lambs (Broster et al. 2010). They have also been used to monitor the contact rates between Eurasian badgers (*Meles meles*) and cattle (Bohm et al. 2009). Loggers are usually attached to animals via neck collars, harnesses, or tags. They transmit a unique signal and record the frequency and duration of contacts when tagged animals come within a pre-set distance of one another.

Data provided by proximity loggers can be used to develop quantitative networks that can be used to predict and manage disease spread (Krause et al. 2011; Hamilton et al. 2020). They can also be used to monitor changes in an animal's sociality that can be used to detect signals of infection (Weber et al. 2013a; Hamilton et al. 2020). In domestic systems, this is important as animals are often housed in large groups and identifying changes in their social behaviour could indicate levels or stress or disease amongst individuals. However, research using proximity loggers in domestic systems has also highlighted the difficulty that animals may have in displaying sickness

behaviours, such as self-isolation or reduced activity when maintained in such high densities (Proudfoot et al. 2012).

The majority of studies monitoring the behaviour of terrestrial mammals use the commercially available IceQube activity monitors (IceRobotics Ltd, Edinburgh, UK) to record animal activity behaviour and Sirtrack proximity loggers (Sirtrack Ltd., Havelock North, New Zealand) to monitor animal social behaviour. IceQubes were developed for use in dairy cattle and have since been validated for use in dairy calves (Trénel et al. 2009), sows (Ringgenber et al. 2010) and more recently lambs (Högberg et al. 2020). However, it is argued that size and age of an animal could affect the accuracy of the system in recording behaviour (Högberg et al. 2020). For instance, the logging of a step by IceQube activity monitors is based on the amount of force used by the animal. Thus, if sensors were deployed into a group of animals of different weights, the data recorded by the IceQubes may not be comparable and if sensors were deployed into a group of animals of different ages, as younger animals grow, this may affect how the sensors record behaviours. Thus, there is a need to assess the capabilities of the loggers in measuring the behaviour of the animal's species that are of similar age and size that the loggers are intended to be used on in future studies.

Sirtrack proximity loggers are commercially available for monitoring the behaviour of multiple animal species (Sirtrack Ltd., Havelock North, New Zealand). Despite the adoption of these sensors in research, loggers can often generate inaccurate data, are prone to failing, and can be unreliable in the field (Drewe et al. 2012). Complete precision is also not possible as radio waves can de reflected, refracted or absorbed by the environment (Patison et al. 2010). However, inaccuracies and failures can be minimized by deploying the loggers into the proposed study system to previously calibrate the loggers ahead of any data collection. This enables exploration of data

processing methods and assesses the capabilities of the loggers in measuring the behaviour of the intended study system.

Here we assess the validity of two remote monitoring systems, to determine their ability to monitor the activity and social behaviour of domesticated lambs *Ovis aries* in future studies (Chapter 3 and Chapter 4). We used IceQube 3D-accelerometers to monitor activity behaviour (IceRobotics Ltd, Edinburgh, UK), and Sirtrack proximity loggers to monitor social contact behaviour (Sirtrack Ltd., Havelock North, New Zealand). Field calibration and deployment tests of remote monitoring sensor technology allows fine tuning of the system and informs deployment protocols in future trials. It also allows for the evaluation of the systems capabilities for studying specific animal behaviour's, provides preliminary data to inform data management, and fine tunes data analysis procedures.

The overall objective of this study was to assess the capabilities of IceQube activity monitors at monitoring lamb activity behaviour and Sirtrack proximity loggers at monitoring lamb social behaviour by comparison to a standard technique (live focal observations). The specific aims of the Chapter are to **(i)** assess the capabilities of the IceQube activity monitors at recording lamb activity behaviour (step count, lying duration, frequency of lying bouts) by comparing activity data between live focal observations and IceQube recordings (experiment 1 and experiment 2), **(ii)** to determine the optimal signal strength (UHF) of the proximity loggers to detect social contacts between lambs that come within 1-1.5m (experiment 3), **(iii)** to determine the accuracy of the optimal signal strength determined in experiment 3 by comparing direct behavioural observations against behavioural observations recorded by the proximity loggers (experiment 4), **(iv)** to determine the capabilities of the proximity system at detecting the changes in patterns of social behaviour (experiment 5) and **(v)** to determine if the longer monitoring periods of the loggers provide different

information from the shorter focal periods (experiment 5). In experiment 5, we will assess the capabilities and utility of the Sirtrack proximity system at studying research questions that are of interest. We use an exemplar study of social contact behaviour in lambs, where we applied a social disturbance treatment that would result in changes in lamb contact behaviour. The Sirtrack proximity system was previously validated in experiment 4 of this Chapter providing evidence that the loggers are a good representation of lamb behaviour during the focal periods, so we next want to determine if the longer monitoring periods of the loggers provide different information from the shorter focal periods. Here, we deploy the system in an experimental manipulation trial to assess if the system will be capable of detecting changes in patterns of social contact behaviour between individuals under an experimental framework. As remote sensors can record multiple animals simultaneously and for longer periods of time, they are capable of collecting more data than live observations. It is therefore expected that the remote sensors will collect more data than live observations which will potentially increase the power of the study (Cohen, 2013) and thus future hypothesis testing in lamb social contact studies (Chapter 4). As the sociality of sheep can be influenced by social group size (Michelena et al. 2008; Jørgensen et al. 2009), we exposed a group of lambs to a social perturbation, whereby members of a social group were removed. Lambs are prey animals, and it has been shown that when individuals are part of a smaller social group spend less time grazing and more time exhibiting vigilant behaviours (Michelena et al. 2009). Therefore, we would expect the remaining groupmates to have increased sociality. By monitoring the behaviour of the lambs before, during and post social disturbance using focal observations and remote sensors, we could assess if the proximity loggers were capable of picking up changes in behaviour earlier than what can be detected using focal observations.

## 2.4 Methods

In all experiments, we used four-month-old Texel x Bluefaced Leister lambs that were selected from a commercial flock. All lambs were marked with a number on their back before experiment start date so they could be easily identified during the live focal observations. During each experiment, lambs were housed indoors, were given silage and concentrate twice daily and had access to water ad libitum.

In experiment 1 and experiment 2, we used IceRobotics IceQube accelerometers to monitor lamb behaviour. IceQube dimensions were 56 x 56 x 27mm and weighed 74g. The IceQubes use a 3-axis accelerometer to continuously capture highly detailed information on the animal's movement behaviour and store the data in 15-minute increments of time with a 9-day memory. Prior to use, the IceQubes were activated and configured via an IceReader (a wireless communication device controlled by the IceManager software). On activation, the loggers were synchronized to the clock of the connected computer. The IceManger software is then used to retrieve and record the data. Loggers were attached to the rear ankle of the lambs with a velcro strap and secured with veterinary self-adherent bandage (Vetrap™, 3 M, St. Paul, *USA).*

In experiment 3, 4 and 5, we used Sirtrack proximity loggers (Sirtrack Ltd., Havelock North, New Zealand) to record the social contacts between lambs. The proximity loggers use an ultra-high frequency (UHF) to send out signals to other loggers using a unique code, while receiving signals from other loggers. Once a contact is detected by a logger, a contact is recorded until one of the loggers in the contact fails to receive a signal for longer than the separation time. When two lambs came into contact with each other, time, date, logger ID and duration of the contact was recorded by the proximity loggers.

Analyses for all experiments were performed in R version 4.0.3 (R Core Team, 2020). Final model formulae and definitions of fixed and random effects are listed in Appendix A, Table S1.1 and S1.2.

## Experiment 1: Assessing the capabilities of activity monitors at recording step count, frequency of lying bouts and lying duration of lambs.

Eighteen lambs were selected based on sex (9 males and 9 females) and weight (live mean weight ± standard deviation 30 ± 0.13Kg). The lambs were housed indoors in a straw-bedded pen 10x16m for 13 days. An IceQube activity logger was fitted to the rear ankle of each lamb and was activated on day one of the experiment. IceQubes continuously record four activity behaviours, including step count (the number of times the lamb lifts their 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). However, as motion index is calculated from step count and as such does not need to be validated in addition to step count, for the purpose of this study we were only interested in step count, lying time and lying bouts.

During the 13 days, we also monitored the activity behaviour of each lamb using live focal observations. As the IceQubes report activity in 15-minute periods, live behaviour observations were conducted in 15-minute bouts of time to enable comparison between focal observations and IceQube recordings. Behaviours recorded during the live focal observations included step count (the number of times the lamb lifts their leg), lying time (the period of time when the lamb is lying down) and lying bouts (the number of times the lamb transitions from standing to lying). To

ensure an accurate method and minimise observer error we recorded the lying bout transition times during a separate experiment that enabled us to monitor animal behaviour using video recordings (experiment 2).

### Experiment 2: Validation of lying transitions recorded by the activity monitors.

Four lambs were housed in a straw bedded pen of 6x6m² for five days. An IceQube activity monitor was fitted to the rear ankle of each lamb on day one and replaced each day so that a total of 18 activity monitors were used in the study (Data from two IceQubes was lost). A video camera was set up that enabled the continuous recording of lamb behaviour. The time stamp of the video camera and the IceQubes were synced, and lamb behaviour was recorded for 5 days between 11:00 and 16:00 generating approximately 24 hours' worth of footage. During the recording period, the time and date of every transition from each animal was recorded to compare with the transition times recorded by the IceQubes. At the end of each day the IceQube was removed, and data downloaded from the tag.

### Experiment 3: Calibration of proximity logger signal strength.

Six lambs were selected based on sex (three males and three females) and weight (live mean weight ± standard deviation 28.0 ± 0.11Kg). Lambs were housed in a straw bedded pen 6x6m² for 1 week. Each day two pairs of animals were selected to participate in the range testing of the Sirtrack proximity loggers. A proximity logger was attached to the neck of one lamb in each pair and the second lamb in the pair was used to reduce the anxiety of the first lamb during the range testing. The signal strength of the collar was set at a level that we predicted would be close to 1-1.5m in detection distance. Pair one was kept stationary in a holding pen. Pair 2 was kept apart from pair 1 at set differences by a wooden gate to test the signal strength of the collars at different distances. During each test, the distance between the two pairs

was reduced by moving the gates closer and moving pair 2 closer to pair 1. This was repeated until the proximity loggers came into contact (confirmed visually by a flash on the logger), thus giving a distance between lambs in relation to the signal strength of that collar.

### Experiment 4: Validation of contact time and duration recorded by proximity loggers.

Eighteen lambs were selected based on sex (9 males and 9 females) and weight (live mean weight ± standard deviation 30 ± 0.13Kg). The lambs were housed indoors in a straw-bedded pen 10x16m for 13 days.

Each lamb was fitted with a Sirtrack proximity data logger on a neck collar to record close proximity contacts with other individuals in the group. The detection distance was set to 1-1.5m (UHF range was pre-determined in experiment 3) to allow detection of a close-contact situation (Ozella et al. 2020). The loggers were activated on day one of the experiment to record the contact behaviour of each lamb. Each day the social contact behaviour (frequency and duration) of every lamb was also recorded for 20 minutes using live focal observations. When the animal under observation came into contact with another animal the date, time, recipient animal ID and duration of the contact was recorded. This data would be compared to the data recorded by the proximity loggers.

### Experiment 5: Assessing the capabilities of the proximity system at detecting subtle changes in behaviour

To evaluate the capabilities of the proximity system to be used in future hypothesis testing, we carried out a mini behaviour trial whereby a group of lambs were subject to a social disturbance. By monitoring lamb behaviour before and after the social disturbance we can assess the capabilities of the system at monitoring lamb contact

behaviour, but also determine the ability of the system at detecting any changes in behaviour. Social contacts between lambs were recorded using Sirtrack proximity loggers (Sirtrack Ltd., Havelock North, New Zealand).

Eighteen lambs were divided into one of two treatment groups, (i) Control; lambs were kept in a stable social group (*n* = 6), and (ii) Removal; lambs were exposed to a social disturbance whereby a proportion of the group were trickle removed during the trial (*n* = 12). The two groups were balanced for sex (equal number of males and females in each group) and weight (live mean weight ± standard deviation 30 ± 0.13Kg). The groups were not replicated as the main aim of this trial was not to answer a specific research question but to test the deployment of the Sirtrack system and evaluate their capabilities for studying social behaviour. Each group were housed in a straw bedded pen measuring 5x8m, that were separated by bales of hay to keep each pen out of view from the other.

The experimental timetable (a total of 13 days) was divided into three phases: Phase A (Days 1- 4), a period when a collection of baselines measurements would be recorded on the social contact behaviour of lambs in each group; Phase B (Days 5-9), a period when the removal group would experience the trickle removal of 2 animals over 3 time points (Day 5, 7 and 9) reducing the group of 12 animals to 6 animals. During the removal days, the control group experienced a disruption of a similar timing without the removal of any animals; Phase C (Days 10-13), a period monitoring the behaviour of both groups following the removal of animals from the removal group.

Every lamb in each group was fitted with a proximity data logger on a neck collar to record close proximity contacts with other individuals in the group. The detection distance was set to 1-1.5m (UHF range was pre-determined in experiment 3) to allow detection of a close-contact situation. The logger was activated on day one of the

experiment and continuously recorded the contact behaviour of each lamb 24 hours per day. Any contacts that were recorded before loggers were placed on the lambs or occurred while animals were being handled (i.e., when animals were removed from the treatment group) during the experiment were not included in the analysis. All contacts of 1 second or less were removed, as it is believed these may represent weak collar signals (Drewe et al. 2012), or detection signals at the edge of the detection range (Prange et al. 2006). As reciprocal contact data from two different collars are not completely symmetrical due to reflection, refraction and absorption of radio waves (Patison et al. 2010), the contact duration between two loggers was defined as starting when one logger recorded a contact and ending when either logger failed to maintain a contact (Hamede et al. 2009; Patison et al. 2010; Smith et al. 2019). The behaviour of the lambs was also monitored using live focal observations. Social contact behaviour (frequency and duration) of each lamb was recorded for 20 minutes each day using live focal observations. When the animal under observation came into contact with another animal the date, time, recipient animal ID and duration of the contact was recorded. Behaviour observations were recorded 30 minutes after any disruptions to lamb behaviour (i.e., feeding, bedding).

## Statistical analysis

In experiment 1, we used linear models to assess the level of agreement between the activity behaviour (step count, lying duration and frequency of lying bouts) recorded using IceQubes and the activity behaviour recorded using live focal observations. We ran three statistical models in total. Activity behaviour recorded using the IceQubes were included in the model as a response variable (i.e. step count, frequency of lying bouts, lying duration). Activity recorded by focal observations (Step OB, Lying Duration OB and Lying Bouts OB) were fitted as continuous fixed effects in the appropriate model.

In experiment 2, the rate of false positive (transition recorded by IceQube but not by focal observation) and false negative (transition recorded by focal observation but not by IceQubes) lying bouts recorded by the IceQubes was calculated by comparing how many transitions were recorded using focal observations with the transitions recorded by the IceQubes given a threshold of 30 seconds either side of the transition time stamp.

In experiment 3, we plotted proximity signal strength (UHF) against distance that a contact was recorded between two loggers, with a fitted a regression line, to determine the most appropriate UHF signal that could detect contacts between two animals that came with 1-1.5m or each other.

In experiment 4, we used linear models to assess the level of agreement between the contact data (duration of contacts) recorded using Sirtrack proximity loggers and the contact behaviour recorded using live focal observations. Duration of contacts recorded by the loggers was included as a response variable and contact behaviour recorded with focal observations (Duration OB) were fitted as a continuous fixed effect. Linear regression models on activity and contact behaviour were fitted with the packages 'lme4' and lmerTest (Bates et al. 2014).

In experiment 5, we used generalised linear mixed models to assess the impact of a social disturbance on the social contact behaviour between the two treatment groups (Control, Removal). Mixed models were fitted using the package 'R-INLA' (Rue et al. 2009; Martins et al. 2013). We used INLA to run the mixed effects models as two animals occurred within each contact, and we wanted to ensure that both animals were given the same coefficient. In total 6 statistical models were run. Three models were on the contact behaviour recorded using live focal observations and three models were on the contact behaviour recorded using Sirtrack proximity loggers.

Frequency (number of contacts per day), duration (length of a contact) and total duration (total contact length per day) of contacts were fitted as response variables in the models. Animal 1 ID and Animal 2 ID were fitted as random effects. Phase (Phase A, Phase B and Phase C) and Treatment group (Control, Removal) were fitted as fixed effects.

Before running all contact models, the mean-variance relationship was assessed to verify the model structure and to ensure the appropriate distribution was used for each response variable. For frequency, duration and total duration we used mixed models fitted with negative binomial distributed errors. As there is no null model used in Bayesian statistics to determine significance at the 5% level, for contact behaviour models we accept the equivalent to frequentist significance if the 95% credible intervals do not overlap 1. Comparison of the fixed effect estimates from each response variable model can be found in Appendix A, Table S1.5-S1.6 and Figures S1.1-S1.2.

## 2.5 Results

### Experiment 1

There was a significant positive relationship between step count recorded using IceQube activity monitors and step count recorded using live focal observations (Est = 0.324, Intercept = 0.650, $p<0.001$) (Figure 2.1A and See Appendix A, Figure S1.1 for model predictions): IceQube activity monitors recorded around 30% of all steps that were recorded using live observations. There was a significant positive relationship between frequency of lying bouts recorded using IceQube activity monitors and focal observations (Est = 0.33, Intercept = 0.03, $p<0.001$) (Figure 2.1B and See Appendix A, Figure S1.1 for model predictions): IceQube activity monitors recorded around 30% of all lying bouts that were recorded using live observations.

There was also a significant positive relationship between lying duration recorded using IceQube activity monitors and live focal observations (Est = 0.59, Intercept = 252.81 $p<0.001$) (Figure 2.1C and Figure S1.1 for model predictions). However, at times when focal observations recorded lambs as standing (i.e., lying duration = 0), the IceQubes recorded lambs as lying down (Figure 2.1C and Appendix A, Table S1. 3).

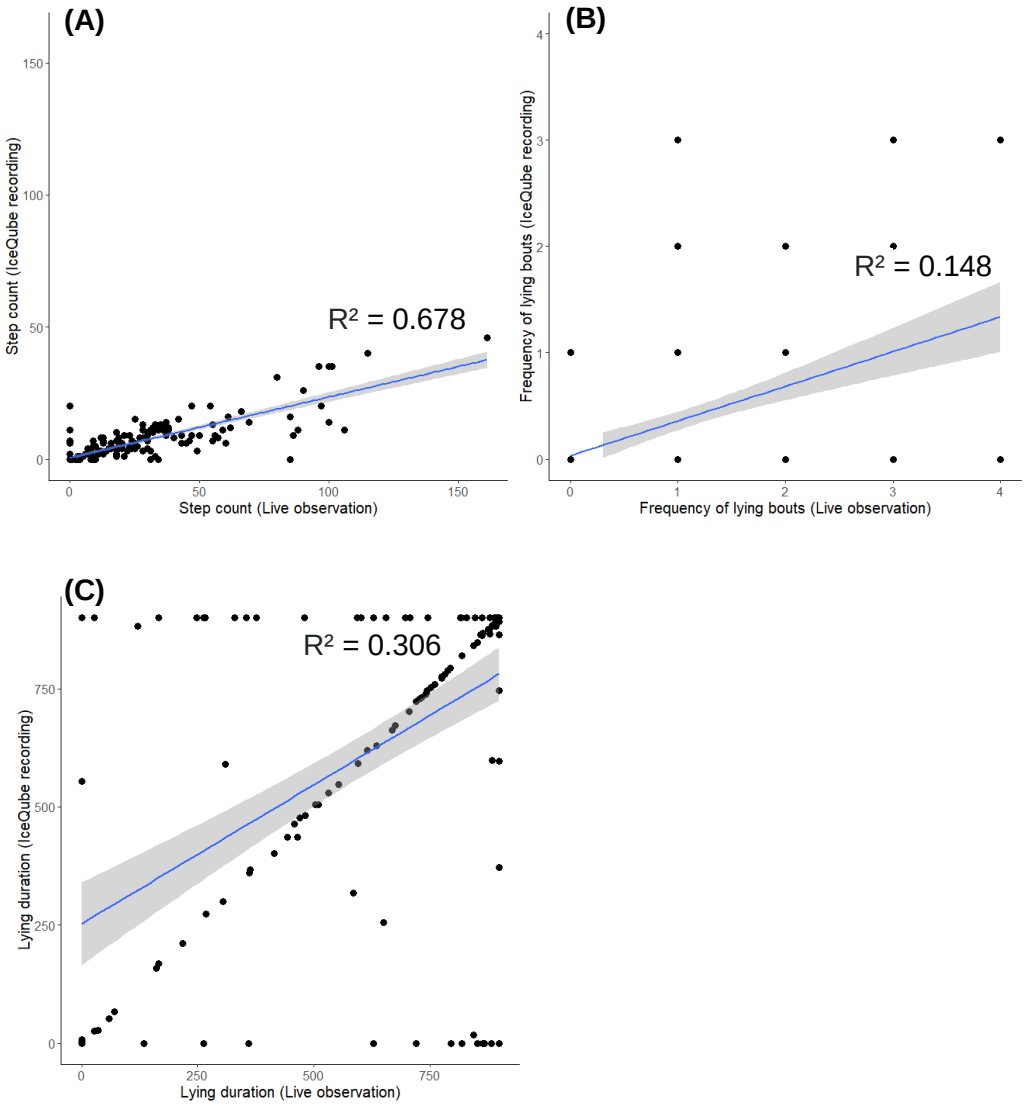


**Figure 2.1.** Relationship between the activity behaviour recorded by the IceQube activity monitors against activity levels recorded using live focal observations. **(A)** Observed step count against step count recorded by IceQube activity monitors, with fitted regression line and R-squared value. **(B)** Observed frequency of lying bouts against frequency of lying bouts recorded by IceQube activity monitors, with fitted regression line and R-squared value. **(C)** Observed lying duration against lying duration recorded by IceQube activity monitors, with fitted regression line and R-squared value.

## Experiment 2

In experiment two, over a 24-hour period 168 transitions were recorded using the IceQubes, and of these transitions, 4% were identified as false positives and 27% were identified as false negatives.

## Experiment 3

There was a negative correlation between UHF range and distance. We found the UHF signal strength of 54 was most consistent at recording contacts when two lambs came within 1-1.5m of each other (Figure 2.2).

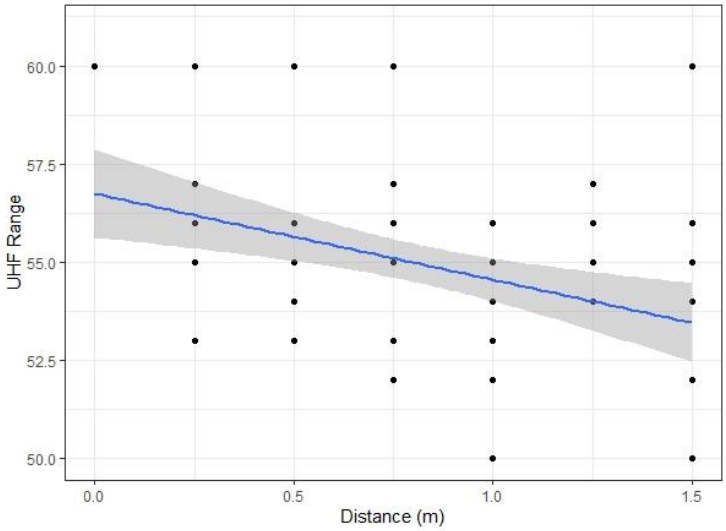


**Figure 2.2.** Sirtrack proximity logger UHF range against distance (metres), fitted with fitted regression line.

## Experiment 4

Using UHF range 54, there was a significant positive relationship between Sirtrack recorded duration of contacts and the duration of contacts recorded by live observations (Est = 0.85, Intercept = 23.84, $p<0.001$) (Figure 2.3 and See Appendix A Figure S1.2 for model predictions): Sirtrack loggers recorded 85% of contact duration recorded by focal observations.

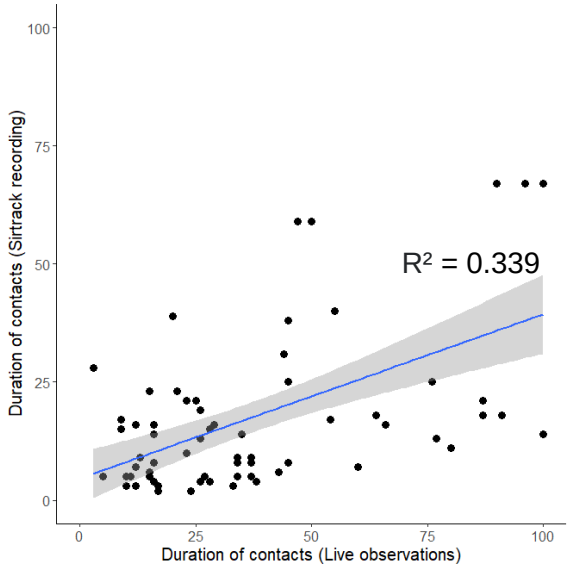


**Figure 2.3.** Relationship between the duration of contacts recorded by the proximity loggers against duration of contacts recorded by live focal observations, with fitted regression line and R-squared value.

Results of experiment 3 and experiment 4 established that the loggers are a good representation of lamb behaviour during the focal periods, so now we want to determine if the longer monitoring periods of the loggers provide different information from the shorter focal periods.

## Experiment 5

**Live focal observations:** When social behaviour was recorded using live focal observations, there was no change in the mean frequency of contacts between individuals in the removal group during the three phases of the study, compared to individuals in the control group (Figure 2.5A and Appendix A, Table S1.5). However, there was an increase in the mean contact duration (CI 0.24, 0.927) (Figure 2.5B), and total contact duration (CI 0.15, 0.922) (Figure 2.5C) between individuals in the removal group during Phase C, compared to individuals in the control group (Appendix A, Table S1.5).

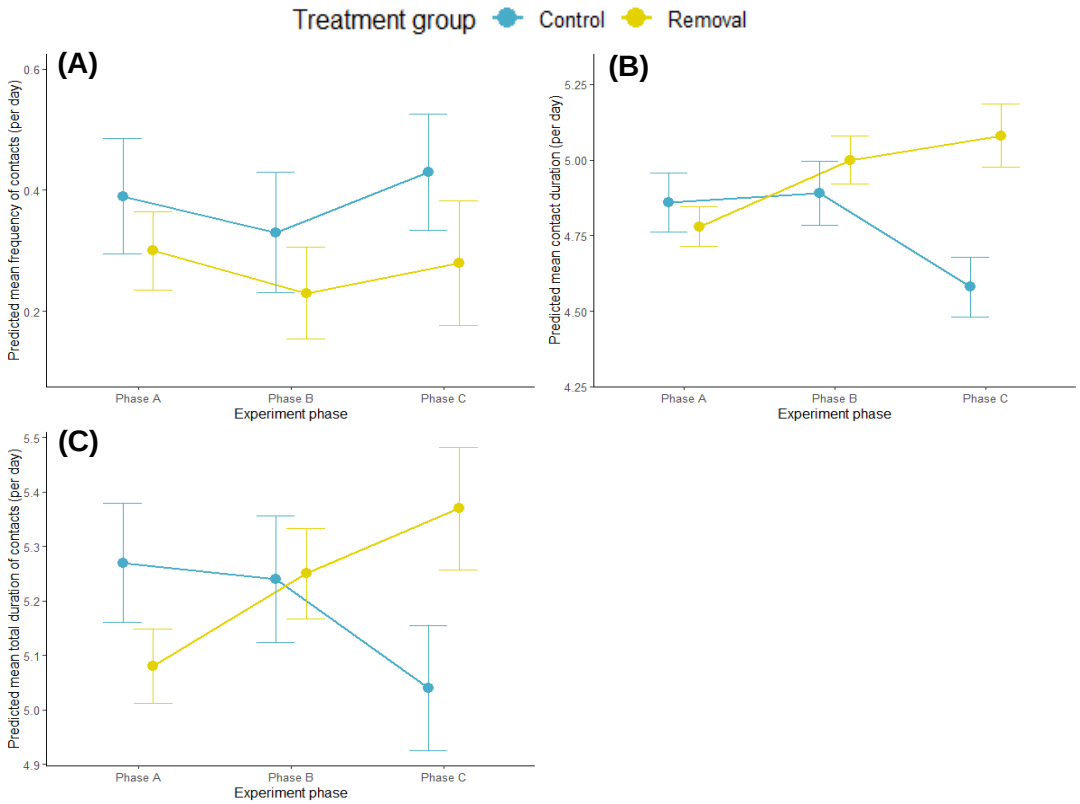


**Figure 2.5**. Model predicted mean ± standard error contact behaviour per day (recorded using live focal observations) of individuals in each treatment group (Control (blue; *n* = 6) and Removal (yellow; (Phase A, *n* = 12), (Phase B, day 5-6, *n* = 10, day 7-8, *n* = 8, day 9, *n* = 6), (Phase C, *n* = 6))) during each phase of the study. **(A)** Model predicted mean frequency of contacts per day. **(B)** Model predicted mean contact duration. **(C)** Model predicted mean total duration of contacts per day.

**Proximity logger observations:** When social behaviour was recorded using Sirtrack proximity loggers: There was no change in the mean frequency of contacts and total duration of contacts between individuals in the removal group during the three phases of the study, compared to individuals in the control group (Figure 2.6A and 2.6C) (Appendix A, Table S1.6). However, there was an increase in the mean contact duration recorded between individuals in the removal treatment group during Phase B (CI 0.02, 0.96) and Phase C (CI 0.081, 0.17), compared to the control group (Figure 2.6B).

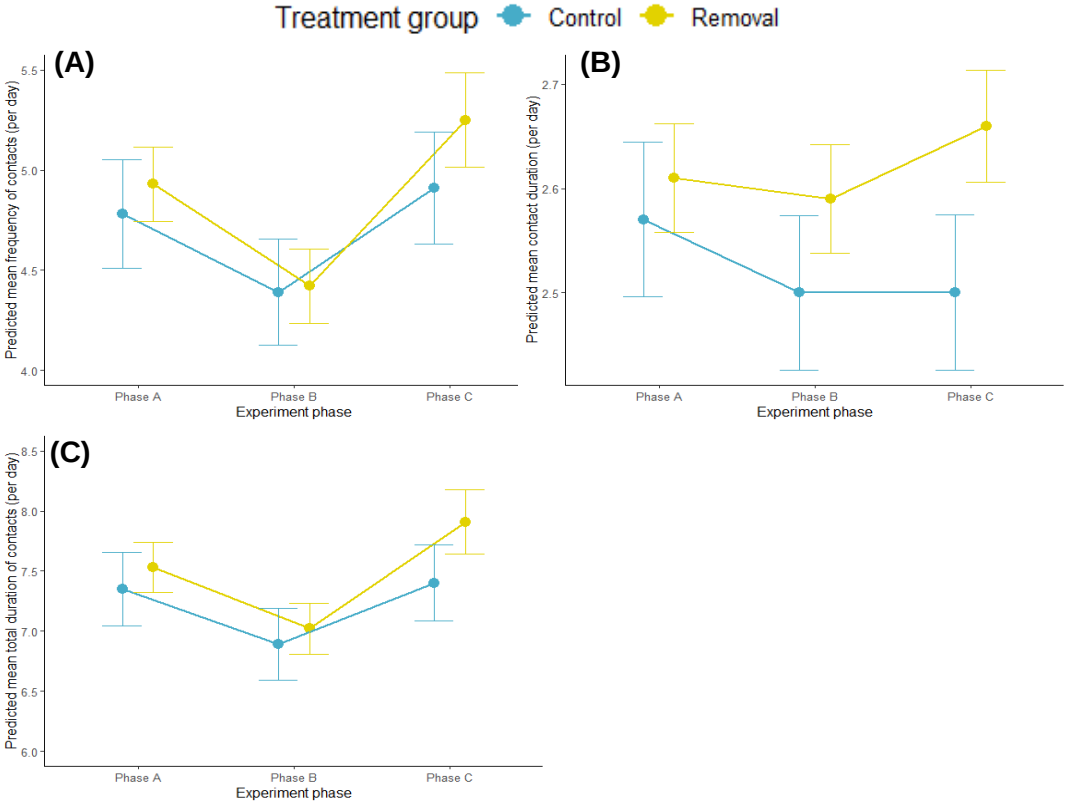


**Figure 2.6**. Model predicted mean ± standard error contact behaviour per day (recorded using Sirtrack proximity loggers) of individuals in each treatment group (Control (blue; *n* = 6) and Removal (yellow; (Phase A, *n* = 12), (Phase B, day 5-6, *n* = 10, day 7-8, *n* = 8, day 9, *n* = 6), (Phase C, *n* = 6))) during each phase of the study. during each phase of the study. **(A)** Model predicted mean frequency of contacts per day. **(B)** Model predicted mean contact duration. **(C)** Model predicted mean total duration of contacts per day.

## 2.6 Discussion

The aim of this study was to assess the validity of two remote monitoring systems, to determine their ability to monitor the activity and social behaviour of lambs in future studies. In order to validate both systems, the data collected by the device was compared with live behavioural observations in order to determine if both systems were accurately measuring the behaviours. We found a positive relationship between activity behaviour (step count, lying duration and frequency of lying bouts) recorded using IceQubes loggers and live focal observations and between contact duration recorded using proximity loggers and live focal observations (experiment 1 - 4). However, both sensors were not good at picking up absolute levels of behaviour and were found to underestimate true values, but data recorded by the sensors were found to provide a good index of activity and contact behaviour as a consistent correlation was found across a range of behaviours. As we were aiming for a representative coverage of behaviours, i.e., we were looking to determine if sensors could be used to monitor patterns in behaviour and not absolute values, we believe the sensors can be used in future hypothesis testing of lamb behaviour (Chapter 3 and Chapter 4).

The underestimation of IceQubes at recording lamb step count is in line with the findings of Högberg et al. (2021) and is something that would need to be considered when using the loggers if the exact values of lamb behaviour is important. Although IceQubes picking up 30% of total steps appears low, as the difference is a consistent scaling issue it is still feasible for the activity monitors to be used to study changes in the pattern of activity. To find treatment effects, changes in patterns of activity are more important than exact step counts, therefore the IceQubes can be used in future trials to monitor changes in lamb behaviour (Chapter 3).

Through the validation work, we found the IceQubes were not sensitive to lamb lying duration behaviour. The results suggest there is potentially a lag in lying duration. As the IceQubes were inaccurate at measuring lying duration, the reliability of the loggers at measuring lying duration is something that we will have to take into consideration when analysing IceQube recorded lying duration data in future trials (Chapter 3). For instance, as IceQubes record data in 15-minute bouts (900 seconds), one way to address the problem would be to convert the data to fit a binomial distribution, were lying duration would be coded as 1 = lambs were lying $\geq$ 450 seconds per 15-minutes, and 0 = lambs were lying < 450 seconds per 15 minutes and analysed with a binomial distributed error.

IceQube activity monitors are a commercially available product designed for use in dairy cattle regarding size and activity algorithms (IceRobotics Ltd, Edinburgh, UK). The loggers record a step when the animal lifts its leg, but the logging of a step is based on the amount of force used by the animal. Therefore, the difference in recorded behaviours between the IceQubes and live focal observations could be explained by the size difference between cattle and lambs, as the average weight of a UK dairy cow is approximately 650kg (Beattie, 2020), compared to the lambs in the current study that weighed around 30kg. As the activity monitors were validated for their use in future studies to monitor behaviour changes in parasitised lambs (Chapter 3), it could be argued that infected animals weigh less, and would therefore have lower activity levels compared to non-infected animals. However, the effect of lamb bodyweight on the ability of IceQubes at monitoring activity behaviour was addressed by Högberg et al (2020) who showed differences between the weights of lambs did not influence behaviour changes recorded by the IceQubes.

There was a negative correlation between Sirtrack UHF range and distance (experiment 3). This was in line with previous work that set out to determine optimal

UHF range to detect contacts between two cows that came with a desired distance (Boyland et al. 2013). There was also a good level of agreement between the data recorded by the proximity loggers with the data recorded using live focal observations (experiment 4). We found the Sirtrack loggers recorded around 85% of contact duration recorded by focal observations. The difference between the two methods could be associated with logger inaccuracies. We know logger radio waves can be absorbed by the environment (Patison et al. 2010), thus, it could be, that during times of observation the loggers missed some of the interactions between the lambs. The difference between the two methods could also be associated with the way the proximity data is pre-processed before analysing. For instance, as reciprocal contact data from two different collars are not completely symmetrical (Patison et al. 2010), the contact duration between two loggers was defined as starting when one logger recorded a contact and ending when either logger failed to maintain a contact (Hamede et al. 2009; Patison et al. 2010; Smith et al. 2019). Furthermore, 1 second contacts were removed from the logger data as it is believed these may represent weak collar signals (Drewe et al. 2012), or detection signals at the edge of the detection range (Prange et al. 2006). Although it would be useful in some studies to record absolute values, as there is no systematic bias with the loggers and the difference between proximity logger data and focal observation data is scaling issue, it is feasible to use the proximity loggers to study changes in the pattern of lamb social behaviour in future studies (Chapter 4) to find treatment effects, as changes in patterns may be more important than exact values.

