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Sensing the worms

Automated behaviour monitoring for detection of parasitism in grazing livestock

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Abstract

Gastrointestinal nematodes (GIN) are common in grazing livestock and is a major cause of impaired health and productivity. Current control practices of GIN infections depend largely on the use of anthelmintic drugs. However, misuse of anthelmintic drugs has led to a widespread development of anthelmintic resistance. Behavioural monitoring has been suggested as a novel method to detect parasite infection in grazing livestock, enabling targeted (selective) treatment, where only infected groups or individual animals within a group are treated. The aim of this thesis was to investigate how multispecies GIN parasite infections affect behavioural patterns in grazing livestock on a group level using different on-animal sensors. The effect of subclinical GIN infection on activity and rumination patterns in first season grazing steers were investigated in contrasting groups during two grazing seasons, using different commercial sensors. The results indicate that untreated steers exposed to a higher GIN level had an increased lying time, lower activity level and affected rumination patterns over time, compared with dewormed steers. To enable the assessment of behavioural responses in lambs, the validity of two sensors for cattle for use in lambs were first evaluated. The effect of subclinical GIN infection on activity in lambs around weaning was then investigated in a replicated grazing trial with treated and untreated groups. The results indicate untreated lambs had a shorter daily lying time over time as well as a lower activity level, compared with dewormed lambs. In conclusion, this thesis supports that behavioural patterns are affected by subclinical GIN infections and the results demonstrate the potential use of automated behavioural observations as a diagnostic tool.

Keywords: activity, behaviour, cattle, gastrointestinal nematodes, sheep, sickness behaviour, parasitism, pasture, sensor, validation.

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Sammanfattning

Mag-tarmnematoder (GIN) är vanliga hos betesdjur och en viktig orsak till nedsatt hälsa och produktivitet. Nuvarande kontrollmetoder av GIN-infektioner bygger främst på användandet av avmaskningsmedel. Dock har överanvändning av avmaskningsmedel lett till en omfattande utveckling av anthelmintikaresistens. Automatiska beteendeobservationer har föreslagits som en möjlig metod för att upptäcka parasitinfektion hos betesdjur. Det skulle möjliggöra en riktad selektiv behandling, där endast infekterade grupper eller enstaka djur inom en grupp behandlas. Syftet med denna avhandling var att undersöka om GIN-infektioner påverkar beteendemönster på gruppnivå hos betesdjur och om avvikelser hos infekterade individer kan registreras med sensorer. Effekten av subkliniska GIN-infektioner på aktivitets- och idisslingsmönster hos förstagångsbetande stutar undersöktes under två betessäsonger med olika kommersiella sensorer. Resultaten indikerar att obehandlade stutar hade en ökad liggtid, en lägre aktivitetsnivå och förändrat idisslingsmönster, under den första tiden av infektionen jämfört med avmaskade. För att möjliggöra automatiska mätningar av beteendeförändringar hos lamm utvärderades validiteten av två sensorer, framtagna för nötkreatur, för att användning på lamm. Effekten av subklinisk GIN-infektion på aktivitet hos lamm kring avvänjning undersöktes därefter i en infektionsstudie. Resultaten indikerar att obehandlade lamm hade en kortare daglig liggtid samt en lägre aktivitetsnivå jämfört med avmaskade. Sammanfattningsvis stödjer resultaten i denna avhandling att beteendemönster påverkas av subkliniska GIN-infektioner. Resultaten visar även på den potentiella användningen av automatiserade beteendeobservationer som ett diagnostiskt verktyg för påvisande av GIN-infektioner.

Keywords: aktivitet, bete, beteende, får, mag-tarmparasiter, nötkreatur, parasitism, sensor, sjukdomsbeteende, validering

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List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Högberg, N.*, Lidfors, L., Hessle, A., Arvidsson Segerkvist, K., Herlin, A., Höglund, J. (2019). Effects of nematode parasitism on activity patterns in first-season grazing cattle. *Veterinary Parasitology: X*, 1, 100011. https://doi.org/10.1016/j.vpoa2019.100011

II. Högberg, N.*, Hessle, A., Lidfors, L., Baltrušis, P., Claerebout, E., Höglund, J. (2021). Subclinical nematode parasitism affects activity and rumination patterns in first-season grazing cattle. *Animal*, in press. https://doi.org/10.1016/j.animal.2021.100237

III. Högberg, N.*, Höglund, J., Carlsson, A., Saint-Jeveint, M., Lidfors, L. (2020). Validation of accelerometers to automatically record postures and number of steps in growing lambs. *Applied Animal Behaviour Science*, 229, 105014. https://doi.org/10.1016/j.applanim.2020.105014

IV. Högberg, N.*, Hessle, A., Lidfors, L., Enweji, N., Höglund, J. (2021). Nematode parasitism affects lying time and overall activity patterns in lambs following pasture exposure around weaning. Submitted manuscript.

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The contribution of Niclas Högberg to the papers included in this thesis was as follows:

I. Performed all data cleaning and analysis. Drafted the manuscript and finalised it together with the co-authors. Corresponded with the journal.

II. Actively participated in the planning of the study and data collection. Performed all data cleaning and analysis. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

III. Actively involved in formulating the research idea. Actively participated in the planning of the study and data collection. Performed all data cleaning and analysis. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

IV. Actively participated in the planning of the study and data collection. Performed all data cleaning and analysis. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

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Abbreviations

AR	Anthelmintic resistance
BWG	Body weight gain
CI	Confidence interval
ddPCR	Droplet digital PCR
DWG	Daily weight gain
EPG	Eggs per gram
FEC	Faecal egg count
FECR	Faecal egg count reduction
FECRT	Faecal egg count reduction test
GIN	Gastrointestinal nematode
L1 – L5	First to fifth stage larvae
LB_{10}	Lying bout of durations >10 s
LB_{30}	Lying bout of durations >30 s
PGE	Parasitic gastroenteritis
PLF	Precision Livestock Farming
PPR	Periparturient relaxation
TST	Targeted selective treatment
TT	Targeted treatment

1. Introduction

Gastrointestinal nematode (GIN) infections are common in grazing ruminants worldwide, including both cattle and sheep. In sheep especially, they are arguably one of the major causes of impaired health and productivity and thereby an important constraint on efficient domestic livestock production (Sutherland & Scott 2010). GIN of the highest economic and animal health importance in northern Europe include *Cooperia oncophora* and *Ostertagia ostertagi* in cattle, and *Haemonchus contortus*, *Teladorsagia circumcincta*, and *Trichostrongylus* spp in sheep (Charlier et al. 2014a).

Production losses are generally linked with subclinical, mixed infections and occasionally clinical signs, such as diarrhoea, dull hair coat, anorexia, loss of body weight and exceptionally mortality. Moreover, the economic impact is also connected to the cost of veterinary advice and anthelmintics. The combined annual cost for major helminth infections in European livestock has recently been estimated to € 1.8 billion (Charlier et al. 2020a). The control of GIN parasitism is vital to the sustainability of pastoral systems, which today is primarily based on preventive or curative use of anthelmintic drugs on group level. However, misuse of these drugs has led to widespread development of drug-resistant worm populations (Vercruysse et al. 2018).

Targeted (selective) treatment (T(S)T), where only infected groups (TT) or individual animals within a group are treated (TST), opposed to the whole group, has been proposed as a sustainable long-term strategy to yield individual benefits to animal health and welfare and at the same time decrease the risk for the selection of AR. However, the implementation of TST approaches in practice is today limited by the lack of user-friendly, reliable and affordable animal-side indicators (Charlier et al. 2014b).

Monitoring of deviations in activity and behaviour has been suggested as an applicable indicator of disease in cattle. For example physical activity (resting and movements) and feeding behaviour (rumination and grazing time), can provide specific information about an animals health and welfare status (Weary et al. 2009). The recent advancements of Precision Livestock Farming (PLF) nowadays enable real-time monitoring of animal activity and behaviour (Berckmans 2017). However, the knowledge of such responses in relation to parasite infections is today limited and PLF-systems therefore have to be developed and explored for parasite management to be integrated (Vercruysse et al. 2018; Morgan et al. 2019).

2. Background

2.1 Gastrointestinal nematodes

Grazing livestock are infected with a community of GIN, where mixed species infections usually have a greater impact on the host rather than monospecific infections. The outcome of mixed infections are generally referred to as PGE (Charlier et al. 2020b; Zajac & Garza 2020). Clinical PGE in both in sheep and cattle is characterized by watery diarrhoea, dull hair coat, anorexia and a loss of general body condition (Charlier et al. 2020b). In sheep, anaemia and submandibular oedema may also occur due to *H. contortus* infection (Sutherland & Scott 2010). However, today GIN are more commonly associated with a subclinical course of infection, with adverse effects on production parameters such as weight gain.

2.1.1 Life cycle

All ruminant GINs have a direct life cycle (Fig.1). Eggs are passed in the faeces and develop into the infective L3 on pasture. The larval development occurs in the faecal pat through moults from hatched L1 via L2, to infective L3. The time and success of development is dependent on temperature and weather conditions and the process takes one to two weeks if conditions are optimal. The optimum temperature for development is generally at moist conditions around 25°C. At higher temperatures mortality of larvae increases and at temperatures below 10°C nematode biology is slowed down and eventually development and activity ceases (reviewed by Sutherland & Scott 2010). At moist conditions, L3 migrate from the faeces on to the surrounding herbage. As a consequence, grazing animal ingests L3 as they feed. After ingestion, the L3 exsheat in the rumen or abomasum and further development takes place in the mucosa of the abomasum or in the upper part of the small intestine, depending on the species. After two additional moults, from L3 to L5, adult worms emerge on the mucosal surface (Charlier et al. 2020b). The pre-patent period is defined as the time it takes from ingestion of L3 to the sexual reproduction of the adult stage and the start of egg shedding. For the most common GINs eggs first appear in the faces of infected animals after approximately three weeks, although the time may vary depending on the species (Sutherland & Scott 2010).

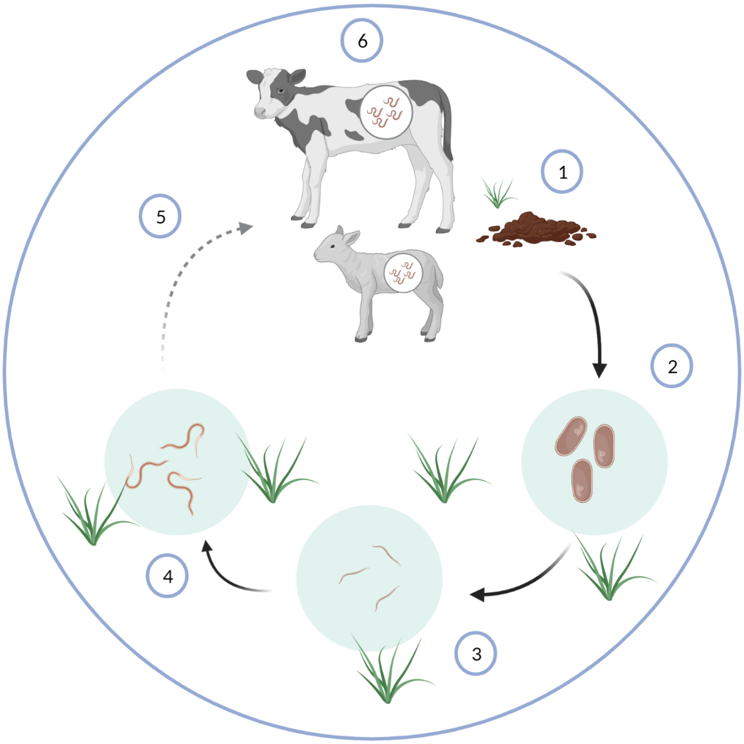


Figure 1. Life cycle of gastrointestinal nematodes in ruminants: 1) nematode eggs pass in faeces, 2) eggs develop and hatch, 3-4) larvae develop (L1-L2-L3) to infective stage and migrate up blades of grass, 5) grazing ruminants ingest infective larvae, 6) larvae develop (L4-L5) to adult egg laying worms. Illustration: Niclas Högberg (Created with BioRender.com).

2.1.2 Epidemiology of gastrointestinal nematodes

The epidemiology of GIN in grazing livestock is determined by climatic factors, environmental conditions, host susceptibility and status, and management of the livestock. The host-parasite interaction mainly determine the course of infection and the potential for disease to occur, whereas the host-environment and parasite-environment interaction influences the transmission of infection (Sutherland & Scott 2010).

The FEC and worm burdens are not normally distributed within a herd. Instead, FEC tends to be overdispersed, with a few animals in a group carrying the majority of parasites (Genchi et al. 1989; Sréter et al. 1994; Warburton & Vonhof 2018). This affects the opportunities for parasite transmission and has important implications for diagnosis and control (Stromberg & Gasbarre 2006; Charlier et al. 2020b).

Moreover, the establishment, development and reproduction of worms occur in a density dependent fashion. Many larvae in the same location will lead to competition between worms of the same as well other species. When an animal ingests a large number of larvae, the worm burden will be higher but the proportion of larvae succeeding to establish and reproduce will be lower (Smith et al. 1987; Charlier et al. 2020b).

Under certain conditions, a proportion of the ingested larvae become arrested in their development at the early fourth stage (L4). This is referred to as hypobios or inhibited development. Various factors have been linked as triggers for entering and leaving the hypobiotic stage. However, they are only partly understood (Sutherland & Scott 2010; Charlier et al. 2020b). Several studies have demonstrated a seasonal or climatic effect where reduced temperature and day length triggers the arrested development (Langrová & Jankovska 2004; Langrová et al. 2008). Moreover, the host immune status contributes in both cattle and sheep nematodes (Eysker 1993, 1997). Resumption of the arrested stages is usually observed during late pregnancy and early lactation and is believed to be partly associated with a PPRI, but possibly also to environmental and seasonal factors (Falzon et al. 2013a). Nevertheless, this causes a rise in FEC (Sutherland & Scott 2010).