In experiment 5, we wanted to determine the capabilities of the proximity system at detecting the changes in patterns of social behaviour and determine if there is a difference in detecting changes in patterns of behaviour change, between data from the remote sensing system and live observations. The removal treatment did create

a social perturbation effect, with both the focal observations and the proximity loggers detecting a change in social behaviour of the remaining group members. Removing individuals from the group, increased the duration of contacts between remaining group members. The mechanisms underpinning an individual's response to social perturbations has been relatively unexplored. In this study, the fact that lambs appear to increase their sociality upon losing their conspecifics may represent a rapid behavioural response to compensate for the loss of connectedness (Firth et al. 2017). In response to a potential threat, flocking is the instinctive behavioural response of sheep (Keeling, 2001), which would be reflected in the length of contacts increasing (Figure 2.6B). Social associations are known to be valuable to individuals (Krause and Ruxton, 2002). For instance, lambs are highly social prey animals, and despite the risk of predation being low on farms, lambs maintain a strong anti-predator behavioural response (Estevez & Andersen, 2007). Maintaining high numbers of group members can protect against predation (Krause and Ruxton, 2002), and while group size decreases, it simultaneously increases the chances of predation of remaining group members, causing individuals to form more social associations. Therefore, it could be hypothesized that increased contact duration between individuals in the removal group, following the removal of group members, are due to lambs recognizing the loss of their associates, and interpreting this as a cue of high predation conditions (Firth et al. 2017).

Interestingly, the proximity loggers detected a change in behaviour earlier than the focal observations. Following the removal of individuals from the removal treatment group, there was an increase in contact duration between the remaining individuals that was detected during Phase B of the study, which was earlier than when the behaviour change was detected using live focal observations (during Phase C). Phase B of the study was the period when the removal group experienced the trickle

removal of two animals over three time points. To identify subtle changes in animal behaviour requires long observation periods (Mathews et al. 2016). Therefore, it could be, that the removal of individuals from the group affected the remaining groups behaviour in Phase B, but the change in behaviour was so subtle that it could not be detected using the shorter observation periods conducted using live focal observations. Proximity loggers record more continuous data than live observations, and as we did find a difference in the behaviour between the two treatment groups using focal observations but just at a later point in the study, suggests this is due to the higher number of data samples recorded by the loggers and not the effect of the observer. These results demonstrate that the remote sensors offer high levels of precision and resolution of data that can detect subtle and otherwise undetectable behaviour changes compared to other behavioural monitoring techniques.

We also found the total duration of contacts was higher between individuals in the removal group during Phase C, compared to individuals in the control group. However, these behaviour changes were only detected in the data recorded using focal observations. These findings suggest that overall; lambs were spending more time together when they were being observed. Although lambs used in the study were domesticated and relatively used to human interactions, lambs do maintain a strong anti-predator behavioural response (Estevez & Andersen, 2007). Thus, this behaviour may have occurred because animals were more nervous around the observer following the removal of their groupmates, which would therefore indicate a degree of observer effect on lamb behaviour (Schneirla, 1950).

The results of this study demonstrate a good level of agreement between the proximity system and focal observations. However, activity monitors do not directly detect all incidences of activity but provide a reliable index of behaviour. Proximity loggers detect the majority of observable contacts and may be more reliable as can detect

changes over a longer time period so may detect changes earlier than observations at limited time point may allow. We show that the proximity loggers can be used to identify changes in animal behaviour and that these behaviour changes occur earlier than what would be detected using focal observations. The results of this chapter therefore provide evidence for the importance of using remote sensors, demonstrating the suitability of the devices at monitoring changes in lamb behaviour and show they can be used for hypothesis testing in an experimental framework in future experiments (Chapter 3 and 4). However, in subsequent Chapters (Chapter 3 and Chapter 4), when we talk about changes in behaviours that were recorded using the sensors, it is in relation to the data recorded by the sensors and not the true values.

# Chapter 3

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

## 3.1 Lay summary

Parasitism can have a major impact on the health of domestic and wild animals. Here we show that parasitism reduces the activity behaviour of infected lambs during the earliest stages of infection. However, the extent of this change can be affected by the infection status of other animals in their group, showing an animal's social environment can affect how these behavioural cues are expressed.

## 3.2 Abstract

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 test, firstly, whether parasitism reduces the activity behaviour of lambs, secondly, whether this occurs prior to other observed

costs of parasitism, and thirdly 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, (i) Parasitised; all lambs were infected with the parasitic nematode *Teladorsagia circumcincta*, (ii) Non-parasitised; all lambs were dosed with water, (iii) Mixed; part of the group were infected and part of the group were dosed with water. Activity behaviour was monitored using IceQube activity monitors, before, during and post-parasite infection. Parasitised 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 to a lesser degree compared to animals in the fully parasitised groups during the patent-parasite phase. Activity levels remained low until lambs were treated with anthelmintic when activity levels of the groups that had been parasitised returned to the same level as non-parasitised 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.

## 3.3 Introduction

Parasites are ubiquitous in the environment and can have a major impact on the health of both wild and domesticated animal populations (Poulin, 1999; Marcogliese, 2004; Hudson et al. 2006; Lafferty et al. 2006; Charlier et al. 2014). 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 (Hart, 1988; Bilbo et al. 2002; Moore, 2002; Kelley et al. 2003; Dantzer, 2004; Ayres and Schneider, 2009; Lopes et al. 2012). It has been hypothesised 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 and Wingfield, 2006), protection of offspring (Aubert et al. 1997; Weil et al. 2006), territorial defense (Friedman et al. 1996) and maintenance of social status (Cohn and 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 (Kiesecker et al. 1999; Behringer et al. 2006; Tobler and 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 with facial tumours decreased as tumour load increased (Hamilton et al. 2020), and also that TB test-positive badgers were socially isolated from their own groups (Weber et al. 2013b). Accelerometers and activity loggers have shown sheep treated with anthelmintics to remove any naturally occurring parasites had higher activity levels than their untreated counterparts (Burgunder et al. 2018; Ikurior at al. 2020; Högberg

et al. 2021). Randomised 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 experimentally infected with African swine fever virus (Martínez-Avilés et al. 2017), and the use of accelerometers demonstrated cows experimentally infected with the roundworm *Ostertagia* o*stertagia* had reduced step rate and increased frequency of lying bouts (Högberg et al. 2019). These 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 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 parasitised and non-parasitised 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 parasitised animals.

Understanding how animals balance the costs and benefits of sickness behaviours across different social environments will aid in understanding 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 non-invasive 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 and 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 (van Wyk, et al. 2002; Kenyon et al. 2009; Stafford et al. 2009). 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 and in a practical application.

Here we investigate 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 *Ovis aries*, experimentally infected with the gastrointestinal nematode *Teladorsagia circumcincta*, a common parasite of both economic and welfare importance (Papadopoulos et al. 2012). Specifically, we ask: (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? (3) Are these behaviours affected by the infection status of group members through social modulation? We predict experimental infection will lead to a reduction in the activity levels of parasitised lambs, as animals become more lethargic through the course of the infection. We also predict that infected individuals in the mixed groups will modulate their behavioural response to infection, with infected lambs in the mixed groups maintaining higher activity levels compared to infected lambs in the single-parasitic state groups as they maintain coordination with the healthy individuals in their social groups.

## 3.4 Methods

### 3.4.1 Animals and experimental design

*Same experimental design was used for Chapter 3 and Chapter 4.*

Sixty 12-week-old Texel x Bluefaced Leicester lambs were selected randomly from a commercial flock of sheep that had been reared indoor 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 4 replicate groups of 5 lambs within each treatment. These were (i) Parasitised: all lambs were infected with parasitic nematode *T. circumcincta* and were of the same parasitic status, (ii) Non-parasitised: all lambs were dosed with water, remained parasite naïve, and were of the same parasitic status and (iii) Mixed: a group containing animals of mixed parasitic status, where three animals were dosed with water and two were dosed with *T. circumcincta* larvae. Each replicate group was standardised for sex (three females and two males per group) and weight (live mean weight ± standard deviation 27.6 ± 0.13Kg). Given the small number of replicate groups, it was decided not to randomise 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, 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, there were no siblings allocated in the same group. One week before the experiment start date groups were put onto pasture in individual plots laid out in a six by two grid, with each plot measuring 30x30m and separated by sheep netting. All plots had been free from grazing ruminants for the previous three years and animals were given ad lib 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; pre-parasite (week 1), a period when all lambs would be kept parasite naïve; pre-patent (weeks 2-4), a period when lambs identified for infection would be parasitised but not yet showing any pathological physiological effects of parasitism and are not yet shedding eggs; patent-parasite (weeks 5-7), a period when lambs show physiological responses to infection and are shedding eggs in their faeces; post-parasite (weeks 8-9), a period when all lambs would be dosed with anthelmintic, and considered parasite free. On the first day of week two, lambs identified for infection, which included all lambs in the parasitised groups and two out of five lambs in the mixed groups received an oral dose of 5,000 L3 stage *T. circumcincta* larvae, and lambs identified to remain non-infected 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 3 times per week for 6 weeks. The trickle infection chosen (5,000 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, 1ml/10Kg) and infections were cleared.

### 3.4.2 Activity behaviour

Activity behaviour of lambs in all groups was continuously and simultaneously recorded 24 hours per day, using IceRobotics IceQube activity monitors (IceRobotics Ltd, Edinburgh) (previously validated in Chapter 2). One week prior to the start date

of the experiment, an activity monitor was fitted to the rear ankle of each lamb and was activated on day one of the experiment. The IceQubes use a 3-axis accelerometer to continuously capture highly detailed information on the animal's movement behaviour and store the data in 15-minute increments of time. The IceQubes recorded four activity behaviours including step count (the number of times the lamb lifts their leg), motion index (a broader measurement of the animals 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) (IceRobotics Ltd, Edinburgh).

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

### 3.4.3 Animal measurements

On the first day of each week rectal faecal samples were taken from all sixty lambs within their plots to estimate the number of nematode eggs per gram of faeces (epg) using a modified salt-flotation method (Jackson, 1974) (See Appendix B for faecal egg count methodology). 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 pre-parasite, patent-parasite and post-parasite phases) to measure serum pepsinogen level (an indication of parasite induced gut damage) using a sheep pepsinogen ELISA assay kit (BlueGene Biotech, Shanghai, China). At the end of the experiment, a faecal sample and weight measurement was taken from every animal, to assess the final weights and parasite load of the lambs.

Following collection, faecal samples were weighed out and separated into subsamples; 1g was stored at 4°C for faecal egg counts. Blood samples were spun within two hours of collection at 3660 r.p.m at 4°C for 15 minutes, the serum was removed and stored at -20°C.

### 3.4.4 Statistical analysis

All analyses were performed in R version 4.0.3 (R Core 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 B, Table S2.1 and S2.2.

All activity data were aggregated on an hourly level. Using generalized linear mixed models, 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 (pre-parasite, pre-patent, patent-parasite, and post-parasite phases) on the activity levels of the three treatment groups (non-parasitised, parasitised and mixed), and between animals in the mixed and single-state groups. Data were also analysed for a week effect 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 fit 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 B, Figure S2.1), 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 (pre-parasite, pre-patent, post-patent,

post-parasite), Week, Parasitic status (infected or non-infected), Group type (mixed-state groups or single-state groups) and Sex. To avoid confounding the fixed effects 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 p-value, however, this approach results in a model which 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 running all models, 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 B, Figure S2.2A and S2.2B) and for lying bouts we used mixed models fitted with Poisson distributed errors (Appendix B, Figure S2.2C). As lying data had a U shape distribution, lying data was converted to fit a binomial distribution (1 = lambs were mostly lying $\geq$ 1800 seconds per hour, and 0 = lambs were mostly not lying < 1800 seconds per hour) and analysed using mixed models with a binomial distributed error. We found abnormally large data spikes at precisely 15, 30 and 45 minutes during each hour within the lying duration data due to a technical malfunction of the equipment, so these data points were not included in the analysis. The abnormal data in lying duration data is something that was identified as problematic in Chapter 2, and something that we were prepared to address during the current study. As lambs were likely to spend more time lying time 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 B, Figure S2.2D) and to compare 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 B, Figure S2.2E) 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-parasitised treatment group, and the referent time point was the pre-parasite 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 do not expect a significant effect of treatment as a main effect. Similarly, the main effect of time is to describe the trajectory of non-parasitised 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 Appendix B. 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 within the main body of text to a discussion of these interactions.

## 3.5 Results

### 3.5.1 Measures of infection and associated physiological costs

When lambs were put onto pasture, all faecal egg counts were zero and the faecal egg counts remained zero for all non-infected animals throughout the experiment (Figure 3.1). Faecal egg counts of all infected lambs increased to 603.6 ± 137.6 (mean

± SE) epg by week 5 of the patent period, three weeks after they were first dosed with larvae (Appendix B, Figure S2.3). Within the treatment groups faecal egg counts of infected animals in the parasitised groups increased to 631.2 ± 177.4 (mean ± SE) and faecal egg counts of infected lambs in the mixed groups increased to 534.6 ± 202.8 (mean ± SE) (Figure 3.1). Faecal egg counts of all infected lambs remained high until lambs were dosed with anthelmintic at the start of week 8 when faecal egg counts returned to zero by week 9. Serum pepsinogen concentrations of infected lambs were significantly higher by the patent-parasite sampling day (Week 7) (Est = 0.42, p = 0.02) (Figure 3.2), whereas non-infected lamb 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 non-infected lambs (Appendix B, Table S2.3).

The average weight of infected and non-infected lambs in each treatment group during each week of the experiment is shown in Figure 3.3. Although there was no significant interaction between treatment group and week on liveweight (Appendix B, Table S2.4) there was a significant interaction between week and parasitic status on the liveweight of the lambs ($F = 3.62$, $df = 9$, $p<0.001$): Overall, the mean weight of infected lambs was significantly lower than non-infected lambs on the final day of the experiment (Est = -1.74, p = 0.04) (Appendix B, Table S2.5). 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 (Est = -4.19, p = 0.053), but this was not significant at the 5% level (Figure 3.3 and Appendix B, Table S2.6).

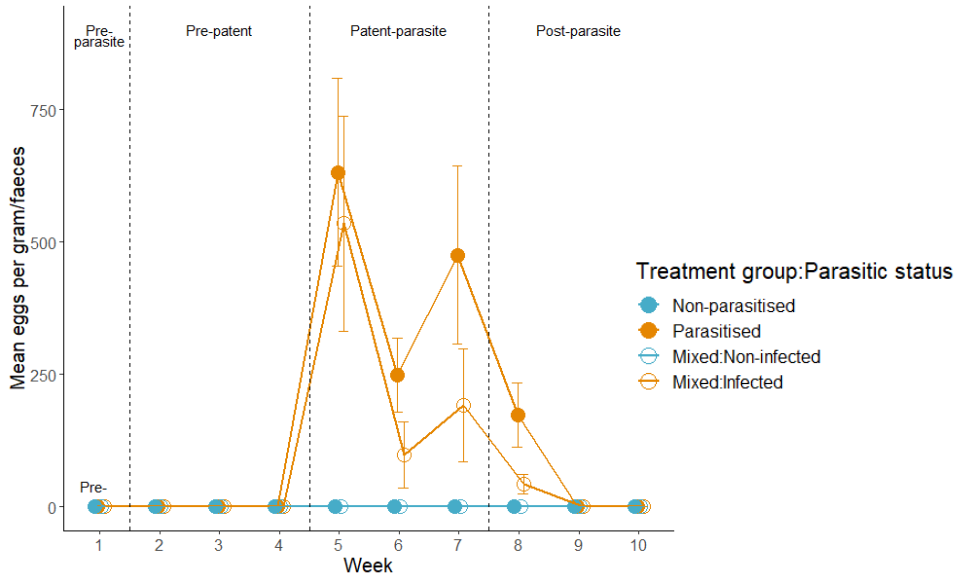


**Figure 3.1.** Mean ± standard error faecal egg counts (eggs per gram) of infected and non-infected lambs in each treatment group, Non-parasitised (solid blue; *n* = 4), Parasitised (solid orange; *n* = 4) and Mixed (clear blue (Non-infected) and clear orange (Infected); *n* = 4) during each week of the experiment, including the final sampling day at the beginning of week 10. The dashed lines separate the experiment into the four phases (pre-parasite, pre-patent, 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.

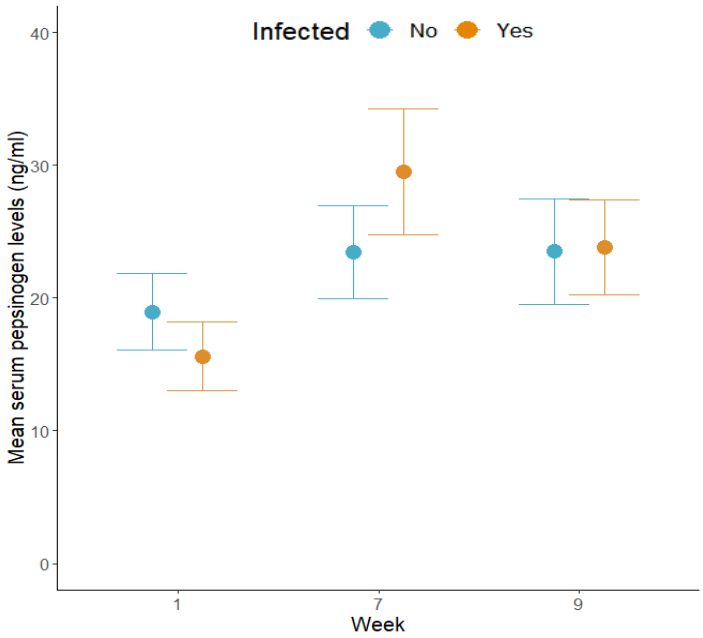


**Figure 3.2.** Mean ± standard error serum pepsinogen levels (ng/ml) of infected (orange; *n* = 14) and non-infected (blue; *n* = 14) lambs during the three blood sampling weeks. Blood samples were taken during the pre-parasite (Week 1), patent-parasite (Week 7) and post-parasite phase (Week 9).

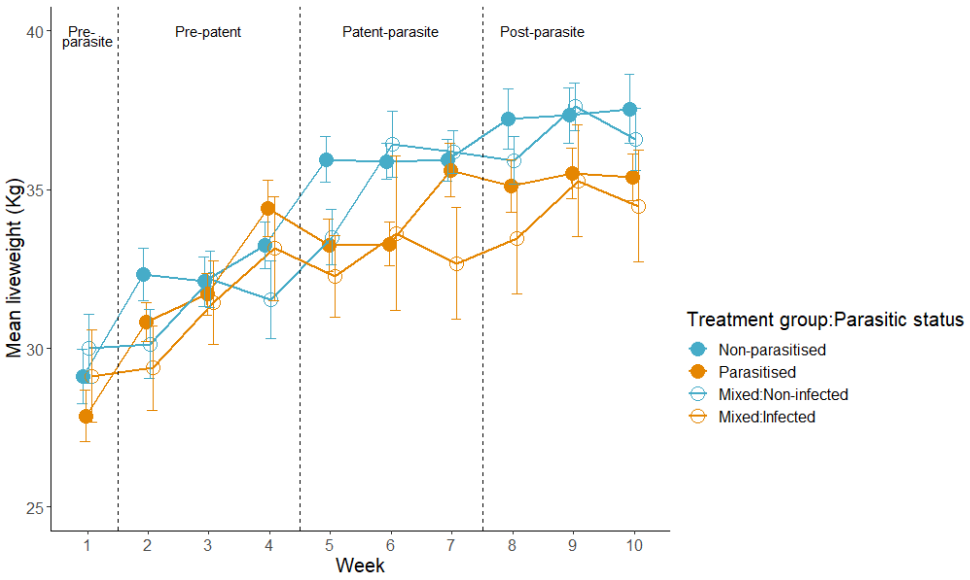


**Figure 3.3.** Mean ± standard error liveweight of infected and non-infected lambs in each treatment group, Non-parasitised (solid blue; *n* = 4), Parasitised (solid orange; *n* = 4) and Mixed (clear blue (Non-infected) and clear orange (Infected); *n* = 4) during each week of the experiment, including the final weighing day at the beginning of week 10. The dashed lines separate the experiment into the four phases (pre-parasite, pre-patent, patent-parasite and post-parasite).

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 non-infected animals were zero throughout demonstrating the expected/predicted difference between infected and non-infected animals, thus creating the required framework to investigate the questions being addressed. We next investigated whether changes in activity could be detected in both single-parasitic state and mixed-parasitic state groups and whether these effects were observable prior to the patent period when the physiological costs of parasitism could be measured.

### 3.5.2 Impact of parasitism on the activity behaviour of the three treatment groups.

**Motion index**: There was a significant interaction between treatment group and phase on motion index (Wald (W) = 33.08, df = 6, p<0.001): Parasitised groups had significantly lower motion index than the non-parasitised groups during the pre-patent (Est = -0.09, p<0.001) and patent-parasite (Est = -0.07, p = 0.015) phases of infection compared to non-parasitised groups (Figure 3.4A). The mixed groups also had reduced motion index during the pre-patent phase of infection (Est = -0.05, p = 0.059), compared to non-parasitised groups, but this was not significant at the 5% level. There was no significant difference in the motion index between the three treatment groups during the pre-parasite phase when all lambs were parasite naïve and following treatment with anthelmintic during the post-parasite phase (Figure 3.4A and Appendix B, Table S2.7). Analysis on a finer scale (e.g., weekly) demonstrated that the drop in motion index in the parasitised groups was consistent throughout all weeks of the pre-patent and patent-parasite phases (see Appendix B, Table 2.8).

**Step count:** There was a significant interaction between treatment group and phase on step count (Wald (W) = 45.60, df = 6, p<0.001): Parasitised groups had significantly lower step counts during the pre-patent (Est = -0.11, p<0.001) and patent-parasite (Est = -0.11, p<0.001) phases of infection compared to the non-parasitised groups (Figure 3.4B). The step count of the mixed groups was also significantly lower than the non-parasitised groups during the pre-patent phase of the study (Est = -0.07, p = 0.033) (Figure 3.4B and Appendix B, Table S2.7). There was no significant difference in step count between the three treatment groups during the pre-parasite phase when all lambs were parasite naïve and following treatment with anthelmintic during the post-parasite phase (Figure 3.4B and Appendix B, Table S2.7). Analysis on a finer

scale demonstrated that the decrease in step count in the parasitised groups was consistent throughout all weeks of the pre-patent and patent-parasite phases (see Appendix B, Table 2.8).

**Frequency of lying bouts:** There was a significant interaction effect between treatment group and phase on frequency of lying bouts (Wald (W) = 15.37, df = 6, p = 0.018): The frequency of lying bouts of the parasitised groups was significantly reduced during the pre-patent (Est = -0.06, p = 0.043), patent-parasite (Est = -0.09, p = 0.004) and post-parasite (Est = -0.07, p = 0.036) phases compared to the non-parasitised groups (Figure 3.4C). There was no significant difference, in the frequency of lying bouts between the mixed and non-parasitised groups during each phase of the experiment (Figure 3.4C and Appendix B, Table S2.7). However, the frequency of lying bouts of the mixed groups was significantly lower than the non-parasitised groups in week 4 (Est = -0.08, p = 0.028) and week 7 (Est = -0.08, p = 0.036).

**Lying duration:** There was no significant interaction effect between phase and treatment group on lying duration (night data: Wald (W) = 6.15, df = 6, p = 0.406, day data: Wald (W) = 4.41, df = 6, p = 0.621) (Appendix B, Table S2.7). However, there was an interaction effect between treatment group and week as well as a diurnal effect on lying duration (night data: Wald (W) = 26.01, df = 16, p = 0.054) (Appendix B, Table S2.8): The parasitised groups were found to spend more time lying down during the night in week 4 (Est = 0.50, p = 0.021) (Figure 3.4D), compared to the non-parasitised groups.

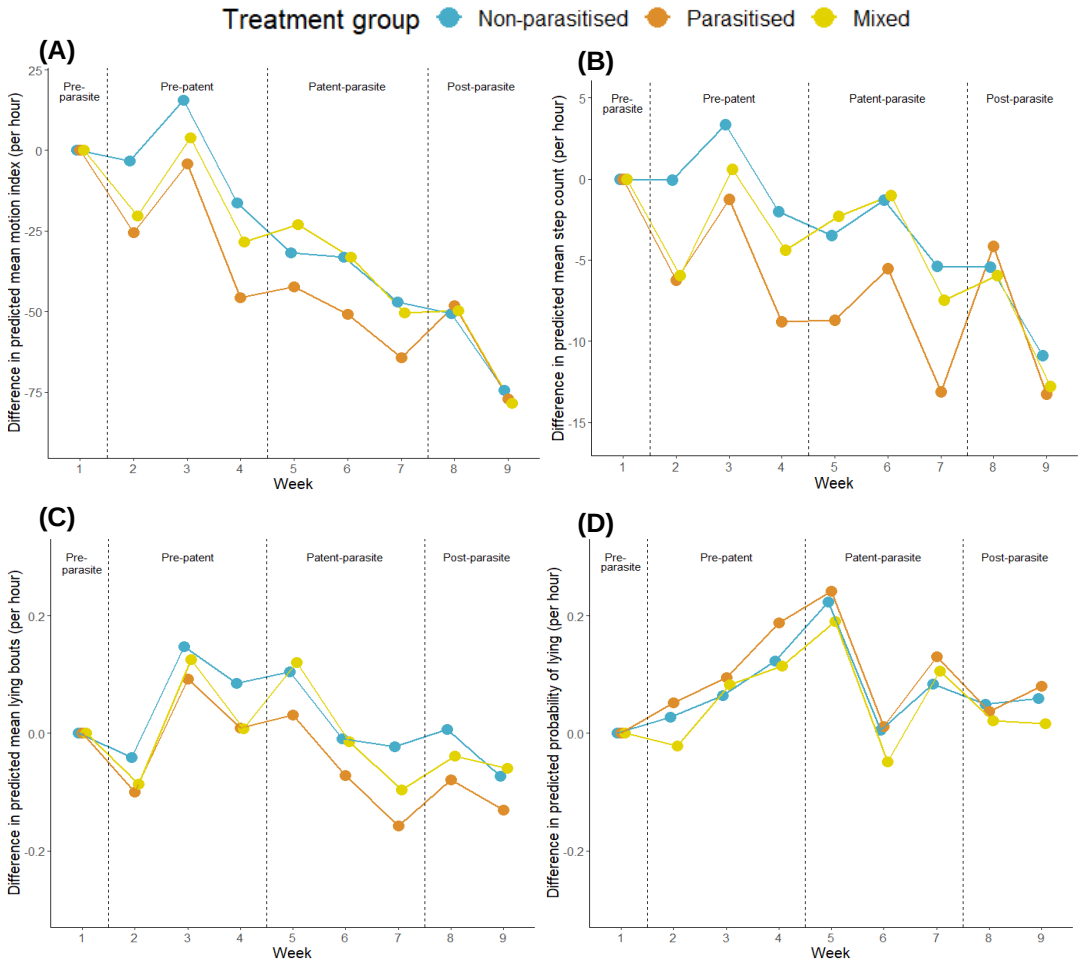


**Figure 3.4.** Difference in activity behaviour of individuals in each treatment group (Non-parasitised (blue; *n* = 4), Parasitised (orange; *n* = 4) and Mixed (yellow; *n* = 4)), during each week of the study compared to the pre-parasite phase (week 1). The dashed lines separate the experiment into the four phases (pre-parasite, pre-patent, patent-parasite and post-parasite). **(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).

### 3.5.3 Impact of parasitism on the activity behaviour of lambs within mixed-state groups.

**Motion index:** There was no significant interaction between parasitic status, group type and phase on motion index (Wald (W) = 3.48, df = 3, p = 0.32) (Appendix B, Table S2.9), or between parasitic status, group type and week on motion index (Wald (W) = 9.67, df = 8, p = 0.299) (Appendix B, Table S2.10). Thus, the pattern of behaviour found between infected lambs in mixed and single parasite state groups (Figure 3.5A), and between non-infected lambs in the mixed and single-state groups did not differ.

**Step count:** There was no significant interaction between parasitic status, group type and phase on step count (Appendix B, Table S2.9), 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 (W) = 32.82, df = 8, p<0.001) (Figure 3.5B and Appendix B, Table S2.10): The step count of non-infected lambs in the mixed-state groups was significantly lower than non-infected lambs in the single-state groups in week 2 (Est = -0.13, p = 0.004) (Figure 3.5B) and the step count of infected lambs in the mixed-state groups was significantly higher than infected lambs in the single-state groups during week 2 (Est = 0.17, p = 0.01) (Figure 3.5B). There was also a difference in week 8 following treatment with anthelmintic where the step count of infected lambs in the mixed-parasitic state groups was significantly lower than infected lambs in the single-parasitic state groups (Est = -0.15, p = 0.023) (Figure 3.5B). Individuals in the single-parasitic state group returned to the level of non-infected individuals following anthelmintic treatment but previously infected individuals in the mixed group did not.

**Frequency of lying bouts:** There was no interaction between parasitic status, group type and phase on frequency of lying bouts (Wald (W) = 4.65, df = 3, p = 0.19) (Appendix B, Table S2.9). However, again there was an interaction between parasitic status, group type and week on frequency of lying bouts (Wald (W) = 14.51, df = 8, p = 0.06), although this was not significant at the 5% level. The frequency of lying bouts of infected lambs in the mixed-state groups was higher than infected lambs in the single-state groups in week 6 (Est = 0.13, p = 0.057) and week 7 (Est = 0.18, p = 0.007) (Figure 3.5C) and the frequency of lying bouts of non-infected lambs in the mixed-state groups was lower than non-infected lambs in the single-state groups in week 7 (Est = -0.09, p = 0.036).

**Lying duration:** There was no significant interaction between parasitic status, group type and phase on lying duration (night data: Wald (W) = 4.66, df = 3, p = 0.19, day data: Wald (W) = 1.99, df = 3, p = 0.58) (Appendix B, Table S2.9), and no significant interaction between parasitic status, group type and week on lying duration (night data: Wald (W) = 7.23, df = 8, p = 0.51, day data: Wald (W) = 5.57, df = 8, p = 0.70) (Appendix B, Table S2.10) (Figure 3.5D).

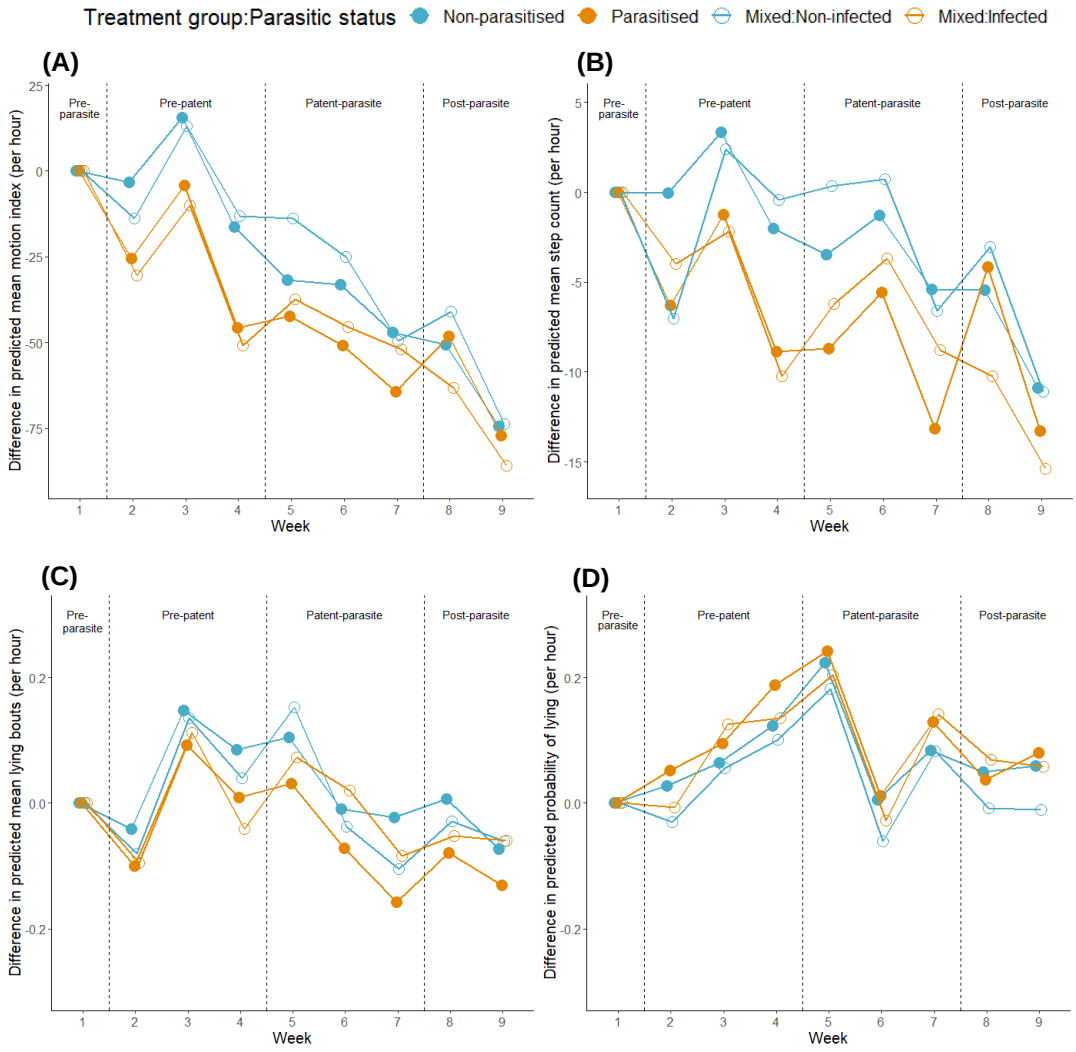


**Figure 3.5**. Difference in activity behaviour between infected and non-infected individuals in each treatment group (Non-parasitised (solid blue; *n* = 4), Parasitised (solid orange; *n* = 4) and Mixed (clear blue (Non-infected) and clear orange (Infected); *n* = 4), during each week of the study compared to the pre-parasite phase (week 1). The dashed lines separate the experiment into the four phases (pre-parasite, pre-patent, patent-parasite and post-parasite). **(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).

## 3.6 Discussion

Here we show that parasitism can induce detectable changes in behaviour early in the pre-patent period of infection and that these can be detected in both single-parasitic state and mixed-parasitic state groups. However, in mixed groups, social modulation of behaviour alters the activity behaviour of all group members, potentially distributing the costs of infection among both infected and uninfected members of the group.

In this study we successfully established experimental treatments groups, detected parasitism and induced measurable costs of infection. We found faecal egg counts of infected lambs were detectable three weeks after the initial infection dose with *T. circumcincta* larvae consistent with other studies suggesting a pre-patent 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 non-infected animals from week 5 through to the end of the study. The parasite infection model therefore successfully established clear pre-parasitised, pre-patent, patent-parasite and post-parasite phases across the treatment groups.

We were able to identify behavioural changes during both the pre-patent and patent-parasite phases of infection. During the pre-patent phase, infected lambs in both the single-parasitic state (parasitised 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 parasitised groups spent less time transitioning between standing and lying during the pre-patent, patent-parasite and post parasite phases and spent more time lying down during week 4 of the pre-patent phase of infection. These pre-patent observations are in line with classic sickness behaviours exhibited by parasitised animals during the patent stage of infection across both domestic and wild systems (Hutchings et al. 2000; Szyszka and Kyriazakis, 2013; Ghai et al. 2015; Besier et al. 2016). Following treatment with anthelmintic there was no difference in activity (motion index, step count, lying duration) between the three treatment groups.

Behaviour changes following parasite infection usually comprise of lower activity levels, reduced feed intake, and changes to sociality (Hart, 1988; Poulin, 1995; Kyriazakis et al. 1998; Moore, 2002; Gauly et al. 2007; Szyszka and Kyriazakis, 2013; Ghai et al. 2015, Kazlauskas et al. 2016). Treatment with anthelmintics to remove naturally occurring parasites has previously been demonstrated to lead to an increase in activity of lambs infected with natural parasite infections (Besier et al. 2016; Grant et al. 2020; Ikurior et al. 2020) suggesting parasitism to be a direct cause of this change. However, the possibility that naturally infected individuals may be a biased subset of the population that may be driving these patterns cannot be excluded, for example, individuals that are naturally more active could be exposed to higher levels of infection while feeding. Experimental infection minimises 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. Through our experimental design, we believe that these behaviour changes can be directly attributed to parasitism and occur during the first week of infection, three weeks before any

measure of parasitism (faecal egg count) or noticeable impact of parasitism (weight loss) was observed.