In temperate areas, where arrested development occurs during winter, both cattle and sheep deposit parasite eggs in the beginning of the pasture season, followed by an increase in FEC, until the onset of immunity (Gasbarre et al. 1990; Vlassoff et al. 2001). Nevertheless, larval pasture contamination gradually increase during the grazing season until the animals are stabled. At the same time, pastures are ungrazed during winter and early spring, resulting in a decrease in larval pasture contamination. However, in temperate areas, there is for most species a high rate of overwintering L3, driving the epidemiology of the infection (Sutherland & Scott 2010). In sheep, the increase in FEC in periparturient animals further contribute to the increase of the L3 population on pasture (Michel et al. 1979; Chartier et al. 1998). In cattle, PPRI is less pronounced, and therefore contributes less to the pasture contamination (Sutherland & Scott 2010).

First season grazing animals, that not yet have a sufficient immunity, are more susceptible to infection and disease than older animals. (Nansen et al. 1989; Sutherland & Scott 2010; Besier et al. 2016). Effective immunity develops at 4-7 months of age and may develop even in the absence of parasitism, decreasing the opportunities for establishment of adult worms (Armour 1980).

2.1.3 Gastrointestinal nematodes in cattle

More than 20 different species within the superfamily Trichostrongyloidea are described. However, the importance and prevalence differ between climatic regions and may vary due to host age and acquired immunity (Charlier et al. 2020b). In temperate regions, such as Northern Europe, the most important and prevalent GINs in cattle include the small intestinal worm *Cooperia oncophora* and the more pathogenic abomasal worm *Ostertagia ostertagi.* They are usually present as mixed infections in grazing cattle (Höglund et al. 2010; Roeber et al. 2017a).

O. ostertagi is considered the most important parasite of cattle in temperate regions. It is well adapted to cooler climates and survive well over winter as L3 on pasture or as arrested larvae inside the animal. (Sutherland & Scott 2010). Adults are slender, reddish brown worms. Males measure 6-8 mm and females 8-9 mm in length. After ingestion, the L3 burrows into the abomasal glands, where it moults into L5, and thereafter return to the lumen, where it develops into the adult stage. The developing larvae damages the parietal cells, which reduces the functional gastric glands, thereby contributing to clinical or subclinical PGE. Clinical signs include watery diarrhoea, anorexia, loss of body weight and reduced weight gain (Anderson et al. 1965; Taylor et al. 2007).

Cooperia spp. in the small intestine are generally considered to be of mild pathogenicity in calves. Arrested development is common during winter as well as L3 surviving the winter on pasture (Dimander et al. 2000). Adults are slender and pinkish white. Males measure around 5-9 mm and females 6-8 mm in length. Ingested L3 burrows into the intestinal wall where they undergo two moults before adults develop on the surface of the intestinal mucosa. Adult *C. oncophora* are mucosal browsers and do not affect the enzymatic digestive function to the same extent as *O. ostertagi*. Like *O. ostertagi*, *C. oncophora* infection will cause a loss of appetite and reduced uptake of nutrients associated with poor weight gain due to the damage to the intestinal mucosa. Furthermore, heavy infections can cause intermittent diarrhoea (Taylor et al. 2007).

2.1.4 Gastrointestinal nematodes in sheep

Grazing sheep are infected with a somewhat more complex community of nematodes than cattle, of which some species are regarded as more pathogenic than others. In temperate regions, such as Northern Europe including Sweden, the five most prevalent GIN species include *H. contortus*, and *Teladorsagia circumcincta* in the abomasum, as well as *Trichostrongylus vitrinus, Oesophagostomum venulosum* and *Chabertia ovina* dwelling in the intestine (Halvarsson & Höglund 2021). *O. venulosum* and *C. ovina* are considered as apathogenic (Taylor et al. 2007; Sutherland & Scott 2010) and will not be covered in this thesis.

H. contortus is the largest of the abomasal parasites and is also considered as the most pathogenic as the adult feeds on blood (Sutherland & Scott 2010). It can cause major problems especially in growing lambs, but occasionally also in pregnant ewes (Waller & Chandrawathani 2005). It is not as adopted to overwintering on pasture in temperate regions as the other GINs affecting ruminants. Consequently surviving pasture contamination is of little practical significance in transmission between grazing seasons in temperate regions (Troell et al. 2005). Instead, the majority of ingested larvae become arrested in the abomasum as L4 and do not complete development until the following spring. The late larval stages moult twice in connection to the gastric glands, whereas adults move freely on the abomasal mucosa. Adults measure 20-30 mm and have a distinctive barber pole appearance. The prepatent period is 2-3 weeks (Troell et al. 2005). Adult worms sucks blood from mucosal vessels using a piercing lancet. Each worm removes about 0.05

ml of blood from the host daily through ingestions and seepage from the lesions. Acute haemonchosis therefore leads to a progressive anaemia and is characterised by variable levels of oedema, lethargy, dark coloured faeces and falling wool (Taylor et al. 2007). Heavy infections may lead to acute severe haemorrhagic gastritis with sudden deaths in apparently healthy sheep. Chronic haemonchosis is associated with weight loss and general weakness, without severe anaemia nor general oedema, whereas diarrhoea is not a general feature (Taylor et al. 2007). It should be noted that *H. contortus* is a highly fecund GIN and a single female can produce up to 10.000 eggs per day (Sutherland & Scott 2010).

T. circumcincta occupies the same niche in sheep as *O. ostertagi* does in cattle and likewise it is an important component of PGE in sheep (Stear et al. 2003; Sutherland & Scott 2010). It can over-winter, both as arrested larvae as well as on pasture, in temperate regions (Waller et al. 2004). Adults are slender and reddish brown. Males measure 6-8 mm and females 8-10 mm. Ingested L3 burrows into the abomasal glands, where they moult into L5, before returning to the lumen where it develops into the adult stage. Similar to *O. ostertagi* in cattle, the developing larvae damages the parietal cells and thereby reduces the functional gastric glands (Taylor et al. 2007). It causes a marked reduction in appetite that, together with losses of plasma proteins into the gastrointestinal tract, leads to a reduction in weight gain (Fox 1997). Clinical signs also include intermittent diarrhoea with stained hindquarters (Taylor et al. 2007).

T. vitrinus is a small, reddish brown, hair like worm found in the small intestine (Sutherland & Scott 2010). Males measure 4-6 mm and females 6-8 mm (Taylor et al. 2007). Infective L3 can survive the winter on pasture. However, hypobiosis is also important for the transmission between grazing seasons (Makovcová et al. 2009). *T. vitrinus* penetrate the mucosa of the small intestine. After two moults, L5 are present under the intestinal epithelium. The majority of adult worms are then found in the lumen or in tunnels between epithelial cells and the lamina propria (Taylor & Pearson 1979). The prepatent period is 2-3 weeks. The infiltrative stage causes enteritis in the small intestine, with oedema and villi atrophy, causing an impairment in the protein metabolism. At low infection levels, this leads to inappetence and a reduction in weight gain. High infection levels can cause weight loss and diarrhoea (Taylor et al. 2007).

2.2 Diagnosis and treatment strategies

2.2.1 Diagnosis

The diagnosis of GIN infections is usually confirmed by the detection of nematode eggs in faeces (Charlier et al. 2009; Vercruysse et al. 2018). Typically, the McMaster technique with a detection limit of 50 EPG is used. The method uses a specific counting chamber to examine microscopically a known volume of a faecal suspension for helminth eggs and coccidian oocysts. However, various modifications of the method such as FECPAK and FLOTAC can also be applied (Bosco et al. 2014). For species identification, different techniques of larvae cultures and identifications have traditionally been used (Taylor et al. 2007). In some laboratories, the presence of *H. contortus* can also be detected by microscopic identification of eggs (Ljungström et al. 2018). Moreover, molecular techniques are improving the detection and determination of nematode parasites in livestock. The different assays available, including qPCR (Demeler et al. 2013) and MT-PCR (Roeber et al. 2017b), and modifications thereof, rely on the amplification of internal transcribed spacer (ITS) regions between the 18S and 5.8S subunits of the ribosomal encoding genes (Gasser et al. 2008). In Sweden, methods using a ddPCR assay has been established for the most important genera (Höglund et al. 2013a, 2019; Elmahalawy et al. 2018; Baltrušis et al. 2019).

The use of FEC on an individual level is limited by the cost of sampling as well as feasibility of sampling. Moreover, the interpretation of FEC may also be interpreted differently depending on the local context, e.g. dominant parasite species, sampling season and climatic and environmental factors (Sargison 2013). FEC data have also been criticised as a treatment indicator because they are often poorly correlated with nematode infection level and animal performance (Greer & Sykes 2012; Sargison 2013). Therefore, the use of indirect methods of diagnosis has been suggested. These include evaluation of weight gain, body condition score and diarrhoea score in small ruminants, and pepsinogen levels as well as *Ostertagia* bulk milk tank ELISA for detection of specific antibodies in cattle (Kenyon & Jackson 2012).

2.2.2 Anthelmintics

Anthelmintics are drugs containing substances that are active against helminth infections, usually showing broad-spectrum activity against GIN. The principal aim of anthelmintic treatment is to decrease pasture contamination and thereby prevent clinical or subclinical disease. Anthelmintics are available in various forms and can be given orally or through parenteral administrations, e.g. injectables and pour-on preparations (Sutherland & Scott 2010).

There are three major anthelmintic substance classes licensed in northern Europe for the control of GIN in cattle: 1) benzimidazoles (e.g. fenbendazole), 2) imidazothiazoles (e.g. levamisole), and 3) macrocylic lactones (e.g. ivermectin) (Vande Velde et al. 2018). However, only benzimidazoles and macrocylic lactones are registered in Sweden (FASS Djurläkemedel, www.fass.se/LIF).

For sheep, there are two substance classes registered in Sweden for the control of GIN. These include: 1) macrocylic lactones, 2) benzimidazoles. Through a license approved by the Swedish Medical Products Agency, there is also access to 3) imidazothiazoles (e.g. levamisole) and 4) the amino-acetonitrile derivate monepantel. Levamisole is mainly used in the case of resistance to macrocyclic lactones and benzimidazoles, whereas monepantel is hardly used at all in Sweden (Höglund et al. 2020).

2.2.3 Anthelmintic resistance

Anthelmintic resistance (AR) is defined as "when a greater frequency of individuals in a parasite population, usually affected by a dose or concentration of compound, are no longer affected, or a greater concentration of drug is required to reach a certain level of efficacy" (Wolstenholme et al. 2004).

In practical terms, those parasites surviving anthelmintic treatment will pass on resistance-associated genes to their offspring. With further selection and reproduction, the resistance associated genes will increase within the parasite population (Sangster et al. 2018). Parasitic nematodes have several biological and genetic features that may favour the development of AR, including short life cycles, high reproductive rates and large population sizes with a high genetic variation (Anderson et al. 1998). When resistance is present, it is difficult to reverse, and therefore the best approach is to implement strategies to prevent it (Sangster et al. 2018).

Extensive use of anthelmintics has led to a global spread of AR in both sheep and cattle, in multiple nematode species, against most drug classes and sometimes against multiple different classes (Sutherland & Leathwick 2011; Kaplan & Vidyashankar 2012; Rose et al. 2015). When it comes to nematode parasites in sheep, the situation is particularly severe. AR has been reported from most sheep-rearing countries in the world (Van Wyk et al. 1999; Waghorn et al. 2006; Höglund et al. 2009a; Falzon et al. 2013b), including Sweden (Areskog et al. 2013; Höglund et al. 2015, 2020). In several countries, studies show that the situation has worsened (Veríssimo et al. 2012; Falzon et al. 2013b; Lamb et al. 2017; Ploeger & Everts 2018). According to a recent review (Vineer et al. 2020), reports of AR were widespread throughout Europe, particularly to benzimidazoles but also to the macrocylic lactone ivermectin. At the same time, AR in cattle nematodes is understudied in many regions. In general, levels of AR are considered as less severe in GIN of cattle than in sheep (Kaplan 2020). Still there are indications that resistance is present and relatively widespread, also in cattle (Soutello et al. 2007; Suarez & Cristel 2007; Rendell 2010; Areskog et al. 2014; Waghorn et al. 2016).

2.2.4 Treatment and control strategies

Control of GIN parasitism is vital to the sustainability of pastoral systems. There are numerous variations of control strategies across the world and most have depended on a combination of two basic principles, 1) the use of anthelmintics and 2) grazing management practices (Sutherland & Scott 2010). In this thesis, grazing management practices will not be covered.

The increasing prevalence and spread of AR worms has forced the industry to include the knowledge of nematode epidemiology into the control strategies (Kenyon et al. 2017; Greer et al. 2020). Frequent treatment administrations have been shown to select for AR (Kenyon et al. 2013). Two concepts for treatment strategies have therefore been introduced: 1) TT, where the whole flock is treated, based on knowledge of the risk, or parameters that quantify the severity of infection (Table 1), and 2) TST, where individual animals within the herd are treated, based on a single, or a combination of, treatment indicators (Kenyon & Jackson 2012).

The aim of both TT and TST is to effectively control nematode infection levels and production impact and at the same time reduce the risk of AR, as it will maintain populations of nematodes in refugia (i.e. parasitic stages in the environment unexposed to anthelmintics) (van Wyk 2001; Kenyon & Jackson 2012; Charlier et al. 2014b; Greer et al. 2020). However, the implementation of these approaches in practice is today somewhat limited by the lack of user-friendly, reliable and affordable animal-side indicators (Charlier et al. 2014b). In this thesis, the implementation of a T(S)T-system has not been assessed and will therefore not be covered further. Nevertheless, as the strategies are based on parasite diagnostics serving as the treatment indicator it is worth mentioning.

Table 1. Potential treatment indicators for targeted treatment (TT) and targeted selective treatments (TST) in grazing livestock (adapted after Greer et al. 2020).