Motion index gives an indication of 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 (Murray and Murray 1979; Hart 1988; Kyriazakis et al. 1996; Hutchings et al. 2000; Adamo et al. 2010; Hite et al. 2020). While we did not measure forage intake during this study, we did find that overall, infected lambs had consistently lower weights than non-infected animals during the patent-parasite and post-parasite 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 in order 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 (Dantzer, 2004; Adelman et al. 2009; Lopes et al. 2012; Lopes, 2017; 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 (Houdijk et al. 2005; Henderson and Stear, 2006; Halliday et al. 2007). Alternatively, changes in host behaviour may also result as a side effect of the pathology associated with infection (Holland and Cox, 2001; Klein, 2003), by the physical presence of the parasite (Lafferty and Shaw, 2013; Jolles et al. 2020) or be a response to pathogen host signalling through molecular mechanisms (Claycomb et al. 2017).

Behavioural responses were also affected by the parasite status of other individuals in a group. Both infected and non-infected 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 non-infected lambs in the mixed-state groups was lower than non-infected lambs in the single-state groups suggesting that non-infected 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 pre-patent 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 (Figure 3.4). These findings indicate that parasitism had an effect on the behaviour of lambs in both single-state and mixed-state group, but the effect of parasitism on the behaviour of individuals in the mixed-state groups were modulated by the non-infected lambs in those groups, as infected individuals increased their activity in the presence of more active non-infected individuals. These findings indicate 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 in which animals engage in different sickness behaviours can often vary depending on their environment, and during certain circumstances infected animals could adjust the expression of sickness behaviours to other behaviours that may be more beneficial at the time (Cohn and de Sá-Rocha, 2006; Willette et al. 2007; Lopes et al. 2012; Lopes et al. 2021). Like most grazing herbivores, lambs are highly social prey animals that will benefit from being a part of a large social group (Hamilton, 1971; Lima, 1995; Krause and Ruxton, 2002). Previous studies have shown that sheep will choose to graze with members of their social group over grazing in more favourable areas, and when part of a larger group will exhibit a lower frequency of vigilant behaviours and increase the time spent foraging (Penning et al. 1993; Sevi et al. 1999; Dumont and Boissy, 2000). It has also recently been suggested that animals may benefit from group living and use social behaviour to increase parasite tolerance

(Almberg et al. 2015; Ezenwa and 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), parasitised individuals could also lose the associated benefits of group living (Kiesecker et al. 1999; Krause and Ruxton, 2002; Behringer et al. 2006; Tobler and Schlupp, 2008). Thus, the higher activity levels exhibited by infected lambs in the mixed groups during the patent-parasite phase could indicate social facilitation occurring, with infected animals overcoming their sickness behaviours to stay with other members of the group. However, as sickness behaviours are believed to have evolved as an adaptive response to fight infection, non-expression of these behaviours may have damaging effects on the health of the animal (Lopes, 2014). Interestingly we found the liveweight of infected lambs in the mixed groups tended to be lower than the liveweight of infected animals in the single-state parasitised groups towards the end of the patent-parasite phase. This suggests not expressing these sickness behaviours may have led to a more severe consequence on the health of the animals in the mixed groups compared to lambs in the single-state parasitised groups.

Behaviour of infected animals returning to normal levels after treatment with anthelmintic was consistent with parasite removal experiments that have shown rapid changes in behaviour in previous work (Hutchings et al. 2002; Gauly et al. 2007; Szyszka and Kyriazakis, 2013; Sharma et al. 2016). Furthermore, by removing the experimental treatment, we lose 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 parasitised 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 parasitised lambs have been reported to have increased

bodyweight gain following treatment with anthelmintic (Sharma et al. 2016), animals could be spending more time grazing and less time transitioning between standing and lying post anthelmintic treatment.

We show that parasitism can impact behaviour at the very early stages of infection. These changes in behaviour occur immediately after exposure to parasites, at an earlier stage than any classical indicators of parasitism e.g., faecal egg counts, indicators of gut wall damage and changes in liveweight. Thus, there is the potential to use these parasite-induced changes in behaviour for early infection detection to inform targeted parasite control strategies. Moreover, we show that the behavioural response of an individual can be modulated by their social environment, as both infected and non-infected 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 parasite burdens in single-parasitic state groups. These findings demonstrate the importance of taking into account the parasitic status of all animals within a social group as certain social contexts may limit the expression of behaviours that are optimal for fitness in both infected and non-infected members of the group.

# Chapter 4

# Parasitism affects social group behaviour but not social network structure

## 4.1 Lay summary

Parasitism is known to influence animal behaviour and social interactions; however, groups of animals will often contain individuals of different parasitic status. Understanding the behavioural response of groups of animals that contain individuals of different parasitic status is important to predict the consequences of infection throughout a population. We show, under experimental conditions, that parasitism reduces the frequency of contacts between infected lambs at the earliest stages of infection in both mixed and single-parasitic state groups, but the degree of behaviour change exhibited by infected animals is influenced by the parasitic status of other individuals within the group. We also show that although infected animals in mixed-state groups had reduced contact frequency, there was no change in social networks

of the group as non-infected animals maintained pre-infection levels of social interactions.

## 4.2 Abstract

Understanding how parasitism influences social behaviour, and how these changes alter group social networks is important for disease transmission. We explore the impact of parasitism on the social contact behaviour of lambs that were part of one of three treatment groups that differed in proportion of individuals that were infected, (i) Parasitised; all lambs were experimentally infected with gastrointestinal nematode *Teladorsagia circumcincta*, (ii) Non-parasitised; all lambs were non-infected, (iii) Mixed; a group containing animals of mixed parasitic status, were part of the group were experimentally infected and part of the group remained non-infected. Here we test, firstly, whether parasitism reduces the social contact behaviour of groups of fully infected lambs, secondly, whether infected and non-infected individuals change their behaviour according to proportion of group infected, and thirdly, whether parasitism effects the network architecture of the social groups. Using proximity loggers, the contact behaviour of each individual was monitored during four phases of infection; pre-parasite, a period when all lambs would be kept parasite naïve; pre-patent, a period when lambs identified for infection would be parasitised but not yet showing any pathological physiological effects of parasitism and are not yet shedding eggs; patent-parasite, a period when lambs show physiological responses to infection (e.g. weight loss and gut wall damage) and are shedding eggs in their faeces; post-parasite, a period when all lambs would be dosed with anthelmintic, and considered parasite free. All individuals in the parasitised groups had reduced contact frequency during the pre-patent, patent-parasite and post-parasite phases, and increased duration of contacts during the pre-patent phase. Infected individuals had reduced contact frequency in the mixed groups but only in relation to their interactions with other

infected individuals. We found that the drop in social interactions between infected individuals and other infected and non-infected animals did not affect the network structure of the mixed groups, as non-infected individual's maintained pre-parasite levels of social interactions with their infected conspecifics, indicating a degree of social network robustness to environmental disruptions. We also found infected animals in the mixed-state groups altered their contact behaviour to a lesser degree during the patent-parasite phase compared to animals with similar parasite burdens in single-state groups. These results demonstrate how the effect of parasitism is not simply driven by changes in the behaviour of parasitized individuals and that infection can impact and be impacted by the wider social behaviour of animals within a group. The expression of behavioural change may therefore depend on the relative parasitic status of all group members.

## 4.3 Introduction

Parasitism modifying the behaviour of animals is well documented across almost all animal taxa (Poulin, 1994; Moore, 2002). These behaviour changes are thought to benefit the host by reducing the impact of infection or benefit the parasite by increasing transmission to susceptible hosts (Hart, 1988; Poulin, 1995; Kyriazakis et al. 1998; Moore, 2002; Poulin, 2010). However, the extent to which infected hosts alter their behaviour in response to parasitism can depend on multiple factors. This includes parasite virulence/load, the energetic costs of a given infection, environmental conditions (including social environment), and the importance of that behaviour for maintaining host overall fitness (Hart, 1988; Bilbo et al. 2002; Owen-Ashley and Wingfield, 2006; Lopes et al. 2012; Lopes, 2014; Ezenwa et al. 2016b; Stephenson, 2019).

Behavioural changes in response to parasitism have been observed in both infected and uninfected individuals (Hart, 1990). Healthy individuals may actively avoid infected conspecifics through the use of chemical and visual cues (Dugatkin et al. 1994; Kiesecker et al. 1999; Behringer et al. 2006), exhibit behaviours such as ear twitching or tail swatting to reduce parasite exposure (Moore, 2002), or show changes in their sociality following behavioural alterations of their infected conspecifics (Edwards, 1988; Bouwman and Hawley, 2010). Whereas infected individuals may undergo parasite induced behavioural changes which may occur through manipulation of host behaviour by the parasite (Klein, 2003), self-isolation by the host (Cremer et al. 2007; Shorter and Rueppell, 2012), or through the expression of sickness behaviours (Hart, 1988; Hawley et al. 2021).

Sickness behaviours are a collective suite of behaviour changes exhibited by infected individuals in response to parasite infection (Hart, 1988; Moore, 2002; Bilbo et al. 2002; Kelley et al. 2003; Ayres and Schneider, 2009; Lopes et al. 2015). Common behavioural changes include reduced feed and water intake and reduced activity levels (Hart, 1988; Ayres and Schneider, 2009; Lopes et al. 2012). These behavioural changes are thought to be associated with energy conservation by the host to mount an immune response (Kyriazakis et al. 1998; Kelley et al. 2003; Dantzer, 2004), or to promote host tolerance to infection (Holland and Cox 2001; Klein, 2003; Kelley et al. 2003; Lafferty and Shaw, 2013). By reducing overall activity, sickness behaviours could reduce the rate of social interactions between hosts (Lopes et al. 2016; Hawley et al. 2021), which is important to consider in social networks to predict the spread of a parasite through a social group.

The extent to which infected hosts alter their behaviour is likely to depend on parasite virulence and the impact of that behaviour on host overall fitness (Willette et al. 2007; Fairbanks et al. 2015; Ezenwa et al. 2016b; Ezenwa and Worsley-Tonks, 2018;

Powell et al. 2020; Stockmaier et al. 2020). For gregarious species, sickness behaviours could reduce the sociality of infected individuals and remove them away from their social groups, and thus the associated benefits of group living (Krause and Ruxton, 2002). Therefore, infected individuals in a highly social setting may adjust the expression of any sickness symptoms to participate in social opportunities, even if this may have a negative impact on the health of the individual (Lopes et al. 2012). However, parasitism is often overdispersed within groups, and groups may contain individuals of different parasitic states (Woolhouse et al. 1997). While there is evidence that parasitism can affect the behaviour of infected individuals, it is unknown how the infection status of individuals within a 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 infected and non-infected members (Granroth-Wilding et al. 2015). Furthermore, previous work has shown that while an individual's position in a social group network may rely upon its own attributes and behaviour, it can also depend on other individuals of which they interact (Wey et al. 2008). Therefore, parasite induced changes in behaviour of one individual in the group could alter the behaviour of uninfected conspecifics with which they interact, which in turn could affect the social network of the group and have an impact on social group dynamics and parasite transmission (Ezenwa et al. 2016b; Lopes et al. 2016).

Understanding how parasitism influences the associations between social groups of animals is therefore a fundamental question of group living and will aid in understanding the impact of infection on the social structure of animal populations. Which in turn will help us to understand the environmental conditions in which parasitism induces changes in animal social behaviour and enhance our understanding of how infections are likely to spread within and between animal

populations. Recent developments in animal-monitoring technologies and statistical methodologies have allowed for the assessment of interactions between individuals within a social group (Krause et al. 2007; Krause et al. 2011), and have meant animal social structures can be quantified as a social network (Croft et al. 2008; Sih et al. 2009; Brent, 2015). Contact networks have since been applied to a wide variety of wild animal societies to describe behavioural patterns within animal social systems (Krause et al. 2013), and more recently, are now being used for hypotheses testing in an experimental framework (Smith et al. 2019).

Here we investigate the effect infection with the gastrointestinal nematode *Teladorsagia circumcincta* (a nematode of economic and welfare importance across the world (Papadopoulos et al. 2012)) has on the social contact behaviour of lambs that are part of mixed-parasitic state and single-parasitic state groups. Domestic sheep *Ovis aries* provide a model organism for testing our proposed hypotheses as they are naturally gregarious animals that develop stable social relationships with other members of the flock (Keller et al. 2011). Furthermore, by identifying parasite infections through behavioural changes in a domestic system also offers a non-invasive indicator of infection in individual animals that may be used exploited in agriculture as an early indicator of disease (Kenyon et al. 2009). Such a strategy may be beneficial in tackling the global problem of anthelmintic resistance in agriculture, by reducing the intensive use of anthelmintic drugs to control parasitism.

We used proximity loggers to continuously record the contact behaviour between lambs that were part of single-parasitic state and mixed-parasitic state social groups. Specifically we ask: 1) Does parasitism lead to a reduction in social interactions between groups of parasitised animals? 2) Will infected and non-infected individuals change their behaviour according to proportion of group infected? 3) Will experimental infection affect the network architecture of the social groups? We predict that

parasitism will lead to a reduction in social interactions between groups of parasitised animals as a consequence of infected animals becoming more lethargic following infection and coming into contact with other individuals less. We also predict that infected and non-infected individuals will change their behaviour according to proportion of group that are infected, as infected animals in the mixed groups will try to maintain coordination with healthy individuals and thus have higher contact rates compared to infected animals in the single state groups. We also predict that the network architecture of the social groups will be affected by experimental infection of group members, following the change in social contacts between infected animals with other individuals in their social groups.

## 4.4 Methods

### 4.4.1 Animals and experimental design

*Same experimental design was used for Chapter 3 and Chapter 4.*

Sixty 12-week-old Texel x Bluefaced Leicester lambs were selected randomly from a commercial flock of sheep that had been reared indoor 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 4 replicate groups of 5 lambs within each treatment. These were (i) Parasitised: all lambs were infected with parasitic nematode *T. circumcincta* and were of the same parasitic status, (ii) Non-parasitised: all lambs were dosed with water, remained parasite naïve, and were of the same parasitic status and (iii) Mixed: a group containing animals of mixed parasitic status, were three animals were dosed with water and two were dosed with *T. circumcincta* larvae. Each replicate group was balanced for sex (three females and two males per group) and weight (live mean weight ± standard deviation 27.6 ± 0.13Kg). In order to choose the individuals within the mixed group to be infected a

structured approach was chosen whereby the smallest female and largest male in all groups were infected. This approach was chosen to account for any potential effect of sex and weight, and so reduce the residual variation, 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, there were no siblings allocated in the same group. One week before the experiment start date groups were put onto pasture in individual plots laid out in a six by two grid, with each plot measuring 30x30m and separated by sheep netting. All plots had been free from grazing ruminants for the previous three years and animals were given ad-lib access to water. To minimise any effects of plot differences on behaviour, 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; pre-parasite (week 1), a period when all lambs would be kept parasite naïve; pre-patent (weeks 2-4), a period when lambs identified for infection would be parasitised but not yet showing any pathological physiological effects of parasitism and are not yet shedding eggs; patent-parasite (weeks 5-7), a period when lambs show physiological responses to infection (e.g. weight loss and gut wall damage) and are shedding eggs in their faeces; post-parasite (weeks 8-9), a period when all lambs would be dosed with anthelmintic, and considered parasite free. On the first day of week two, lambs identified for infection, which included all lambs in the parasitised groups and two out of five lambs in the mixed groups received an oral dose of 5,000 L3 stage *T. circumcincta* larvae, and lambs not identified for infection 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 3 times per week (Monday, Wednesday, and Friday) for 6 weeks. The trickle infection

chosen (5,000 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, 1ml/10Kg) and infections were cleared.

### 4.4.2 Contact behaviour

Contacts between lambs in each social group were continuously recorded using proximity loggers (Sirtrack Ltd., Havelock North, New Zealand) (previously validated in Chapter 2). Each lamb in the study was fitted with a proximity data logger on a neck collar to record close proximity contacts with any other individual in their social group. The proximity loggers use an ultra-high frequency (UHF) to send out signals to other loggers using a unique code, while receiving signals from other loggers. The detection distance was set to 1-1.5m (pre-determined in Chapter 2), to allow detection of a close-contact situation, during which social interactions might occur (Ozella et al. 2020). Once a contact is detected by a logger, a contact is recorded until one of the loggers in the contact fails to receive a signal for longer than the separation time, which was set at 10 seconds. When two lambs came into contact with each other, time, date, logger ID and duration of the contact was recorded by the proximity loggers.

Any contacts that were recorded before loggers were placed on the lambs or occurred while animals were being handled during the experiment were not included in the analysis. All contacts of 1 second or less were removed, as it is believed these may represent weak collar signals (Drewe et al. 2012), or detection signals at the edge of the detection range (Prange et al. 2006). To reduce inter-logger variation that has been associated with proximity loggers (Drewe et al. 2012; Boyland et al. 2013), loggers were rotated between social groups twice weekly. As reciprocal contact data

from two different collars are not completely symmetrical due to reflection, refraction and absorption of radio waves (Patison et al. 2010), the contact duration between two loggers was defined as starting when one logger recorded a contact and ending when either logger failed to maintain a contact (Hamede et al. 2009; Patison et al. 2010; Smith et al. 2019).

### 4.4.3 Animal measurements

On the first day of each week faecal samples were taken from all sixty lambs within their plots to check for presence/absence of faecal eggs and to estimate the number of nematode eggs per gram of faeces (epg) in positive samples using a modified salt-flotation method (Jackson, 1974) (See Appendix B for faecal egg count methodology). Blood samples were taken by jugular venepuncture at the start of weeks 1, 7 and 9 (one measurement during pre-parasite, patent-parasite and post-parasite phases) to measure serum pepsinogen level (an indication of parasite induced gut damage) using a sheep pepsinogen ELISA assay kit (BlueGene Biotech, Shanghai, China). To assess the impact of infection, lambs were weighed to measure weekly weight gain. At the end of the experiment, a faecal sample and weight measurement was taken from every animal, to assess the final weights and parasite load of the lambs.

Following collection, faecal samples were weighed out and separated into subsamples; 1g was stored at 4°C for faecal egg counts. Blood samples were spun within two hours of collection at 3660 r.p.m at 4°C for 15 minutes, the serum was removed and stored at -20°C.

### 4.4.4 Social network analysis

Social network analysis was used to investigate whether parasite induced changes in social behaviour of both infected and non-infected animals in the mixed groups affected the overall social network architecture. The frequency (number of contacts

per hour), duration (length of a contact) and total duration (total contact length per hour) of contacts per animal per phase were used to produce weighted adjacency matrices to create the networks (Figure 4.2-Figure 4.4 and Appendix C, Figures 3.6-3.11). Weighted networks not only give you the binary presence and absence of a contact between individuals, but also give you the strength of the contact between two individuals. Social network analysis was carried out using R version 4.0.3 (R Core Team, 2020). Social network graphs were created, and network metrics calculated using the 'igraph' package (Csárdi and Nepusz, 2006). The networks consisted of nodes (individual lambs) and edges and were non-directed such that the adjacency matrix was symmetric. Each network graph was drawn using the 'layout_nicely' function in 'igraph', which sorts nodes into a layout for presentation purposes and does not imply spatial location of an individual. To understand if parasitism affected the network architecture of the mixed-state groups, we assessed the change in centrality closeness of infected animals during each phase of the experiment. Centrality closeness is one measure of centrality that provides an index to how central an individual is within a network and is the mean geodesic distance (shortest path) between an individual to all other individuals. The more central an individual is within a network, the greater the potential that animal has in facilitating parasite transmission (Corner et al. 2003).

### 4.4.5 Statistical analysis

All analyses were performed using R version 4.0.3 (R Core Team, 2020). Final model formulae and definitions of fixed and random effects are listed in Appendix C, Table S3.1 and S3.2.

Statistical analyses on the contact behaviour (frequency, duration and total duration) were carried out using the Integrated Nested Laplace Approximation (INLA) and were

fitted using the linear modelling package R-INLA (Rue et al. 2009; Martins et al. 2013). We used INLA to run the mixed effects models as two animals and two loggers occurred within each contact and wanted to ensure that both animals and both loggers were given the same coefficient. Using generalised linear mixed models (GLMM), the impact of parasitism on contact behaviour was assessed by analysing a phase and week effect on the social contact behaviour between the three treatment groups (non-parasitised, parasitised and mixed) and between individuals in the mixed treatment groups. We fitted Animal 1 ID, Animal 2 ID, Logger 1 ID, Logger 2 ID, Plot and Group ID as random effects in all contact models. Fixed effects considered included, Treatment group (non-parasitised, parasitised and mixed), Contact type (Infected - Infected (I-I), Non-Infected - Non-Infected (N-N), Infected - Non-infected (I-N)), Phase, Week (week was included to give greater resolution than phase, and to account for differences in time periods between the phases) and Sex (Male-Male, Male-Female, Female-Female). To avoid confounding, Phase and Week were not fitted in the same model.

After running mixed models on the social contact behaviour, we ran Pearson correlation tests to compare the activity behaviour (Chapter 3) of the lambs with their social contact behaviour throughout the experiment (See Appendix C for results).

Before running all models, the mean-variance relationship was assessed to verify the model structure and to ensure the appropriate distribution was used for each response variable. For frequency, duration and total duration we used mixed models fitted with negative binomial distributed errors (Appendix C, Figure 3.1). Each model explanatory variable formulae fitted using INLA were compared using Deviance Information Criterion (DIC). A change in 2 DIC was selected to distinguish between models and select the most parsimonious model (Spiegelhalter et al. 2002). As there is no null model used in Bayesian statistics to determine significance at the 5% level, for contact

behaviour models we accept the equivalent to frequentist significance if the 95% credible intervals do not overlap 1. Comparison of the fixed effect estimates from each response variable model can be found in Appendix C, Table S3.3-S3.6 and Figures S3.2-S3.5.

Statistical analyses on centrality closeness and animal measurement models (weight and pepsinogen) were fitted with the package 'lme4' (Bates et al. 2015). We tested for statistically significant differences in the properties of the individuals within the networks by using closeness centrality as a response variable in linear mixed models (LMM) with a Gaussian distributed error after a logit transformation (Appendix C, Figure S3.1). We analysed each mixed social group separately to compare the centrality closeness of infected and non-infected individuals during the four experimental phases. We fitted Logger ID and Plot as random effects, and Individual parasitic status (categorical variable with 5 levels describing the infection status of each individual), Phase and Sex as fixed effects. To avoid confounding Animal ID was not fitted as a random effect as it was accounted for in the models by fitting Individual parasitic status as a fixed effect (See Appendix C, Table S3.2 for definitions of fixed effects).

LMM's were used to assess the impact of parasitism on the weight of the lambs fitted with a Gaussian distributed error (Appendix C, Figure S3.1) and compared the liveweights between lambs in the mixed and single-state groups by analyzing data containing animals that were exposed to the same treatment. We also used GLMM's with a Poisson distributed error (Appendix C, Figure S3.1) to assess the impact of parasitism on blood serum pepsinogen levels. The best fit models for animal measurements and centrality closeness were 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. Coefficients described as being statistically significant are those where the calculated p-value was less than 0.05 throughout.

In all contact models that were used to analyse the behaviour between the treatment groups, the referent treatment group was the non-parasitised group, and the referent time point was the pre-parasite phase (week 1). Similarly, in all the contact models that were used to analyse the behaviour of individuals in the mixed-state groups, the referent contact type was the non-infected – non-infected contact, and the referent time point was the pre-parasite phase (week 1). The main effect of treatment reported for the models is therefore the difference in treatment groups and the difference in contact types in the pre-parasite phase (week 1), i.e., prior to being infected with parasites. We therefore do not expect a significant effect of treatment or contact type as a main effect. These results are not discussed, but are available in the Appendix C. The effect of interest in these models is therefore the interaction between treatment group and time (either phase or week), and contact type and time (either phase or week), as this describes how differences between treatment groups and between individuals in the mixed-state groups develop over time. We restrict the results in this paper to a discussion of these interactions.

## 4.5 Results

### 4.5.1 Measures of parasitism

Faecal egg counts of all lambs at the start of the experiment were zero, and the faecal egg counts remained zero for all non-infected animals throughout the experiment (Chapter 3, Figure 3.1). By week 5 of the experiment, faecal egg counts of infected animals in the parasitised groups increased to 631.2 ± 177.4 (mean ± SE) and faecal egg counts of infected lambs in the mixed groups increased to 534.6 ± 202.8 (mean ± SE) (Chapter 3, Figure 3.1). Faecal egg counts of all infected lambs remained high

until lambs were dosed with anthelmintic at the start of week 8 when faecal egg counts returned to zero by week 9. Serum pepsinogen concentrations of infected lambs increased by the patent-parasite sampling day (Est = 0.33, p = 0.018) (Chapter 3, Figure 3.2), whereas non-infected lamb concentrations showed no significant change. Before parasitism and following treatment with anthelmintic there was no statistically significant difference in the serum pepsinogen levels between infected and non-infected lambs (Chapter 3, Figure 3.2).

The average weight of infected and non-infected lambs in each treatment group during each week of the experiment is shown in Chapter 3, Figure 3.3. There was no statistically significant interaction between treatment group and week on liveweight (Appendix B, Table S2.4). However, there was a statistically significant interaction between week and parasitic status on the liveweight of the lambs ($F = 3.62$, $p < 0.001$): Overall, the mean weight of infected lambs was statistically significantly higher than non-infected lambs in week 4 (Est = 1.74, p = 0.039) (Appendix B, Table S2.5) but statistically significantly lower than non-infected lambs on the final day of the experiment (Est = -1.74, p = 0.04). Infected lambs in mixed-state groups had lower liveweights than infected lambs in single-state groups during week 7 of the patent-parasite phase (Est = -4.19, p = 0.053) (Appendix B Figure S2.3 and Appendix B, Table S2.6).

### 4.5.2 Effect of parasitism on the social contact behaviour of the three treatment groups.

The contact behaviour differed between the three treatment groups following parasite infection (Appendix C, Table S3.3 and S3.4). There was a reduction in the mean frequency of contacts between individuals in the parasitised groups during the pre-patent (CI -0.111, -0.021), patent-parasite (CI -0.128, -0.038) and post-parasite phases (CI -0.124, -0.027), compared to the non-parasitised groups (Figure 4.1A)

(Appendix C, Table S3.7). Individuals in the mixed groups had reduced mean frequency of contacts during the pre-patent (CI -0.135, -0.046) and post-parasite phases (CI -0.108, -0.01), compared to the non-parasitised groups (Figure 4.1A). Weekly analysis that accounts for the different lengths of time in each phase gives the same results. See Appendix C.

There was an increase in the mean contact duration between individuals in the parasitised groups during the pre-patent phase of infection (CI 0.036, 0.116), compared to the non-parasitised groups (Figure 4.1B). This increase in contact duration was consistent across all weeks of the pre-patent phase, but also occurred in week 5 of the patent-parasite phase (CI 0.023, 0.118) (Figure 4.1B). There was no statistical evidence of a difference in the mean duration of contacts between the mixed and non-parasitised groups at the phase level (Appendix C, Table S3.3).

Overall, the mean total duration of contacts between individuals in the parasitised groups decreased during the patent-parasite (CI -0.177, -0.034) and post-parasite phases (CI -0.198, -0.046), compared to the non-parasitised groups (Figure 4.1C), and the mean total duration of contacts between individuals in the mixed groups decreased during the pre-patent phase (CI -0.17, -0.03), compared to non-parasitised groups. There was no statistical evidence of a difference in the frequency, duration or total duration of contacts between each treatment group, before lambs were infected with *T. circumcincta* larvae during the pre-parasite phase and following treatment with anthelmintic during week 9 of the post-parasite phase (Appendix C, Table S3.3).

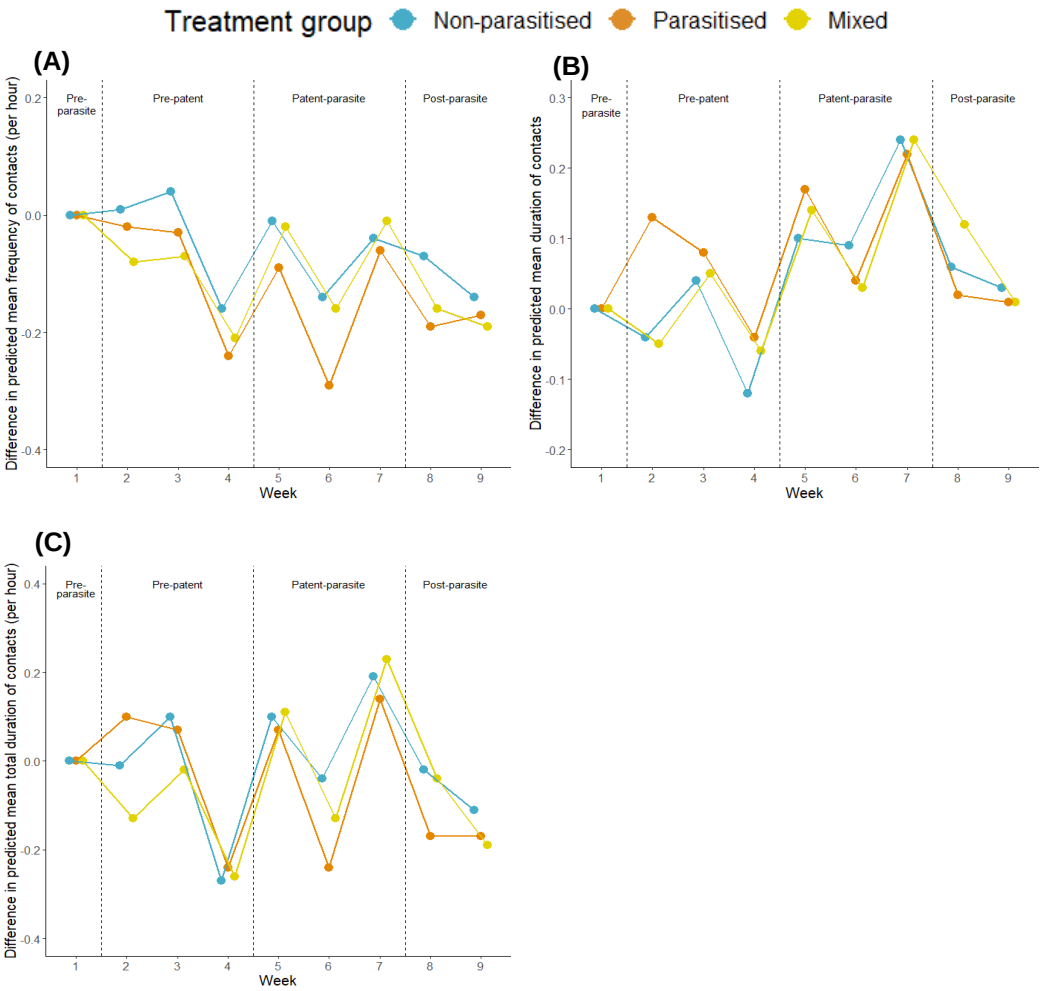


**Figure 4.1.** Difference in contact behaviour between individuals in each treatment group, (Non-parasitised (blue; *n* = 4), Parasitised (orange; *n* = 4) and Mixed (yellow; *n* = 4)), during each week of the study compared to the pre-parasite phase (week 1). The dashed lines separate the study into the four experimental phases (pre-parasite, pre-patent, patent-parasite and post-parasite). **(A)** Difference in model predicted mean frequency of contacts per hour. **(B)** Difference in model predicted mean duration of contacts. **(C)** Difference in model predicted mean total duration of contacts per hour.

### 4.5.3 Effect of parasitism on the social contact behaviour between individuals of different parasite status in the mixed-parasitic state groups.

All individuals in each group were in contact with all other individuals during each phase of the study. Figure 4.2, Figures 4.3 and 4.4 compares the visualizations of the social networks created using the frequency, duration and total duration of contacts of the mixed-state groups.

We found the contact behaviour differed between different combinations of individuals in the mixed groups throughout the study (Appendix C, Table S3.5 and S3.6). There was a reduction in the mean frequency of contacts between two infected animals (I-I) during the pre-patent (-0.35, -0.112), patent-parasite (CI -0.235, 0.000) and post-parasite phases (CI -0.464, -0.192) (Figure 4.2 and Figure 4.5A), compared to two non-infected animals (N-N). There was also a decrease in the frequency of contacts between infected and non-infected animals (I-N), compared to N-N contacts, but only during the post-parasite phase (CI -0.174, -0.026). There was no statistical evidence of a change in the frequency of contacts between I-N animals during the pre-patent (CI -0.098, -0.034) and patent-parasite phases (CI -0.112, -0.02), compared to N-N animals.

There was no statistical evidence of a difference in the frequency, duration and total duration of contacts between each contact type in the mixed-state groups before lambs were infected with *T. circumcincta* larvae during the pre-parasite phase of infection (Figure 4.5).

There was an increase in the mean duration of contacts between I-I (CI 0.048, 0.164) and I-N animals (CI 0.028, 0.237) compared to contacts between N-N individuals though only during the patent-parasite phase (Figure 4.5B). Overall, the total duration of contacts between I-I decreased compared to N-N animals during the pre-patent (CI -0.40, -0.023) and post-parasite phases (CI -0.57, -0.153) (Figure 4.5C). There was no statistical evidence for a difference in the total mean duration of contacts between I-N and N-N individuals at the phase level (Appendix C, Table S3.10). However, the total mean duration of contacts between I-N was higher than N-N animals during weeks 5, 6 and 8 and lower in week 9 (Appendix C, Table S3.6).

There was no significant change in centrality closeness of infected and non-infected individuals in the mixed groups, when closeness was calculated using frequency of contacts (Mixed Group 1: $F = 0.35$, $df = 12$, $p = 0.977$, Mixed Group 2: $F = 0.36$, $df = 12$, $p = 0.973$, Mixed Group 3: $F = 0.26$, $df = 12$, $p = 0.994$, Mixed Group 4: $F = 0.25$, $df = 12$, $p = 0.994$) (Figure 4.2) and when closeness was calculated using total duration of contacts (Mixed Group 1: $F = 1.72$, $df = 12$, $p = 0.064$, Mixed Group 2: $F = 1.41$, $df = 12$, $p = 0.17$, Mixed Group 3: $F = 1.85$, $df = 12$, $p = 0.05$, Mixed Group 4: $F = 0.82$ $df = 12$, $p = 0.64$) (Figure 4.4). Analysis of centrality closeness in the non-parasitised and parasitised groups gives the same results (See Appendix C, Figures S3.6 – S3.11). However, when closeness was calculated using duration of contacts, we found a statistically significant interaction between individual parasitic status and phase (Mixed Group 1: $F = 2.49$, $df = 12$, $p = 0.004$, Mixed Group 2: $F = 2.46$, $df = 12$, $p = 0.004$, Mixed Group 3: $F = 4.78$, $df = 12$, $p < 0.001$, Mixed Group 4: $F = 2.07$, $df = 12$, $p = 0.02$) (Figure 4.3 and Appendix C, Table S3.7-S3.10 for full model output). Analysis of centrality closeness in the non-parasitised and parasitised groups also gave the same results (See Appendix C, Figures S3.6 – S3.11).

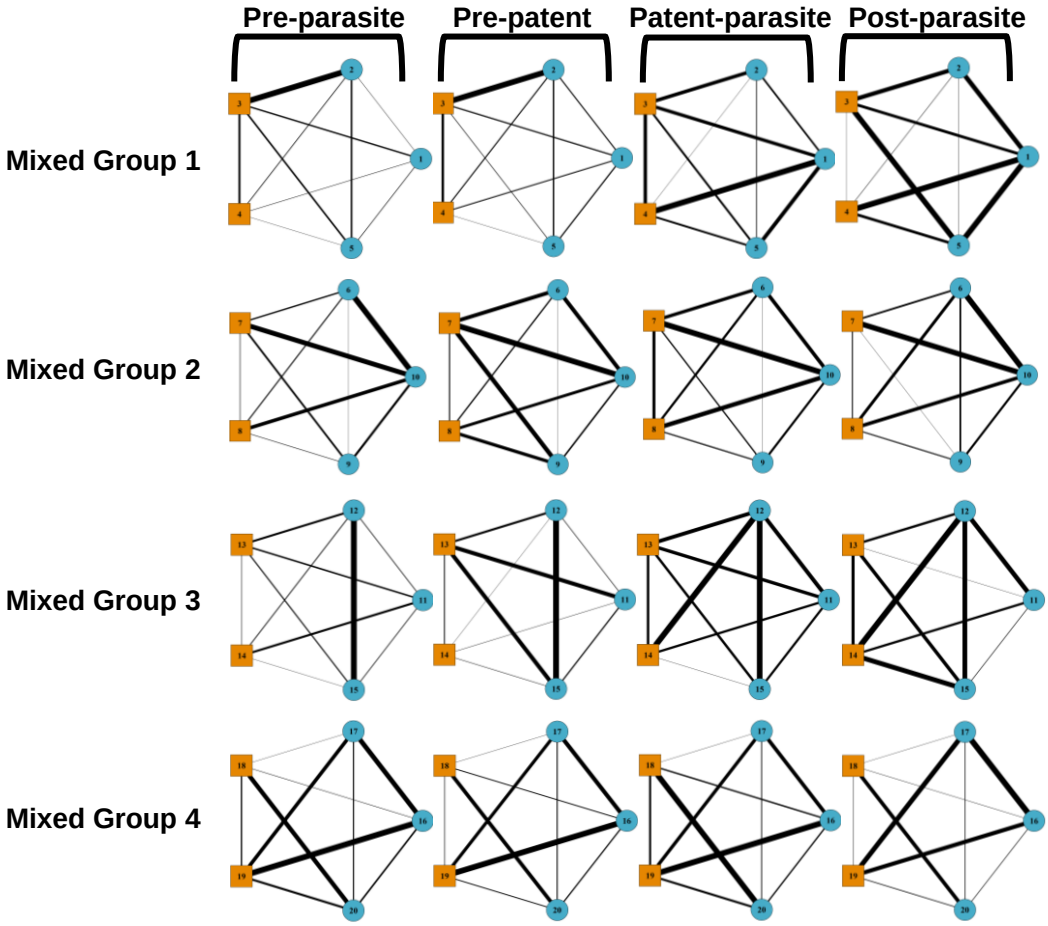


**Figure 4.2**. Social network graphs created using frequency of contacts of lambs in the mixed-state treatment groups (*n* = 4) for each phase of the study. Pre-parasite (week 1), Pre-patent (weeks 2-4), Patent-parasite (weeks 5-7) and Post-parasite (weeks 8-9). Blue circles represent non-infected individuals and orange squares represent infected individuals. Line thickness represents the strength of association between two individuals based on frequency of contacts per phase.