	Regime Type	**Indicator**	**Reference**
Calves	TT	Mean faecal egg counts	(Areskog et al. 2013)
	TT	Mean plasma pepsinogen levels	(Charlier et al. 2011)
	TST	Live weight gain	(Höglund et al. 2009b)
Lambs	TT	Pooled faecal egg counts	(Höglund et al. 2019)
	TST	Live weight gain	(Stafford et al. 2009)
	TST	Production efficiency	(Greer et al. 2009)
	TST	FAMACHA[1]	(Van Wyk & Bath 2002)
	TST	Diarrhoea score	(Bentounsi et al. 2012)

[1]Faffa Malan Chart (FAMACHA) – suitable indicator in the case of infections with *H. contortus*.

2.3 Behaviour of grazing livestock

2.3.1 Normal behaviour

The definition of what constitutes normal behaviour is not straightforward (Kilgour et al. 2012). Over the years, there have been numerous studies trying to provide information of the range of behaviours conducted of cattle at pasture. No complete time budget has been suggested and studies have mainly focused on the major behaviours of grazing, resting and rumination. Cattle have an extensive repertoire of behaviour, including over 40 identifiable categories (not covered here). Grazing tends to be the most common behaviour, followed by ruminating and resting. Together they constitute between 90-95% of an animal’s day. Most of the grazing is performed during the hours of daylight with peaks of grazing activity in connection to sunrise and sunset (Hughes & Reid 1951; Krysl & Hess 1993; Kilgour 2012). The amount of time spent on grazing varies depending on a variety of factors including breed and season (Hessle et al. 2008), body weight (Rook et al. 2004), amount of daylight (Rutter 2006), health status (see 2.3.2) and postural synchrony (Stoye et al. 2012). Daily grazing times have been reported to vary between 5 and 13 hours (Krysl & Hess 1993; Fraser & Broom 1997; Kilgour et al. 2012; Ungar et al. 2018). Rumination generally occurs while cattle are lying. The average rumination time per 24 h has been reported to vary between 4.7 and 10.2 h (reviewed by Kilgour, 2012). In ruminants, resting is a highly prioritised behavioural need (Plesch et al. 2010). It is hard to determine the preferred lying time for cattle, since it is affected by many factors, but it is probably around ten hours per day (Phillips 2002). This was confirmed in a more recent study which showed an average lying time of about ten hours per 24 h (Lee et al. 2013).

Besides rumination, grazing tends to be the most common behaviour of sheep on pasture (Gonyou 1984). Sheep used in farming exhibit a circadian pattern of feeding that includes a major grazing period at, or soon after, sunrise. The grazing pattern throughout the day is influenced by feed availability, weather conditions, topography and social factors (Arnold 1960, 1962; Hinch 2017). Tobler at al. (1991) showed that sheep kept on pasture become active in connection to dawn, with a first maximum of activity towards noon, and a second peak in the evening that is followed by decline after dusk. Moreover, extensively managed ewes in Scotland were mainly active for 60% during daytime and spent 60% of the active time grazing. The rest of the time was classified as inactive and mainly recumbent. In contrast to cattle, sheep tend to divide rumination time equally between standing and lying (Pokorná et al. 2013). Sheep have been reported to graze between 8 and 13 h per day (Arnold 1962; Lynch et al. 1992; Zupan et al. 2010).

2.3.2 Sickness behaviour and behavioural responses to disease

As mentioned, ruminants exhibit distinctive behavioural patterns, where grazing, ruminating and resting are the most common at pasture. The behavioural patterns may be altered by several external factors, including changes in weather, pasture availability and quality, but also emerging disease. This means that if deviations can be measured, they can potentially identify individual animals at risk of developing health issues. However, external factors always need to be taken into account to avoid misinterpretations of sickness related behavioural patterns (Weary et al. 2009). Behavioural patterns affected by disease is collectively called sickness behaviour (Hart 1988; Weary et al. 2009; Harden et al. 2015).

It has been suggested that the release of inflammatory cytokines, such as TNF-α, IL-1β, IL-6, as a response to pathogens and tissue damage, will trigger the behavioural responses linked to sickness behaviour (Tizard 2008; Hart & Hart 2019). However, sickness behaviour is also related to non-inflammatory states such as metabolic disorders, where the inflammatory response is not necessarily given as with infectious disease. Also, mechanical injuries such as lameness triggers sickness related behaviour changes. In the case of lameness, the behavioural changes can be linked to pain avoidance (Dittrich et al. 2019).

Both activity and feeding behaviour play major roles in the health, productivity and welfare of livestock (Weary et al. 2009). The majority of activity behaviour can be divided into resting and physical activity. Resting can be divided into lying and standing, whereas physical activity mostly includes walking. Feeding behaviour patterns can be divided into feeding duration and feeding bouts as well as rumination (Dittrich et al. 2019). Alterations in activity are thought to protect the individuals' energy deposits that are needed to fight the disease (Tizard 2008; Hart & Hart 2019). It has also been suggested that the cytokine response linked with disease, such as IL-1, generates a reduction of appetite leading to a reduction in feed intake (Broom 2006). Moreover, the monitoring of activity and rumination behaviour provides specific information about health and welfare. It is also one of the most widely used methods to detect oestrus in dairy cows today (Roelofs et al. 2010). For sickness behaviour, the general behavioural changes seen are, a prolongation of resting behaviour, a decrease in physical activity and an impairment of general feeding behaviour (Dittrich et al. 2019).

The above described behavioural changes linked to sickness behaviour are very general and tend to occur irrespective of the underlying disease. Therefore, linking mentioned deviations to specific health and welfare disorders are one of the major challenges. It can therefore be necessary to include additional information about the animal or herd, such as production parameters and historical health records to be able to determine the cause of the deviation (Dittrich et al. 2019).

2.4 Behavioural monitoring

2.4.1 Precision livestock farming

The use of sensor technologies in livestock production, often referred to as PLF is a relatively new phenomenon. PLF is defined as management of livestock by continuous automated real-time monitoring of production, reproduction, health and welfare of livestock, and environmental impact. Continuous monitoring enables real-time warnings when something with the animals deviates so that the farmer can undertake immediate action. The monitoring can be done with, for example, microphones with real-time sounds analysis, cameras with real-time image analysis, or with sensors on or around the animal (Berckmans 2017). The use of sensors have the possibility to increase surveillance in larger herds and remote pastures (Rutten et al. 2013; Richeson et al. 2018).

2.4.2 Sensors

A general approach to collect real-time data from animals is by the use of various sensors. The sensor continuously monitors bio-signals from the animal, or events in its environment (Berckmans 2017). Such bio-signals can include measuring physical, physiological or behavioural indicators of the state of the animal, such as body temperature, position, activity, rumination etc.

Animal-based sensors are attached to the animal on ear tags, collars, leg straps, or internally as boluses or implants. There is a variety of commercially available sensors that can be used for animal health and welfare monitoring (Rutten et al. 2013; Caja et al. 2016). They are usually developed for use in indoor systems, but ongoing developments of sensor technologies and infrastructure for data transfer enables gradual application to grazing animals. Accelerometers have been extensively used in dairy production systems to detect oestrus and health and welfare issues, such as mastitis and locomotion problems (Rutten et al. 2013; Norton & Berckmans 2017). However, the use in grazing livestock is less explored.

Most on-animal sensors currently uses a three-axis (3D) accelerometers that continuously logs data, in different frequencies, and summarises it in a user friendly manner (Richeson et al. 2018). All three axes records movement and posture changes simultaneously and depending on the placement of the sensor on the animal different behaviours or activates can be measured. Sensors attached around the neck or in the ear of the animal often provide information on activity alone, i.e. total amount of movement, whereas sensors attached around the animal's legs can provide information on number of steps, lying and standing times and number of lying bouts (reviewed by Caja et al., 2016; Richeson et al., 2018; Rutten et al., 2013).

There are also a number of commercially available sensors that can record chewing and rumination activity. The systems mostly use either an accelerometer to detect movement or a microphone to detect sound. The sensors can be mounted in ear tags or on collars (reviewed by Beauchemin, 2018).

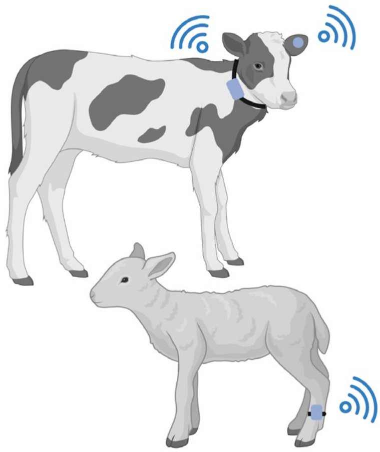


Figure 2. Different sensor placements. Illustration: Niclas Högberg (created with BioRender.com).

In contrast, there are few commercially available sensors that have been validated for use in small ruminants. However, there are currently many applications of sensors that are at a proof-of-concept stage, enabling future commercialisation of the technology (Fogarty et al. 2018). A reason for the delay in development could be the combination of large herds, low individual profits and a relatively high sensor cost (Caja et al. 2020). Nevertheless, PLF technologies developed for cattle has the potential to be adapted also for use in sheep production. Both leg and neck/ear mounted accelerometers (as mentioned above) should technically work to monitor sheep (Rutter 2017). Like for cattle, most applications of sensors in sheep research have been conducted over short periods of time, with a small number of animals and mainly on housed animals (Fogarty et al. 2020). Moreover, there is little information on the use of sensors on sheep under grazing conditions (Caja et al. 2020).

2.4.3 Health monitoring

Sensor systems have been shown to be able to detect a variety of infectious diseases, metabolic disorders and lameness (Table 2). Several of the conditions can also be detected at an earlier stage than by manual monitoring.

To date there are only a handful of studies assessing activity and behavioural patterns in relation to GIN infections with a sensor approach. Studies on housed Holstein-Friesian calves experimentally trickle infected with 300000 *O. ostertagi* L3 for three weeks showed a decrease in number of steps taken as well as a decrease in number of standing and lying bouts 21 days post infection (Szyszka et al. 2013). Moreover, heifer calves naturally challenged with GIN for 12 weeks showed a decrease in grazing time 70 days after turn out (Forbes et al. 2000), whereas dairy cows naturally infected with GIN showed a decrease in eating time, meal duration and total bites (Forbes et al. 2004).

Studies of sheep naturally infected with GIN exhibited a smaller behavioural complexity compared with dewormed animals, suggesting that organizational patterns of their behaviour changes with GIN infections (Burgunder et al. 2018). More recently, sheep naturally infected with strongyles had a lower activity level compared with dewormed animals after 42-46 days on pasture (Ikurior et al. 2020).

2.4.4 Validity of sensor output

A validation process assesses the accuracy and appropriateness of a sensor within a specific framework and is required before the use of a sensor and its output. So far, there is no standardized validation protocol with an agreement on methodology. However, a guideline has been developed within the SmartCow project (Bouchon et al. 2019), including a checklist to consult before a study to assess the validity of a sensor. Aspects that needs to be considered includes a description of the equipment used, data output and handling, the test environment, animals included, positioning of the sensor, measurement of the *gold standard*, sample size and analysis and robustness (Bouchon et al. 2019).

Table 2. Examples of health issues in cattle and sheep where behavioural changes can be detected using sensor systems.

	Health issue	Behavioural change	Change	Reference
Cattle	Hypocalcaemia	Lying duration	↑	(Sepúlveda-Varas et al. 2014)
	Ketosis	Standing duration	↑	(Itle et al. 2015)
		Rumination duration	↓	(King et al. 2018)
	Lameness	Lying duration	↑	(Thorup et al. 2015)
		Rumination duration	↓	(Miguel-Pacheco et al. 2014)
	Metritis	Lying duration	↑	(Sepúlveda-Varas et al. 2014)
		Lying bout duration	↑	(Neave et al. 2018)
		Physical activity	↓	(Stangaferro et al. 2016b)
		Rumination duration	↓	(Steensels et al. 2017)
	Mastitis	Lying duration	↓	(Cyples et al. 2012; Fogsgaard et al. 2015)
		Rumination duration	↓	(Fogsgaard et al. 2012; King et al. 2018)
	Parasite infection	Lying duration	↑	(Szyszka et al. 2013)
		Grazing time	↓	(Forbes et al. 2004)
Sheep	Lameness	Physical activity	↓	(Barwick et al. 2018)
	Parasite infections	Physical activity	↓	(Ikurior et al. 2020)

3. Aim and objective

The overall aim of this thesis was to investigate if multispecies GIN parasite infections affect behavioural patterns in grazing livestock and to identify and test commercially available sensor systems recording behavioural and activity patterns.

The specific objectives of Papers I-IV was as follows:

I. To investigate activity patterns and standard diagnostic indicators in first season grazing cattle when exposed to two different levels of *Ostertagia ostertagi* and *Cooperia oncophora* under pasture conditions.

II. To further investigate how activity patterns and rumination patterns change along with standard diagnostic indicators in first season grazing cattle when exposed to two different levels of *O. ostertagi* and *C. oncophora* under pasture conditions.

III. To assess the validity of two commercially available loggers, i.e. IceTag® and IceQube®, in lambs.

IV. To investigate how activity patterns along with standard diagnostic indicators in naive grazing lambs are influenced when exposed to contrasting levels of GIN under pasture conditions.

4. Materials and methods

An overview of materials and methods used in this thesis is presented below. More details can be found in Papers I-IV.

4.1 Ethical permission

The studies in Papers I-IV were approved by the Committee on Animal Experiments in Gothenburg (registration numbers 187-2014 and 824-2017).

4.2 Experimental location

Papers I, II and IV took place at Götala Beef and Lamb Research Centre, Skara, Sweden (58° 42′N, 13° 21′E; elevation 150 masl). Paper III took place at a commercial sheep farm near Skara, Sweden (58° 28′20.4″N, 13° 19′57.9″E; elevation 75 masl).

4.3 Animals

4.3.1 Cattle

First season grazing steers of Swedish Holstein and Swedish Red were used in Papers I-II. In Paper I, the animals were purchased at 3-5 months of age, from the same commercial farm. In Paper II, the animals were purchased at 3-7 months of age, from ten commercial farms. Before turn-out, animals were housed in an uninsulated building on deep straw bedding and fed a forage based total mixed rations at *ad libitum* intake. The age at turn-out was 6-13 months in Paper I, and 9-13 months in Paper II.

4.3.2 Sheep

In Paper III, lambs of Texel (M) x (Swedish Fine wool x Dorset) (F) crossbreeds from a commercial farm were used. In paper IV, lambs of Swedish Finewoll (M) x (Swedish Fine wool x Dorset) (F) from a nearby commercial farm, were used.

4.4 Experimental design

Paper I, II and IV all included infection studies where we followed behavioural responses to different exposure levels of mixed GIN infections. In Paper III, a validation study of two commercially available 3D-accelerometers' accuracy to monitor activity in lambs was conducted.

Paper I involved in total 63 steers, allocated into different grazing groups exposed to two different levels of GIN. At turn-out, the animals were allocated to one of two treatment groups grazing in different enclosures. Activity patterns, FEC, SPC and body weight were monitored in 18 steers during three two-week periods.

In Paper II, animals were allocated to one of four experimental groups, exposed to two different levels of GIN. Activity and rumination patterns, FEC, SPC and body weight were monitored in 60 steers for 140 days.

Paper III was a validation study where the activity of ten lambs, divided into two groups, was recorded and analyses of the lambs body posture and number of steps per second from 50 h of video recordings were used as a gold standard to determine the accuracy of the two loggers.

In Paper IV, ewes and their twin-born lambs were let out into two permanent pasture enclosures. At weaning, lambs were allocated to one out of four groups based on sex and GIN exposure level. Activity patterns were monitored from seven days pre-weaning until 49 days post-weaning. Body weight and FEC was investigated continuously.

4.4.1 Infection levels and worm isolates

In Papers I-II, all steers were naturally exposed to overwintering strongyle nematode larvae at pasture. Furthermore, steers in the high exposure groups (HP) were primed at turn-out with, Paper I) 5000, and Paper II), 10000, L3 of *O. ostertagi* and *C. oncophora* (1:1). The isolates used were obtained from Tierärztliche Hochschule, Hannover (TiHo). The parasite isolates had no history of previous exposure to macrocyclic lactones or of being refractory to treatment with any anthelmintics. Before each study, two calves, that were not included in the study, were used as "incubators" to propagate L3 for priming in the studies.

In contrast, steers in the low exposure groups (LP) were treated with an ivermectin pour-on solution, Paper I) ivermectin (Noromectin®) 0.5 mg per kg body weight, Paper II) ivermectin (Ivomec®) 0.5 mg per kg body weight, at four-week intervals from turn-out until the end of the trial.

In Paper IV, the sheep were let out on semi-natural pastures, naturally contaminated with GIN the previous year, thereby exposing them to overwintering strongyle larvae.

The AR status of the GIN was characterised by a FECRT according to the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al. 2006). FECR was calculated using a two samples paired model, using the shiny-eggCounts web interface (Torgerson et al. 2014). The level of susceptibility within the flock was calculated to be 100% (95% CI 98% - 100%), thereby being susceptible to ivermectin.

Lambs in the low exposure groups (LP) were dewormed four weeks after turn-out with 0.2 mg ivermectin (Ivomec® vet, oral suspension) per kg body weight and thereafter at four-week intervals.

4.5 Behavioural monitoring

A brief overview of the sensors used in Papers I-IV, including IceTag®, IceQube® and Heatime® HR-LD, are listed below. Prior to Papers II-IV, an overview of available commercial sensors was made. Inclusion criteria's included the possibility to use at pasture, price, adaptation for use in lambs and collaboration with the manufacturer.

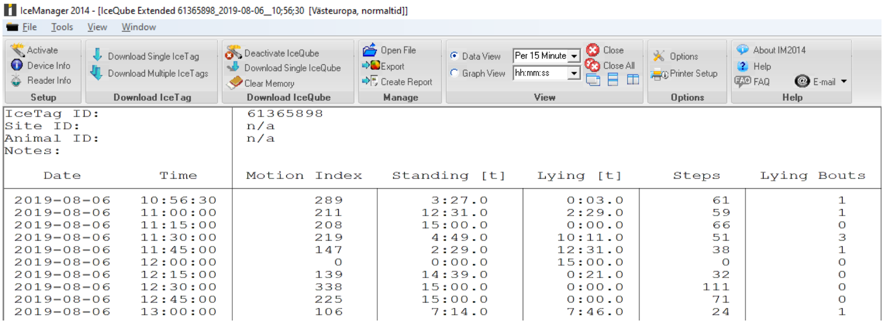
4.5.1 IceTag

In Papers I and III, IceTag® 3D-accelerometers (IceRobotics Ltd, Edinburgh, UK; validated for cattle by Ungar et al. 2017), were used to monitor animal activity patterns. Sensor dimensions in Paper I, were 65×60×30 mm and 197 g with the thermoplastic rubber strap. In Paper III, the thermoplastic cover was removed and dimensions were 65×55×30 mm and 102 g. The tri-axial accelerometer operates using a sample rate of 16 Hz with a time resolution of 1 s. It continuously recorded **lying** (indicates whether the animal is lying down or not), **steps** (number of steps/leg movements), **lying bouts** (indicates the start of a lying bout) and **motion index** (the measured net acceleration, indicates total activity). Behavioural recordings from IceTags®, expressed as minutes per hour and 24 h and numbers of steps and lying bouts per hour and 24 h, were downloaded at the end of the study using the download station IceReader (Fig. 3) and IceManager software (Fig. 4). Data was finally exported as comma separated value files (.csv).

Figure 3. Data downloaded from an IceTag® senor using the IceReader download station. Photo: www.icerobotics.com.

4.5.2 IceQube

In Papers II, III and IV, IceQube® 3D-accelerometers (IceRobotics Ltd, Edinburgh, UK; validated for cattle by: Borchers et al., 2016; Kok et al., 2015; Ungar et al., 2018) were used (Fig.5). Sensor dimensions were 55×55×27 mm and 75 g. The tri-axial accelerometer operates using a sample rate of 4 Hz with a time resolution of 15 min, with a 9-day internal memory. It continuously recorded **lying** (indicates whether the animal is lying down or not), **steps** (number of steps/leg movements), **lying bouts** (indicates the start of a lying bout) and **motion Index** (the measured net acceleration, indicates total activity). Behavioural recordings from IceQubes®, expressed as minutes per hour and 24 h and numbers of steps and lying bouts per hour and 24 h, were downloaded at the end of the study using the download station IceReader (Fig. 3) and IceManager software (Fig. 4). Data was finally exported as comma separated value files (.csv).



Date	Time	Motion Index	Standing [t]	Lying [t]	Steps	Lying Bouts
2019-08-06	10:56:30	289	3:27.0	0:03.0	61	1
2019-08-06	11:00:00	211	12:31.0	2:29.0	59	1
2019-08-06	11:15:00	208	15:00.0	0:00.0	66	0
2019-08-06	11:30:00	219	4:49.0	10:11.0	51	3
2019-08-06	11:45:00	147	2:29.0	12:31.0	38	1
2019-08-06	12:00:00	0	0:00.0	15:00.0	0	0
2019-08-06	12:15:00	139	14:39.0	0:21.0	32	0
2019-08-06	12:30:00	338	15:00.0	0:00.0	111	0
2019-08-06	12:45:00	225	15:00.0	0:00.0	71	0
2019-08-06	13:00:00	106	7:14.0	7:46.0	24	1

Figure 4. Example of IceQube® sensor data presented in the IceManager software.

4.5.3 Heatime

In Paper II, Heatime® HR-LD activity and rumination collar (SCR Engineers Ltd., Netanya, Israel; validated for cattle by: Burfeind et al., 2011; Schirmann et al., 2009), were used (Fig.5). The sensor measured neck **activity** and **rumination time** using a 3D-accelerometer and a microphone containing a microprocessor, respectively. Data was calculated and summarized at 2-h intervals and automatically downloaded via a long-distance antenna to the Heatime® HR system (SCR Engineers Ltd., Netanya, Israel), which continuously gives the raw rumination time (min) and general activity level, scaled from 0 to 255 at 2-h intervals. In addition, the system calculates **activity change** and **rumination change**, determining the individual behavioural change by comparing raw data of the given 2-h interval to data from the corresponding 2-h interval of previous days, presented as the change in standard deviations adjusted to a -100 to 100 scale. Data was provided in csv-format by the company. Activity and rumination data, raw and change respectively, was summarized on a 24 h basis at the end of the study. It should be noted that the data processing is not fully transparent due to intellectual property rights.

Figure 5. A grazing steer in Paper II illustrating the placement of the leg attached IceQube® sensor and the collar used to attach the Heatime® sensor. Photo: Karin Wallin.

4.6 Parasitological examination

4.6.1 Faecal egg counts

In Papers I-II, nematode FEC was determined according to a modified McMaster technique based on 5 g of faeces with a minimum detection level of 20 EPG. In Paper IV, the same method was used, but instead based on 3 g faeces dispersed in 42 mL saturated NaCl, providing a minimum detection level of 50 EPG.

4.6.2 Nematode determination

In Paper II, pooled faecal group samples were mixed with Vermiculite® to culture the eggs at room temperature under moist conditions. After one week larvae were harvested using the Petri-dish method (Elmahalawy et al. 2018). Total DNA was then extracted using the NucleoSpin XS Tissue kit (Macherey Nagel, Germany). The proportion of ITS2 DNA copies of *O. ostertagi* and *C. oncophora* were then determined in duplex reactions using a ddPCR assay (BioRad), as described by Baltrušis et al. (2019).

In Paper IV, total DNA from duplicated individual faecal slurries (1:408) were extracted using the NucleoSpin DNA Stool kit® (Macherey Nagel, Germany). The proportions of *Haemonchus* and *Teladorsagia* ITS2 copies were then determined in duplex reactions using a ddPCR assay (BioRad), as described earlier by Elmahalawy et al. (2018).

4.6.3 Serum pepsinogen concentration (SPC)

To evaluate the level of abomasal mucosal damage caused by *O. ostertagi*, SPC expressed as IU tyrosin was analysed in Papers I-II according to a micro method described by Charlier et al. (2011). This method is only available for cattle and not applicable for sheep.

4.6.4 Body weight

The body weight of steers in Papers I and II was manually recorded at turn-out (start of experiment) and at housing (end of experiment), as well as every fortnight in between. In addition, BWG between every pair of consecutive weighings were calculated.

Body weights of lambs in Paper IV were registered manually from three weeks prior to weaning and thereafter every week for ten weeks. The BWG between every pair of consecutive weighings were then calculated.

4.6.5 Pasture

In Paper I and II, the pasture consisted of 28 ha of mainly open, permanent semi-natural pastures. For Paper I, the pasture was split up into two similar enclosures, whereas in Paper II, it was split into four similar enclosures. In both studies, the same area was grazed throughout the grazing period.

The pasture used in early summer for ewes and lambs in Paper IV consisted of 7.5 ha mainly open, permanent semi-natural pasture, split up into two similar enclosures. The weaned lambs then grazed a grass-dominated ley, split up into four similar enclosures, each of 1 ha.

In Papers I, II and IV, sward height and nutrient concentration of the pasture herbage were measured every four weeks from turn-out to housing, to ensure similar conditions for the respective exposure group. Sward height measurements were made according to (Frame 1993) and nutrient concentration was determined according to Dumas (1831), Chai and Udén (1998) and Lindgren (Lindgren 1979).

4.6.6 Validation procedure

In Paper III (Fig. 6), 50 h of video data per animal was recorded using a Media Recorder system (Noldus Technology Ltd, the Netherlands). Two observers scored the behavioural measures using a predefined ethogram (see Paper III for details) with definitions of specific behaviours. Five hours of data per animal was scored on a 1-second time scale, using The Observer XT 11.5 (Noldus Technology Ltd). The manually scored data set was then compared with sensor output to assess the validity.

4.6.7 Statistical methods

All statistical analyses (Papers I-IV) were performed using R (v. 3.4.3 – v. 4.0.3). In addition, all graphical illustrations (Papers I-IV) were made using the ggplot2 package (Wickham 2016).

Activity and rumination data, BWG, FEC (Papers I, II and IV) and SPC (Papers I-II), were analysed using mixed models in the nmle package (Pinhero et al. 2020). Continuous time covariates (corCar1) were fitted to account for autocorrelation in the models. Final model selections were based on the Akaike Information Criterion.

In Paper III, the positive predictive value, sensitivity and specificity was calculated to assess the validity of IceTag® and IceQube® output data to record lamb activity. Furthermore, sensor performance was also evaluated through Bland-Altman plots, using the Bland-AltmanLeh package. Cohen's kappa was calculated using the irr package, to ensure interobserver reliability. In addition, the sensitivity (probability that a sensor scores an event recorded by video), specificity (probability that a sensor does not score an event recorded by video) and positive predictive value (PPV) (probability that sensor scores a real event) were calculated for lying and standing time. In addition, computed indications, for the start of a lying bout of durations >10 s (LB_{10}) and >30 s (LB_{30}), respectively, was performed.

Additional analyses in Papers I, II and III were made with the R base package.

Figure 6. Lamb during validation data collection (Paper III). Photo: Niclas Högberg.

5. Results and discussion

The general findings made in Papers I-IV of this thesis are briefly described and discussed below. Results are presented as arithmetic mean and standard deviation if nothing else is stated. Detailed information of the results can be found in the corresponding section within each paper.

5.1 Parasitology and animal performance (I, II, IV)

To evaluate the levels of GIN infection and its composition, standard diagnostic methods including FEC, body weight gain (BWG) and SPC were used. The compositions of major parasite genera were analysed using two ddPCR assays on group level in Paper II and on individual level in Paper IV.