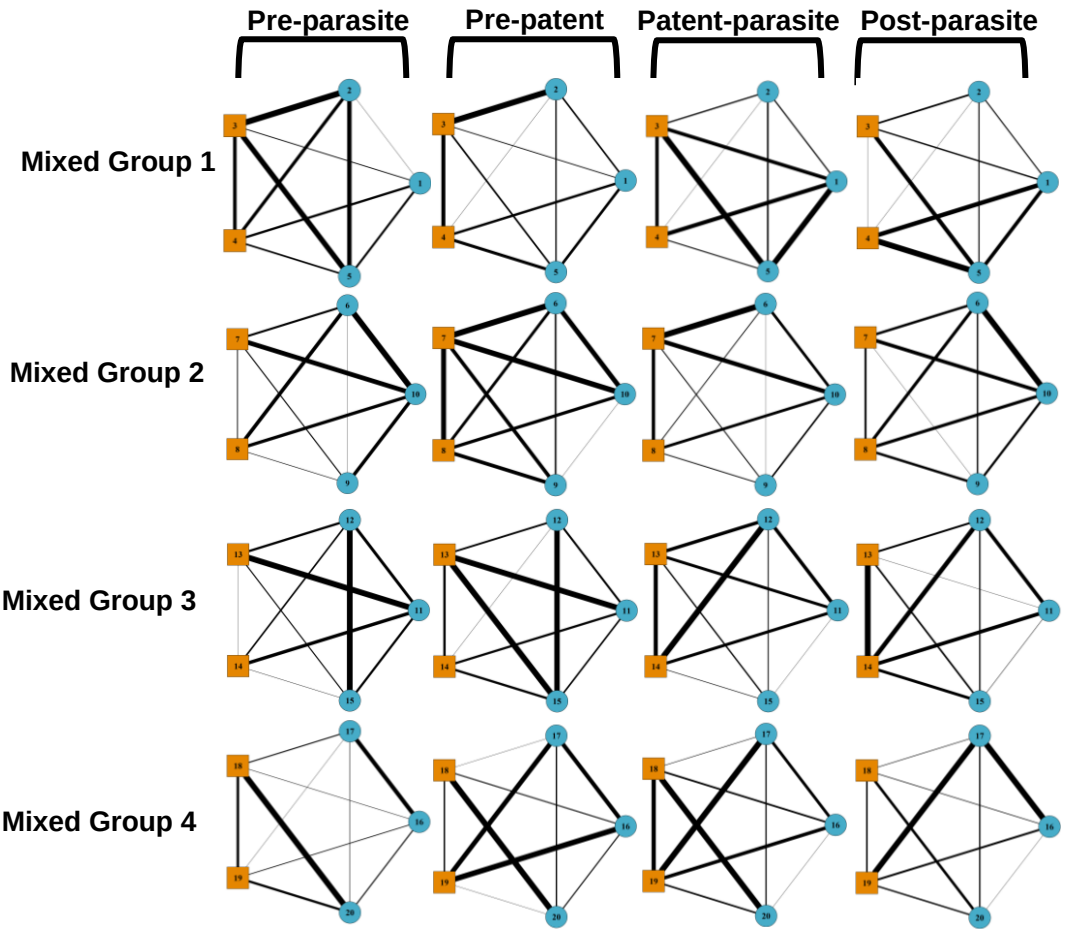


**Figure 4.3**. Social network graphs created using duration of contacts of lambs in the mixed-state treatment groups (*n* = 4) for each phase of the study. Pre-parasite (week 1), Pre-patent (weeks 2-4), Patent-parasite (weeks 5-7) and Post-parasite (weeks 8-9). Blue circles represent non-infected individuals and orange squares represent infected individuals. Line thickness represents the strength of association between two individuals based on frequency of contacts per phase.

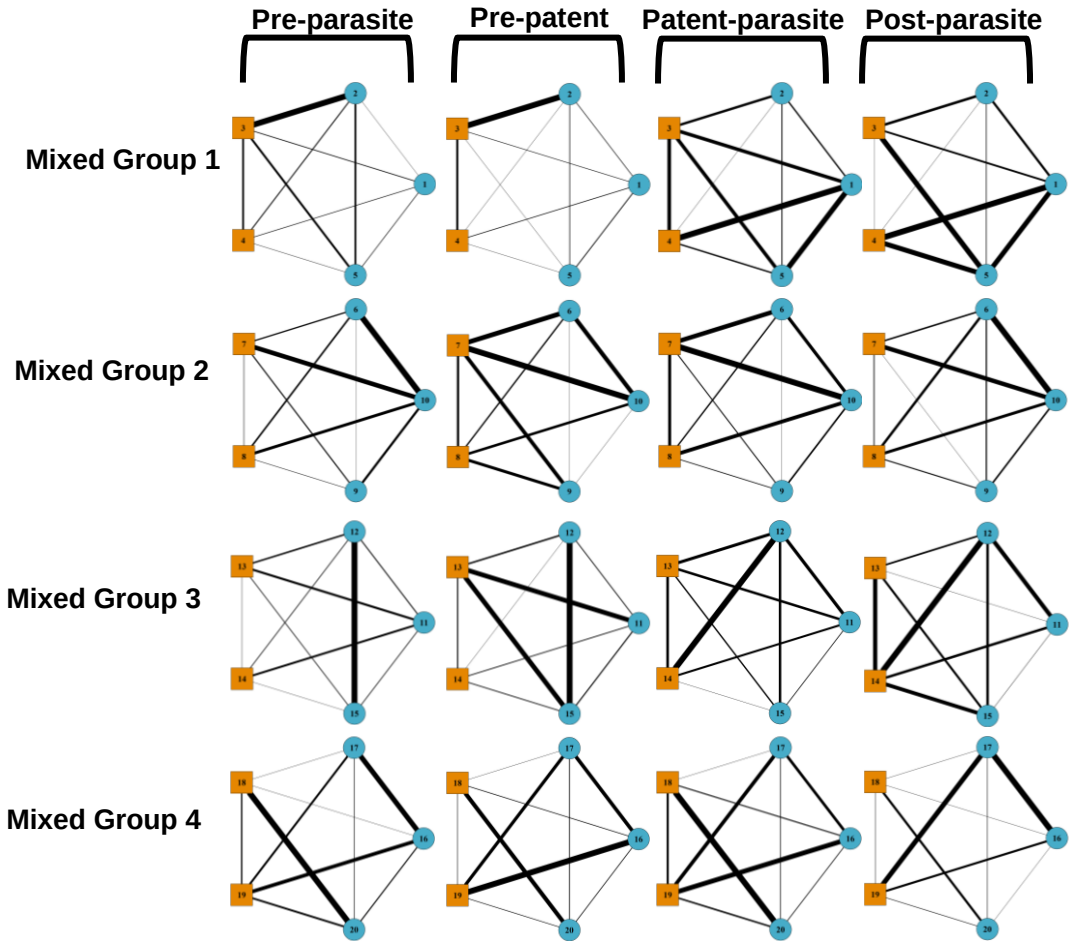


**Figure 4.4**. Social network graphs created using total duration of contacts of lambs in the mixed-state treatment groups (*n* = 4) for each phase of the study. Pre-parasite (week 1), Pre-patent (weeks 2-4), Patent-parasite (weeks 5-7) and Post-parasite (weeks 8-9). Blue circles represent non-infected individuals and orange squares represent infected individuals. Line thickness represents the strength of association between two individuals based on frequency of contacts per phase.

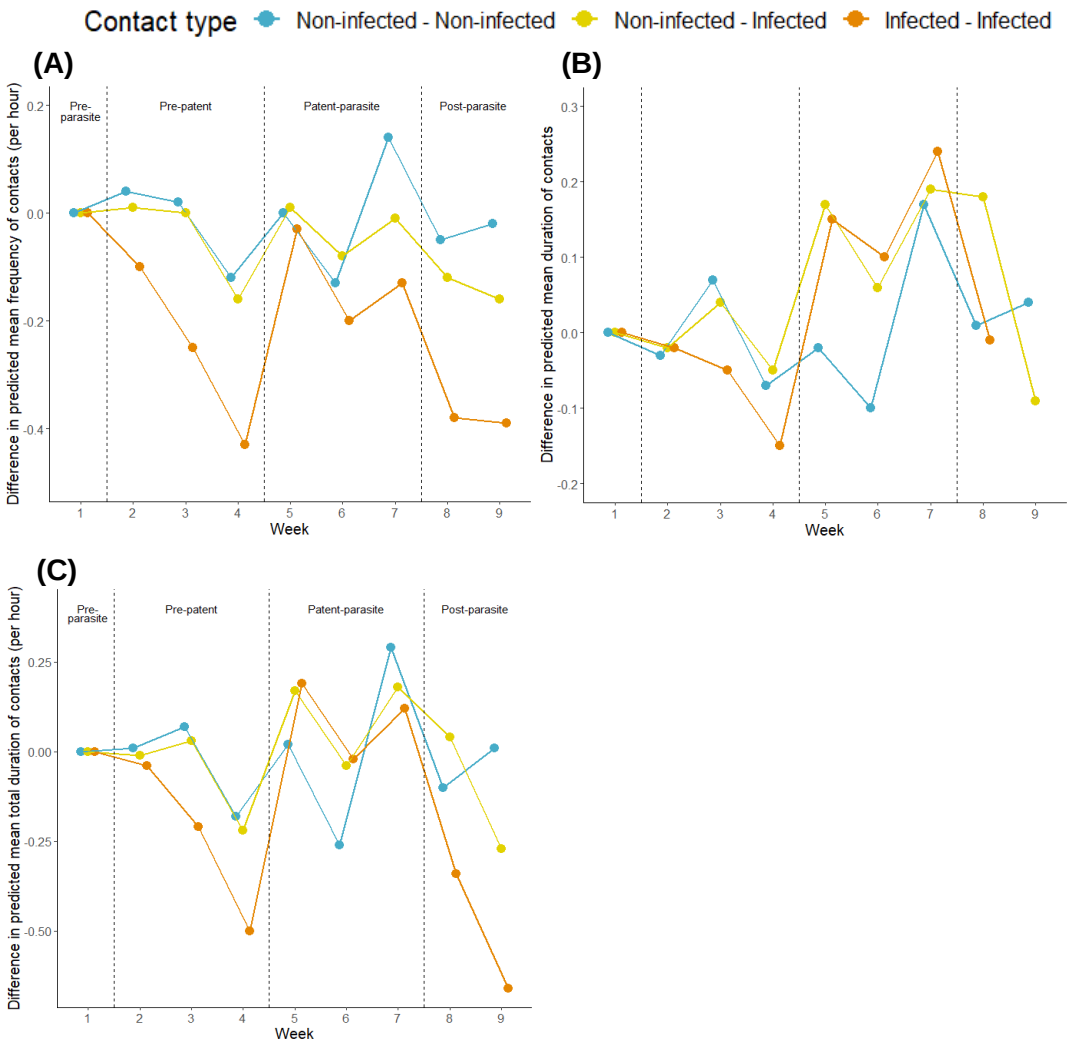


**Figure 4.5.** Difference in contact behaviour between each pair contact type in the mixed treatment groups, during each week of the study compared to the pre-parasite phase (week 1). Colours denote the pair contact type (blue: Non-infected - Non-infected, orange: Infected - Infected, yellow: Non-infected – Infected). The dashed lines separate the experiment into the four experimental phases (pre-parasite, pre-patent, patent-parasite and post-parasite). **(A)** Difference in model predicted mean frequency of contacts per hour. **(B)** Difference in model predicted mean duration of contacts. **(C)** Difference in model predicted mean total duration of contacts per hour.

## 4.6 Discussion

We show experimentally that parasitism can affect the social contact behaviour of infected individuals in single-parasitic state and mixed-parasitic state groups. However, in mixed-parasitic state groups the parasitic status of other group members can socially modulate contact behaviour of both infected and non-infected individuals. We also show that although infected animals in mixed-state groups had reduced contact frequency, there was no change in the social network structure of the group, as non-infected animals maintained pre-infection levels of social interactions.

The first step in this study was to create the three experimental treatment groups (non-parasitised, parasitised and mixed). We found faecal egg counts of infected lambs were detectable three weeks after the initial dose and remained high until lambs were treated with anthelmintic. There was an increase in the serum pepsinogen levels of infected lambs during the patent-parasite phase, an indication of gut wall damage by late larval and adult stages of *T. circumcincta* (Scott et al. 2000). We also found infected lambs had lower liveweights than non-infected lambs from the patent-parasite through to the end of the experiment. Thus, the parasite infection model was successful, with lambs identified for infection becoming infected and lambs identified to remain parasite free remaining clear of parasites throughout the study

Individuals in the parasitised groups had reduced contact frequency during the pre-patent, patent-parasite and post-parasite phases and increased duration of contacts during the pre-patent phase relative to the non-parasitised groups. This suggests infected individuals were less likely to come into contact, but when they did come into contact were less likely to break connections than individuals in the non-parasitised groups, and for any one contact spend longer together. These findings are in line with previous work, whereby animals will show changes in their sociality following parasitism (Dugatkin et al. 1994; Croft et al. 2011; Weber et al. 2013b; Lopes et al.

2016; Hamilton et al. 2020; Stockmaier et al. 2020; Hawley et al. 2021), and support the idea that individuals will reduce the frequency of their contacts but increase the mean duration of contacts following parasite infection. This behaviour change is likely due to a reduction in activity due to the parasite infection (Hutchings et al. 2000; Ikurior et al. 2020), and is consistent with previous studies, that have demonstrated how reduced frequency, but increased duration of contacts is a behaviour change associated with reduced activity of parasitised animals (Michelena et al. 2008; Kerckhove et al. 2013; Lopes et al. 2016; Müller-Klein et al. 2019; Ripperger et al. 2020; Stockmaier et al. 2020) (See Appendix C for correlation between lamb activity behaviours and social contact behaviours).

Following treatment with anthelmintic there was no difference in the contact behaviour between the three treatment groups by week 9 of the post-parasite phase. These results add to the growing body of evidence that the removal of parasite infections can alter the behaviour of infected animals (Hutchings et al. 2000; Besier et al. 2016; Sharma et al. 2016; Grant et al. 2020; Ikurior et al. 2020), and provide further evidence of the effect of parasitism on social contact behaviour. However, the reversal of behaviour did not occur until one week after parasite clearance, suggesting that parasitised animals may have spent the first week after treatment compensating with increased activity (i.e., grazing) (Sharma et al. 2016) (Chapter 3), prioritising feed intake after the period of anorexia.

Overall, individuals in the mixed groups had reduced contact frequency during the pre-patent and post-parasite phases and reduced total duration of contacts during the pre-patent phase. However, during the pre-patent phase this was driven by a drop in contacts between infected animals with other infected individuals only. During the post-parasite phase, the reduction in contact frequency was driven by contacts between infected individuals with other infected and non-infected animals.

Additionally, we also found the frequency of contacts between two infected individuals was lower than contacts between non-infected animals with other infected and non-infected individuals during the patent-parasite phase. These results indicate that non-infected animals during this study were not actively avoiding their infected conspecifics during periods of infection (pre-patent and patent-parasite phases), a contrast to what has been found in many taxa (Kiesecker et al. 1999; Behringer et al. 2006; Zylberberg et al. 2013), and also that healthy individuals maintained pre-parasite levels of social interactions with infected conspecifics following the drop in sociality from their infected groupmates.

We know social behaviour comes with both costs and benefits (Alexander, 1974; Loehle, 1995), and the most documented cost of group living is increased transmission of parasites through close proximity contact with other group members (Altizer et al. 2003). However, if exposure of the parasite is likely to be equal for all group members regardless of social contact, for example, if route of transmission is faecal-oral rather than direct contact, continuing social behaviours may be more beneficial for group members than avoidance of infected individuals (Hart, 1990, Loehle, 1995). As with most social animals, grazing herbivores will benefit from being part of a group (Hamilton, 1971; Lima, 1995). Maintaining large group sizes can protect against predators (Krause and Ruxton, 2002) and studies have shown that group size may affect foraging efficiency, with smaller groups interrupting foraging periods more to scan the environment (Berger, 1978; Ezenwa and Worsley-Tonks, 2018). As gastrointestinal nematodes are not directly transmitted between hosts, it is unlikely that reducing contacts between other members of the group will affect the transmission rate of the parasite. Therefore, it is likely, that the overall benefits of social interactions outweighed the benefits of avoiding sick individuals where indirect transmission was inevitable (Fairbanks et al. 2015; Stockmaier et al. 2020). Another

possibility is that the increase in social interactions from non-infected animals towards their infected conspecifics was a consequence of infected animals becoming more docile and healthy animals taking a more dominant role in the group (Bouwman and Hawley, 2010). However, without monitoring the behaviour of the lambs using focal observations or video recordings, we cannot state why non-infected animals increased their social interactions towards infected conspecifics. Yet, the resulting changes in social behaviour of non-infected animals in response to their infected conspecifics are important, as they demonstrate that although sick individuals may reduce their own social interactions, the effect this has on parasite transmission may be less effective than predicted if the non-infected group members increase their social connections in response to their behavioural change.

In contrast to the fully parasitised group, where the change in social interactions was consistent throughout all phases of infection, we found that during the patent-parasite phase, the contact frequency between two infected animals in the mixed groups was reduced to a lesser degree compared to the pre-patent phase. This suggests that the behavioural response of infected individuals was affected by the parasitic status of an individual's social group, as infected lambs in the mixed groups increased their social interactions in the presence of non-infected individuals. The behaviour of individuals in response to parasitism can vary depending on their social environment (Lopes et al. 2012), and under certain conditions infected individuals may modulate their behavioural response to infection (Cohn and de Sá-Rocha, 2006; Lopes et al. 2012). When lambs are infected with gastrointestinal nematodes, they exhibit changes in their activity levels (Chapter 3) and may become anorexic (Hutchings et al. 1998). These behaviour changes can lead to individuals having reduced social interactions (Hart, 1988; Lopes et al. 2016; Hawley et al. 2021), and thus, the associated benefits of group living (Hart 1990; Penning et al. 1993; Kiesecker et al. 1999; Sevi et al. 1999;

Dumont and Boissy, 2000; Krause and Ruxton, 2002; Behringer et al. 2006; Tobler and Schlupp, 2008) (See Appendix C for correlation between activity and contact behaviours). However, it may also be that being part of a larger group could minimize the effects of infection (Almberg et al. 2015). For example, Ezenwa and Worsley-Tonks (2018) suggested, that association with larger groups benefits individuals infected with gastrointestinal nematodes as it allowed infected hosts to better ameliorate the costs associated with infection-induced anorexia. As infected hosts that were part of a larger group could spend more time grazing and reduce the costs of anorexia. We also know that infected individuals across many social taxa experience higher levels of predation (Alzaga et al. 2008; Stephenson et al. 2016). Therefore, gregariousness may be a common mechanism for parasite tolerance under certain environmental conditions, and it could be, that although parasitism had an effect on the behaviour of infected lambs in the mixed and single-parasitic state groups, social modulation and social group potentially altered the behavioural response of individuals in the mixed-state groups.

Unlike individuals in the single-parasitic state groups, the frequency of social contacts between infected individuals with other infected and non-infected animals in the mixed groups decreased following treatment with anthelmintic. This behavioural change could be associated with the difference in severity of infection between animals in the mixed and single-state groups. For example, infected lambs in the mixed-state groups had lower liveweights than infected lambs in the single-state groups during the patent-parasite phase, which may be associated with lambs coping with the stressors of infection and maintaining high levels of interactions with group mates. Thus, after parasite removal, lambs in the mixed groups may have exhibited compensatory grazing (Sharma et al. 2016), prioritizing their feeding behaviour over their social behaviours.

Changes in social interactions between infected individuals did not affect the network architecture of the mixed groups, as the centrality closeness (when calculated using frequency of contacts) of both infected and non-infected animals remained unchanged despite infected animals reducing their associations with other group members (Figure 4.2). These results indicate a degree of social network robustness to environmental disruptions (i.e., parasitism), which is something that is common amongst highly social systems (Goldenberg et al. 2016; Lopes et al. 2016; Firth et al. 2017). Furthermore, contact behaviour is not independent and can be affected by other members of an individual's group (Krause et al. 2015), and although infected lambs in the mixed groups reduced their social contacts, non-infected individuals maintained the associations with their infected conspecifics. When closeness was calculated using frequency and total duration of contacts, we found no change in the centrality of individuals in all three treatment groups. However, when closeness was calculated using duration of contacts, there was more variability in the centrality closeness of individuals in all three treatment groups (non-parasitised, parasitised and mixed). These results demonstrate how methodological differences represent a potential source of heterogeneity in results observed across social network analysis studies, as discussed in Briard and Ezenwa (2021). These results also highlight the importance of using a network measurement that matches parasite biology to understand how parasitism affects host social networks. For instance, in the current study we know parasite infection reduces host activity levels (Chapter 3) which is likely to impact the frequency of social interactions, thus using frequency to calculate closeness centrality is the most sensible metric as this is likely to explain how infection is affecting host position in the network.

Our results show that parasitism can affect the social contact behaviour of infected individuals in both mixed and single-parasitic state groups at the very earliest stages

of infection. However, we show that the level of behaviour change of infected and non-infected individuals can be affected by an individual's social environment, as animals in the mixed-state groups altered their contact behaviour to a different degree compared to animals with similar parasite burdens in single-state groups. These findings are important, as although some parasite infections may reduce the sociality of infected individuals, and thus disease spread, this may not always be the case. As certain social contexts may affect the expression of expected behaviours, which may affect the spread of infection between and within animal populations. This highlights the importance of taking into account the behaviour and infection status of all members of a social group in response to parasitism, to gain a better understanding of a group's response to infection and improve the ability to predict the consequences of infection.

# Chapter 5

## 5. General Discussion

### 5.1 Thesis summary

This thesis aimed to explore the impact of parasitism on host behaviour, with a particular focus on the effect an individual's social environment can have on their behavioural response to infection. I experimentally infected domesticated lambs *Ovis aries* with the gastrointestinal nematode *Teladorsagia circumcincta,* that were part of one of three treatments that differed in proportion of individuals that were infected. The aim was to investigate the effect of parasitism on the activity and social behaviour of groups of lambs, to understand what stage of infection these behavioural changes occur, and to determine what effect the infection status of an individual's social group can have on their behavioural response to infection.

In Chapter 2, I carried out a series of experiments to assess the capabilities of two remote sensors (activity monitors and proximity loggers) at monitoring lamb activity and social contact behaviour that would be used in future experimental work. I compared the behaviour data recorded by the two remote sensors to behaviour data recorded using live focal observations. I found a positive correlation between the remote sensor data and data recorded by using focal observations, and although the remote sensors recorded lower levels of overall activity than what was observed by direct observations, the level recorded was directly proportional across varying levels

of activity, therefore providing a useful and reliable index of activity level. As we were looking to determine if sensors could be used to monitor patterns in behaviour and not absolute values, the sensors could be used in future hypothesis testing of lamb behaviour. There were also a number of additional advantages that remote sensors offered. For instance, by using proximity loggers that enabled the continuous recording of lamb behaviour 24 hours per day, I found that I could detect subtle changes in lamb behaviour that would otherwise be missed using focal observations.

I then carried out a large-scale field trial to investigate how parasitism affects the activity (Chapter 3) and social contact behaviour (Chapter 4) of lambs that were part of groups containing individuals of different parasitic status. The aim of the study was to understand at what stages of infection behavioural alterations occur and what effect the infection status of an individual's social group has on their behavioural response to infection. Social groups of lambs were part of one of three treatments: Parasitised; all lambs were experimentally infected with the gastrointestinal nematode *T. circumcincta*, Non-parasitised; all lambs were non-infected, Mixed; part of the group were infected and part of the group were non-infected. Faecal samples and blood samples were taken to measure levels of parasitism and ensure the parasite model was successful. Using the previously validated remote sensors (Chapter 2) I monitored the activity and social contact behaviour of lambs during four phases of infection (pre-parasite, pre-patent, patent-parasite and post-parasite).

Faecal egg counts of infected lambs were detectable three weeks after the initial infection dose with *T. circumcincta* and remained high until infections were cleared (Chapter 3 and Chapter 4). Faecal egg counts of non-infected lambs remained at zero throughout the experiment. Infected lambs had increased serum pepsinogen during the patent-parasite phase. I also found infected lambs had lower liveweights than non-infected animals during the patent-parasite and post-parasite phases of the study.

These results showed that the parasite infection model worked and successfully established clear pre-parasitised, pre-patent, patent-parasite and post-parasite phases across the treatment groups.

In Chapter 3, I showed that infected individuals had reduced activity levels immediately after parasite exposure; three weeks before any measure or noticeable impact of parasitism were observed. However, the extent of this behaviour change was affected by the infection status of an individual's social group, as infected lambs in the mixed parasitic-state groups reduced their activity to a lesser degree during the patent-parasite phase compared to infected lambs in the fully parasitised groups. I also found following treatment with anthelmintic, the behaviour of infected lambs returned to pre-parasite levels, providing further evidence of the effect of parasitism on activity behaviour.

In Chapter 4, I showed that all infected individuals in the parasitised groups had reduced contact frequency during the pre-patent, patent-parasite and post-parasite phases, but increased duration of contacts during the pre-patent phase. I also showed that there was a decrease in the frequency of contacts in the mixed groups relative to the non-parasitised groups. However, this was driven by a reduction in contacts between infected individuals only, as there was no change in the social contact behaviour between infected and non-infected animals. I also found infected animals in the mixed-state groups altered their contact behaviour to a lesser degree compared to animals with similar parasite burdens in single-state groups during the patent-parasite phase. Furthermore, although infected animals had reduced contact frequency, I found no change in the network architecture of the group as non-infected animals maintained pre-infection levels of social interactions with their infected conspecifics.

The results from Chapter 3 and 4 show that parasitism can affect the activity and social behaviour of infected individuals. However, in mixed-parasitic state groups the parasitic status of other group members can socially modulate the behaviour of both infected and non-infected individuals. Moreover, given the social effects of parasitism and the impact on traits related to host fitness as well as on behaviour, this research highlights that parasite-mediated behavioural changes can vary due to an individual's social environment.

In this final discussion chapter, I will discuss the broader implications of my research for its use in domestic systems to identify early indicators of parasitism. I will discuss how these findings can have implications for our understanding of how sickness behaviours can affect disease dynamics, which in turn will aide our understanding of how infections are likely to impact a population. Finally, I will discuss limitations of the research presented, and future avenues of work that could follow on from this thesis to complement the results.

## 5.2 Relationship between activity and social behaviour

When looking at the results presented in Chapter 3 and Chapter 4, I found the change in activity behaviour corroborated with the change in contact behaviour of infected lambs in the mixed and single-state groups. During the pre-patent and patent-parasite phases, infected lambs in the mixed and single-state groups had reduced activity levels (step count and motion index) (Figure 3.4), reduced contact frequency and increased duration of contacts with other individuals (Figure 4.2). Together, these results indicate that during the less active periods, infected lambs were less likely to come into contact, but when they did come into contact were less likely to break connections than individuals in the non-parasitised groups.

During the patent-parasite phase of infection, the activity levels and social interactions of all individuals in the parasitised groups remained low. Again, demonstrating that lambs in the parasitised groups were less likely to come into contact as a result of moving around less. In the mixed groups, I found infected lambs had reduced activity levels and reduced social interactions, but both behavioural alterations were to a lesser degree during the patent-parasite phase compared to animals with similar parasite burdens in single-state groups. This difference in behaviour between infected animals in the mixed and single-state groups during the patent-parasite phase further demonstrates the level of alignment between these two behaviours.

Whether infection induced behavioural changes influenced social behaviour, or changes in social behaviour impacted activity levels would need to be further investigated. As sickness behaviours are potentially an adaptive response by the host to reallocate energy resources to fight off infection (Hart, 1988; Hutchings et al. 1998), it could be argued that social behaviour is not a sickness behaviour but rather a consequence of infection induced lethargy. There are studies where infected animals have shown changes in their sociality following infection without reports of animals exhibiting changes in activity levels (Croft et al. 2011; Weber et al. 2013a; Hamilton et al. 2020; Ripperger et al. 2020). However, these findings may be associated with the fact that activity and social behaviour levels were not simultaneously monitored during the time that these studies were carried out. Whereas studies that have monitored both social behaviour and activity behaviour simultaneously, have shown infection induced lethargy can influence social behaviour (Michelena et al. 2008; Kerckhove et al. 2013; Lopes et al. 2016; Müller-Klein et al. 2019; Stockmaier et al. 2020).

During my experiment, lambs had reduced activity levels during the pre-patent phase. It could be, that during this phase of infection, the decrease in activity levels reduced

the social interactions of infected individuals in the mixed and single-state groups (Lopes et al. 2016). However, in the mixed groups during the patent-parasite phase infected lambs had increased activity and social interactions. These changes in sociality could have been driven by changes in lamb activity levels. However, it could also be argued that the social pull for infected animals to seek out those social connections, increased the activity levels of the infected animals.

In Chapter 3 and Chapter 4, I found the liveweight of infected lambs in the mixed groups was lower during the patent-parasite phase compared to infected lambs in the single-state groups (Figure 3.3). The patent-parasite phase was the period of infection when infected lambs in the mixed groups had higher activity levels and social interactions compared to animals with similar parasite burdens in single-state groups. If lambs were increasing their activity levels to graze, we might expect the weight of the lambs to reflect this alteration in behaviour. However, as infected lambs in the mixed groups had lower liveweights than infected lambs in the single-state groups during the patent-parasite phase, this suggests that during this period lambs were prioritising their social behaviour, which may have had an influence on their activity behaviour. Overall, the results presented in Chapter 3 and Chapter 4 suggest, that although infection induced lethargy can reduce sociality as shown in previous work (Michelena et al. 2008; Kerckhove et al. 2013; Lopes et al. 2016; Müller-Klein et al. 2019), animals seeking out social interactions may simultaneously influence host activity behaviour.

## 5.3 Gastrointestinal nematodes in domestic sheep

Research of sickness behaviours in livestock has been of interest given the economic implications of disease outbreaks on farms. Early detection of parasitism through changes in lamb behaviour has potential to be used as a non-invasive tool to identify

and treat infected individuals (Kenyon et al. 2009). Target control strategies that treat individuals within a group based on a biological indicator of infection, have proven successful in slowing down the rate of anthelmintic resistance in agriculture systems whilst keeping parasite burdens low (van Wyk, 2001; Kenyon et al. 2009). The results presented in Chapter 3 and Chapter 4, show lambs infected with gastrointestinal nematodes exhibit changes in their behaviour immediately after parasite exposure during the pre-patent phase of infection. These behaviour changes occur earlier than any other biological indicator of parasitism, such as faecal egg counts or changes in liveweight, and can be identified using two remote monitoring systems. These findings show that there is a behaviour change that can be associated with gastrointestinal parasitism in lambs that could be exploited for use in a commercial setting.

Monitoring of activity behaviour in a commercial setting could provide detailed information about specific animals within a group and could be used to target infected individuals based on a reduction in activity levels, and monitoring changes in sociality may also provide information about sub-groups of infected animals. I found infected lambs had reduced activity levels one week after lambs were first dosed with *T. circumcincta* larvae, three weeks before lambs were shedding eggs in their faeces. These findings suggest there is potential to use behaviour changes in parasitised lambs to identify and treat animals before parasite infection intensifies, which would reduce welfare implications, and may also clear infections before animals start shedding eggs reducing contamination on pasture (Kenyon et al. 2009). However, it must be noted that the behaviour changes identified in Chapter 3 and Chapter 4 were of lambs that were part of social groups containing individuals of known parasitic status. I also dosed the lambs used in the study with a known amount of parasite larvae that would ensure a subclinical infection would be established (Coop et al. 1982; Wood et al. 1995). This combined meant that I knew the exact time point when lambs

were first infected, which I could later use as a point of reference in the data analysis. Furthermore, sickness behaviours in lambs and across most taxa, are not disease specific, so it would not be practical for a farmer to treat lambs with anthelmintic following a change in activity or social behaviour. These are all factors that may impact whether these behaviours can actually be exploited for used in an agriculture setting.

There are also practical considerations to take into account when using the two remote systems to monitor sickness behaviours of animals in an agriculture setting. For instance, as changes in behaviour were identified using both remote systems, only one system would need to be deployed into an agriculture setting. Although proximity logger data provides interesting information about lamb behaviour, the IceQube activity monitors are more user friendly, both in the field, as sensors are not required to be removed from the animal to download data, and in terms of data processing. Proximity data has reciprocal data points that must be appropriately dealt with before data can be analysed. Thus, if the results of this thesis were going to be taken up by agriculture systems, monitoring activity behaviour with the IceQubes activity monitors would be the more practical route to take.

## 5.4 Methodological limitations to study

Although I sought to design an experiment to assess the impact of parasitism on the behaviour of hosts to allow for robust conclusions, there were several limitations in this work that should be considered.

### 5.4.1 Experimental design

Experimental work presented in this thesis (Chapter 3 and Chapter 4) was designed to maximise replicates of social groups within each treatment, ensure behavioural changes associated with parasitism could be identified in infected lambs, whilst simultaneously keeping non-infected lambs' parasite free. The timeline of the

experiment had to take into account potential contamination of pasture from previous grazing years and as social groups were rotated between plots weekly, the design had to control for any potential contamination between treatment groups. This entailed experimentally infecting lambs in week two, which meant the pre-parasite phase of the study was comparatively shorter than other phases of the study. However, this difference in phase length was controlled for by including both Phase and Week as fixed effects in the mixed models (Chapter 3 and Chapter 4). Infected lambs remained infected for six weeks, with all lambs treated with anthelmintic at the start of week 8, ensuring parasite exposure of non-infected lambs was kept at a minimum. Although there was a period that non-infected lambs were exposed to infective larvae on pasture (between weeks 5-7), faecal egg counts of non-infected lambs in week 8 were zero suggesting that non-infected lambs remained parasite free.

### 5.4.2 Remote sensors

In my experiment, I used remote sensors to monitor the behaviour of the lambs. Remote sensors enable the continuous and simultaneous monitoring of behaviour for longer periods of time than an observer could manage (Krause et al. 2007, Krause et al. 2013), generating large datasets that can be used to identify subtle changes in animal behaviour that may otherwise be missed (Proudfoot et al. 2012). However, one of the main limitations of relying solely on remote sensors to monitor animal behaviour is that the sensors cannot detect the context of social interactions. For instance, in Chapter 4, I found non-infected lambs maintained pre-infection levels of social interactions with their infected conspecifics. Although, I discussed possible reasons to explain these behaviour alterations, as no focal observations were conducted, I was not able to draw any final conclusions from the data. Similarly, in Chapter 3, I found infected lambs had reduced motion index during the pre-patent and patent-parasite phases of infection. As motion index gives an indication of total

amount of energy used (IceRobotics Ltd, Edinburgh) and reduced feed intake and anorexia are associated with parasite infections (Murray and Murray 1979; Hart, 1988; Kyriazakis et al. 1998; Hutchings et al. 2000; Hite et al. 2020), a decrease in motion index could be associated with a reduction in behaviours such as forage intake. Yet, without measuring forage intake or conducting focal observations it is not possible to state if lambs in the study did reduce their feed intake. A combination of focal observations with remote sensors may have provided a more detailed representation of lamb behaviour within this thesis. However, due to the size of the trial, focal observations would have provided limited information as the observation period of the sample size would have been very small. Furthermore, video recording of animals in the field was also not practically possible due to the size of the plot's groups were housed in (each group was housed in a 30x30m plot) and the difficulty that would have posed for identification of animals and clarity of recordings. Therefore, due to these reasons and the potential of observer effect (Carpenter, 1934; Schneirla, 1950) it was decided that the addition of live focal observations would not benefit the study.

Another limitation of using remote sensors is the amount of data that has to be removed during the period of time when animals are handled. However, as the loggers record data 24 hours per day the amount of data the user is left with is still much larger than what would be obtained using live focal observations. Furthermore, remote sensors also record all animals simultaneously, which you would not be able to do with focal observations. Another important point to consider is that if animals are handled for longer periods of time during certain parts of the study, the amount of data removed could disproportionally affect the amount of time animals are observed during each time period. For instance, animal measurements (faecal samples/weight) were taken once per week from every individual. It was decided that liveweight and faecal samples would be taken on the same day, to reduce the amount of time animals

were handled. However, this also meant that as we were in the field for a longer period of time for one day each week a larger amount of data would have to be removed from the sensor data during that day. Although, this did not affect the results of the study as the same data were removed for all groups, it is something that may need to be considered when using remote sensors.

### 5.4.3 Parasite infections

In my experiment, the primary parasitological output I used to determine whether animals were infected was intensity of infection (faecal egg counts). Although faecal egg counts do not always give an indication of true worm burdens in some systems (Granroth-Wilding et al. 2015), a positive correlation between egg counts and worm burdens has been reported in domestic sheep (Caberet et al. 1998). However, as I did not measure actual worm burden of the sheep, true parasite infection levels of the lambs remain unknown.

While the relationship between parasite burden and behaviour change would be an interesting avenue to explore in the future, it was not the objective of this study. In my experiment, the main purpose of using faecal egg counts to measure parasitological output was to ensure lambs identified for infection became infected, and lambs identified to remain parasite free, were clear of parasites throughout the study. I also wanted to ensure that the levels of parasitism of the lambs were of similar levels to what you might expect on UK farms based on faecal egg counts (average on farm faecal egg counts range between 200-630 epg (Learmount et al. 2016; Williams et al. 2021)). As the faecal egg counts of infected lambs fell within the average range of UK farms, (Figure 3.1) I could confirm that the parasite infection model used in this experiment was successful.

## 5.5 Potential areas for future research

This study has opened up interesting questions about the role of using behaviour change as an early indicator of parasite infection, including more specific questions regarding the influence an individual's social environment has on the expression of sickness behaviours. Here I discuss future research areas I believe would be most rewarding and informative to follow on from the findings within this thesis.

The most fundamental, but also interesting to address, is how different social group structures can affect an individual's behavioural response to parasitism. Sociality of sheep can be influenced by a variety of factors including breeding period (Norton et al. 2012), age (Doyle et al. 2016), group size (Michelena et al. 2008) and individual characteristics such as temperament or personality (Michelena et al. 2009; Doyle et al. 2016). Thus, an interesting avenue to explore would be to determine if the sickness behaviours that were identified during the experiment presented in this thesis were exhibited when lambs were part of different social group structures. Changes to lamb social structure could include social groups of different sizes, social groups containing different combination of sexes, social groups containing different sheep breeds, or social groups containing different proportions of infected and non-infected animals. Having social groups that contain different proportions of infected and non-infected animals would be most interesting as previous work in other animal taxa, for instance in sticklebacks, has shown when infected and non-infected animals are housed in a 1:1 ratio, the activity levels of non-infected fish were lower compared to non-infected fish housed with other healthy animals (Jolles et al. 2020), this study also showed that the activity behaviour of infected fish housed with healthy fish was higher than the activity behaviour of two unhealthy fish housed together. Understanding how the ratio of infected and non-infected animals in a group impacts the behaviour change of animals in a social group is also important when understanding the use of behaviour

as an indicator of infection in an agriculture setting. The main findings of this thesis show that infected animals housed in a 2:3 ratio with non-infected lambs have reduced activity and sociality following parasite infection. However, for these behaviour changes to be used in a commercial setting, we would need to understand if these behavioural alterations also occur when animals are part of a more natural sized group. Sheep farms in the UK usually house between 60-200 animals. So a natural step to follow on from the work presented in this thesis would be to run a similar experiment, using one large social group of lambs rather than replicated smaller groups. This would then determine if these behaviour changes occurred in a more natural domestic setting, and whether identifying parasitism based on behaviour in lambs is feasible in an agriculture setting.

The robustness of a social group has also been shown to have an effect on the impact of parasitism on host behaviour. For instance, a study looking at the impact of LPS injections on mouse social behaviour found changes in social connectivity were mainly localized around the infected individuals, while the rest of the group network remained unaffected (Lopes et al. 2016). In comparison, the entire social network of a group of female guppies (*Poecillia reticulate*), was affected by the addition of a diseased fish (Croft et al. 2011). The main difference between the outcomes of the two studies is thought to be associated with social group robustness. The social groups of the guppies were artificially formed and given 24 hours to acclimatize, whereas the mice social groups were naturally formed. It is has also been shown that high levels of kinship between groupmates can alter the degree in which animals exhibit sickness behaviours in response to parasitism (Stockmaier et al. 2020). In my experiment, I purposely sorted siblings into different social groups to ensure all lambs had the same social experience before carrying out the experiment. Thus, an interesting area of research to explore would be to determine how resilient the

sickness symptoms identified in this study are to changes to the social group experience of the lambs. Understanding how resilient these behaviours are to a social disturbance would aide in our understanding of how disease may affect the impact of disease on a population. It would also aide our understanding of whether behaviour change is a feasible measure of infection on a farm setting, as farmers would rarely separate siblings, and thus, understanding how the behaviour of an individual is affected by parasitism in the presence of a sibling would be important before using behaviour as an indicator of parasitism on farms.

Another interesting research avenue to explore is associated with the size of the parasite challenge. In my experiment, lambs were trickle dosed with 5,000 *T. circumcincta* larvae 3 times per week. The trickle infection chosen ensured a subclinical infection was established and also represented a level similar to that encountered naturally by sheep naturally when grazing on contaminated pastures (Coop et al. 1982; Wood et al. 1995). It has been hypothesized that the onset of behavioural changes in animals are related to the size of the health challenge such as parasite dose (Szyszka and Kyriazakis, 2013). This idea was tested during a study on cattle, where animals were given either high, medium or low doses of gastrointestinal nematode *Ostertagia ostertagia* to induce a subclinical infection. The study reported changes in behaviour of animals who received a high infection dose (Szyszka and Kyriazakis, 2013). Therefore, an interesting avenue to explore, would be to vary parasite dose to investigate if there was a difference between the expressions of sickness behaviours exhibited by lambs of different parasite exposure.

## 5.6 Broader implications

Embracing the role of behaviour in facilitating how parasite infections spread can have important implications beyond biological understanding. For instance, the importance

of understanding the relationship between parasitism and host behaviour was highlighted during the recent COVID-19 pandemic (Moya et al. 2020), when the initial suggested policies that were put in place to slow down the spread of the virus required the immediate and extensive change of human behaviour.

Results presented in Chapter 3 and Chapter 4, show infected animals often behave in ways that are different to healthy individuals. In Chapter 4, I demonstrated how infected individuals in the mixed groups had reduced contact frequency but only in relation to their interactions with other infected animals. These sickness symptoms could serve to reduce disease spread within a social group, as infected animals become less central within the social network. However, on further investigation, I found non-infected individuals maintained pre-parasite levels of social interactions with their infected conspecifics. These findings demonstrate the importance of understanding the behavioural response of both infected and non-infected individuals within a group for understanding the impact of infection on a population which in turn can be used to predict and control disease spread.

## 5.7 Concluding remarks

Using an experimentally infected domestic sheep system, I was able to investigate the effect of parasitism on host behaviour and how an individual's social environment can influence their response to parasitism. Remote sensor technology and advanced statistical techniques enabled me to assess the activity and social behaviour of lambs that were part of social groups of mixed parasitic status during four phases of parasite infection. This work represents to the best of my knowledge the earliest demonstration of lamb behavioural change in replicated groups of animals that can be directly associated with gastrointestinal nematode infection. Through the experimental design, I showed parasitism can influence the behaviour of infected hosts immediately after

parasite exposure. I also showed that these behavioural changes can be modulated by an individual's social environment, and that the behaviour of non-infected animals can also be influenced by the presence of infected animals within a social group. Overall, the results from this thesis show that although some parasite infections may reduce the activity and sociality of infected hosts, and thus potentially reduce disease spread, this is dependent on an individual's social environment and may therefore not always be the case. Certain social contexts may affect the expression of sickness symptoms, which may influence how a disease spreads within a population. These results highlight the importance of taking into account not only the behaviour of infected animals but also the infection status of their social group to gain a better understanding of how a social group is likely to respond to infection and improve the ability to predict how parasite infection is likely to spread within and between animal populations.

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# Appendix A: Supplementary material for Chapter 2

## 1. Model description and output

**Table S1.1.** Model formulae for analyses of activity behaviour (step count, frequency of lying bouts and lying time) and contact behaviour (frequency, duration and total duration of contacts) during experiment 1, 3 and 5.

| Model Group | Response | Model Class | Model Family | Fixed effects and interactions | Random Effects |
|---|---|---|---|---|---|
| **Experiment 1** | Step count | LM | Gaussian | Step count (OB) | |
| | Frequency of lying bouts | LM | Gaussian | Lying duration (OB) | |
| | Lying time | LM | Gaussian | Lying bouts (OB) | |
| **Experiment 4** | Duration of contacts | LM | Gaussian | Duration of contacts (OB) | |
| **Experiment 5** | Frequency of contacts (focal observations) | GLMM | Negative Binomial | Treatment:Phase | Animal 1 ID + Animal 2 ID |
| | Duration of contacts (focal observations) | GLMM | Negative Binomial | Treatment:Phase | Animal 1 ID + Animal 2 ID |
| | Total duration of contacts (focal observations) | GLMM | Negative Binomial | Treatment:Phase | Animal 1 ID + Animal 2 ID |
| | Frequency of contacts (Sirtrack recordings) | GLMM | Negative Binomial | Treatment:Phase | Animal 1 ID + Animal 2 ID |
| | Duration of contacts (Sirtrack recordings) | GLMM | Negative Binomial | Treatment:Phase | Animal 1 ID + Animal 2 ID |
| | Total duration of contacts (Sirtrack recordings) | GLMM | Negative Binomial | Treatment:Phase | Animal 1 ID + Animal 2 ID |

**Table S1.2.** Description of fixed and random effects.

| Term | Class | Description |
|---|---|---|
| **Step count (OB)** | Continuous | Step count of lambs recorded using live focal observations |
| **Lying duration (OB)** | Continuous | Lying duration of lambs recorded using live focal observations |
| **Lying bouts (OB)** | Continuous | Frequency of lying bouts recorded using live focal observations |
| **Duration (OB)** | Continuous | Duration of contacts recorded using live focal observations |
| **Phase** | Factor (3 levels) | **Phase A** (Days 1-4 a period when a collection of baselines measurements would be recorded on the social contact behaviour of lambs in each group), **Phase B** (Days 5-9 a period when the removal group would experience the trickle removal of 2 animals over 3 time points), **Phase C** (Days 10-13**,** a period monitoring the behaviour of both groups of animals following the removal of animals from the removal group) |
| **Treatment group** | Factor (2 levels) | **Control** (group of lambs that were part of a stable social group throughout the trial), **Removal** (group of lambs that were exposed to a social disturbance) |
| **Animal 1 ID** | Factor (18 levels) | ID of Lamb 1 |
| **Animal 2 ID** | Factor (18 levels) | ID of Lamb 2 |

**Table S1.3.** Model estimates for fixed effects of linear regression models on activity levels of lambs recorded using live focal observations and IceQube activity monitors (experiment 1). AIC values are presented from final models. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| **Step count** | | | | |
| Step count (OB) | **0.333** | **0.016** | **20.407** | **<0.001** |
| (Intercept) | 0.650 | 0.016 | 20.407 | 0.097 |
| | | | | AIC = 1156.0 |
| **Frequency of lying bouts** | | | | |
| Lying bouts (OB) | **0.33** | **0.056** | **5.867** | **<0.001** |
| (Intercept) | 0.03 | 0.075 | 0.444 | 0.657 |
| | | | | AIC = 371.4 |
| **Lying duration** | | | | |
| **Lying (OB)** | **0.59** | **0.06** | **9.35** | **<0.001** |
| (Intercept) | 252.81 | 43.93 | 5.76 | <0.001 |
| | | | | AIC = 2843.0 |

**Table S1.4.** Model estimates for fixed effects of linear regression models on contact behaviour of lambs recorded using live focal observations and Sirtrack proximity loggers (experiment 3). AIC values are presented from final models. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| **Duration of contacts** | | | | |
| Duration (OB) | **0.85** | **0.14** | **5.88** | **<0.001** |
| (Intercept) | 23.84 | 4.37 | 5.45 | <0.001 |
| | | | AIC = | 733.6434 |

**Table S1.5.** Effect estimates associated with fixed effects in models of contact behaviour (frequency, duration and total duration) of each treatment group during each experimental phase recorded using **focal observations** (experiment 5). Estimates include the posterior mean, standard error and lower and upper 95% credibility intervals. Estimates are displayed in Appendix A, Figure S1.3. DIC values presented from final model. **Bold represent significant estimates (i.e., estimates that did not overlap with zero).**

| Fixed Effect | mean | se | lower | mode | upper |
|---|---|---|---|---|---|
| **Frequency of contacts** | | | | | |
| Treatment, Removal | -0.084 | 0.114 | -0.306 | -0.085 | 0.143 |
| Phase, B | -0.053 | 0.137 | -0.323 | -0.053 | 0.215 |
| Phase, C | 0.041 | 0.135 | -0.224 | 0.041 | 0.305 |
| Treatment, Removal: Phase B | -0.024 | 0.169 | -0.356 | -0.024 | 0.307 |
| Treatment, Removal: Phase C | -0.069 | 0.181 | -0.426 | -0.068 | 0.285 |
| (Intercept) | 0.386 | 0.095 | 0.195 | 0.388 | 0.568 |
| | | | | DIC =1523.39 | |
| **Duration of contacts** | | | | | |
| Treatment, Removal | -0.087 | 0.111 | -0.306 | -0.087 | 0.129 |
| Phase, B | 0.027 | 0.134 | -0.236 | 0.026 | 0.291 |
| Phase, C | -0.282 | 0.13 | -0.537 | -0.283 | -0.027 |
| Treatment, Removal: Phase B | 0.2 | 0.165 | -0.124 | 0.2 | 0.523 |
| Treatment, Removal: Phase C | **0.583** | **0.175** | **0.24** | **0.583** | **0.927** |
| (Intercept) | 4.863 | 0.097 | 4.672 | 4.863 | 5.054 |
| | | | | DIC = 9187.06 | |
| **Total duration of contacts** | | | | | |
| Treatment, Removal | -0.196 | 0.124 | -0.442 | -0.195 | 0.047 |
| Phase, B | -0.031 | 0.151 | -0.326 | -0.032 | 0.265 |
| Phase, C | -0.24 | 0.15 | -0.534 | -0.24 | 0.055 |
| Treatment, Removal: Phase B | 0.199 | 0.182 | -0.159 | 0.2 | 0.556 |
| Treatment, Removal: Phase C | **0.536** | **0.197** | **0.15** | **0.535** | **0.922** |
| (Intercept) | 5.275 | 0.11 | 5.058 | 5.275 | 5.491 |
| | | | | DIC = 6963.3 | |

**Table S1.6.** Effect estimates associated with fixed effects in models of contact behaviour (frequency, duration and total duration) of each treatment group during each experimental phase recorded using **Sirtrack proximity loggers** (experiment 5). Estimates include the posterior mean, standard error and lower and upper 95% credibility intervals. Estimates are displayed in Appendix A Figure S1.4. DIC values presented from final model. **Bold represent significant estimates (i.e., estimates that did not overlap with zero).**

| Fixed Effect | mean | se | lower | mode | upper |
|---|---|---|---|---|---|
| **Frequency of contacts** | | | | | |
| Treatment, Removal | 0.149 | 0.329 | -0.504 | 0.149 | 0.801 |
| Phase, B | -0.387 | 0.131 | -0.646 | -0.387 | -0.131 |
| Phase, C | 0.132 | 0.147 | -0.156 | 0.131 | 0.422 |
| Treatment, Removal: Phase B | -0.118 | 0.153 | -0.417 | -0.118 | 0.183 |
| Treatment, Removal: Phase C | 0.188 | 0.22 | -0.24 | 0.187 | 0.622 |
| (Intercept) | 4.778 | 0.272 | 4.238 | 4.778 | 5.316 |
| | | | | DIC =6972.03 | |
| **Duration of contacts** | | | | | |
| Treatment, Removal | 0.032 | 0.091 | -0.148 | 0.032 | 0.211 |
| Phase, B | -0.069 | 0.017 | -0.102 | -0.069 | -0.036 |
| Phase, C | -0.072 | 0.017 | -0.105 | -0.072 | -0.04 |
| Treatment, Removal: Phase B | **0.058** | **0.02** | **0.02** | **0.058** | **0.096** |
| Treatment, Removal: Phase C | **0.125** | **0.023** | **0.081** | **0.125** | **0.17** |
| (Intercept) | 2.573 | 0.074 | 2.427 | 2.573 | 2.721 |
| | | | | DIC = 567350.77 | |
| **Total duration of contacts** | | | | | |
| Treatment, Removal | 0.178 | 0.373 | -0.564 | 0.178 | 0.916 |
| Phase, B | -0.457 | 0.146 | -0.745 | -0.457 | -0.173 |
| Phase, C | 0.054 | 0.164 | -0.267 | 0.053 | 0.377 |
| Treatment, Removal: Phase B | -0.051 | 0.17 | -0.384 | -0.051 | 0.285 |
| Treatment, Removal: Phase C | 0.332 | 0.245 | -0.145 | 0.331 | 0.818 |
| (Intercept) | 7.349 | 0.308 | 6.739 | 7.349 | 7.96 |
| | | | | DIC =10232.23 | |

## 1.1 Additional figures

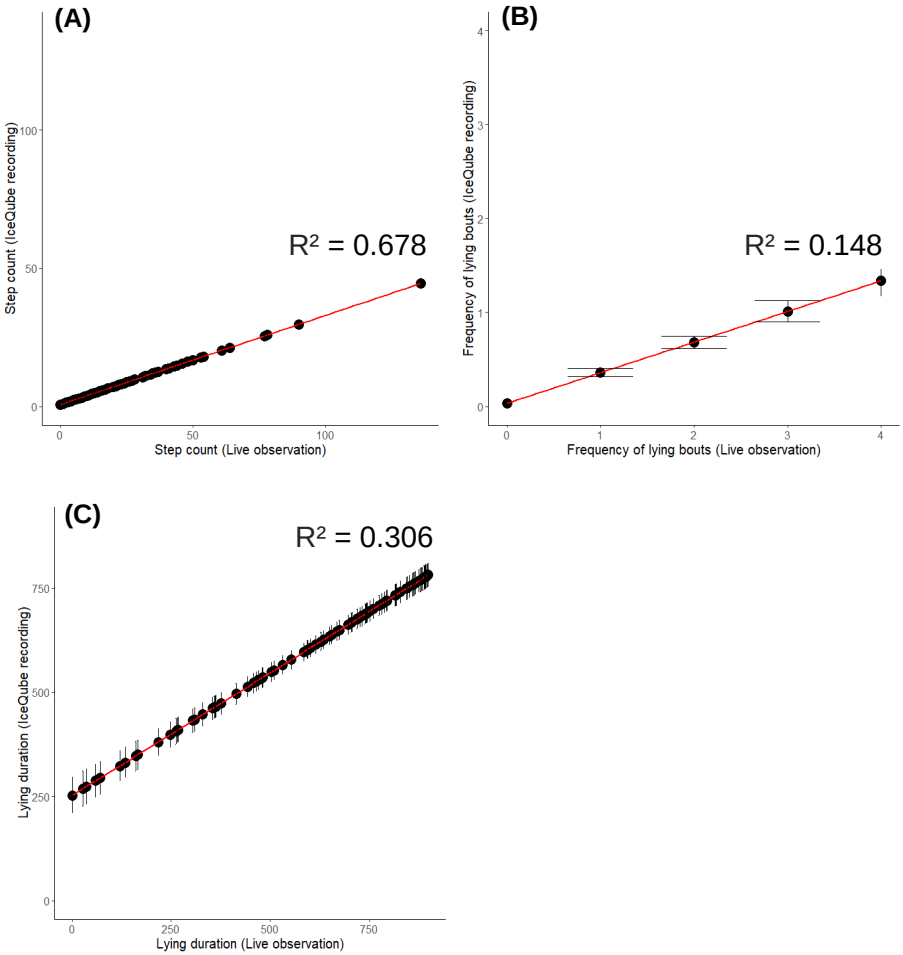


**Figure S1.1.** Model predicted mean ± standard error activity behaviour recorded by the IceQube activity monitors against activity levels recorded using live focal observations. **(A)** Observed step count against step count recorded by IceQube activity monitors, with fitted regression line. **(B)** Observed frequency of lying bouts against frequency of lying bouts recorded by IceQube activity monitors, with fitted regression line. **(C)** Observed lying duration against lying duration recorded by IceQube activity monitors, with fitted regression line.

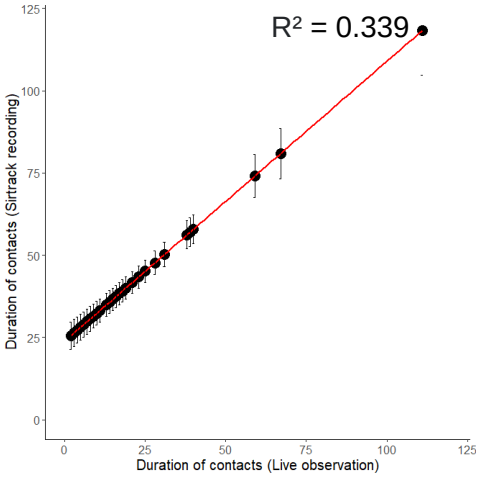


**Figure S1.2.** Model predicted mean ± standard error contact duration recorded by the proximity loggers against duration of contacts recorded by live focal observations, with fitted regression line.

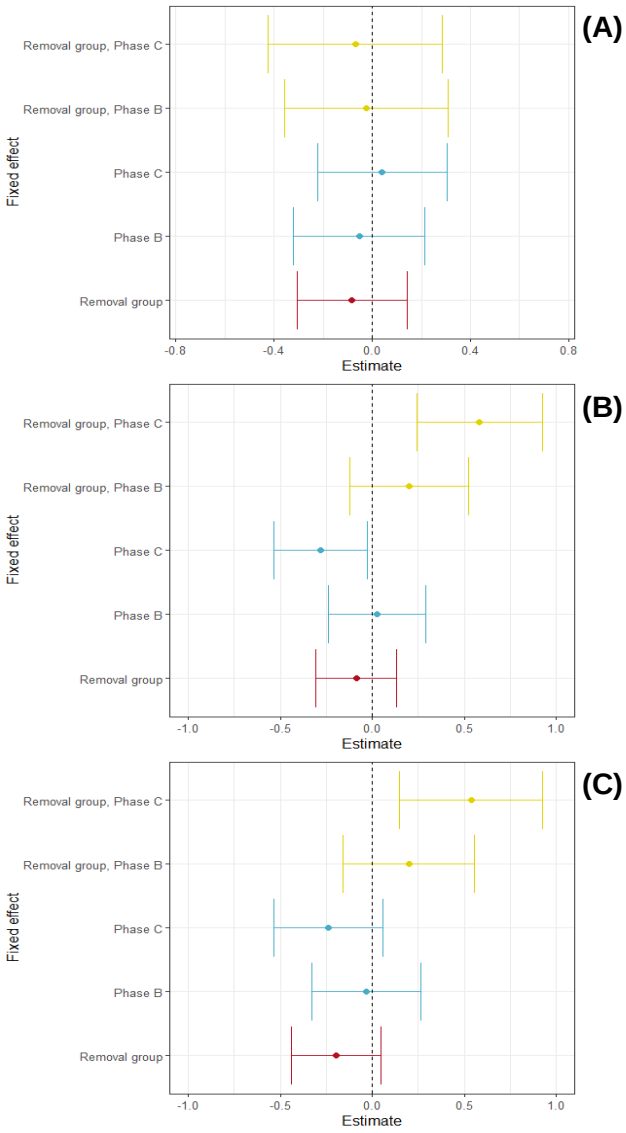


**Figure S1.3**. Comparison of the fixed effect estimates from each response variable model fitted on contact data recorded using live **focal observations**. Points denote the mean effect estimate and bars represent the 0.025 and 0.975 quantiles. Plot **(A)** denotes the estimates from the frequency model including Treatment group x Phase interaction effect. Plot **(B)** denotes the estimates from the duration model including Treatment group x Phase interaction effect. Points of different colours denote the results from different levels of the explanatory variables. Plot **(C)** denotes the estimates from the total duration model including Treatment group x Phase interaction effect. Points of different colours denote the results from different levels of the explanatory variables.

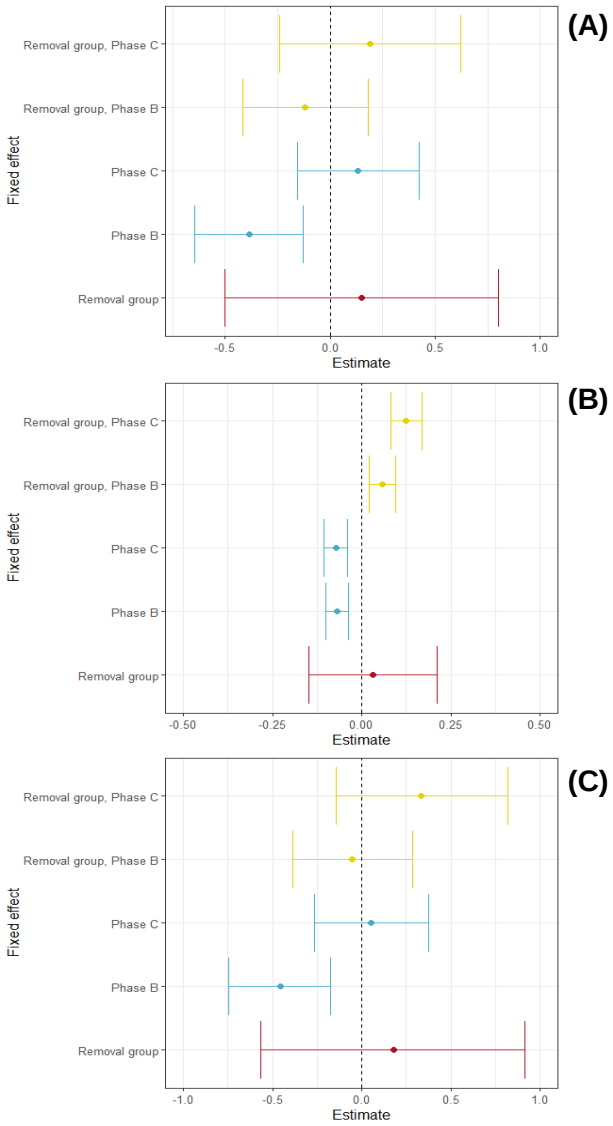


**Figure S1.4**. Comparison of the fixed effect estimates from each response variable model fitted on contact data recorded **Sirtrack proximity loggers**. Points denote the mean effect estimate and bars represent the 0.025 and 0.975 quantiles. Plot **(A)** denotes the estimates from the frequency model including Treatment group x Phase interaction effect. Plot **(B)** denotes the estimates from the duration model including Treatment group x Phase interaction effect. Points of different colours denote the results from different levels of the explanatory variables. Plot **(C)** denotes the estimates from the total duration model including Treatment group x Phase interaction effect. Points of different colours denote the results from different levels of the explanatory variables.

# Appendix B: Supplementary material for Chapter 3

## 2. Model description and output

**Table S2.1.** Model formulae for analyses of activity behaviour (step count, motion index, frequency of lying bouts and lying time) and animal measurements during the experiment. Bold indicates terms that were included in the minimal model.

| Model Group | Response | Model Class | Model Family | Fixed Effects | Interactions | Random Effects |
|---|---|---|---|---|---|---|
| **Activity Behaviour** | Step count | GLMM | Negative Binomial | Sex + **IceQube** | **Treatment Group:Phase** | **Group ID/Animal ID + Plot** |
| | | | | Sex + **IceQube** | **Treatment Group:Week** | **Group ID/Animal ID + Plot** |
| | | | | Sex + **IceQube** | **Parasitic status:Group type:Phase** | **Group ID/Animal ID + Plot** |
| | | | | Sex + **IceQube** | **Parasitic status:Group type:Week** | **Group ID/Animal ID + Plot** |
| | Motion index | GLMM | Negative Binomial | Sex | **Treatment Group:Phase** | **Group ID/Animal ID + IceQube ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Group ID/Animal ID + IceQube ID + Plot** |
| | | | | Sex | **Parasitic status:Group type:Phase** | **Group ID/Animal ID + IceQube ID + Plot** |
| | | | | Sex | **Parasitic status:Group type:Week** | **Group ID/Animal ID + IceQube ID + Plot** |
| | Frequency of lying bouts | GLMM | Poisson | Sex | **Treatment Group:Phase** | **Group ID/Animal ID + IceQube ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Group ID/Animal ID + IceQube ID + Plot** |
| | | | | Sex | **Parasitic status:Group type:Phase** | **Group ID/Animal ID + IceQube ID + Plot** |
| | | | | Sex | **Parasitic status:Group type:Week** | **Group ID/Animal ID + IceQube ID + Plot** |
| | Lying time | GLMM | Binomial | Sex | **Treatment Group:Phase** | **Group ID/Animal ID + IceQube ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Group ID/Animal ID + IceQube ID + Plot** |
| | | | | Sex | **Parasitic status:Group type:Phase** | **Parasitic status:Group type:Week** |
| | | | | Sex | **Parasitic status:Group type:Week** | **Group ID/Animal ID + IceQube ID + Plot** |
| **Animal measurements** | Weight | GLMM | Gaussian | Sex | **Parasitic status:Week** | **Group ID/Animal ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Group ID/Animal ID + Plot** |
| | | | | Sex | **Infected Group:Week** | **Group ID/Animal ID + Plot** |
| | Pepsinogen | GLMM | Poisson | Sex | **Parasitic status:Week** | **Group ID/Animal ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Group ID/Animal ID + Plot** |

**Table S2.2.** Description of fixed and random effects.

| Term | Class | Description |
|---|---|---|
| **Parasitic status** | Factor (2 levels) | **Infected** (dosed with parasites); **Non-infected** (dosed with water) |
| **Treatment Group** | Factor (3 levels) | **Non-parasitised** (social groups containing non-infected lambs); **Parasitised** (social groups containing infected lambs); **Mixed** (social groups containing a mixture of infected and non-infected lambs) |
| **Phase** | Factor (4 levels) | **Pre-parasite** (first week of experiment when all lambs were parasite naïve); **pre-patent** (weeks 2-4 when lambs are infected but not shedding eggs); **patent-parasite** (weeks 5-7 when infected lambs are shedding eggs); **post-parasite** (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 part of a group containing infected and non-infected lambs), **Single-parasitic state** (individual is in the parasitised or non-parasitised group) |
| **Sex** | Factor (2 levels) | Male or Female |
| **Animal ID** | Factor (60 levels) | ID of Animal |
| **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 S2.3.** Model estimates for fixed effects of final generalised linear mixed models on mean serum pepsinogen levels of infected and non-infected lambs during the three blood sampling days. Blood samples were taken during the pre-parasite (Week 1), patent-parasite (Week 7) and post-parasite phase (Week 9). AIC values are presented from final models. Bold indicates significant results.