5.1.1 Cattle

The nematode egg count in Paper I and II (Fig. 7) had a similar dynamic with a peak in FEC four weeks after turn-out, composing a natural infection dynamic. Furthermore, the FEC were elevated in the high exposure groups compared with the dewormed animals until the end of the studies (Paper I: $P = 0.0038$; Paper II: $P < 0.0001$). The FEC in both papers were comparable to reported field levels in Sweden, 4-6 weeks after turn-out (Areskog et al. 2013). However, the FEC levels were lower than in comparable previous trials on the same study location (Höglund et al. 2013b). The molecular investigation showed that animals in both groups were exposed to different levels of *O. ostertagi* and *C. oncophora* throughout the grazing season. However, the proportion of *O. ostertagi* and *C. oncophora* (Ost/Coop) in HP shifted from approximately 1:2 at day 31 to 4:1 at day 141 and were in line with precious studies in Sweden (Dimander et al. 2003).

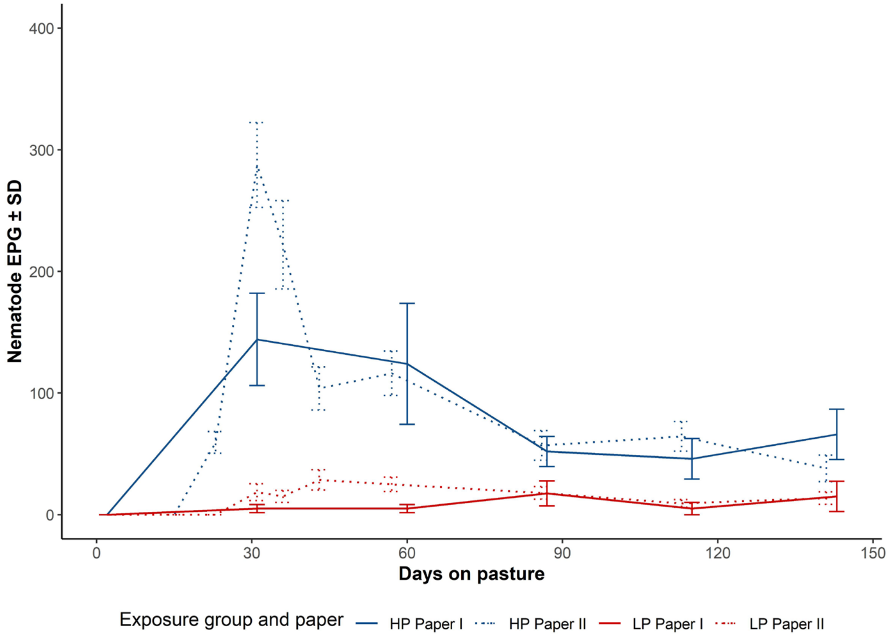


Figure 7. Mean gastrointestinal nematode faecal egg counts (EPG) ± SD in two exposure treatments of first season grazing steers during two years. One treatment (HP) was primed at turn-out with, I) ≈ 5000, II) ≈ 10000, infective third stage larvae of *Ostertagia ostertagi* and *Cooperia oncophora* (1:1) and thereby exposed to a high parasite challenge, whereas the other treatment (LP) was dewormed with ivermectin (0.5 mg kg^{-1}) monthly, thus being exposed to a lower parasite challenge.

Corresponding differences in SPC levels between high exposure groups and dewormed groups (Paper I: $P < 0.0001$; Paper II: $P < 0.0001$) were observed in both papers (Fig. 8). However, SPC levels in HP were lower in Paper II compared with Paper I. Levels higher than 3.5 IU tyrosin, indicating clinical ostertagiosis (Charlier et al. 2011), were only observed at three occasions in different animals in HP in Paper I, and on one occasion in HP in Paper II. Levels from both studies are lower than in a comparable study on the same study location (Höglund et al. 2013b). SPC levels tend to increase throughout the grazing period and is usually used for diagnostic purposes on farms in August and September (Ploeger et al. 1994). The patterns of SPC levels observed in Paper I and II may therefore reflect the relatively high initial response to priming at let-out, and a relatively low level of continuous pasture exposure to *O. ostertagi*. Nevertheless, the proportion of *O. ostertagi* and *C. oncophora* in HP were in line with previous studies in Sweden (Dimander et al. 2003).

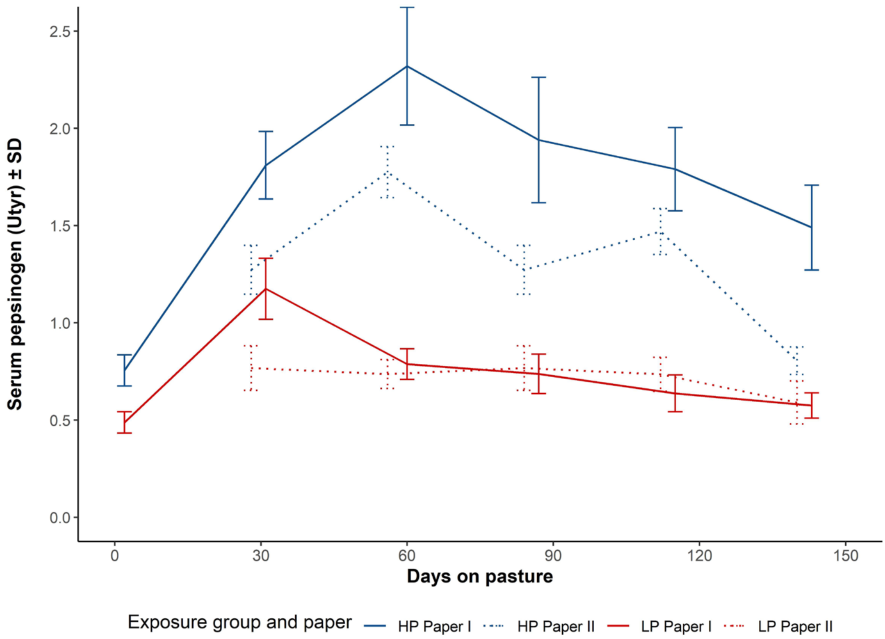


Figure 8. Mean serum pepsinogen (international units of tyrosine) ± SD in two exposure treatments of first season grazing steers during two years. One treatment (HP) was primed at turn-out with, I) ≈ 5000, II) ≈ 10000, infective third stage larvae of *Ostertagia ostertagi* and *Cooperia oncophora* (1:1) and thereby exposed to a high parasite challenge, whereas the other treatment (LP) was dewormed with ivermectin (0.5 mg kg^{-1}) monthly, thus being exposed to a lower parasite challenge.

Corresponding differences in BWG between LP and HP groups were only observed in Paper I ($P = 0.037$), but not in Paper II ($P = 0.97$). The difference in body weight (Fig. 9) at turn-out, with animals in Paper I having a higher start weight compared with Paper II, can be explained by the age difference of the experimental animals at the start of the experiments between the two studies. Subclinical GIN infections in Sweden have repeatedly been associated with decreased BWG in similar trials (Dimander et al. 2003; Larsson et al. 2007; Höglund et al. 2013b). In Paper I, animals exposed to higher levels of GIN had a 15% lower DWG (542 ± 29 g) compared with animals exposed to low levels of GIN (636 ± 29 g). In contrast, no difference in DWG was observed between animals exposed to high levels of GIN (283 ± 13 g) and animals exposed to low levels of GIN (281 ± 13 g), in Paper II. The initial decrease in body weight seen during the first two weeks, in both Paper I and II, is primarily associated with a reduce in feed intake and change in rumen content, rather than a reduction in body mass (Tayler et al. 1957). Performance in grazing cattle is a complex interaction between, among other, genetics and pasture availability and quality (Lawrence et al. 2012). The low weight gains in Paper II could be explained by severe drought during the summer of 2018 (SLU Fältforsk 2020) and lower nutrient concentration in the herbage, compared to previous studies at the same pasture (Höglund et al. 2013b), outweighing a possible detrimental effect from parasite infection.

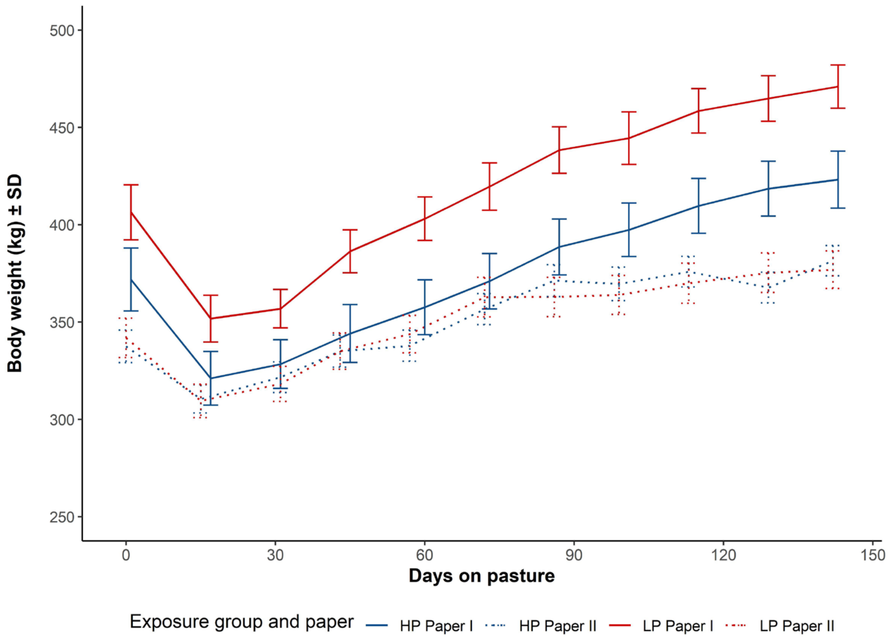


Figure 9. Mean body weight ± SD in two exposure treatments of first season grazing steers during two years. One treatment (HP) was primed at turn-out with, I) ≈ 5000, II) ≈ 10000, infective third stage larvae of *Ostertagia ostertagi* and *Cooperia oncophora* (1:1) and thereby exposed to a high parasite challenge, whereas the other treatment (LP) was dewormed with ivermectin (0.5 mg kg^{-1}) monthly, thus being exposed to a lower parasite challenge.

In summary, we detected effects on the standard diagnostic indicators FEC, SPC and BWG in animals exposed to higher levels of GIN, with the exception of BWG in Paper II. Clinical signs of parasitism were not observed in any of the papers. Although no differences in BWG was observed in Paper II (Fig.9), the findings indicates either a low level of parasite exposure in HP, and/or that that the full growth potential of the animals in the treated group was not fully exploited. Furthermore, the differences in both FEC and SPC clearly indicates a difference in parasite exposure levels between exposure groups, especially since FEC reflects infection levels in cattle during the first two months of exposure (Ploeger, et al., 1994). Together, this implies a subclinical course of disease in animals exposed to a higher level of GIN, in both papers.

5.1.2 Sheep

In Paper IV, strongyle nematode eggs were observed in both groups three weeks prior to weaning. However, FEC were significantly higher in HP ($P <$ 0.0001) compared with LP throughout the study. The highest FECs, in HP (646 ± 412 EPG) and LP (365 ± 250 EPG), respectively, were observed 21 days prior to weaning. The molecular investigation showed that animals were predominantly infected with *Teladorsagia* spp., combined with low proportions of *Haemonchus* spp. (0-15 ± 19%). This was in contrast to a recent study of the nemabiome composition in Swedish sheep by Halvarsson and Höglund (2021) where *H. contortus* was the predominant species. *Teladorsagia* was more abundant in both experimental groups, appearing at day -21, and initially contributing to a high proportion of eggs shed. From then on, *Haemonchus* was present in both groups, but at a higher rate in HP compared with LP animals. The relatively low EPG levels in combination with predominantly low proportions of *Haemonchus*, indicates moderate infections also in HP animals. There are no publications on the seasonal dynamics of EPG in lambs in Sweden. However, according to Halvarsson and Höglund (2021) EPG values varied between approximately 100 to 1500 in 61 commercial sheep flocks. The FEC in sheep is to a higher extent dependent on species composition, compared with cattle. This is especially true regarding *H. contortus,* as it is highly fecund (Sutherland & Scott 2010).

Differences in BWG ($P < 0.0001$) were observed throughout the study period and were on average 19% higher in LP (365 ± 5 g) compared with HP

(304 ± 6 g) from three weeks prior to weaning until seven weeks post weaning.

Like in the cattle trials, no clinical signs of parasitism were observed. Together, the differences in FEC, nematode composition and differences in BWG implies a moderate subclinical course of disease also in HP.

5.2 The effect of GIN infection on activity (Papers I, II, IV)

To evaluate activity patterns, two types of commercially available sensors were used. In Paper I, IceTag® 3D-accelerometers were used, whereas IceQube® 3D-accelerometers were used in Papers II and IV. The main difference consists of the sensors using a different sampling rate, with IceTag® recording at 16 Hz compared with IceQube® that records at 4 Hz. This may affect the output and interpretation of the data as discussed below. Moreover, the internal memory differs resulting in differences in recorded data length. However, the data output and time resolution used in Papers I, II and IV correspond and enables comparisons. In addition, Heatime® activity and rumination collars were used in Paper II to measure general activity and activity change.

In Paper I we predicted that steers exposed to a higher level of GIN would have a longer daily lying time, take fewer steps, have a lower motion index and have fewer lying bouts, compared with dewormed steers. In contrast, based on the results in Paper I, we predicted that the number of lying bouts would increase in steers exposed to a higher level of GIN, in Paper II. In Paper IV, we predicted that motion index and number of lying bouts would decrease whereas lying time would increase in lambs exposed to a higher level of GIN.

5.2.1 Cattle

There was an interaction effect of exposure level and period on average daily lying time (Paper I: $P < 0.0001$; Paper II: $P < 0.0001$) (Fig. 10). However, HP steers in Paper I had a shorter daily lying time compared with LP animals, whereas HP had a longer daily lying time in Paper II. It should be noted that the largest difference in lying time in Paper II was seen during the first 40 days ($P = 0.037$), whereas the same pattern was not observed in Paper I. The lying time also differed between the trials, with steers in Paper I having a longer daily lying time in comparison to steers in Paper II. However, this was not tested statistically, as comparisons are difficult, as the resolution of data differed between the trials. This underlines that it is hard to determine what a *normal* behaviour is, as outlined in section 2.3.1. There was also a tendency ($P = 0.069$) of exposure level and period effect on diurnal lying time patterns in Paper I. Although it is hard to generalize how the lying time in cattle is affected by the exposure to subclinical levels of GIN, our results confirm the manifestation of sickness behaviour.

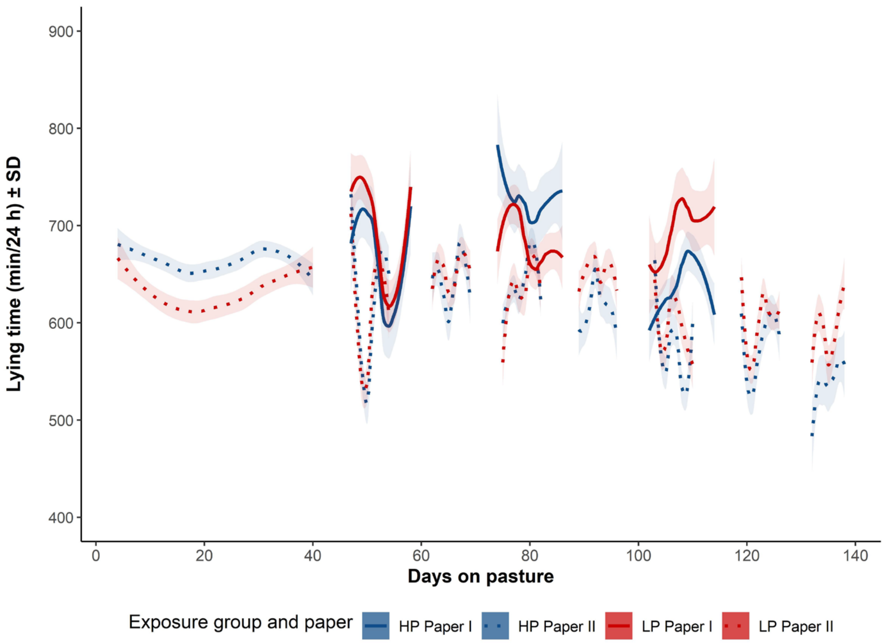


Figure 10. Average daily lying time ± SD in two exposure treatments of first season grazing steers during two years. One treatment (HP) was primed at turn-out with, I) ≈ 5000, II) ≈ 10000, infective third stage larvae of *Ostertagia ostertagi* and *Cooperia oncophora* (1:1) and thereby exposed to a high parasite challenge, whereas the other treatment (LP) was dewormed with ivermectin (0.5 mg kg^{-1}) monthly, thus being exposed to a lower parasite challenge.

It can be argued that the behavioural responses may be connected to the mucosal phase of *O. ostertagi*. As discussed in section 5.1.1, the infection levels were low in both studies and the responses in FEC and SPC reflect the initial response to the priming of the HP groups at turn-out. Upon ingestion the L3 of *O. ostertagi* enter the gastric glands after 6 six hours, resulting in swelling and oedema after 12-24 hours (Osborne et al. 1960). In FSG they then leave the mucosa following moulting to L4, after approximately three weeks and with the majority of glands in the fundic region of the mucosa normalising after seven weeks. Therefore, the longer lying time the first 40 days, in HP in Paper II, more likely reflects a behavioural response in connection to the priming rather than a more gradual pasture exposure. This could also explain the discrepancy of effect on lying time between the papers.

There was an interaction effect of exposure level and period ($P < 0.0001$), as well as a pairwise difference ($P = 0.038$) between exposure groups day 74-86, on average number of daily lying bouts (Fig. 11) in Paper I, with HP animals conducting more lying bouts than LP. Likewise, there was a similar pattern on the diurnal pattern of lying bouts, with an interaction effect of exposure level and period ($P < 0.0001$), and a pairwise difference ($P = 0.0035$) between exposure groups day 74-86, with HP animals conducting more lying bouts than those in LP.

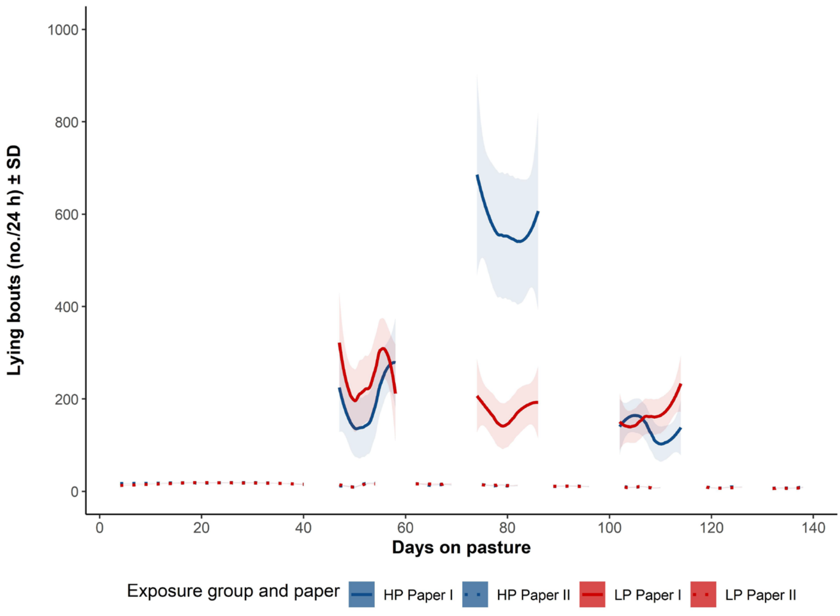


Figure 11. Average number of daily lying bouts ± SD in two exposure treatments of first season grazing steers during two years. One treatment (HP) was primed at turn-out with, I) ≈ 5000, II) ≈ 10000, infective third stage larvae of *Ostertagia ostertagi* and *Cooperia oncophora* (1:1) and thereby exposed to a high parasite challenge, whereas the other treatment (LP) was dewormed with ivermectin (0.5 mg kg^{-1}) monthly, thus being exposed to a lower parasite challenge.

In contrast, for the number of lying bouts no such differences were observed in Paper II. The increase in lying bouts observed at day 74-86 in Paper I probably reflects what can be seen as *false* lying bouts (Ungar et al. 2018), i.e. leg movements trigger the recording of a lying bout rather than a change in posture.

There was an interaction effect of exposure level and period ($P < 0.0001$) on the average number of daily steps (Fig. 12) as well as on the diurnal pattern of steps ($P < 0.0001$), in Paper I. In contrast, there was only a tendency ($P = 0.057$) of difference in average daily number of steps between exposure groups in Paper II. However, there was a highly significant difference in recorded number of steps ($P < 0.0001$) day 62-69, with steers in HP taking 913 ± 148 more steps per day than those in LP.

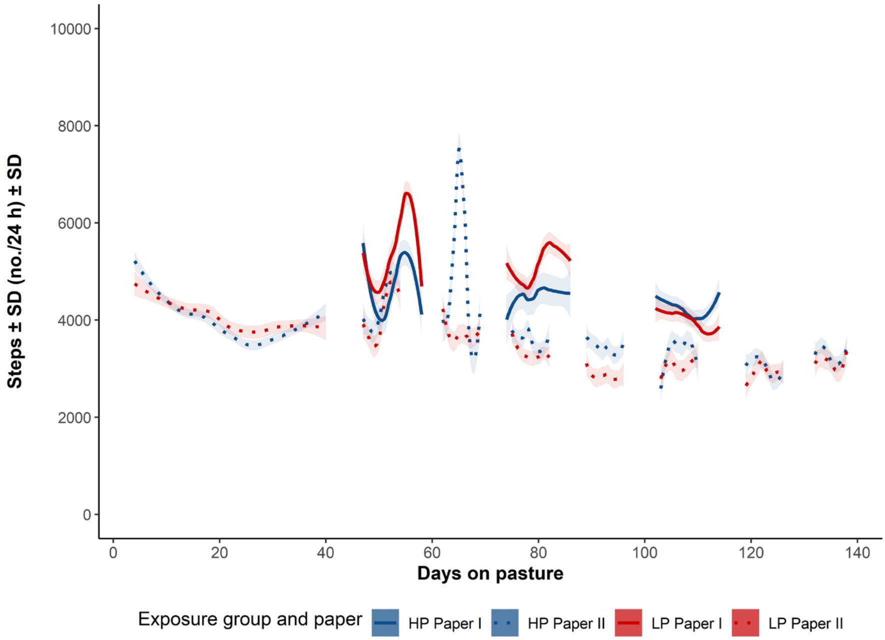


Figure 12. Average number of daily lying bouts ± SD in two exposure treatments of first season grazing steers during two years. One treatment (HP) was primed at turn-out with, I) ≈ 5000, II) ≈ 10000, infective third stage larvae of *Ostertagia ostertagi* and *Cooperia oncophora* (1:1) and thereby exposed to a high parasite challenge, whereas the other treatment (LP) was dewormed with ivermectin (0.5 mg kg^{-1}) monthly, thus being exposed to a lower parasite challenge.

In line with the high number of recorded lying bouts day 74-86 in Paper I, this probably reflects an increase in leg movement, possibly abdominal kicking, rather than an increase in locomotor movement (*i.e.* a leg movement used to move from one place to another).

Moreover, there was an interaction effect of exposure level and period ($P < 0.0001$) on average daily motion index (Fig. 13) as well as the diurnal pattern of motion index ($P = 0.006$) in Paper I. The motion index measures the total movement of the sensor and this finding indicate that HP steers had a generally lower activity compared to dewormed animals.

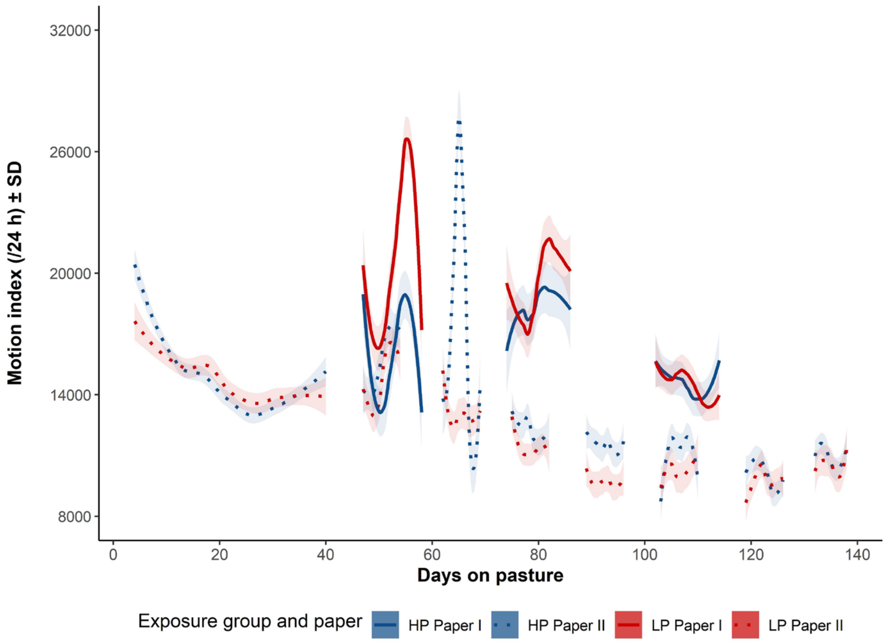


Figure 13. Average daily motion index ± SD in two exposure treatments of first season grazing steers during two years. One treatment (HP) was primed at turn-out with, I) ≈ 5000, II) ≈ 10000, infective third stage larvae of *Ostertagia ostertagi* and *Cooperia oncophora* (1:1) and thereby exposed to a high parasite challenge, whereas the other treatment (LP) was dewormed with ivermectin (0.5 mg kg^{-1}) monthly, thus being exposed to a lower parasite challenge.

In contrast, in Paper II there was a tendency ($P = 0.051$) of parasite exposure effect on average daily motion index, with a highly significant ($P < 0.0001$) difference day 62-69. HP steers were showing a daily increase in motion index with 3148 ± 539 compared with those in LP. On the other hand, in Paper II, no such effect of parasite exposure was observed on activity (P = 0.86), or activity change (P = 0.49). Still, an interaction effect (P = 0.0009) on activity and a tendency (P = 0.065) of a difference in activity change between the two parasite exposure levels was recorded during the first 40 days. The increase in motion index in HP day 62-69 are in line with the corresponding increase in number of recorded daily steps and, as stated earlier, probably reflects an increase in leg movement rather than an increase in general activity. This is further highlighted by the results that no difference in activity (Heatime®) was observed during the same period.

Pearson's correlations coefficients between the mean activity measures and associated EPG and SPC were calculated in Paper I. Only a moderate positive correlation (r = 0.55) between SPC and number of lying bouts at day 74-86, coinciding with the highest SPC levels observed (see section 5.1.1), was found. This further indicates that the behavioural response may be connected to the mucosal phase of *O. ostertagi* and the associated inflammatory response. However, combined these findings indicates that the use of behaviour responses to predict parasite fecundity resulting in pasture contamination, probably is limited.

5.2.2 Sheep

There was an interaction effect of parasite exposure level and period (P = 0.0013) on average daily lying time. In contrast to the observations in cattle (Paper II), HP lambs had a 10 ± 31 min shorter daily lying time compared with LP. The lambs were primarily infected with *T. circumcincta* that inhabit the same niche in sheep as *O. ostertagi* in cattle, with similar pathological effects on the host (Sutherland & Scott 2010). Steers in Paper II, were primarily exposed to *O. ostertagi* and *C. oncophora* and were, in contrast to the lambs in Paper IV, primed at turn-out. Moreover, HP steers had a longer daily lying time compared with LP steers. This underlines the possibility of a connection between nematode induced mucosal damage in the abomasum and deviation in host activity patterns. However, it also shows that the deviations in host activity patterns may vary between species.

Moreover, there was an interaction effect of exposure level and period (P = 0.0001) on average daily motion index with lambs in group HP having a lower average daily motion index of 567 ± 539 compared with LP. This is in agreement with the findings of Ikurior et al. (2020), that also detected a reduced activity in sheep challenged with subclinical levels of GIN.