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| Parasitic status, Infected | -0.19 | 0.21 | -0.92 | 0.360 |
| Phase, Patent-parasite | 0.21 | 0.11 | 1.92 | 0.055 |
| Phase, Post-parasite | 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, Post-parasite | 0.21 | 0.23 | 0.92 | 0.359 |
| (Intercept) | 2.94 | 0.15 | 19.51 | <0.001 |
| | | | | AIC = 614.8 |

**Table S2.4.** Model estimates for fixed effects of final generalised linear mixed models on mean weight of the three treatment groups (non-parasitised, parasitised and mixed) during each week of the experiment. AIC values are presented from final models. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| Group, Parasitised | -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, Parasitised:Week, 2 | -0.21 | 1.73 | -0.12 | 0.903 |
| Group, Parasitised:Week, 3 | 0.89 | 0.99 | 0.91 | 0.366 |
| Group, Parasitised:Week, 4 | 2.46 | 1.73 | 1.42 | 0.175 |
| Group, Parasitised:Week, 5 | -1.42 | 1.73 | -0.82 | 0.424 |
| Group, Parasitised:Week, 6 | -1.32 | 0.99 | -1.33 | 0.184 |
| Group, Parasitised:Week, 7 | 0.97 | 1.73 | 0.56 | 0.582 |
| Group, Parasitised:Week, 8 | -0.83 | 1.73 | -0.48 | 0.639 |
| Group, Parasitised:Week, 9 | -0.53 | 0.99 | -0.54 | 0.592 |
| Group, Parasitised: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 |

**Table S2.5.** Model estimates for fixed effects of final generalised linear mixed models on mean weight of infected and non-infected lambs during each week of the experiment. AIC values are presented from final models. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| 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 |

**Table S2.6.** Model estimates for fixed effects of final generalised linear mixed models on mean weight of infected lambs in the mixed and single state groups during each week of the experiment. AIC values are presented from final models. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| 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 |

**Table S2.7.** Model estimates for fixed effects of final generalised linear mixed models on activity behaviour of the three treatment groups during each experiment phase. AIC values are presented from final models. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| **Motion index** | | | | |
| Group, Parasitised | 0.01 | 0.04 | 0.16 | 0.873 |
| Group, Mixed | 0.03 | 0.04 | 0.63 | 0.531 |
| Pre-patent | -0.14 | 0.02 | -7.18 | <0.001 |
| Patent-parasite | 0.00 | 0.02 | -0.14 | 0.889 |
| Post-parasite | -0.25 | 0.02 | -12.04 | <0.001 |
| Group, Parasitised:Phase, Pre-patent | **-0.09** | **0.03** | **-3.40** | **<0.001** |
| Group, Parasitised:Phase, Patent-parasite | **-0.07** | **0.03** | **-2.42** | **0.015** |
| Group, Parasitised:Phase, Post-parasite | -0.01 | 0.03 | -0.19 | 0.849 |
| Group, Mixed:Phase, Pre-patent | **-0.05** | **0.03** | **-1.89** | **0.059** |
| Group, Mixed:Phase, Patent-parasite | 0.01 | 0.03 | 0.23 | 0.821 |
| Group, Mixed:Phase, Post-parasite | -0.01 | 0.03 | -0.18 | 0.855 |
| (Intercept) | 5.65 | 0.03 | 174.75 | <0.001 |
| | | | | AIC = 1087531.2 |
| **Step count** | | | | |
| Group, Parasitised | 0.04 | 0.06 | 0.64 | 0.524 |
| Group, Mixed | 0.02 | 0.06 | 0.39 | 0.698 |
| Pre-patent | 0.01 | 0.02 | 0.49 | 0.625 |
| Patent-parasite | -0.06 | 0.02 | -2.73 | 0.006 |
| Post-parasite | -0.15 | 0.02 | -6.54 | <0.001 |
| Group, Parasitised:Phase, Pre-patent | **-0.11** | **0.03** | **-3.49** | **<0.001** |
| Group, Parasitised:Phase, Patent-parasite | **-0.11** | **0.03** | **-3.59** | **<0.001** |
| Group, Parasitised:Phase, Post-parasite | -0.01 | 0.03 | -0.38 | 0.701 |
| Group, Mixed:Phase, Pre-patent | **-0.07** | **0.03** | **-2.14** | **0.033** |
| Group, Mixed:Phase, Patent-parasite | -0.07 | 0.03 | -2.23 | 0.026 |
| Group, Mixed:Phase, Post-parasite | -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, Parasitised | 0.07 | 0.03 | 2.04 | 0.041 |
| Group, Mixed | 0.05 | 0.03 | 1.36 | 0.174 |
| Pre-patent | 0.07 | 0.02 | 3.09 | 0.002 |
| Patent-parasite | 0.03 | 0.02 | 1.14 | 0.254 |
| Post-parasite | -0.04 | 0.02 | -1.64 | 0.101 |
| Group, Parasitised:Phase, Pre-patent | **-0.06** | **0.03** | **-2.02** | **0.043** |
| Group, Parasitised:Phase, Patent-parasite | **-0.09** | **0.03** | **-2.90** | **0.004** |
| Group, Parasitised:Phase, Post-parasite | **-0.07** | **0.03** | **-2.10** | **0.036** |
| Group, Mixed:Phase, Pre-patent | **-0.05** | **0.03** | **-1.73** | **0.085** |
| Group, Mixed:Phase, Patent-parasite | -0.02 | 0.03 | -0.76 | 0.447 |
| Group, Mixed:Phase, Post-parasite | -0.02 | 0.03 | -0.53 | 0.597 |
| (Intercept) | -0.10 | 0.03 | -3.84 | <0.001 |
| | | | | AIC = 239872.5 |
| **Lying duration (night data)** | | | | |
| Group, Parasitised | -0.12 | 0.15 | -0.81 | 0.419 |
| Group, Mixed | 0.07 | 0.15 | 0.49 | 0.623 |
| Pre-patent | 0.43 | 0.11 | 3.87 | <0.001 |
| Patent-parasite | 0.63 | 0.11 | 5.63 | <0.001 |
| Post-parasite | 0.30 | 0.11 | 2.71 | 0.007 |
| Group, Parasitised:Phase, Pre-patent | 0.23 | 0.16 | 1.43 | 0.154 |
| Group, Parasitised:Phase, Patent-parasite | 0.12 | 0.16 | 0.73 | 0.466 |
| Group, Parasitised:Phase, Post-parasite | 0.01 | 0.16 | 0.07 | 0.943 |
| Group, Mixed:Phase, Pre-patent | -0.05 | 0.16 | -0.30 | 0.768 |
| Group, Mixed:Phase, Patent-parasite | -0.11 | 0.16 | -0.66 | 0.507 |
| Group, Mixed:Phase, Post-parasite | -0.19 | 0.16 | -1.22 | 0.224 |
| (Intercept) | 1.01 | 0.11 | 9.61 | <0.001 |
| | | | | AIC = 239872.5 |
| **Lying duration (day data)** | | | | |
| Group, Parasitised | -0.03 | 0.10 | -0.29 | 0.776 |
| Group, Mixed | -0.07 | 0.10 | -0.67 | 0.501 |
| Pre-patent | -0.33 | 0.07 | -4.79 | <0.001 |

| | | | | |
|---|---|---|---|---|
| Patent-parasite | -0.28 | 0.07 | -3.98 | <0.001 |
| Post-parasite | -0.62 | 0.08 | -7.92 | <0.001 |
| Group, Parasitised:Phase, Pre-patent | -0.05 | 0.10 | -0.49 | 0.626 |
| Group, Parasitised:Phase, Patent-parasite | -0.02 | 0.10 | -0.21 | 0.836 |
| Group, Parasitised:Phase, Post-parasite | -0.01 | 0.11 | -0.11 | 0.911 |
| Group, Mixed:Phase, Pre-patent | -0.13 | 0.10 | -1.31 | 0.191 |
| Group, Mixed:Phase, Patent-parasite | -0.01 | 0.10 | -0.13 | 0.898 |
| Group, Mixed:Phase, Post-parasite | 0.01 | 0.11 | 0.08 | 0.938 |
| (Intercept) | -0.30 | 0.07 | -4.16 | <0.001 |
| | | | | AIC = 38668.2 |

**Table S2.8.** Model estimates for fixed effects of final generalised 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 | Std.error | z | p-value |
|---|---|---|---|---|
| **Motion index** | | | | |
| Group, Parasitised | 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, Parasitised:Week, 2 | **-0.08** | **0.04** | **-2.29** | **0.022** |
| Group, Parasitised:Week, 3 | **-0.07** | **0.03** | **-2.08** | **0.038** |
| Group, Parasitised:Week, 4 | **-0.12** | **0.03** | **-3.46** | **<0.001** |
| Group, Parasitised:Week, 5 | **-0.04** | **0.04** | **-1.19** | **0.235** |
| Group, Parasitised:Week, 6 | **-0.07** | **0.03** | **-2.09** | **0.037** |
| Group, Parasitised:Week, 7 | **-0.08** | **0.03** | **-2.17** | **0.030** |
| Group, Parasitised:Week, 8 | 0.01 | 0.04 | 0.30 | 0.761 |
| Group, Parasitised: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, Parasitised | 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, Parasitised:Week, 2 | **-0.11** | **0.04** | **-2.70** | **0.007** |
| Group, Parasitised:Week, 3 | **-0.08** | **0.04** | **-2.08** | **0.037** |
| Group, Parasitised:Week, 4 | **-0.12** | **0.04** | **-3.26** | **0.001** |
| Group, Parasitised:Week, 5 | **-0.09** | **0.04** | **-2.37** | **0.018** |
| Group, Parasitised:Week, 6 | **-0.07** | **0.04** | **-1.91** | **0.056** |
| Group, Parasitised:Week, 7 | **-0.15** | **0.04** | **-3.81** | **<0.001** |
| Group, Parasitised:Week, 8 | 0.03 | 0.04 | 0.66 | 0.511 |
| Group, Parasitised: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, Parasitised | **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, Parasitised:Week, 2 | -0.06 | 0.04 | -1.52 | 0.128 |
| Group, Parasitised:Week, 3 | -0.06 | 0.04 | -1.66 | 0.097 |
| Group, Parasitised:Week, 4 | **-0.08** | **0.04** | **-2.15** | **0.031** |
| Group, Parasitised:Week, 5 | **-0.08** | **0.04** | **-1.96** | **0.050** |
| Group, Parasitised:Week, 6 | -0.07 | 0.04 | -1.68 | 0.093 |
| Group, Parasitised:Week, 7 | **-0.15** | **0.04** | **-3.85** | **<0.001** |
| Group, Parasitised:Week, 8 | **-0.09** | **0.04** | **-2.32** | **0.021** |
| Group, Parasitised: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, Parasitised | -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, Parasitised:Week, 2 | 0.12 | 0.21 | 0.59 | 0.555 |
| Group, Parasitised:Week, 3 | 0.16 | 0.20 | 0.83 | 0.410 |
| Group, Parasitised:Week, 4 | **0.50** | **0.21** | **2.32** | **0.021** |
| Group, Parasitised:Week, 5 | -0.02 | 0.30 | -0.05 | 0.959 |
| Group, Parasitised:Week, 6 | 0.03 | 0.18 | 0.15 | 0.881 |
| Group, Parasitised:Week, 7 | 0.27 | 0.19 | 1.40 | 0.163 |
| Group, Parasitised:Week, 8 | -0.08 | 0.19 | -0.44 | 0.660 |
| Group, Parasitised: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, Parasitised | -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, Parasitised:Week, 2 | -0.14 | 0.13 | -1.12 | 0.262 |

| | | | | |
|---|---|---|---|---|
| Group, Parasitised:Week, 3 | 0.01 | 0.12 | 0.08 | 0.938 |
| Group, Parasitised:Week, 4 | -0.04 | 0.12 | -0.37 | 0.708 |
| Group, Parasitised:Week, 5 | -0.02 | 0.13 | -0.19 | 0.852 |
| Group, Parasitised:Week, 6 | 0.04 | 0.13 | 0.30 | 0.765 |
| Group, Parasitised:Week, 7 | -0.11 | 0.12 | -0.92 | 0.358 |
| Group, Parasitised:Week, 8 | -0.08 | 0.14 | -0.61 | 0.544 |
| Group, Parasitised: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 S2.9.** Model estimates for fixed effects of final generalised linear mixed models on the activity behaviour of infected and non-infected lambs in the mixed and single parasitic state groups during each phase. AIC values are presented from final models. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| **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, Pre-patent | **-0.14** | **0.02** | **-7.18** | **<0.001** |
| Phase, Patent-parasite | 0.00 | 0.02 | -0.14 | 0.886 |
| Phase, Post-parasite | **-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, Pre-patent | **-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, Post-parasite | -0.01 | 0.03 | -0.19 | 0.853 |
| Group type, Mixed:Phase, Pre-patent | 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, Post-parasite | 0.02 | 0.03 | 0.49 | 0.621 |
| Parasitic status, Infected:Group type, Mixed:Phase, Pre-patent | 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, Post-parasite | -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, Pre-patent | 0.01 | 0.02 | 0.49 | 0.626 |
| Phase, Patent-parasite | -0.06 | 0.02 | -2.73 | 0.006 |
| Phase, Post-parasite | -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, Pre-patent | -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, Post-parasite | -0.01 | 0.03 | -0.38 | 0.700 |
| Group type, Mixed:Phase, Pre-patent | -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, Post-parasite | 0.01 | 0.04 | 0.35 | 0.723 |
| Parasitic status, Infected:Group type, Mixed:Phase, Pre-patent | 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, Post-parasite | -0.08 | 0.06 | -1.52 | 0.130 |
| (Intercept) | 4.11 | 0.05 | 78.54 | <0.001 |
| | | | | AIC =818278.3 |
| **Frequency of lying bouts** | | | | |
| Parasitic status, Infected | 0.07 | 0.03 | 2.04 | 0.041 |
| Group type, Mixed | 0.03 | 0.04 | 0.72 | 0.470 |
| Phase, Pre-patent | 0.07 | 0.02 | 3.09 | 0.002 |
| Phase, Patent-parasite | 0.03 | 0.02 | 1.14 | 0.254 |
| Phase, Post-parasite | -0.04 | 0.02 | -1.64 | 0.101 |
| Parasitic status, Infected:Group type, Mixed | -0.02 | 0.06 | -0.43 | 0.669 |
| Parasitic status, Infected:Phase, Pre-patent | -0.06 | 0.03 | -2.02 | 0.043 |
| Parasitic status, Infected:Phase, Patent-parasite | **-0.09** | **0.03** | **-2.90** | **0.004** |
| Parasitic status, Infected:Phase, Post-parasite | **-0.07** | **0.03** | **-2.10** | **0.036** |
| Group type, Mixed:Phase, Pre-patent | -0.04 | 0.04 | -1.03 | 0.302 |
| Group type, Mixed:Phase, Patent-parasite | -0.02 | 0.04 | -0.63 | 0.526 |
| | -0.01 | 0.04 | -0.35 | 0.724 |

| | | | | |
|---|---|---|---|---|
| Group type, Mixed:Phase, Post-parasite | **0.02** | **0.05** | **0.41** | **0.683** |
| Parasitic status, Infected:Group type, Mixed:Phase, Pre-patent | 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, Post-parasite | **0.07** | **0.03** | **2.04** | **0.041** |
| (Intercept) | -0.10 | 0.03 | -3.85 | <0.001 |
| | | | AIC = 239877.7 | |
| **Lying duration (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, Pre-patent | **0.43** | **0.11** | **3.87** | **<0.001** |
| Phase, Patent-parasite | **0.63** | **0.11** | **5.63** | **<0.001** |
| Phase, Post-parasite | **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, Pre-patent | 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, Post-parasite | 0.01 | 0.16 | 0.07 | 0.948 |
| Group type, Mixed:Phase, Pre-patent | -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, Post-parasite | -0.35 | 0.18 | -1.90 | 0.058 |
| Parasitic status, Infected:Group type, Mixed:Phase, Pre-patent | 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, Post-parasite | 0.39 | 0.28 | 1.41 | 0.159 |
| (Intercept) | 1.01 | 0.11 | 9.62 | <0.001 |
| | | | AIC = 19119.3 | |
| **Lying duration (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, Pre-patent | -0.33 | 0.07 | -4.77 | <0.001 |
| Phase, Patent-parasite | -0.27 | 0.07 | -3.88 | <0.001 |
| Phase, Post-parasite | -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, Pre-patent | -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, Post-parasite | -0.01 | 0.11 | -0.11 | 0.915 |
| Group type, Mixed:Phase, Pre-patent | -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, Post-parasite | -0.04 | 0.13 | -0.34 | 0.735 |
| Parasitic status, Infected:Group type, Mixed:Phase, Pre-patent | 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, Post-parasite | 0.15 | 0.19 | 0.77 | 0.440 |
| (Intercept) | -0.30 | 0.07 | -4.21 | <0.001 |
| | | | AIC= 38580.2 | |

**Table S2.10.** Model estimates for fixed effects of final generalised linear mixed models on the activity behaviour of infected and non-infected lambs in the mixed and single parasitic state groups during each week. AIC values are presented from final models. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| **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 |
| 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 duration (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 |
| 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, 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 duration (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 |
| (Intercept) | 0.30 | 0.07 | -4.09 | <0.001 |
| | | | | AIC = 38517.4 |

## 2.1 Additional figures

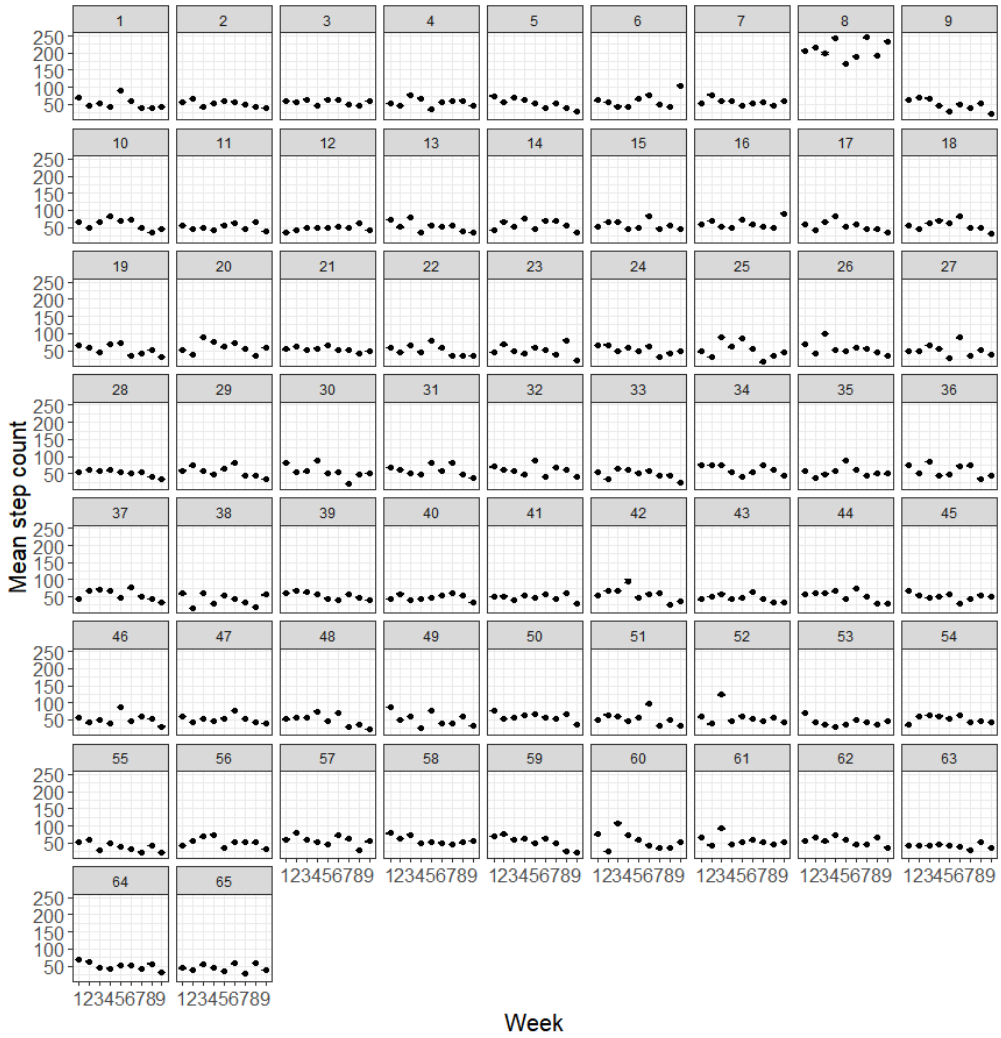


**Figure S2.1**. Mean step count recorded by each IceQube (n=65) during each week of the experiment. As one IceQube (IceQube 8) was more sensitive at recording step count than all others and consistently recorded a higher step count each week, IceQube ID was included in the GLMM's for step count as a fixed effect to explain the variance rather than control for it.

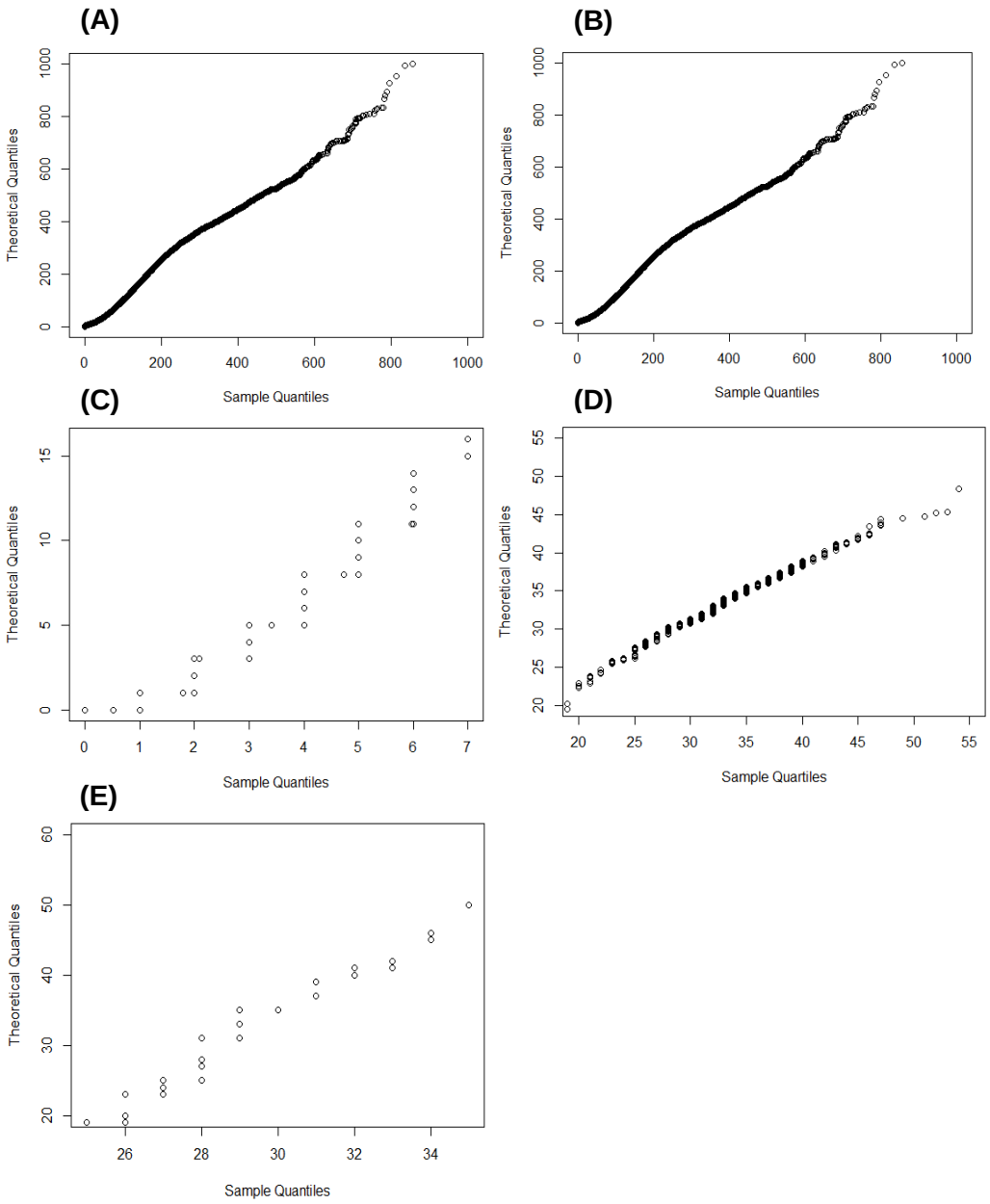


**Figure S2.2**. Quantile-quantile plots showing the empirically observed quantiles of **(A)** step count and **(B)** motion index as a function of quantiles expected from a negative binomial distribution, the empirically observed quantiles of **(C)** lying bouts as a function of quantiles from a Poisson distribution, the empirically observed quantiles of **(D)** weight as a function of quantiles from a Gaussian distribution and the empirically observed quantiles of **(E)** serum pepsinogen as a function of quantiles from a Poisson distribution.

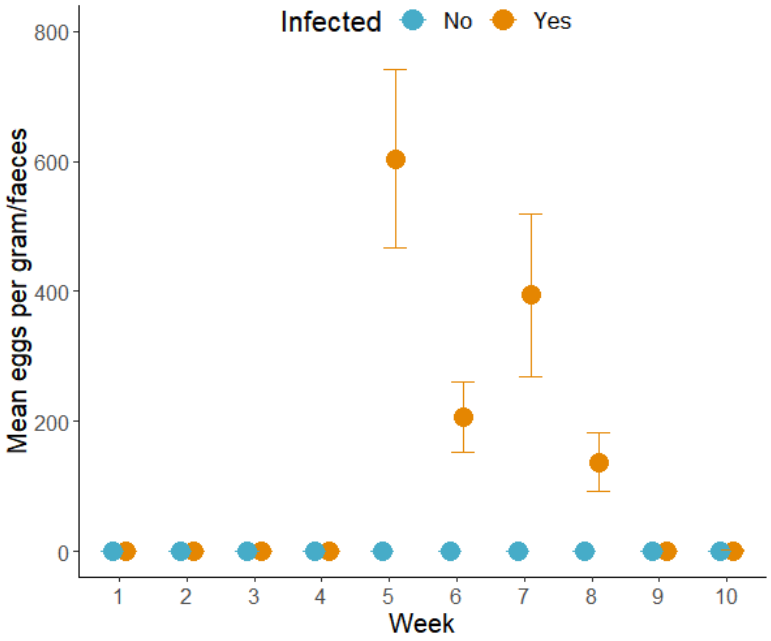


**Figure S2.3.** Mean ± standard error faecal egg counts (epg) of infected (orange; *n* = 28) and non-infected (blue; *n* = 32) lambs each week of the experiment, including the final sampling day at the beginning of week 10. Lambs were dosed with *T. circumcincta* larvae at the start of week 2 and infections were cleared at the start of week 8 after final faecal samples were collected.

## 2.2 Results continued

**Motion index:** There was a statistically significant interaction between treatment group and week on motion index (Wald (W) = 30.38, df = 16, p = 0.001): The parasitised groups had statistically significantly lower motion index between weeks 2 and 7 compared to non-parasitised groups (Figure 3.3A and Appendix B, Table S2.4). There was no statistically significant difference in motion index between mixed and non-parasitised groups during each week of the study (Appendix B, Table S2.4).

**Step count:** There was a statistically significant interaction between treatment group and week on step count (Wald (W) = 63.31, df = 16, p<0.001): Parasitised groups had statistically significantly lower step counts than the non-parasitised groups between

weeks 2 and week 7 (Figure 3.3B and Appendix B, Table S2.4), and the step count of the mixed groups was statistically significantly lower than the non-parasitised groups during week 2 (Est = -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 (W) = 29.23, p=0.02): Parasitised groups had statistically significantly lower frequency of lying bouts during week 4 (Est = -0.08, p = 0.031), week 7 (Est = -0.15, p<0.001) and week 8 (Est = -0.09, p = 0.021) (Figure 3.3C) compared to the non-parasitised groups. Mixed groups also had statistically significantly lower frequency of lying bouts during week 4 (Est = -0.08, p = 0.028) and week 7 (Est = -0.08, p = 0.036) compared to the non-parasitised 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 (Figure 3.3C Appendix B, Table S2.4).

## 2.3. Lab assays

### Faecal egg counts

Faecal egg counts were conducted using a modified salt-flotation method (Jackson, 1974). One day after sample collection, 1g of faeces was weighed out and placed in a fresh bag with 10ml of water and emulsified. The sample was taken and dispensed through a 1mm sieve into a beaker, with the retenate washed into the beaker with an additional 5ml of water. The retenate was transferred to a 15ml cellulose acetate tube and centrifuged at 1000 rpm for 2 minutes. The supernatant was removed using a vacuum line, and the faecal pellet was suspended in 10ml saturated sodium chloride solution and centrifuged at 1000 rpm for 2 minutes. 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. 1ml of NaCl solution was used to rinse the upper chamber of the tube

and added to the cuvette. The cuvette was inverted to homogenise 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 1epg (eggs per gram).

# Appendix C: Supplementary material for Chapter 4

## 3. Model description and output

**Table S3.1.** Model formulae for analyses of contact behaviour (frequency of contacts, duration of contacts and total duration of contacts) and animal measurements of lambs during the experiment. **Bold indicates terms that were included in the minimal model.**

| Model Group | Response | Model Class | Model Family | Fixed Effects | Interactions | Random Effects |
|---|---|---|---|---|---|---|
| **Contact Behaviour** | Frequency of contacts | GLMM | Negative Binomial | Sex | **Treatment Group:Phase** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | | | | Sex | **Contact Type:Phase** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | | | | Sex | **Contact Type:Week** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | Duration of contacts | GLMM | Negative Binomial | Sex | **Treatment Group:Phase** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | | | | Sex | **Contact Type:Phase** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | | | | Sex | **Contact Type:Week** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | Total duration of contacts | GLMM | Negative Binomial | Sex | **Treatment Group:Phase** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | | | | Sex | **Contact Type:Phase** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| | | | | Sex | **Contact Type:Week** | **Animal 1 ID + Animal 2 ID + Logger 1 ID + Logger 2 ID + Group ID + Plot** |
| **Animal measurements** | Pepsinogen | GLMM | Poisson | Sex | **Parasitic Status:Week** | **Animal ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Animal ID + Plot** |
| | Weight | GLMM | Gaussian | Sex | **Parasitic Status:Week** | **Animal ID + Plot** |
| | | | | Sex | **Treatment Group:Week** | **Animal ID + Plot** |
| **Network metrics** | Closeness | LMM | Gaussian | Sex | **Individual Parasitic Status:Phase** | **Logger ID + Plot** |

**Table S3.2.** Description of fixed and random effects.

| Term | Class | Description |
|---|---|---|
| **Treatment Group** | Factor (3 levels) | **Non-parasitised** (social groups of non-infected lambs); **Parasitised** (social groups of infected lambs); **Mixed** (social groups of a mixture of infected and non-infected lambs) |
| **Parasitic Status** | Factor (2 levels) | **Infected** (dosed with parasites); **Non-infected** (dosed with water) |
| **Contact Type** | Factor (3 levels) | **Non-infected – Non-infected** (contact between two non-infected animals), Infected **– Infected** (contact between two infected animals), Infected **– Non-infected** (contact between one infected and one non-infected animal) |
| **Phase** | Factor (4 levels) | **Pre-parasite** (first week of experiment when all lambs were parasite naive); **pre-patent** (weeks 2-4 when lambs are infected but not shedding eggs); **patent-parasite** (weeks 5-7 when infected lambs are shedding eggs); **post-parasite** (weeks 8-9 after lambs are treated with anthelmintic) |
| **Week** | Factor (9 levels) | Week of experiment (week 1-9) |
| **Sex** | Factor (3 levels) | Male-Male, Female-Female, Male-Female |
| **Individual parasitic status** | Factor (5 levels) | Animal.1:Non-infected, Animal.2:Non-infected, Animal.3:Infected, Animal.4:Infected, Animal.5:Non-infected |
| **Animal 1 ID** | Factor (60 levels) | ID of Animal 1 |
| **Animal 2 ID** | Factor (60 levels) | ID of Animal 2 |
| **Group ID** | Factor (12 levels) | ID of the social groups |
| **Plot** | Factor (12 levels) | ID of Plot |
| **Logger 1 ID** | Factor (65 levels) | ID of Proximity logger worn by Animal 1 |
| **Logger 2 ID** | Factor (65 levels) | ID of Proximity logger worn by Animal 2 |

**Table S3.3.** Effect estimates associated with fixed effects in our models of contact behaviour (frequency, duration and total duration of contacts) **of each treatment group,** during each experimental phase. Estimates include the posterior mean, standard error and lower and upper 95% credibility intervals. Estimates are displayed in Appendix C, Figure S3.2. DIC values presented from final model. **Bold represent significant estimates (i.e., estimates that did not overlap with zero).**

| Fixed Effect | mean | se | lower | mode | upper |
|---|---|---|---|---|---|
| **Frequency of contacts** | | | | | |
| Group, Parasitised | 0.097 | 0.05 | -0.001 | 0.097 | 0.195 |
| Group, Mixed | 0.054 | 0.05 | -0.043 | 0.054 | 0.152 |
| Pre-patent | -0.021 | 0.016 | -0.053 | -0.021 | 0.011 |
| Patent-parasite | -0.051 | 0.017 | -0.083 | -0.051 | -0.018 |
| Post-parasite | -0.105 | 0.018 | -0.14 | -0.105 | -0.07 |
| Group, Parasitised:Phase, Pre-patent | **-0.066** | **0.023** | **-0.111** | **-0.066** | **-0.021** |
| Group, Parasitised:Phase, Patent-parasite | **-0.083** | **0.023** | **-0.128** | **-0.083** | **-0.038** |
| Group, Parasitised:Phase, Post-parasite | **-0.075** | **0.025** | **-0.124** | **-0.075** | **-0.027** |
| Group, Mixed:Phase, Pre-patent | **-0.09** | **0.023** | **-0.135** | **-0.09** | **-0.046** |
| Group, Mixed:Phase, Patent-parasite | 0.015 | 0.023 | -0.03 | 0.015 | 0.06 |
| Group, Mixed:Phase, Post-parasite | **-0.059** | **0.025** | **-0.108** | **-0.059** | **-0.01** |
| (Intercept) | 0.891 | 0.062 | 0.77 | 0.891 | 1.012 |
| | | | | DIC = 442725.97 | |
| **Duration of contacts** | | | | | |
| Group, Parasitised | 0.001 | 0.061 | -0.119 | 0.001 | 0.121 |
| Group, Mixed | 0.069 | 0.061 | -0.051 | 0.069 | 0.188 |
| Pre-patent | -0.016 | 0.014 | -0.044 | -0.016 | 0.012 |
| Patent-parasite | 0.158 | 0.015 | 0.129 | 0.158 | 0.187 |
| Post-parasite | 0.05 | 0.016 | 0.019 | 0.05 | 0.081 |
| Group, Parasitised:Phase, Pre-patent | **0.076** | **0.02** | **0.036** | **0.076** | **0.116** |
| Group, Parasitised:Phase, Patent-parasite | -0.006 | 0.02 | -0.046 | -0.006 | 0.035 |
| Group, Parasitised:Phase, Post-parasite | -0.044 | 0.022 | -0.087 | -0.044 | 0 |
| Group, Mixed:Phase, Pre-patent | 0.002 | 0.02 | -0.037 | 0.002 | 0.041 |
| Group, Mixed:Phase, Patent-parasite | -0.001 | 0.021 | -0.041 | -0.001 | 0.039 |
| Group, Mixed:Phase, Post-parasite | 0.018 | 0.022 | -0.025 | 0.018 | 0.062 |
| (Intercept) | 3.22 | 0.08 | 3.063 | 3.22 | 3.376 |
| | | | | DIC = 2681275.01 | |
| **Total duration of contacts** | | | | | |
| Group, Parasitised | 0.086 | 0.093 | -0.098 | 0.086 | 0.269 |
| Group, Mixed | 0.124 | 0.093 | -0.06 | 0.124 | 0.307 |
| Pre-patent | -0.025 | 0.025 | -0.074 | -0.025 | 0.025 |
| Patent-parasite | 0.107 | 0.026 | 0.056 | 0.107 | 0.158 |
| Post-parasite | -0.056 | 0.028 | -0.111 | -0.056 | -0.002 |
| Group, Parasitised:Phase, Pre-patent | 0.008 | 0.036 | -0.063 | 0.008 | 0.078 |
| Group, Parasitised:Phase, Patent-parasite | **-0.105** | **0.036** | **-0.177** | **-0.105** | **-0.034** |
| Group, Parasitised:Phase, Post-parasite | **-0.122** | **0.039** | **-0.198** | **-0.122** | **-0.046** |
| Group, Mixed:Phase, Pre-patent | **-0.1** | **0.035** | **-0.169** | **-0.1** | **-0.03** |
| Group, Mixed:Phase, Patent-parasite | 0.004 | 0.036 | -0.067 | 0.004 | 0.076 |
| Group, Mixed:Phase, Post-parasite | -0.048 | 0.039 | -0.125 | -0.048 | 0.028 |
| (Intercept) | 4.074 | 0.129 | 3.821 | 4.075 | 4.326 |
| | | | | DIC = 1212114.24 | |