Finally, the number of recorded lying bouts was not affected by GIN exposure (P = 0.51) or exposure by period interaction (P = 0.82).

5.3 The effect of GIN infection on rumination (Paper II)

To evaluate rumination patterns in Paper II, Heatime® activity and rumination collars were used. We predicted that steers exposed to a higher dose of GIN would show a decrease in rumination time.

There was no effect of GIN exposure level on average daily rumination time (P = 0.68) or average daily rumination change (P = 0.45). However, there was an interaction effect of exposure level and experimental day, on both rumination (P < 0.0001) and rumination change (P = 0.0008) during the first 40 days. Steers in HP showed a larger variation (0.25 ± 21.9) in rumination change compared with those in LP (-1.31 ± 20.4). This is in contrast to the results of Forbes et al. (2007), where FSG cattle challenged with *O. ostertagi* and *C. oncophora* did not show any difference in rumination time compared with dewormed animals during two 24 hour measurements, 14 weeks after turn-out. The reason for this discrepancy is unclear. Forbes et al. reported a mean FEC of 125 EPG that is comparable to the observed FEC in Paper II. However, in the study by Forbes rumination was only recorded for two days, and the number of animals was smaller than in our study where differences was observed during the first 40 days of grazing. Moreover, different types of sensors were used. Nevertheless, this discrepancy may indicate that the effects are more distinct in connection to the early exposure of parasite naive animals.

5.4 Sensor performance (Paper III)

The objective of Paper III was to assess the validity of two commercially available sensors, i.e. IceTag® and IceQube®, in lambs, by comparison to video observations. We predicted to find a good agreement for standing and lying time. However, it was hypothesised that less accurate agreements would be found for step count and the ability to detect the start of lying bouts.

Based on the interpretation of Bland-Altman comparisons, both sensors can be used to record standing and lying times. Moreover, the PPV, sensitivity and specificity of the IceTag® compared to video recordings, per second, for standing and lying were all higher than 92%. The results are in line with those of Trénel (2009) on dairy calves, where the IceTag® showed a sensitivity and specificity of 0.99 and 0.98 for lying and 0.92 and 0.98 for standing, respectively. Moreover, results of Finney et al. (2018) showed that IceQubes® are reliable for measuring standing and lying time in dairy calves. The majority of errors observed when the animal was in an upright position but scored as lying by the logger. These were mainly caused by a shift of angle of the sensor when animals raised their legs in a horizontal position, e.g. when scratching or grooming. These finding are in line with those of Tolkamp et al. (2010), used on adult cattle. However, no systematic error was observed, and it is therefore reasonable to argue that both sensors can provide reliable standing and lying time in lambs.

IceTag® showed a poor average PPV of 55%, but a higher average sensitivity of 93%, for recorded lying bouts. IceQube® showed a higher average PPV of 94%, but a somewhat lower average sensitivity of 90%, for recorded lying bouts. The performance of IceTag® increased for the computed indications LB_{10}, with average PPV being 92% and average sensitivity being 100%, with an average loss of only 0.3% of true lying time. Moreover, the performance improved further using the computed indication LB_{30}, with average PPV and sensitivity being 100%. However, this filter resulted in a 1% loss of true lying time. Similarly, the performance of IceQube® increased with the computed indications LB_{10}, with an average PPV of 99% and sensitivity of 100%, respectively, with a 1.7% average loss of true lying time. The performance improved further using the computed indication LB_{30}, with an average PPV and sensitivity of 100%, respectively, but with a 2.6% loss of true lying time. Different thresholds have been tested and suggested for cattle (10 s and 60 s, respectively) (Ungar et al., 2018) and dairy calves (5 s) (Trénel et al., 2009). The shift of angle of the sensor, described above, may also record a lying bout generating a false measurement. This is underlined by the increase in performance when filtering computed indications of lying bouts of durations > 10 s and > 30 s, respectively, implying that animals are unlikely to have shorter lying bouts. Even though such filters generates a loss of true lying time, the results suggest that excluding lying bouts shorter than 30 s should be undertaken to increase sensor performance.

Based on Bland-Altman comparisons for both sensors, no agreements between video recordings and sensor recordings could be found for step count and therefore, none of the sensors should be used for step count recording. The results indicate that both sensors underestimate the step counts. In addition, proportional errors were observed, with differences between sensor and video observation data increasing with the number of steps taken. This indicates that the acceleration threshold needed to record a step is not met for all locomotor steps and is probably due to the smaller size of lambs, than of adult dairy cattle.

The lambs included in the validation study weighed from 20 kg and was adapted after the weaning weights in Sweden so that the sensor could be used in Paper IV. However, it is not recommended to use neither of these sensors on lambs lighter than 20 kg.

6. General discussion

All grazing ruminants are exposed to a community of GIN resulting in a high prevalence of, usually, subclinical disease (Charlier et al. 2014a, 2020b; Zajac & Garza 2020). However, the infection levels are not normally distributed within a herd and instead tends to be overdispersed, with a few animals in a group carrying the majority of parasites (Genchi et al. 1989; Sréter et al. 1994; Warburton & Vonhof 2018). Additionally, parasite naive first season grazing animals, that not yet have acquired immunity, are more susceptible to infection and disease (Nansen et al. 1989; Sutherland & Scott 2010; Besier et al. 2016).

Sickness behaviour (a deviation from normal behaviour seen in connection to disease, see section 2.3.2) has been linked to various diseases, including both bacterial and viral infections, metabolic disorders and mechanical injuries such as lameness, in livestock. Most studies have been conducted on housed dairy cattle and for clinical diseases with a, in comparison to subclinical GIN infections, low prevalence within the group.

There is currently a limited knowledge regarding behavioural responses to GIN infection in grazing livestock, especially when exposed under longer periods on pasture (see section 2.4.3). Nevertheless, behavioural monitoring has been suggested as a possible method to detect subclinical parasite infection in grazing livestock (Vercruysse et al. 2018).

Within this section, the most important findings in papers I-IV are generally discussed, as well as some theories of the underlying causes of the behavioural responses observed in connection to GIN infections.

6.1 The effect of GIN infection on activity

Although it is hard to generalise how the lying time in cattle is affected by a GIN infection, the results in Paper I, II and IV, demonstrated that animals challenged with a higher level of GIN exhibits a lower overall activity than treated animals accordingly exposed to a lower level of GIN. The association between behavioural change and proinflammatory cytokines have mainly been linked to the fact that fever and sickness behaviour are simultaneously occurring phenomena (reviewed by Dittrich et al. 2019). Even though GIN infections mediate both inflammatory and cytokine responses in livestock, they usually do not induce fever (Claerebout et al. 2005; Miller & Horohov 2006; Craig et al. 2007). Moreover, the role of cytokines in the behavioural response to GIN infection has not been fully investigated. Irrespectively, behavioural alterations observed in connection with disease are thought to protect the individual energy reserves, which are need to manage the ongoing health impairment (Tizard 2008; Hart & Hart 2019). Therefore, the decrease in activity and increase in resting behaviour seen in HP groups can arguably be seen as a response from the host to conserve energy and thereby manage the ongoing infection. It should be noted that resting is, irrespective of disease, a high priority and an inelastic behavioural need in ruminants (Jensen et al. 2005; Jørgensen et al. 2009). In Paper IV, lambs challenged with a higher level of GIN had a shorter daily lying time compared with dewormed animals. The reason for this deviation from sickness behaviour is not fully understood, although similar discrepancies has been observed in different welfare challenges in sheep. For instance, an observed reduction in lying time has been suggested as an indicator of pain after castration (Thornton & Waterman-Pearson 2002). Moreover, sheep infected with sheep scab (*Psoroptes ovis*) has been observed to exhibited a reduced total lying time, interpreted as a response to skin lesions and associated itch (Berriatua et al. 2001). As covered in section 2.3.1, lying behaviour is affected by several factors, and it is therefore hard to draw conclusions from one study. Therefore, whether different behavioural responses are induced in cattle and sheep following GIN exposure needs to be further investigated.

Postural synchrony, in which animals lie down or stand up at the same time as other animals within their herd or group, occurs in both cattle and sheep at pasture (Benham 1982; Gautrais et al. 2007; Stoye et al. 2012). Stoye et al. (2012) reported that 70% of cattle within a group exhibited the same posture over 93% of the time. Corresponding amounts of synchrony in sheep, has been reported to range from 60 to 80% (Gautrais et al. 2007). However, the degree of synchrony can vary with group composition (Conradt 1998; Ruckstuhl & Neuhaus 2002) and time of day, where cattle are least synchronized in the middle of the day and most synchronized in the morning and evening (Stoye et al. 2012). The mechanisms underlying such synchrony are poorly understood (Estevez et al. 2007), but there are at least two different ways in which synchrony may arise. One is through concurrent responses, where animals makes its own decision to stand up or lie down at the same time as other individuals within the group, due to internal factors (such as a similar need for food or daily rhythms) or external factors (such as arrival of food or the presence of predators) (Stoye et al. 2012). Moreover, cattle show more postural similarity with their nearest neighbours than with a random member of the group. This suggests that the synchronisation of the group includes active collective, allelomimetic, behaviour, i.e. the performance of a behaviour increases the probability of that it is being performed also by other nearby animals (Stoye et al. 2012). As behaviour, including foraging and resting cycles, may be affected by disease, it is arguable that postural synchrony may be interlinked with sickness behaviour. If the prevalence of a disease is low in the group, it can be expected to have a low effect on the synchrony of the group, whereas if the prevalence is high within the group, as in the case with GIN infection, it will have a higher impact on the synchrony of the group. Provided that the sickness behaviour is induced at a relatively low level of infection.

The same pattern of effect may apply to the severity of the disease. It can be expected that a disease with a severe and acute onset will affect the individual animal more compared to a subclinical disease, such as GIN infection, and thereby affect the individual's synchrony with the group to a larger extent. Therefore, both the expected prevalence and the severity of disease needs to be taken into account, when assessing behavioural discrepancies linked to sickness behaviour, especially regarding animals on pasture. This is especially important when the interpreting behavioural responses to GIN infections, as it tend to be overdispersed. However, the general knowledge regarding this connection is limited, and needs to be further investigated.

6.2 The effect of GIN infection on foraging

It has been shown that GIN infections reduce the voluntary feed intake and efficiency of feed utilisation in both cattle and sheep (Fox et al. 1989; Coop & Holmes 1996), and decrease the grazing time in FSG cattle (Forbes et al., 2000). Young dairy heifers treated with eprinomectin had 30% longer daily grazing time than control animals infected with GIN (Forbes et al. 2007). In sheep, Hutchings et al. (2000) observed that parasite infected animals had a 31% reduction in feed intake, which was associated with 40 min less grazing time per day, compared with dewormed animals.

There are two aspects regarding the grazing behaviour that are interesting in connection to GIN infection in livestock ruminants, 1) reduction in appetite and feed intake, as it relates to performance, and 2) faecal avoidance, as it relates to herbage infection and acquisition of GIN infection (Forbes 2021). In connection to 1) reduction in appetite and feed intake, the Heatime® sensors used in Paper II are not validated for the measurement of grazing time. Therefore, only rumination and rumination change were assessed. It has been suggested that cattle can compensate a decrease in eating time with an increase in rumination time (Dado & Allen 1994), and thus rumination time could be affected by GIN infection. However, the compensation is also affected by the fibre concentration in the feed. Thus, a higher negative correlation is expected at semi-natural pastures than on leys. However, this cannot explain the differences between the results in Paper II and those of Forbes et al. (2007). In the study of Forbes et al. (2007), animals were kept on grass sward that enables a higher compensation in rumination time than on a semi-natural pasture. Therefore, the effect of GIN on rumination patterns, at different time points during the grazing season and under different pasture conditions, needs to be further explored. Moreover, there is a normal animal variability of daily rumination time, where the coefficient of variation has been reported to vary between 16% (Dado & Allen 1994) and 48% (Byskov et al. 2015). With this in mind, the effect on rumination change underlines that also individual variations need to be taken into account when evaluating behavioural changes connected to sickness behaviour. This is important as it is known that GIN are overdispersed in their hosts, with a few animals in a group carrying the majority of parasites and where the impact is dose dependent (Charlier et al. 2020b). However, even though the results in Paper II show promise in the use of rumination as an indicator of GIN infection, more studies are needed on the effect of GIN on all foraging behaviours, including rumination and eating time.

There is also a possible link between activity patterns and foraging behaviour that needs to be further investigated. It can be speculated that animals with a decrease in grazing time will exhibit a decrease in general activity, because grazing involves movement in connection with animals walking between different parts of the pasture to graze. At the same time, it can be speculated that the idle time, including lying time, will increase in these animals. However, a reduced time spent grazing could also result from altered behaviour linked to faecal avoidance (Hutchings et al. 2000) or increased diet selectivity (Kyriazakis et al. 1996), and therefore not decrease the activity patterns *per se*.

6.3 Complexity of behavioural responses

As mentioned in section 6.2, most behavioural alterations in connection with disease are thought to protect the individuals' energy reserves. In contrast, we observed an increase of average number of lying bouts at day 74-86 in Paper I, and a similar increase in number of steps at day 62-69 in Paper II, in steers challenged with a higher level of GIN. Comparable discrepancies from sickness behaviour in housed dairy cattle with mastitis has been interpreted as a response to soreness and pain and could therefore be important from a welfare point of view (Fogsgaard et al. 2015).

Signs of colic, although rare in cattle, include kicking at the abdomen (Belknap & Navarre 2000). However, kicking was not included as a sign of pain in The Cow Pain Scale (Gleerup et al. 2015), since it was not observed during the development of the method. Still, an increase in Visual Analog Scale, a subjective method to evaluate pain in animals by observing belly kicking and foot stomping among others, has been observed in untreated castrated calves but not in those treated with meloxicam (Olson et al. 2016). However, the behavioural responses, observed in Papers I and II, are difficult to interpret in terms of relative pain without further investigation.