**Table S3.4.** Effect estimates associated with fixed effects in our models of social contact behaviour (frequency, duration and total duration of contacts) of each treatment group, during each experimental week. Estimates include the posterior mean, standard error and lower and upper 95% credibility intervals. DIC values presented from final model. Estimates are displayed in Appendix C, Figure S3.3. **Bold represent significant estimates (i.e., estimates that did not overlap with zero).**

| Fixed Effect | mean | se | lower | mode | upper |
|---|---|---|---|---|---|
| **Frequency of contacts** | | | | | |
| Group, Parasitised | 0.092 | 0.05 | -0.007 | 0.092 | 0.19 |
| Group, Mixed | 0.058 | 0.05 | -0.041 | 0.058 | 0.156 |
| Week, 2 | 0.009 | 0.019 | -0.028 | 0.009 | 0.047 |
| Week, 3 | 0.042 | 0.018 | 0.006 | 0.042 | 0.078 |
| Week, 4 | -0.162 | 0.02 | -0.202 | -0.162 | -0.123 |
| Week, 5 | -0.007 | 0.019 | -0.045 | -0.007 | 0.031 |
| Week, 6 | -0.14 | 0.02 | -0.18 | -0.14 | -0.1 |
| Week, 7 | -0.035 | 0.02 | -0.073 | -0.035 | 0.004 |
| Week, 8 | -0.069 | 0.02 | -0.109 | -0.069 | -0.029 |
| Week, 9 | -0.138 | 0.02 | -0.178 | -0.138 | -0.098 |
| Group, Parasitised:Week, 2 | -0.034 | 0.028 | -0.089 | -0.034 | 0.02 |
| Group, Parasitised:Week, 3 | **-0.07** | **0.026** | **-0.12** | **-0.07** | **-0.02** |
| Group, Parasitised:Week, 4 | **-0.074** | **0.028** | **-0.129** | **-0.074** | **-0.019** |
| Group, Parasitised:Week, 5 | **-0.081** | **0.028** | **-0.135** | **-0.081** | **-0.027** |
| Group, Parasitised:Week, 6 | **-0.147** | **0.029** | **-0.203** | **-0.147** | **-0.09** |
| Group, Parasitised:Week, 7 | -0.029 | 0.027 | -0.083 | -0.029 | 0.025 |
| Group, Parasitised:Week, 8 | **-0.12** | **0.029** | **-0.178** | **-0.12** | **-0.063** |
| Group, Parasitised:Week, 9 | -0.035 | 0.028 | -0.09 | -0.035 | 0.02 |
| Group, Mixed:Week, 2 | **-0.09** | **0.028** | **-0.145** | **-0.09** | **-0.036** |
| Group, Mixed:Week, 3 | **-0.105** | **0.026** | **-0.155** | **-0.105** | **-0.054** |
| Group, Mixed:Week, 4 | -0.044 | 0.028 | -0.099 | -0.044 | 0.01 |
| Group, Mixed:Week, 5 | -0.009 | 0.027 | -0.062 | -0.009 | 0.045 |
| Group, Mixed:Week, 6 | -0.017 | 0.029 | -0.075 | -0.017 | 0.04 |
| Group, Mixed:Week, 7 | 0.033 | 0.027 | -0.021 | 0.033 | 0.086 |
| Group, Mixed:Week, 8 | **-0.083** | **0.029** | **-0.141** | **-0.083** | **-0.026** |
| Group, Mixed:Week, 9 | -0.048 | 0.029 | -0.105 | -0.048 | 0.008 |
| (Intercept) | 0.889 | 0.062 | 0.767 | 0.889 | 1.011 |
| | | | | | DIC = 442036.74 |
| **Duration of contacts** | | | | | |
| Group, Parasitised | -0.043 | 0.017 | -0.076 | -0.043 | -0.009 |
| Group, Mixed | 0.039 | 0.016 | 0.007 | 0.039 | 0.071 |
| Week, 2 | -0.12 | 0.018 | -0.155 | -0.12 | -0.084 |
| Week, 3 | 0.101 | 0.017 | 0.067 | 0.101 | 0.135 |
| Week, 4 | 0.093 | 0.018 | 0.058 | 0.093 | 0.128 |
| Week, 5 | 0.236 | 0.018 | 0.202 | 0.236 | 0.271 |
| Week, 6 | 0.056 | 0.018 | 0.019 | 0.056 | 0.092 |
| Week, 7 | 0.031 | 0.018 | -0.004 | 0.031 | 0.067 |
| Week, 8 | -0.021 | 0.061 | -0.141 | -0.021 | 0.1 |
| Week, 9 | 0.058 | 0.061 | -0.062 | 0.058 | 0.178 |
| Group, Parasitised:Week, 2 | **0.175** | **0.025** | **0.126** | **0.175** | **0.223** |
| Group, Parasitised:Week, 3 | 0.043 | 0.023 | -0.001 | 0.043 | 0.088 |
| Group, Parasitised:Week, 4 | **0.085** | **0.025** | **0.036** | **0.085** | **0.135** |
| Group, Parasitised:Week, 5 | **0.07** | **0.024** | **0.023** | **0.07** | **0.118** |
| Group, Parasitised:Week, 6 | -0.047 | 0.026 | -0.098 | -0.047 | 0.003 |
| Group, Parasitised:Week, 7 | -0.011 | 0.025 | -0.059 | -0.011 | 0.037 |
| Group, Parasitised:Week, 8 | -0.035 | 0.026 | -0.086 | -0.035 | 0.017 |
| Group, Parasitised:Week, 9 | -0.025 | 0.025 | -0.074 | -0.025 | 0.024 |
| Group, Mixed:Week, 2 | -0.005 | 0.024 | -0.053 | -0.005 | 0.043 |

| | | | | | |
|---|---|---|---|---|---|
| Group, Mixed:Week, 3 | **0.012** | **0.023** | **-0.033** | **0.012** | **0.057** |
| Group, Mixed:Week, 4 | **0.066** | **0.025** | **0.017** | **0.066** | **0.115** |
| Group, Mixed:Week, 5 | 0.036 | 0.024 | -0.011 | 0.036 | 0.084 |
| Group, Mixed:Week, 6 | **-0.065** | **0.026** | **-0.115** | **-0.065** | **-0.014** |
| Group, Mixed:Week, 7 | 0.003 | 0.024 | -0.046 | 0.003 | 0.051 |
| Group, Mixed:Week, 8 | 0.064 | 0.027 | 0.012 | 0.064 | 0.116 |
| Group, Mixed:Week, 9 | -0.024 | 0.026 | -0.074 | -0.024 | 0.027 |
| (Intercept) | 3.23 | 0.08 | 3.072 | 3.23 | 3.387 |
| | | | | DIC = 2680637.43 | |
| **Total duration of contacts** | | | | | |
| Group, Parasitised | -0.016 | 0.03 | -0.075 | -0.016 | 0.043 |
| Group, Mixed | 0.097 | 0.029 | 0.04 | 0.097 | 0.154 |
| Week, 2 | -0.272 | 0.031 | -0.333 | -0.272 | -0.212 |
| Week, 3 | 0.094 | 0.03 | 0.034 | 0.094 | 0.153 |
| Week, 4 | -0.037 | 0.031 | -0.098 | -0.037 | 0.024 |
| Week, 5 | 0.192 | 0.031 | 0.131 | 0.192 | 0.253 |
| Week, 6 | -0.018 | 0.032 | -0.081 | -0.018 | 0.045 |
| Week, 7 | -0.11 | 0.031 | -0.171 | -0.11 | -0.048 |
| Week, 8 | 0.061 | 0.094 | -0.124 | 0.061 | 0.246 |
| Week, 9 | 0.116 | 0.094 | -0.069 | 0.116 | 0.301 |
| Group, Parasitised:Week, 2 | **0.11** | **0.044** | **0.024** | **0.11** | **0.196** |
| Group, Parasitised:Week, 3 | -0.026 | 0.04 | -0.105 | -0.026 | 0.053 |
| Group, Parasitised:Week, 4 | 0.028 | 0.043 | -0.057 | 0.028 | 0.113 |
| Group, Parasitised:Week, 5 | -0.028 | 0.043 | -0.113 | -0.028 | 0.056 |
| Group, Parasitised:Week, 6 | **-0.201** | **0.044** | **-0.287** | **-0.201** | **-0.114** |
| Group, Parasitised:Week, 7 | -0.058 | 0.044 | -0.144 | -0.058 | 0.028 |
| Group, Parasitised:Week, 8 | **-0.151** | **0.046** | **-0.241** | **-0.151** | **-0.061** |
| Group, Parasitised:Week, 9 | -0.066 | 0.043 | -0.151 | -0.066 | 0.019 |
| Group, Mixed:Week, 2 | **-0.114** | **0.043** | **-0.199** | **-0.114** | **-0.029** |
| Group, Mixed:Week, 3 | **-0.113** | **0.04** | **-0.192** | **-0.113** | **-0.034** |
| Group, Mixed:Week, 4 | 0.017 | 0.043 | -0.066 | 0.017 | 0.101 |
| Group, Mixed:Week, 5 | 0.023 | 0.043 | -0.061 | 0.023 | 0.107 |
| Group, Mixed:Week, 6 | **-0.094** | **0.045** | **-0.182** | **-0.094** | **-0.006** |
| Group, Mixed:Week, 7 | 0.041 | 0.044 | -0.045 | 0.041 | 0.127 |
| Group, Mixed:Week, 8 | -0.021 | 0.046 | -0.111 | -0.021 | 0.069 |
| Group, Mixed:Week, 9 | -0.08 | 0.044 | -0.167 | -0.08 | 0.007 |
| (Intercept) | 4.081 | 0.129 | 3.826 | 4.082 | 4.335 |
| | | | | DIC = 1211285.71 | |

**Table S3.5.** Effect estimates associated with fixed effects in our models of social contact behaviour (frequency, duration and total duration of contacts) of lambs in the **mixed-parasitic state groups,** during each experimental phase. Estimates include the posterior mean, standard error and lower and upper 95% credibility intervals. DIC values presented from final model. Estimates are displayed in Appendix C, Figure S3.4. **Bold represent significant estimates (i.e., estimates that did not overlap with zero).**

| Fixed Effect | mean | se | lower | mode | upper |
|---|---|---|---|---|---|
| **Frequency of contacts** | | | | | |
| Contact type, Infected - Infected | 0.068 | 0.08 | -0.09 | 0.068 | 0.226 |
| Contact type, Non-infected - Infected | 0.05 | 0.042 | -0.033 | 0.05 | 0.132 |
| Phase, Pre-patent | -0.025 | 0.03 | -0.083 | -0.025 | 0.034 |
| Phase, Patent-parasite | 0.02 | 0.03 | -0.039 | 0.02 | 0.078 |
| Phase, Post-parasite | -0.048 | 0.033 | -0.112 | -0.048 | 0.016 |
| Contact type, Infected - Infected:Phase, Pre-patent | **-0.231** | **0.061** | **-0.35** | **-0.231** | **-0.112** |
| Contact type, Infected - Infected:Phase, Patent-parasite | **-0.117** | **0.06** | **-0.235** | **-0.117** | **0** |
| Contact type, Infected - Infected:Phase, Post-parasite | **-0.328** | **0.07** | **-0.464** | **-0.328** | **-0.192** |
| Contact type, Non-infected - Infected:Phase, Pre-patent | -0.032 | 0.034 | -0.098 | -0.032 | 0.034 |
| Contact type, Non-infected - Infected:Phase, Patent-parasite | -0.046 | 0.034 | -0.112 | -0.046 | 0.02 |
| Contact type, Non-infected - Infected:Phase, Post-parasite | **-0.1** | **0.038** | **-0.174** | **-0.1** | **-0.026** |
| (Intercept) | 0.812 | 0.08 | 0.653 | 0.813 | 0.969 |
| | | | | DIC = 144682.48 | |
| **Duration of contacts** | | | | | |
| Contact type, Infected - Infected | -0.024 | 0.096 | -0.214 | -0.024 | 0.167 |
| Contact type, Non-infected - Infected | -0.004 | 0.049 | -0.102 | -0.004 | 0.094 |
| Phase, Pre-patent | -0.01 | 0.027 | -0.063 | -0.01 | 0.042 |
| Phase, Patent-parasite | 0.038 | 0.026 | -0.014 | 0.038 | 0.09 |
| Phase, Post-parasite | 0.001 | 0.029 | -0.056 | 0.001 | 0.058 |
| Contact type, Infected - Infected:Phase, Pre-patent | 0 | 0.03 | -0.059 | 0 | 0.059 |
| Contact type, Infected - Infected:Phase, Patent-parasite | **0.106** | **0.03** | **0.048** | **0.106** | **0.164** |
| Contact type, Infected - Infected:Phase, Post-parasite | 0.055 | 0.034 | -0.011 | 0.055 | 0.121 |
| Contact type, Non-infected - Infected:Phase, Pre-patent | -0.053 | 0.054 | -0.159 | -0.053 | 0.053 |
| Contact type, Non-infected-Infected:Phase, Patent-parasite | **0.133** | **0.053** | **0.028** | **0.133** | **0.237** |
| Contact type, Non-infected - Infected:Phase, Post-parasite | -0.108 | 0.063 | -0.231 | -0.108 | 0.016 |
| (Intercept) | 3.34 | 0.086 | 3.171 | 3.34 | 3.508 |
| | | | | DIC = 881057.56 | |
| **Total duration of contacts** | | | | | |
| Contact type, Infected - Infected | -0.019 | 0.156 | -0.328 | -0.02 | 0.289 |
| Contact type, Non-infected - Infected | 0.039 | 0.081 | -0.121 | 0.039 | 0.198 |
| Phase, Pre-patent | -0.035 | 0.047 | -0.127 | -0.034 | 0.057 |
| Phase, Patent-parasite | 0.053 | 0.047 | -0.04 | 0.053 | 0.145 |
| Phase, Post-parasite | -0.085 | 0.051 | -0.186 | -0.085 | 0.014 |
| Contact type, Infected - Infected:Phase, Pre-patent | -0.215 | 0.094 | -0.401 | -0.214 | -0.03 |
| Contact type, Infected - Infected:Phase, Patent-parasite | 0.059 | 0.095 | -0.127 | 0.06 | 0.244 |
| Contact type, Infected - Infected:Phase, Post-parasite | -0.361 | 0.106 | -0.57 | -0.36 | -0.153 |
| Contact type, Non-infected - Infected:Phase, Pre-patent | -0.031 | 0.053 | -0.135 | -0.031 | 0.074 |
| Contact type, Non-infected - Infected:Phase, Patent-parasite | 0.064 | 0.053 | -0.041 | 0.063 | 0.169 |
| Contact type, Non-infected - Infected:Phase, Post-parasite | -0.015 | 0.059 | -0.131 | -0.015 | 0.101 |
| (Intercept) | 4.177 | 0.14 | 3.9 | 4.178 | 4.453 |
| | | | | DIC = 398092.26 | |

**Table S3.6.** Effect estimates associated with fixed effects in our models of social contact behaviour (frequency, duration and total duration of contacts) of lambs in the **mixed-parasitic state groups,** during each experimental week. Estimates include the posterior mean, standard error and lower and upper 95% credibility intervals. DIC values presented from final model. Estimates are displayed in Appendix C, Figure S3.5. **Bold represent significant estimates (i.e., estimates that did not overlap with zero).**

| Fixed Effect | mean | se | lower | mode | upper |
|---|---|---|---|---|---|
| **Frequency of contacts** | | | | | |
| Contact type, Infected - Infected | 0.042 | 0.039 | -0.034 | 0.042 | 0.117 |
| Contact type, Non-infected - Infected | 0.017 | 0.035 | -0.052 | 0.017 | 0.086 |
| Week, 2 | -0.116 | 0.036 | -0.186 | -0.116 | -0.046 |
| Week, 3 | 0 | 0.036 | -0.071 | 0 | 0.07 |
| Week, 4 | -0.128 | 0.04 | -0.206 | -0.128 | -0.05 |
| Week, 5 | 0.146 | 0.036 | 0.076 | 0.146 | 0.216 |
| Week, 6 | -0.045 | 0.039 | -0.122 | -0.045 | 0.032 |
| Week, 7 | -0.022 | 0.037 | -0.096 | -0.022 | 0.051 |
| Week, 8 | 0.077 | 0.084 | -0.09 | 0.077 | 0.243 |
| Week, 9 | 0.053 | 0.044 | -0.034 | 0.053 | 0.14 |
| Contact type, Infected - Infected:Week, 2 | -0.14 | 0.075 | -0.288 | -0.14 | 0.007 |
| Contact type, Infected - Infected:Week, 3 | **-0.268** | **0.071** | **-0.407** | **-0.268** | **-0.128** |
| Contact type, Infected - Infected:Week, 4 | **-0.31** | **0.077** | **-0.461** | **-0.31** | **-0.16** |
| Contact type, Infected - Infected:Week, 5 | -0.027 | 0.069 | -0.163 | -0.027 | 0.109 |
| Contact type, Infected - Infected:Week, 6 | -0.071 | 0.086 | -0.24 | -0.071 | 0.099 |
| Contact type, Infected - Infected:Week, 7 | **-0.276** | **0.07** | **-0.413** | **-0.276** | **-0.139** |
| Contact type, Infected - Infected:Week, 8 | **-0.329** | **0.079** | **-0.483** | **-0.329** | **-0.175** |
| Contact type, Infected - Infected:Week, 9 | **-0.361** | **0.089** | **-0.537** | **-0.361** | **-0.187** |
| Contact type, Non-infected - Infected:Week, 2 | -0.031 | 0.043 | -0.115 | -0.031 | 0.053 |
| Contact type, Non-infected - Infected:Week, 3 | -0.025 | 0.041 | -0.105 | -0.025 | 0.055 |
| Contact type, Non-infected - Infected:Week, 4 | -0.05 | 0.041 | -0.131 | -0.05 | 0.03 |
| Contact type, Non-infected – Infected:Week, 5 | 0.004 | 0.04 | -0.075 | 0.004 | 0.083 |
| Contact type, Non-infected – Infected:Week, 6 | 0.048 | 0.047 | -0.044 | 0.048 | 0.139 |
| Contact type, Non-infected - Infected:Week, 7 | **-0.163** | **0.04** | **-0.241** | **-0.163** | **-0.084** |
| Contact type, Non-infected - Infected:Week, 8 | -0.081 | 0.044 | -0.168 | -0.081 | 0.005 |
| Contact type, Non-infected - Infected:Week, 9 | **-0.139** | **0.045** | **-0.227** | **-0.139** | **-0.051** |
| (Intercept) | 0.859 | 0.074 | 0.713 | 0.859 | 1.005 |
| | | | | DIC = 144528.22 | |
| **Duration of contacts** | | | | | |
| Contact type, Infected - Infected | -0.015 | 0.101 | -0.215 | -0.015 | 0.185 |
| Contact type, Non-infected - Infected | -0.003 | 0.052 | -0.105 | -0.003 | 0.1 |
| Week, 2 | -0.029 | 0.035 | -0.097 | -0.029 | 0.039 |
| Week, 3 | 0.064 | 0.032 | 0.002 | 0.064 | 0.127 |
| Week, 4 | -0.068 | 0.032 | -0.131 | -0.068 | -0.005 |
| Week, 5 | -0.019 | 0.032 | -0.082 | -0.019 | 0.043 |
| Week, 6 | -0.102 | 0.036 | -0.172 | -0.102 | -0.032 |
| Week, 7 | 0.17 | 0.031 | 0.109 | 0.17 | 0.231 |
| Week, 8 | 0.007 | 0.035 | -0.063 | 0.007 | 0.077 |
| Week, 9 | 0.037 | 0.034 | -0.028 | 0.037 | 0.103 |
| Contact type, Infected - Infected:Week, 2 | 0.016 | 0.066 | -0.115 | 0.016 | 0.146 |
| Contact type, Infected - Infected:Week, 3 | -0.109 | 0.064 | -0.235 | -0.109 | 0.016 |
| Contact type, Infected - Infected:Week, 4 | -0.081 | 0.07 | -0.219 | -0.081 | 0.057 |
| Contact type, Infected - Infected:Week, 5 | **0.17** | **0.061** | **0.049** | **0.17** | **0.29** |
| Contact type, Infected - Infected:Week, 6 | **0.203** | **0.078** | **0.051** | **0.202** | **0.355** |
| Contact type, Infected - Infected:Week, 7 | 0.074 | 0.062 | -0.047 | 0.074 | 0.195 |
| Contact type, Infected - Infected:Week, 8 | -0.01 | 0.071 | -0.15 | -0.01 | 0.13 |
| Contact type, Infected - Infected:Week, 9 | **-0.374** | **0.082** | **-0.534** | **-0.374** | **-0.212** |
| Contact type, Non-infected - Infected:Week, 2 | 0.009 | 0.038 | -0.066 | 0.009 | 0.085 |
| Contact type, Non-infected - Infected:Week, 3 | -0.021 | 0.037 | -0.093 | -0.021 | 0.051 |
| Contact type, Non-infected - Infected:Week, 4 | 0.024 | 0.037 | -0.049 | 0.024 | 0.096 |
| Contact type, Non-infected – Infected:Week, 5 | **0.188** | **0.035** | **0.118** | **0.188** | **0.257** |

| | | | | | |
|---|---|---|---|---|---|
| Contact type, Non-infected - Infected:Week, 6 | 0.164 | 0.042 | 0.082 | 0.164 | **0.246** |
| Contact type, Non-infected - Infected:Week, 7 | 0.022 | 0.035 | -0.046 | 0.022 | 0.091 |
| Contact type, Non-infected - Infected:Week, 8 | **0.174** | **0.039** | **0.096** | **0.174** | **0.251** |
| Contact type, Non-infected - Infected:Week, 9 | **-0.128** | **0.04** | **-0.207** | **-0.128** | **-0.049** |
| (Intercept) | 3.332 | 0.088 | 3.159 | 3.332 | 3.505 |
| | | | | DIC = 880800.11 | |
| **Total duration of contacts** | | | | | |
| Contact type, Infected - Infected | 0.006 | 0.157 | -0.303 | 0.006 | 0.316 |
| Contact type, Non-infected - Infected | 0.048 | 0.081 | -0.112 | 0.048 | 0.208 |
| Week, 2 | 0.016 | 0.061 | -0.103 | 0.016 | 0.135 |
| Week, 3 | 0.071 | 0.056 | -0.038 | 0.071 | 0.18 |
| Week, 4 | -0.178 | 0.056 | -0.287 | -0.178 | -0.069 |
| Week, 5 | 0.02 | 0.057 | -0.091 | 0.02 | 0.132 |
| Week, 6 | -0.253 | 0.061 | -0.372 | -0.253 | -0.134 |
| Week, 7 | 0.294 | 0.057 | 0.183 | 0.294 | 0.405 |
| Week, 8 | -0.101 | 0.062 | -0.222 | -0.101 | 0.02 |
| Week, 9 | 0.012 | 0.059 | -0.103 | 0.012 | 0.127 |
| Contact type, Infected - Infected:Week, 2 | -0.06 | 0.117 | -0.291 | -0.06 | 0.17 |
| Contact type, Infected - Infected:Week, 3 | **-0.287** | **0.11** | **-0.503** | **-0.287** | **-0.072** |
| Contact type, Infected - Infected:Week, 4 | **-0.322** | **0.115** | **-0.549** | **-0.322** | **-0.096** |
| Contact type, Infected - Infected:Week, 5 | 0.168 | 0.11 | -0.048 | 0.168 | 0.383 |
| Contact type, Infected - Infected:Week, 6 | 0.228 | 0.131 | -0.028 | 0.227 | 0.485 |
| Contact type, Infected - Infected:Week, 7 | -0.182 | 0.111 | -0.401 | -0.182 | 0.036 |
| Contact type, Infected - Infected:Week, 8 | **-0.244** | **0.119** | **-0.478** | **-0.244** | **-0.01** |
| Contact type, Infected - Infected:Week, 9 | **-0.677** | **0.132** | **-0.936** | **-0.678** | **-0.417** |
| Contact type, Non-infected - Infected:Week, 2 | -0.02 | 0.067 | -0.153 | -0.02 | 0.112 |
| Contact type, Non-infected - Infected:Week, 3 | -0.037 | 0.064 | -0.163 | -0.037 | 0.089 |
| Contact type, Non-infected - Infected:Week, 4 | -0.035 | 0.064 | -0.161 | -0.035 | 0.091 |
| Contact type, Non-infected – Infected:Week, 5 | **0.151** | **0.064** | **0.026** | **0.151** | **0.275** |
| Contact type, Non-infected – Infected:Week, 6 | **0.221** | **0.072** | **0.08** | **0.221** | **0.361** |
| Contact type, Non-infected - Infected:Week, 7 | -0.114 | 0.064 | -0.24 | -0.114 | 0.012 |
| Contact type, Non-infected - Infected:Week, 8 | **0.149** | **0.069** | **0.014** | **0.149** | **0.284** |
| Contact type, Non-infected - Infected:Week, 9 | **-0.274** | **0.07** | **-0.412** | **-0.274** | **-0.136** |
| (Intercept) | 4.157 | 0.141 | 3.878 | 4.158 | 4.435 |
| | | | | DIC = 397836.83 | |

**Table S3.7.** Model estimates for fixed effects of linear mixed models on closeness (calculated using duration of contacts) of individuals in the mixed treatment group **(1)** during each phase of the experiment. AIC values presented from final model. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| **Mixed group 1** | | | | |
| Individual parasitic status, Animal.2 Non-infected | -0.28 | 0.11 | -2.57 | 0.011 |
| Individual parasitic status, Animal.3 Infected | -0.33 | 0.11 | -2.97 | 0.003 |
| Individual parasitic status, Animal.4 Infected | -0.18 | 0.11 | -1.69 | 0.093 |
| Individual parasitic status, Animal.5 Non-infected | -0.40 | 0.11 | -3.62 | 0.000 |
| Pre-patent | -0.46 | 0.14 | -3.25 | 0.001 |
| Patent-parasite | -0.11 | 0.13 | -0.89 | 0.374 |
| Post-parasite | -0.63 | 0.15 | -4.37 | <0.001 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Pre-patent | 0.21 | 0.13 | 1.66 | 0.098 |
| Individual parasitic status, Animal.3 Infected:Phase, Pre-patent | 0.21 | 0.13 | 1.66 | 0.099 |
| Individual parasitic status, Animal.4 Infected:Phase , Pre-patent | 0.23 | 0.13 | 1.84 | 0.068 |
| Individual parasitic status, Animal.5 Non-infected:Phase, Pre-patent | 0.32 | 0.13 | 2.50 | 0.013 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Patent-parasite | **0.43** | **0.13** | **3.43** | **0.001** |
| Individual parasitic status, Animal.3 Infected:Phase, Patent-parasite | **0.25** | **0.13** | **1.94** | **0.053** |
| Individual parasitic status, Animal.4 Infected:Phase, Patent-parasite | 0.13 | 0.13 | 0.99 | 0.323 |
| Individual parasitic status, Animal.5 Non-infected:Phase, Patent-parasite | **0.40** | **0.13** | **3.16** | **0.002** |
| Individual parasitic status, Animal.2 Non-infected:Phase, Post-parasite | 0.24 | 0.13 | 1.78 | 0.077 |
| Individual parasitic status, Animal.3 Infected:Phase, Post-parasite | **0.31** | **0.13** | **2.34** | **0.020** |
| Individual parasitic status, Animal.4 Infected:Phase, Post-parasite | 0.17 | 0.13 | 1.28 | 0.203 |
| Individual parasitic status, Animal.5 Non-infected:Phase, Post-parasite | **0.33** | **0.13** | **2.47** | **0.014** |
| (Intercept) | -4.33 | 0.19 | -23.06 | <0.001 |
| | | | AIC = 145.0867 | |

**Table S3.8.** Model estimates for fixed effects of linear mixed models on closeness (calculated using duration of contacts) of individuals in the mixed treatment group **(2)** during each phase of the experiment. AIC values presented from final model. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| **Mixed group 2** | | | | |
| Individual parasitic status, Animal.2 Non-infected | 0.18 | 0.11 | 1.65 | 0.101 |
| Individual parasitic status, Animal.3 Infected | 0.30 | 0.12 | 2.46 | 0.015 |
| Individual parasitic status, Animal.4 Infected | 0.36 | 0.11 | 3.29 | 0.001 |
| Individual parasitic status, Animal.5 Non-infected | 0.46 | 0.11 | 4.21 | 0.000 |
| Pre-patent | 0.14 | 0.14 | 0.95 | 0.341 |
| Patent-parasite | 0.56 | 0.13 | 4.41 | 0.000 |
| Post-parasite | 0.02 | 0.14 | 0.12 | 0.905 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Pre-patent | -0.26 | 0.13 | -2.05 | 0.041 |
| Individual parasitic status, Animal.3 Infected:Phase, Pre-patent | -0.39 | 0.14 | -2.81 | 0.005 |
| Individual parasitic status, Animal.4 Infected:Phase, Pre-patent | -0.38 | 0.13 | -2.96 | 0.003 |
| Individual parasitic status, Animal.5 Non-infected:Phase, Pre-patent | -0.43 | 0.13 | -3.40 | 0.001 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Patent-parasite | 0.04 | 0.13 | 0.35 | 0.727 |
| Individual parasitic status, Animal.3 Infected:Phase, Patent-parasite | **-0.25** | **0.14** | **-1.80** | **0.074** |
| Individual parasitic status, Animal.4 Infected:Phase, Patent-parasite | **-0.23** | **0.13** | **-1.78** | **0.076** |
| Individual parasitic status, Animal.5 Non-infected:Phase, Patent-parasite | -0.17 | 0.13 | -1.31 | 0.192 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Post-parasite | -0.07 | 0.13 | -0.51 | 0.609 |
| Individual parasitic status, Animal.3 Infected:Phase, Post-parasite | -0.14 | 0.15 | -0.94 | 0.349 |
| Individual parasitic status, Animal.4 Infected:Phase, Post-parasite | **-0.26** | **0.13** | **-1.91** | **0.057** |
| Individual parasitic status, Animal.5 Non-infected:Phase, Post-parasite | **-0.29** | **0.14** | **-2.11** | **0.036** |
| (Intercept) | -4.68 | 0.15 | -30.39 | <0.001 |
| | | | AIC = 139.471 | |

**Table S3.9.** Model estimates for fixed effects of linear mixed models on closeness (calculated using duration of contacts) of individuals in the mixed treatment group **(3)** during each phase of the experiment. AIC values presented from final model. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| **Mixed group 3** | | | | |
| Individual parasitic status, Animal.2 Non-infected | 0.03 | 0.11 | 0.23 | 0.822 |
| Individual parasitic status, Animal.3 Infected | 0.31 | 0.11 | 2.69 | 0.008 |
| Individual parasitic status, Animal.4 Infected | 0.37 | 0.11 | 3.22 | 0.001 |
| Individual parasitic status, Animal.5 Non-infected | 0.21 | 0.11 | 1.84 | 0.066 |
| Pre-patent | 0.26 | 0.15 | 1.79 | 0.074 |
| Patent-parasite | 0.58 | 0.13 | 4.44 | 0.000 |
| Post-parasite | 0.34 | 0.15 | 2.28 | 0.023 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Pre-patent | 0.08 | 0.13 | 0.63 | 0.527 |
| Individual parasitic status, Animal.3 Infected:Phase, Pre-patent | **-0.39** | **0.13** | **-2.98** | **0.003** |
| Individual parasitic status, Animal.4 Infected:Phase, Pre-patent | -0.22 | 0.13 | -1.69 | 0.093 |
| Individual parasitic status, Animal.5 Non-infected:Phase, Pre-patent | -0.16 | 0.13 | -1.24 | 0.215 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Patent-parasite | -0.19 | 0.13 | -1.45 | 0.148 |
| Individual parasitic status, Animal.3 Infected:Phase, Patent-parasite | **-0.33** | **0.13** | **-2.54** | **0.012** |
| Individual parasitic status, Animal.4 Infected:Phase, Patent-parasite | **-0.48** | **0.13** | **-3.63** | **<0.001** |
| Individual parasitic status, Animal.5 Non-infected:Phase, Patent-parasite | -0.16 | 0.13 | -1.22 | 0.224 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Post-parasite | -0.09 | 0.14 | -0.67 | 0.505 |
| Individual parasitic status, Animal.3 Infected:Phase, Post-parasite | -0.32 | 0.14 | -2.29 | 0.023 |
| Individual parasitic status, Animal.4 Infected:Phase, Post-parasite | **-0.64** | **0.14** | **-4.59** | **<0.001** |
| Individual parasitic status, Animal.5 Non-infected:Phase, Post-parasite | -0.18 | 0.14 | -1.30 | 0.195 |
| (Intercept) | -4.90 | 0.17 | -28.42 | <0.001 |
| | | | AIC = 163.7092 | |

**Table S3.10.** Model estimates for fixed effects of linear mixed models on closeness (calculated using duration) of individuals in the mixed treatment group **(4)** during each phase of the experiment. AIC values presented from final model. **Bold indicates significant results.**

| Fixed effect | Estimate | Std.error | z | p-value |
|---|---|---|---|---|
| **Mixed group 4** | | | | |
| Individual parasitic status, Animal.2 Non-infected | 0.04 | 0.11 | 0.37 | 0.713 |
| Individual parasitic status, Animal.3 Infected | -0.16 | 0.11 | -1.47 | 0.144 |
| Individual parasitic status, Animal.4 Infected | -0.21 | 0.11 | -1.88 | 0.061 |
| Individual parasitic status, Animal.5 Non-infected | -0.16 | 0.11 | -1.41 | 0.159 |
| Pre-patent | -0.03 | 0.14 | -0.19 | 0.850 |
| Patent-parasite | -0.14 | 0.13 | -1.10 | 0.274 |
| Post-parasite | 0.26 | 0.15 | 1.80 | 0.073 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Pre-patent | -0.08 | 0.13 | -0.64 | 0.523 |
| Individual parasitic status, Animal.3 Infected:Phase, Pre-patent | 0.19 | 0.13 | 1.46 | 0.145 |
| Individual parasitic status, Animal.4 Infected:Phase, Pre-patent | 0.18 | 0.13 | 1.41 | 0.162 |
| Individual parasitic status, Animal.5 Non-infected:Phase, Pre-patent | **0.29** | **0.13** | **2.25** | **0.025** |
| Individual parasitic status, Animal.2 Non-infected:Phase, Patent-parasite | 0.14 | 0.13 | 1.12 | 0.264 |
| Individual parasitic status, Animal.3 Infected:Phase, Patent-parasite | 0.21 | 0.13 | 1.69 | 0.092 |
| Individual parasitic status, Animal.4 Infected:Phase, Patent-parasite | 0.16 | 0.13 | 1.29 | 0.197 |
| Individual parasitic status, Animal.5 Non-infected:Phase, Patent-parasite | 0.22 | 0.13 | 1.76 | 0.080 |
| Individual parasitic status, Animal.2 Non-infected:Phase, Post-parasite | -0.10 | 0.13 | -0.76 | 0.446 |
| Individual parasitic status, Animal.3 Infected:Phase, Post-parasite | 0.20 | 0.13 | 1.51 | 0.133 |
| Individual parasitic status, Animal.4 Infected:Phase, Post-parasite | 0.01 | 0.13 | 0.10 | 0.918 |
| Individual parasitic status, Animal.5 Non-infected:Phase, Post-parasite | 0.14 | 0.13 | 1.01 | 0.315 |
| (Intercept) | -4.41 | 0.15 | -28.82 | <0.001 |
| | | | AIC = 148.2655 | |

## 3.1 Additional figures

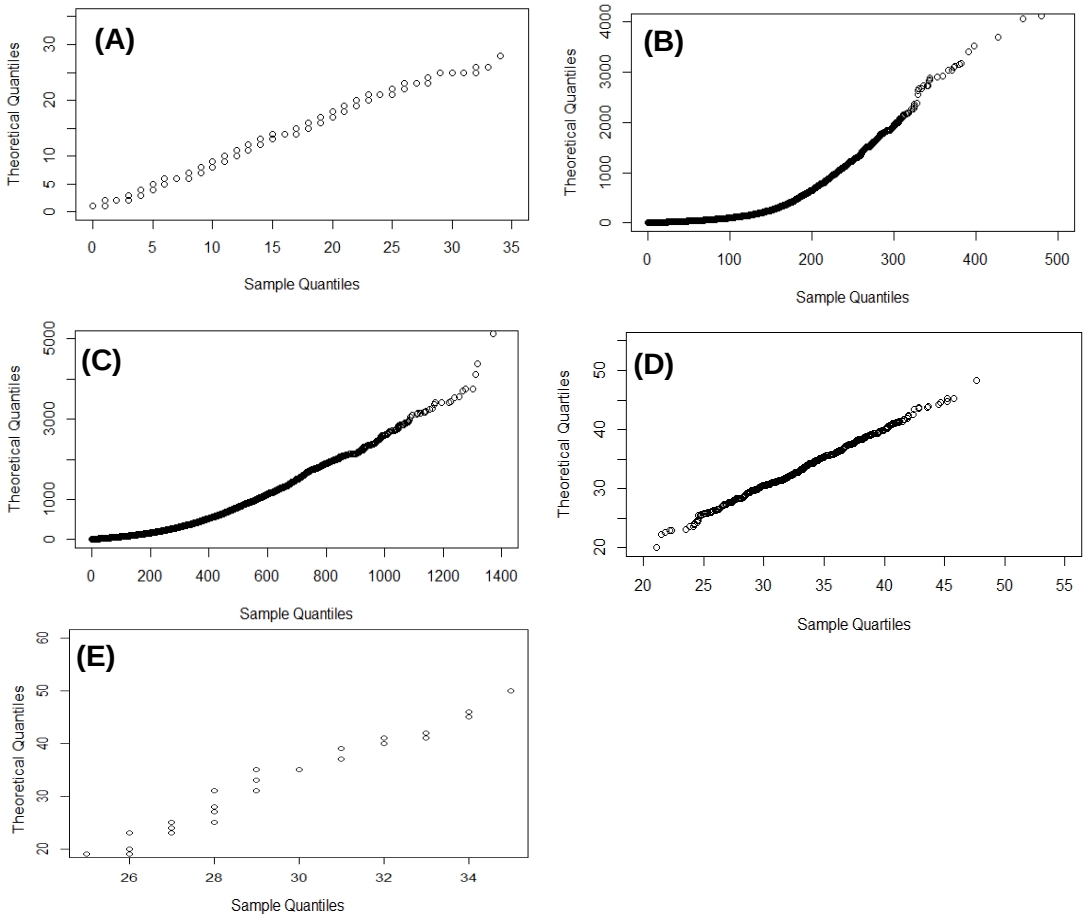


**Figure S3.1**. Quantile-quantile plots showing the empirically observed quantiles of **(A)** frequency of contacts **(B)** duration of contacts and **(C)** total duration of contacts as a function of quantiles expected from a negative binomial distribution, **(D)** weight as a function of quantiles from a Gaussian distribution and **(E)** serum pepsinogen as a function of quantiles from a Poisson distribution.

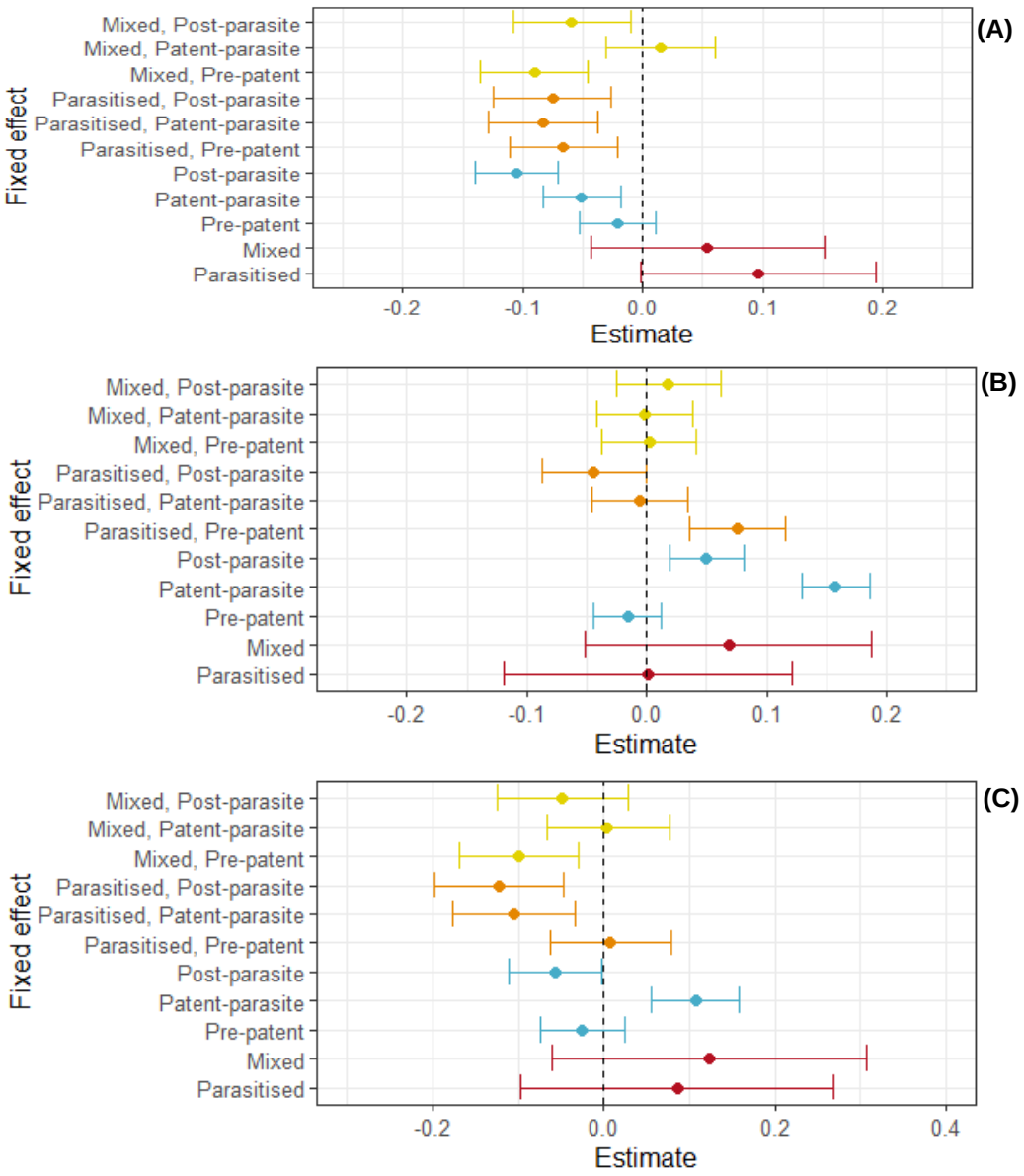


**Figure S3.2**. Comparison of the fixed effect estimates from each response variable model fitted on **all data**. Points denote the mean effect estimate and bars represent the 0.025 and 0.975 quantiles. Plot **(A)** denotes the estimates from the frequency model including Treatment group x Phase interaction effect. Plot **(B)** denotes the estimates from the duration model including Treatment group x Phase interaction effect. Points of different colours denote the results from different levels of the explanatory variables. Plot **(C)** denotes the estimates from the total duration model including Treatment group x Phase interaction effect. Points of different colours denote the results from different levels of the explanatory variables.