It has been described that a complexity of behavioural patterns viewed over time, may be considered as an indicator of stress as a result of health and welfare challenges (Alados et al. 1996; Rutherford et al. 2004). As an example, sheep naturally infected with strongylid nematodes exhibited a smaller behavioural complexity compared with dewormed sheep (Burgunder et al., 2018), providing evidence for the possibility to use behavioural observations as a method for welfare monitoring of GIN infected animals. Moreover, housed young cattle challenged with a single dose of 200 000 L3 *O. ostertagi* showed an increase in lying bout length, suggesting a decrease in behavioural complexity (Szyszka et al. 2012). However, these results are in contrast to those in Paper I and II, where an increase in leg movements were seen day 74-86 and 62-69, respectively. It has been suggested that experienced pain or discomfort can override the motivational state of the animals' sickness behaviour (Siivonen et al. 2011). Therefore, the deviation from sickness behaviour and behaviour complexity may be a result of pain or a general feel of discomfort. However, this needs to be further studied before causality between subclinical GIN infection and pain or discomfort responses can be concluded. Still, the results presented in Papers I and II suggests that the new analytical tools used herein are useful and can be further explored when analysing behavioural responses to GIN infection.

6.4 Implementation into a T(S)T system

The principal aim of anthelmintic treatment is to decrease pasture contamination with nematode eggs and thereby prevent clinical and/or subclinical disease (as covered in section 2.2.2). The results in Papers I, II and IV, suggests possible connection between nematode induced mucosal damage in the abomasum and deviation in host activity patterns. However, the findings showed weak associations between the behaviour responses and FEC levels. It could therefore be argued that the use of behavioural monitoring in a T(S)T-system would be of limited value. Nevertheless, the results in Papers II and IV, indicates that the host behavioural response were connected to the damage caused by the parasites late larval stages, preceding the adult fecund stages resulting in egg shedding. This could therefore enable an early treatment and potentially decrease pasture contamination, and thereby in the long term contribute to improved animal health and productivity. In comparison, the association between FEC and BWG is inconsistent in both cattle (Zapa et al. 2020) and sheep (Sweeny et al. 2011; Sargison 2013). Still, TST strategies based on weight gain have proven to be effective at reducing anthelmintic usage whilst still maintaining animal performance in both cattle (Höglund et al. 2013b) and sheep (Greer et al. 2009). However, none of these TST strategies resulted in a reduced pasture contamination. In case it turns out that it is possible to identify severely parasite infected animals at an early stage with sensor technologies, this is of course a preferable diagnostic tool.

A key step for the implementation of all T(S)T-strategies is determining a suitable threshold level for treatment. The production-based indicators in cattle and sheep, mentioned above, both used predefined thresholds for weight gains. However, as pointed out by Höglund et al. (2013b), suboptimal performance can be caused by many factors, parasitism being just one. This therefore prevents the establishment of universal thresholds. The same probably apply for behavioural responses in relation to GIN infection, as normal behaviour varies depending on a variety of factors, as outlined in section 2.3.1 and 5.2.1, including individual variation. This aspect clearly needs to be further investigated before incorporating automatic behavioural monitoring in a TST system. Nevertheless, several commercial sensor systems (Caja et al. 2016, 2020) have health warnings, where the user is provided an early warning when the system detects a deviation in for example activity. However, it should be noted that these are generally not fully transparent due to intellectual property rights. González et al. (2008) have suggested that changes of 2.5 times the standard deviations from the previous seven day rolling average may have a diagnostic value. In a farm setting, this allowed the detection of more than 80% of cows with acute disorders, such as ketosis and lameness, at least one day before diagnosis by farm staff. However, if this could be used as a threshold also for subclinical GIN infections remains to be investigated.

Finally, the results in Paper II, where differences were observed with both sensor systems, indicates that there may be a possibility to combine data and thereby possibly improve the detection of parasitic diseases. Studies on dairy cattle have showed that the sensitivity for disease detection, including mastitis, metritis and metabolic and digestive disorders, increases when rumination and activity data are combined into a health score system (Stangaferro et al. 2016a; b; c). This shows that the combination of different behavioural data in response to GIN infection must be evaluated. It should be noted that several commercial sensor systems already combine different type of data for health scoring and oestrus detection (Caja et al. 2016). Moreover, the development of sensor systems is an ongoing process and the possibilities to evaluate and combine different types of data will increase.

A limitation in the interpretation of sickness behaviour is that the changes in behaviour are very general and tend to occur irrespectively of the underlying disease. It can therefore be a challenge to link mentioned deviations to a specific health disorder, such as GIN infection. It may be necessary to include other information about the herd, such as production parameters and health records, to be able to determine the cause of deviation (Dittrich et al. 2019). Therefore, more studies regarding specific host responses to different diseases, including GIN infection, are needed.

6.5 Concluding remarks

During the work of this thesis, we have provided new insights in behavioural responses to GIN infections as well as the use of sensors to monitor livestock on pasture. The main conclusions are:

- Behavioural measurements, including lying time, number of lying bouts, step count, motion index, rumination and activity, are affected by subclinical GIN infections and the results demonstrate the potential use of automated behavioural observations as a diagnostic tool.

- Both FSG steers and lambs infected with GIN exhibited sickness behaviour with a decrease in activity in early infection.

- The host response in form of activity change may differ both between host and parasite species. This emphasises the need for species-specific interpretation of associated sickness behaviour for different host-parasite relationships.

- The difference of both activity changes and rumination changes in steers underlines that individual variations need to be taken into account when developing and evaluating PLF-systems for parasite surveillance. The determination of thresholds should therefore be based on individual variation.

- The molecular investigation showed that both hosts were predominantly infected with abomasal nematodes. It is therefore arguable that behavioural differences observed may be linked to mucosal damage connected to these nematodes.

- Sensors developed for cattle can be used for activity monitoring in lambs after assessing the validity. However, measurements, such as step count, that is adapted after the size of the animal, are limited.

7. Future perspectives

Even though the knowledge regarding behavioural responses to GIN infection in grazing livestock and the use of sensors to monitor livestock on pasture has increased, there are still many questions that remain and that should be explored in future studies.

The long-term aim is (if feasible) to implement the use of continuous behavioural monitoring, enabling real-time warnings of GIN infection as a part of a T(S)T regime. During the work of this thesis, we investigated the behavioural response to GIN infections on a contrasting group level. Therefore, there is a need to further investigate:

- If it is possible to determine individual responses within a grazing group with a normal infection dynamic, i.e. not compared to contrasting dewormed animals.
- How different infection levels and nematode compositions affects the behavioural response. This is especially important for highly pathogenic species, such as *H. contortus*.
- How reoccurring larval challenges affect long-term activity and rumination patterns.
- If grazing time and patterns are affected over time in cattle and therefore could be used as an indication of infection.
- If grazing and rumination patterns are affected in sheep.

- How the level of a behavioural response should be interpreted, i.e. if there is a correlation between the infection level and behavioural responses and how a threshold level indicating infection could be determined.

- If the combination of different sensor data, i.e. activity and foraging behaviour, can improve the detection of infection.

- If behavioural responses normalises after deworming, enabling evaluation of treatment success.

Finally, if these questions, among others, can be considered to have been answered, such a system must then be tested and evaluated on farm level. This includes evaluating if it can yield individual benefits to animal health, welfare and production, and at the same time decreasing the total use of anthelmintics, thereby lowering the risk for the development of anthelmintic resistance.

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Popular science summary

Parasitic roundworm infections are common in grazing ruminants worldwide and affects livestock production negatively. All farmed ruminants kept on pasture are exposed to these parasites. Symptoms of clinical disease can include watery diarrhoea, reduced appetite, weight loss and dull hair coat. However, an infection without clinical symptoms, that only affects production and weight gain, is more common today. The control of parasite infections at pasture is primarily based on the use of dewormers at group level. Misuse of these drugs has led to widespread development of drug-resistant worm populations. Targeted selective treatment, where individual animals within a grazing group are dewormed based on for example performance or sickness indicators, have been proposed as a sustainable strategy to yield benefits to animal health and welfare. This will also decrease the risk for overuse of deworming drugs and subsequent resistant worms.

Traditionally, the diagnosis of roundworm infections is usually based on the detection of roundworm eggs in faeces. However, this method is not practically feasible on an individual level. Sickness behaviour is a normal response to disease and include a set of behavioural changes that occur in physically ill animals. These behaviours include lethargy and a loss of appetite, among others. Sickness behaviour has been suggested as an applicable indicator for monitoring disease in animals. Deviating foraging behaviour and general activity has been showed to provide information about an animals' health and welfare status. The advancement of so called precision livestock farming, a collective name for technologies used to continuously monitor the status of animals and their environment, enables real-time monitoring of such behaviours and could potentially be utilized as an indicator of roundworm infections in livestock. However, the knowledge of behavioural responses in relation to roundworm infections in grazing livestock is today limited. Therefore, this needs to be investigated further before it can be integrated in parasite management at farm level.

The aim of this thesis was to increase the knowledge on how roundworm infections affects activity and rumination patterns in grazing livestock. The long-term objective was also to assess if the use of behavioural monitoring using sensors has the potential to be integrated into future parasite control programs. Three infection experiments, including two in cattle and one in sheep, were carried out to assesse if real-time monitoring of animal activity could be used as an indicator of subclinical roundworm infection.

The results suggest that even low levels of gastrointestinal parasite infection affect the activity of livestock. Both steers and lambs infected with roundworm parasites exhibited sickness behaviour with a decrease in general activity compared with dewormed animals. Steers exposed to roundworm infection had an increased daily lying time early in infection, compared with dewormed animals. In contrast, exposed lambs had a decreased daily lying time. Moreover, roundworm infection also affected rumination patterns over time in exposed steers.

In conclusion, the results suggest that even low levels of gastrointestinal parasite infection affect the activity of both cattle and sheep. This in turn shows that there is a potential that sensor based behavioural recordings could be integrated into sustainable parasite control programs.

Populärvetenskaplig sammanfattning

Mag-tarmparasiter (rundmaskinfektion) är vanliga hos betande idisslare över hela världen och är en stor begränsning för en effektiv produktion. Alla idisslare som hålls på bete utsätts för dessa parasiter. Symtom på klinisk sjukdom är bland annat vattnig diarré, aptitlöshet, viktminskning och en matt hårrem. Dock är ett sjukdomsförlopp utan kliniska symptom, som bara påverkar produktionen och tillväxten, vanligare idag. Kontrollen av mag-tarmparasitinfektioner på bete baseras idag främst på användning av avmaskningsmedel på gruppnivå. En överanvändning av dessa läkemedel har dock lett till en omfattande utveckling av resistenta mag-tarmparasiter. Riktad selektiv behandling, där man endast avmaskar de djur inom en betesgrupp som behöver avmaskas, har föreslagits som en hållbar strategi för att tillgodose djurens hälsa och välbefinnande och samtidigt minska risken för överanvändning av avmaskningsmedel och utveckling av resistenta mag-tarmparasiter.

Vanligtvis diagnostiseras mag-tarmparasiter genom påvisandet av maskägg i djurens avföring. Denna metod är dock inte praktiskt genomförbar på individnivå. Djur som blir sjuka förändrar sitt beteende, bland annat blir de slöa och tappar aptiten. Övervakning av sjukdomsbeteenden har föreslagits som en användbar indikator för sjukdom hos djur. Det är sedan tidigare känt att avvikelser i ätbeteende och aktivitet kan ge information om djurens hälso- och välfärdsstatus. Framsteg inom så kallat precisionslantbruk, ett samlingsnamn för teknologier som bland annat används för att kontinuerligt övervaka djurens status och deras miljö, möjliggör övervakning av djurens beteende och aktivitet i realtid. Avvikelser i beteende kan potentiellt användas som en indikator för mag-tarmparasitinfektioner hos betesdjur. Kunskapen om förändringar i beteende kopplade till infektioner med mag-tarmparasiter är idag begränsad. Mer kunskap behövs innan man eventuellt kan använda automatisk beteendeövervakning för att diagnostisera mag-tarmparasiter hos betesdjur.

Syftet med denna avhandling är att öka kunskapen om hur mag-tarmparasiter påverkar aktivitets- och idisslingsmönster hos betesdjur. Det långsiktiga målet är också att bedöma om det finns potential att integrera automatisk beteendeövervakning med sensorer i framtida parasitkontrollprogram. Tre infektionsförsök, varav två på nötkreatur och ett på får, utfördes för att bedöma om sensorövervakning av aktivitet kan användas som en indikator på subklinisk rundmaskinfektion.

Resultaten tyder på att även låga nivåer av rundmaskinfektion påverkar aktiviteten hos förstagångsbetande kalvar och lamm. Båda djurslagen, infekterade med mag-tarmparasiter, uppvisade sjukdomsbeteende med minskad aktivitet jämfört med avmaskade djur. Stutar som utsattes för mag-tarmparasiter hade en ökad daglig liggtid tidigt i infektionen jämfört med avmaskade djur. Däremot hade infekterade lamm en minskad daglig liggtid. Därutöver påverkade infektion med mag-tarmmaskar också idisslingsmönster över tid hos infekterade stutar.

Sammanfattningsvis tyder resultaten på att även låga nivåer av mag-tarmparasiter påverkar aktiviteten hos både nötkreatur och får. Detta visar i sin tur att det finns en potential att sensorbaserad övervakning kan integreras i framtida parasitkontrollprogram.

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Acta Universitatis Agriculturae Sueciae

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This thesis investigates how contrasting levels of major gastrointestinal nematode infections affect activity and rumination patterns in grazing livestock, using on-animal sensors. The results indicate that moderately infected first season grazing steers and lambs exhibit sickness behaviour with a decrease in activity compared with dewormed animals. In conclusion, this thesis supports that activity and rumination patterns are affected by subclinical gastrointestinal nematode infections. Furthermore, the potential use of automated behavioural observations as a diagnostic tool is discussed.

Niclas Högberg received his postgraduate education at the Department of Biomedical Sciences and Veterinary Public Health. He obtained his degree in veterinary medicine in 2013 at the Faculty of Veterinary Medicine and Animal Science, SLU, Uppsala.

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