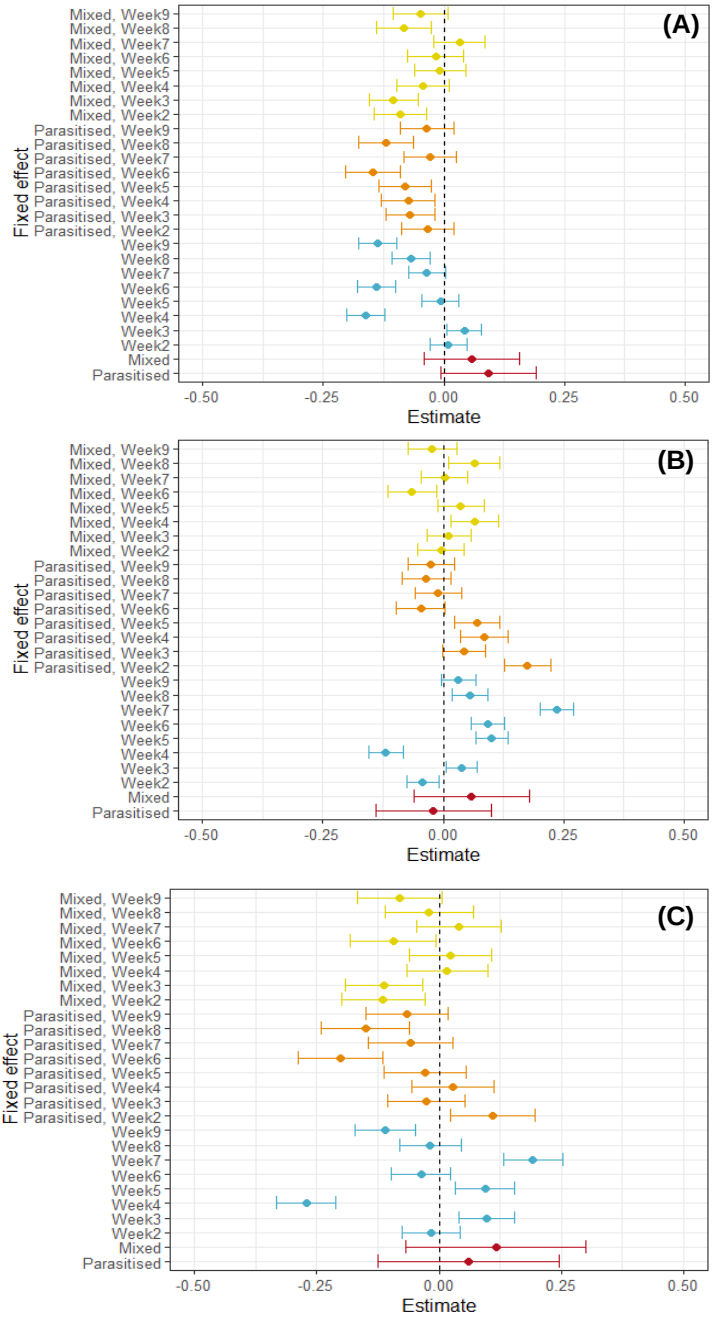


**Figure S3.3**. Comparison of the fixed effect estimates from each response variable model fitted on all data. Points denote the mean effect estimate and bars represent the 0.025 and 0.975 quantiles. Plot **(A)** denotes the estimates from the frequency model including Treatment group x Week interaction effect. Plot **(B)** denotes the estimates from the duration model including Treatment group x Week interaction effect. Points of different colours denote the results from different levels of the explanatory variables. Plot **(C)** denotes the estimates from the total duration model including Treatment group x Week interaction effect. Points of different colours denote the results from different levels of the explanatory variables.

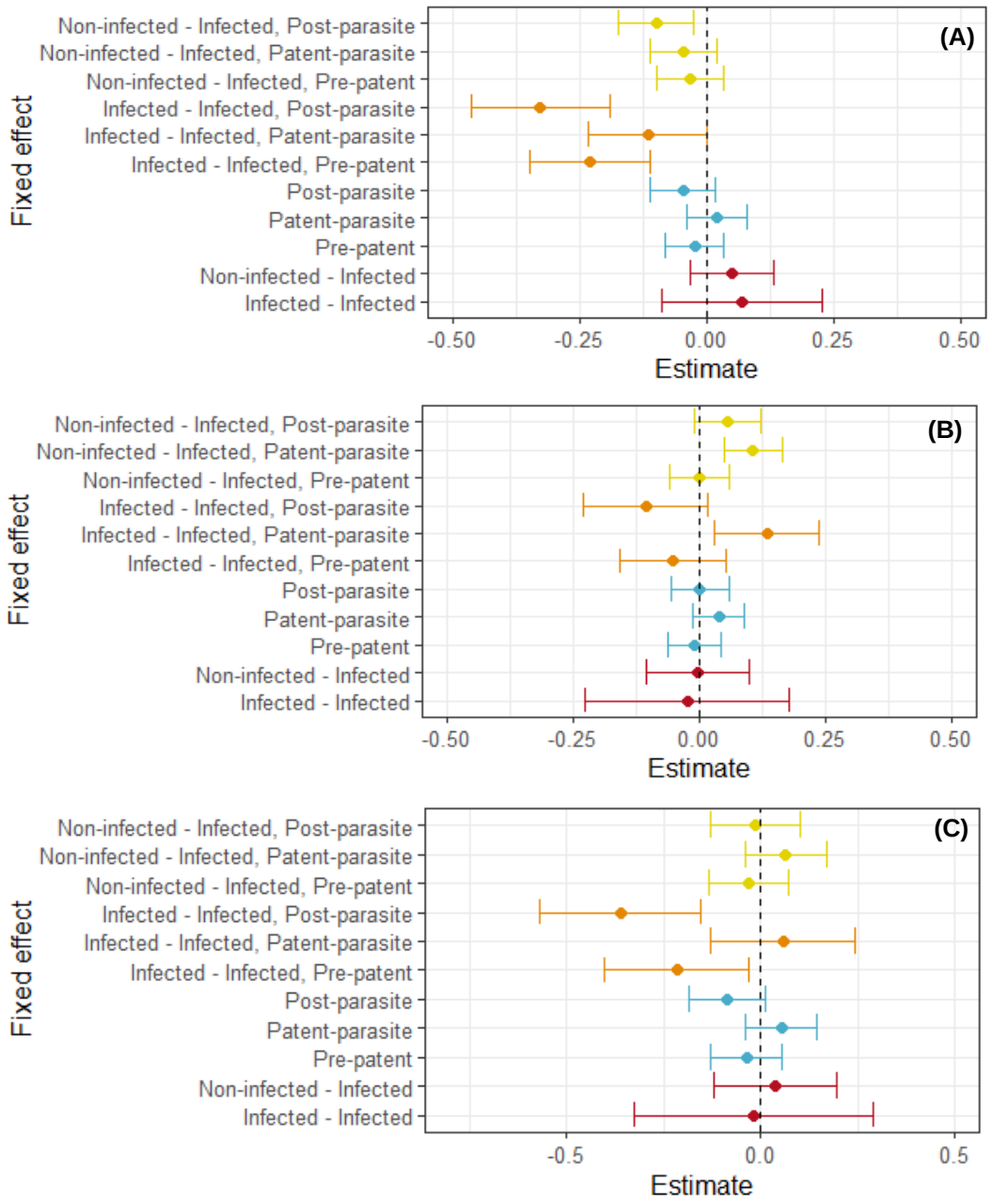


**Figure S3.4**. Comparison of the fixed effect estimates from each response variable model fitted on **mixed group data only**. Points denote the mean effect estimate and bars represent the 0.025 and 0.975 quantiles. Plot **(A)** denotes the estimates from the frequency model including Contact type x Phase interaction effect and Plot **(B)** denotes the estimates from the duration model including Contact type x Phase interaction effect and Plot **(C)** denotes the estimates from the total duration model including Contact type x Phase interaction effect.

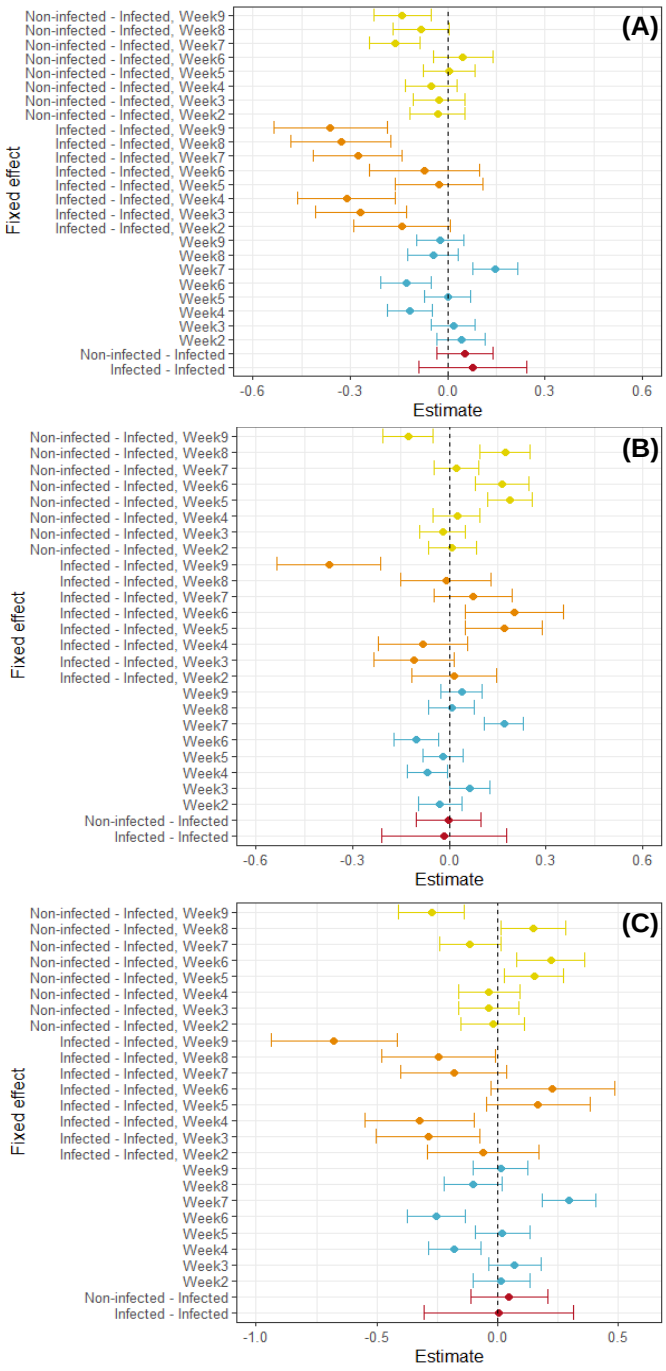


**Figure S3.5**. Comparison of the fixed effect estimates from each response variable model fitted on **mixed group data only**. Points denote the mean effect estimate and bars represent the 0.025 and 0.975 quantiles. Plot **(A)** denotes the estimates from the frequency model including Contact type x Week interaction effect and Plot **(B)** denotes the estimates from the duration model including Contact type x Week interaction effect and Plot **(C)** denotes the estimates from the total duration model including Contact type x Week interaction effect.

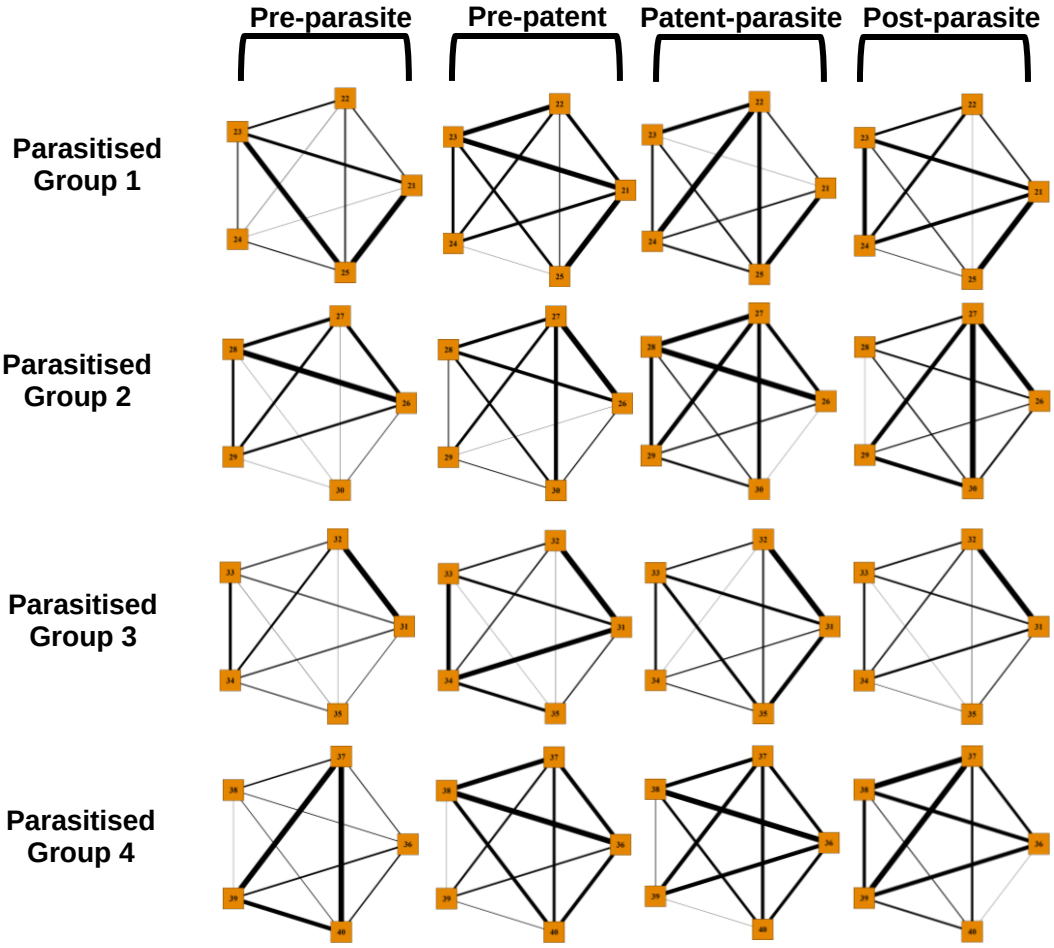


**Figure S3.6**. Social network graphs created using frequency of contacts of lambs in the parasitised treatment groups (*n* = 4) for each phase of the study. Pre-parasite (week 1), Pre-patent (weeks 2-4), Patent-parasite (weeks 5-7) and Post-parasite (weeks 8-9). Orange squares represent the infected individuals in the parasitised groups. Line thickness represents the strength of association between two individuals based on frequency of contacts per phase.

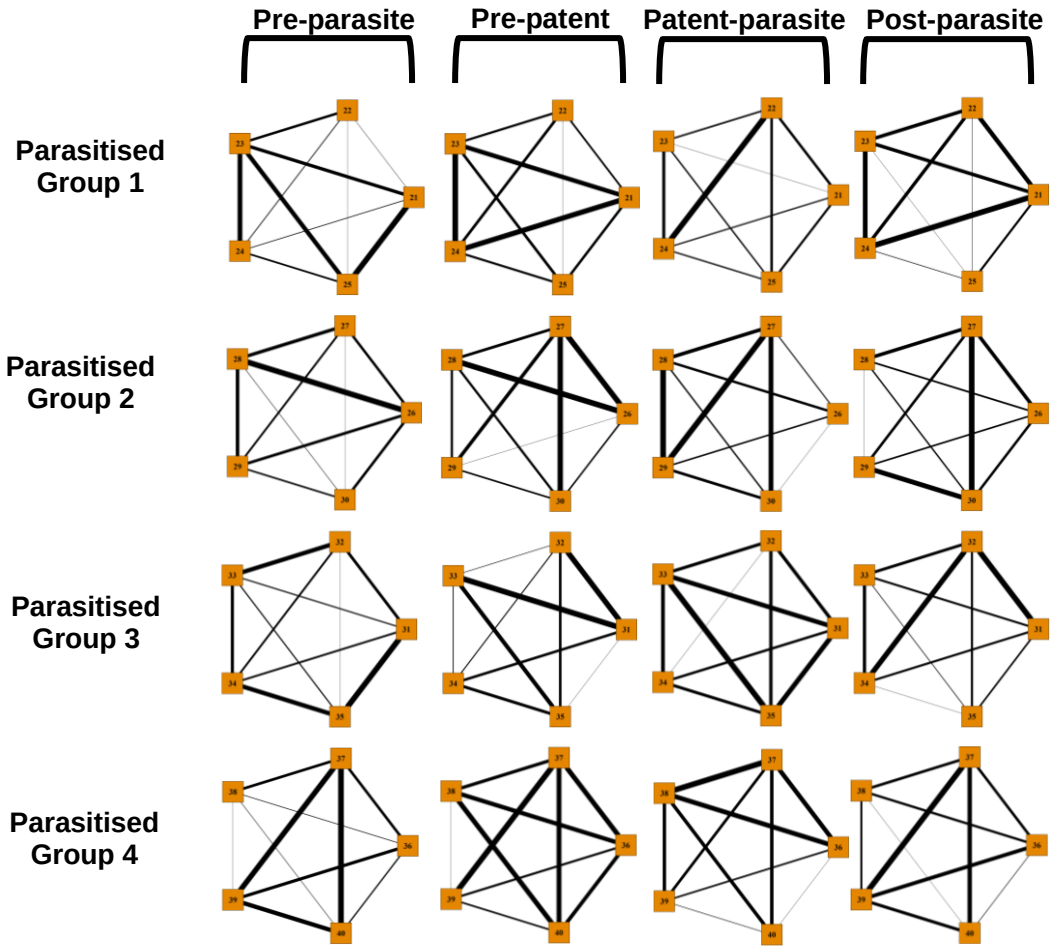


**Figure S3.7.** Social network graphs created using duration of contacts of lambs in the parasitised treatment groups (*n* = 4) for each phase of the study. Pre-parasite (week 1), Pre-patent (weeks 2-4), Patent-parasite (weeks 5-7) and Post-parasite (weeks 8-9). Orange squares represent the infected individuals in the parasitised groups. Line thickness represents the strength of association between two individuals based on frequency of contacts per phase.

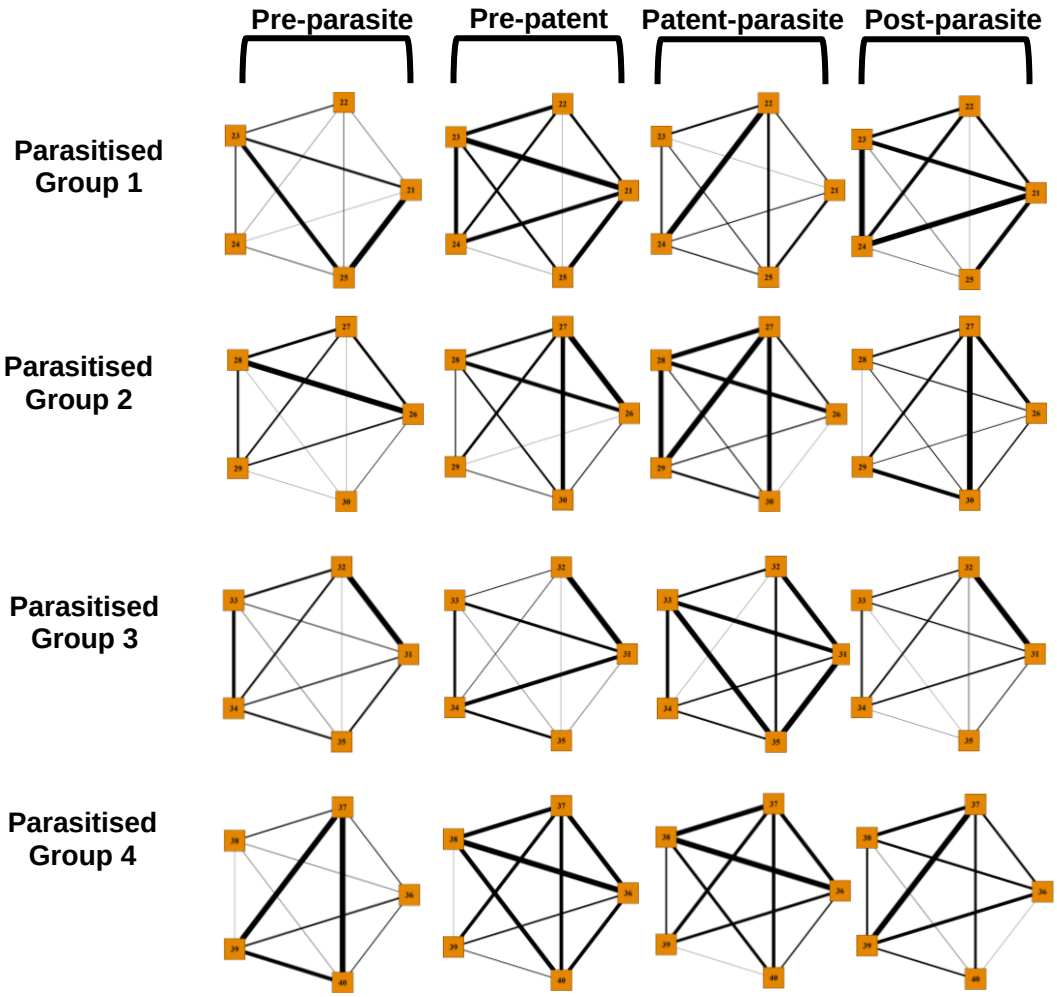


**Figure S3.8.** Social network graphs created using total duration of contacts of lambs in the parasitised treatment groups (*n* = 4) for each phase of the study. Pre-parasite (week 1), Pre-patent (weeks 2-4), Patent-parasite (weeks 5-7) and Post-parasite (weeks 8-9). Orange squares represent the infected individuals in the parasitised groups. Line thickness represents the strength of association between two individuals based on frequency of contacts per phase.

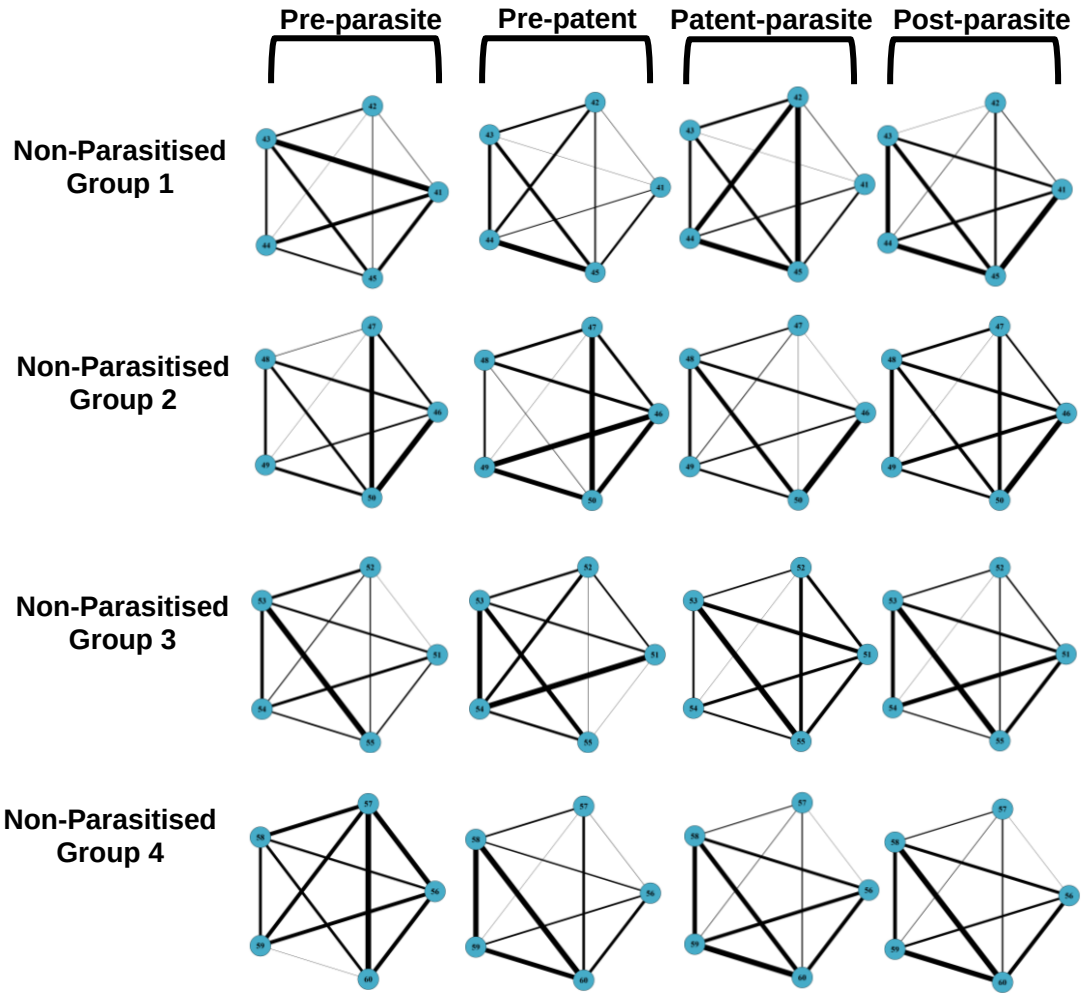


**Figure S3.9**. Social network graphs created using frequency of contacts of lambs in the non-parasitised treatment groups (*n* = 4) for each phase of the study. Pre-parasite (week 1), Pre-patent (weeks 2-4), Patent-parasite (weeks 5-7) and Post-parasite (weeks 8-9). Blue circles represent the non-infected individuals in the non-parasitised groups. Line thickness represents the strength of association between two individuals based on frequency of contacts per phase.

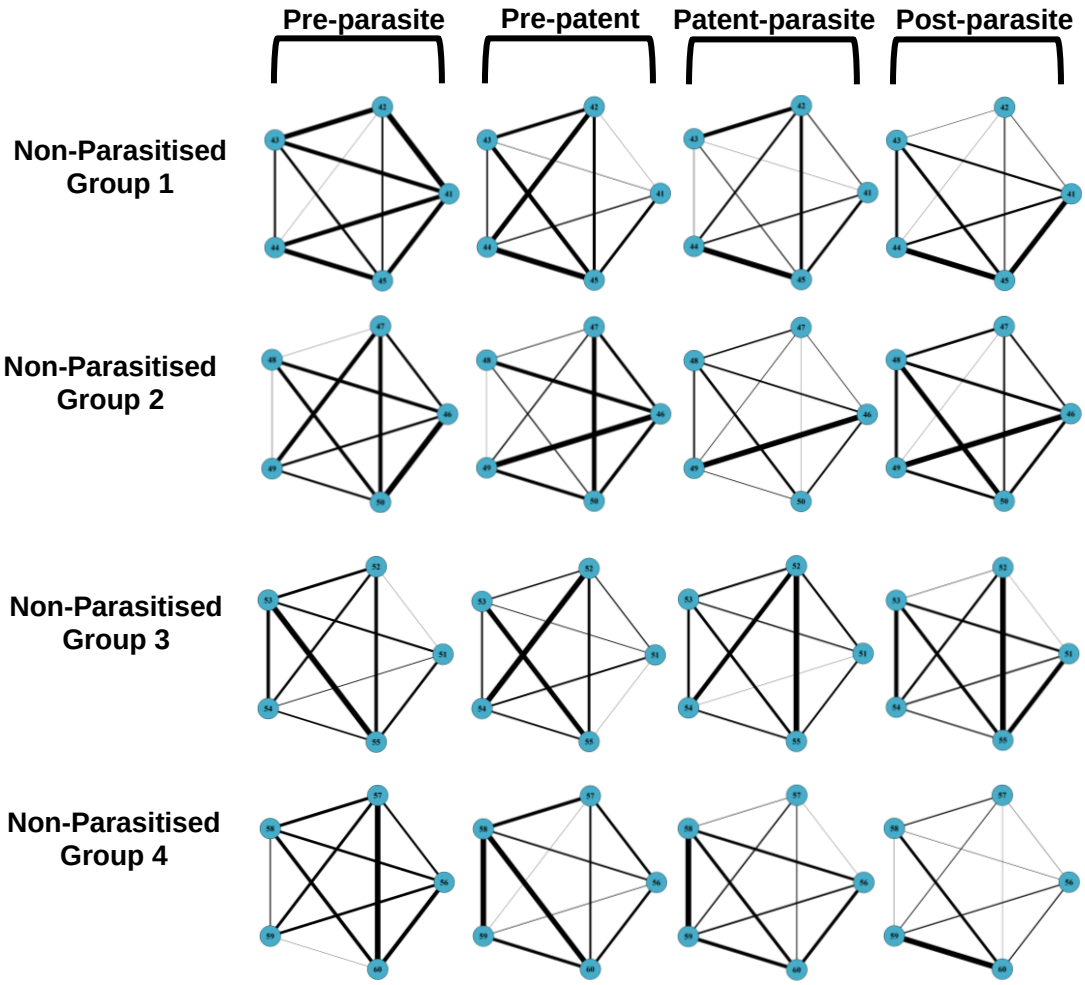


**Figure S3.10**. Social network graphs created using duration of contacts of lambs in the non-parasitised treatment groups (*n* = 4) for each phase of the study. Pre-parasite (week 1), Pre-patent (weeks 2-4), Patent-parasite (weeks 5-7) and Post-parasite (weeks 8-9). Blue circles represent the non-infected individuals in the non-parasitised groups. Line thickness represents the strength of association between two individuals based on frequency of contacts per phase.

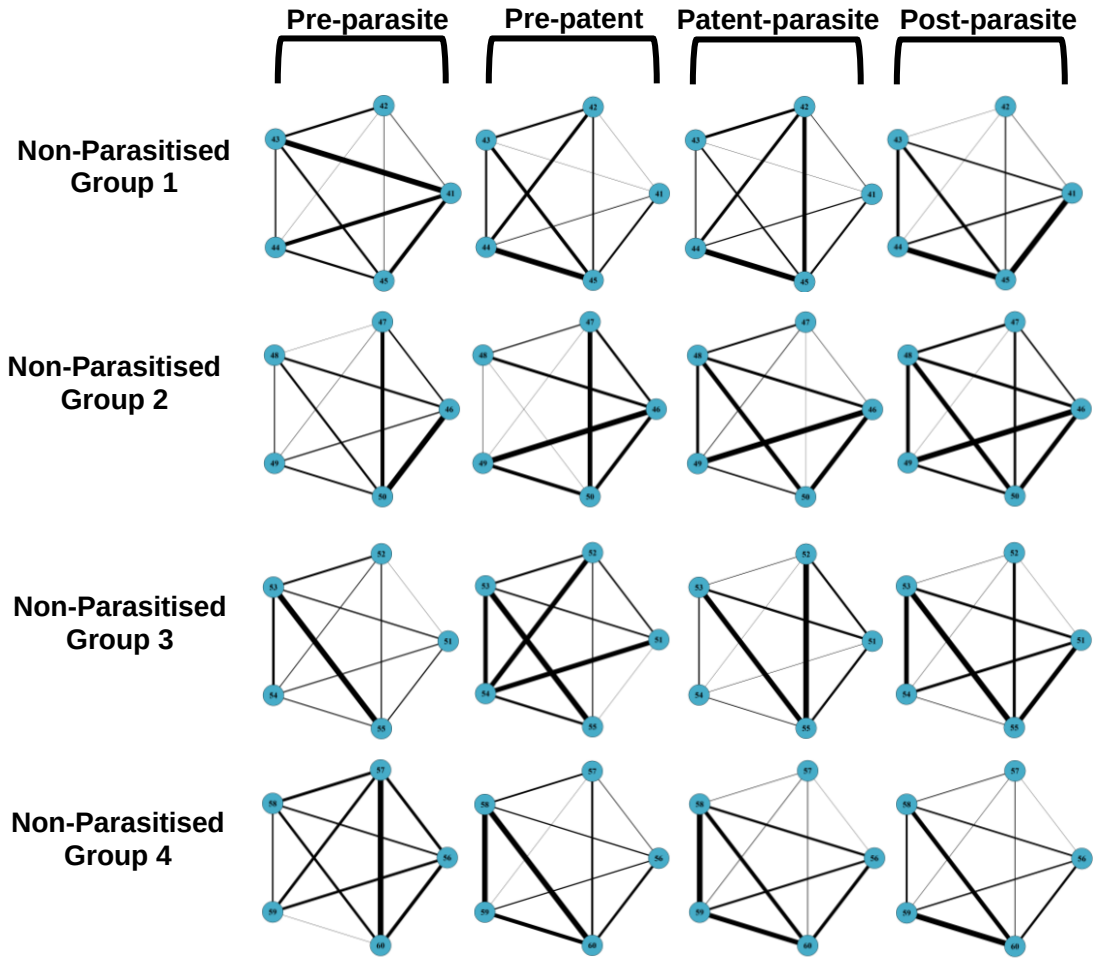


**Figure S3.11**. Social network graphs created using total duration of contacts of lambs in the non-parasitised treatment groups (*n* = 4) for each phase of the study. Pre-parasite (week 1), Pre-patent (weeks 2-4), Patent-parasite (weeks 5-7) and Post-parasite (weeks 8-9). Blue circles represent the non-infected individuals in the non-parasitised groups. Line thickness represents the strength of association between two individuals based on frequency of contacts per phase.

## 3.2 Results continued

**Correlation between activity behaviour (Chapter 3) and social contact behaviour (Chapter 4).**

**Step count**

There was a significant positive correlation between step count and frequency of contacts ($t = 6.46$, $df = 115803$, $p < 0.001$), and a significant negative correlation between step count and duration ($t = -39.48$, $df = 313493$, $p < 0.001$) and total duration of contacts ($t = -27.32$, $df = 119835$, $p < 0.001$).

**Motion index**

There was a significant positive correlation between motion index and frequency of contacts ($t = 15.131$, $df = 115803$, $p < 0.001$), and a significant negative correlation between motion index and duration ($t = -35.052$, $df = 313493$, $p < 0.001$) and total duration of contacts ($t = -22.208$, $df = 119835$, $p < 0.001$).

**Frequency of lying bouts**

There was a significant positive correlation between the frequency of lying bouts and frequency of contacts ($t = 27.46$, $df = 115803$, $p < 0.001$), and a significant positive correlation between frequency of lying bouts and duration ($t = 5.09$, $df = 313493$, $p < 0.001$) and total duration of contacts ($t = 15.52$, $df = 119835$, $p < 0.001$).

**Lying duration**

There was a significant positive correlation between lying duration and frequency of contacts ($t = 36.163$, $df = 115803$, $p < 0.001$), and a significant positive correlation between lying duration and duration ($t = 45.502$, $df = 313493$, $p < 0.001$) and total duration of contacts ($t = 51.182$, $df = 119835$, $p < 0.001$.