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Master Thesis

Investigating the effect of herd and landscape factors on brainworm (*Elaphostrongylus rangiferi*) prevalence and infection intensity in a semi-domesticated reindeer (*Rangifer tarandus tarandus*) herd

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Abstract

The increasing concern about brainworm (*Elaphostrongylus rangiferi*) in reindeer (*Rangifer tarandus*) is driven by climate change. The development of the nematode in its intermediate host – gastropods – is strongly affected by temperature and accelerates with increasing temperatures. Warmer summers therefore result in an increased risk of large disease outbreaks. Whilst there have been a number of studies looking at wild tundra reindeer in southern Norway, there have been no systematic studies of brainworm infections and landscape use in the semi-domesticated reindeer herds bordering these wild reindeer populations. More knowledge about brainworm and the environmental factors involved in its lifecycle is needed to better help herders in developing brainworm management strategies. I hypothesized that brainworm infection prevalence and intensity is not only age- and sex-related but also affected by landscape use of the reindeer, as this determines the measure of intake of brainworm-infected gastropods. Data includes faecal samples from GPS-collared semi-domesticated reindeer in central Norway, taken in winter 2019-2021 as well as in summer and autumn 2020. Faeces were analysed using the Baermann method to count brainworm larval intensity. Landscape use of the reindeer was analysed using GPS data of collared reindeer and vegetation maps. During autumn 2020, I conducted gastropod sampling by visually searching plots for gastropods inside the reindeer grazing areas and these results were integrated in the landscape analysis. Results of faecal analysis show patterns in which infections in all age-classes increase as the year progresses, which corresponds to literature describing the duration of the migratory life cycle of brainworm parasites. Probability of infection and infection intensity decreased with increasing body weight for calves and adult females, which is in contrast with previous literature and may be a sign of brainworm having a sub-clinical impact with reduced weight gain or weight loss in individuals with a high level of infection. Landscape use analysis showed trends in which the availability of, and time spent in, bogs had a positive effect on the infection intensity, whereas increasing altitude lead to a decrease in brainworm infection intensity. However, since the significance level was not reached for these relationships, more research is needed to confirm these trends.

Keywords: *Elaphostrongylus rangiferi*, brainworm, nematode, intermediate host, gastropods, semi-domesticated reindeer, *Rangifer tarandus tarandus*, landscape use, climate change, Norway

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1.   Introduction

The increasing concern about *Elaphostrongylus rangiferi* in reindeer, better known as 'brainworm', is due to current climate change. The development of the parasite in its intermediate host – snails and slugs – is strongly affected by temperature, taking several months to develop to the infectious stage at temperatures below 10°C and less than two weeks above 15°C (Davidson et al., 2020; Halvorsen & Skorping, 1982). Whilst the climatic conditions in Norway are usually cool enough to slow down the larval development periods thereby limiting numbers of infected reindeer, warmer summers, as experienced in recent years and predicted by climate models, are likely to increase the risk of large severe disease outbreaks.

In reindeer the brainworm parasite causes the disease elaphostrongylosis. The late appearance of symptoms of this disease and the lack of treatments other than symptomatic intensive care often results in fatal outcomes. Therefore, an alternative strategy to prevent future outbreaks is urgently needed. Predicting the exposure risk to brainworm by, for example, a mathematical model including weather parameters and parasite biology would allow herders to better manage future outbreaks and to implement measures such as moving reindeer to new grazing areas when infection pressure is predicted to be high to avoid infections. However, more knowledge about the brainworm parasite, its intermediate host and the involved environmental factors is needed to complete this model and thus help the reindeer herders reduce the negative impacts of climate change on traditional reindeer herding practices.

This study focusses on the influences of external factors on the survival and abundance of brainworm parasites and gastropods in relation to brainworm infections in semi-domesticated reindeer. Biological factors such as sex and age of reindeer and environmental factors such as vegetation types and altitude in grazing areas of the semi-domestic reindeer herd are included. Through our larger project (see 1.4), this information will later be linked to local weather parameters with the aim to create a mathematical model to predict infection risk and intensity with brainworm in reindeer.

1.1 Semi-domesticated reindeer in Norway

Reindeer (*Rangifer tarandus*) belong to the deer family (Cervidae) and are distributed around the northern Holarctic region. The species has a high number of subspecies in which a distinction is made between reindeer and caribou. Although both names refer to the same species, caribou is used to refer to all wild specimens in North America, while reindeer includes both wild and semi-domesticated specimens in Eurasia. In this latter region, most reindeer belong to the subspecies Eurasian tundra reindeer (*Rangifer tarandus tarandus*) (Røed et al., 2019).

Reindeer husbandry started on a large scale in Norway in the 16th and 17th centuries, mainly for high quality meat production, skins and antlers (Nieminen, 2019). Presently, the semi-domesticated reindeer herds are still of great importance for the economy, employment and culture of the indigenous Sami people of northern Fennoscandia (Nieminen, 2019; Ministry of Agriculture and Food, 2017). In Norway, the Sami rangelands are divided into six regional grazing areas, which are further divided into 82 reindeer grazing districts, determined by the Reindeer Husbandry Board. Almost 3,000 people in Norway are involved in Sami reindeer husbandry (Ministry of Agriculture and Food, 2017; Landbruksdirektoratet, 2021). Herds consist of reindeer of different owners, who manage them together and collaborate in management groups called "Siida" (Northern Sami) and "Sijte" (Southern Sami). A siida can be seasonally orientated and in recent years there have been around 100 summer siida and 150 winter siida. Grazing areas are not fenced and reindeer within a siida can mix, and must therefore carry an owner's mark (Ministry of Agriculture and Food, 2017; Landbruksdirektoratet, 2021). The reindeer are gathered for certain purposes during the year, as for the marking of new-born calves during summer, dividing into summer or winter siidas and slaughtering during autumn and winter. Supplementary feeding of the reindeer is usually avoided but is practiced when natural grazing resources are scarce or inaccessible. In some cases, supplementary feeding is used as a strategy to, for example, avoid conflicts with infrastructure or predators (Horstkotte et al., 2020).

The total population of semi-domesticated reindeer in Norway today counts ca. 214,000 animals, of which 70% is located in the northernmost county of Finnmark. Reindeer husbandry covers about 40% of the Norwegian land area. Yearly ca. 75,000 animals are slaughtered, a total meat production of about 1,100 tonnes (Hætta, 2020).

1.2 Brainworm parasite

Elaphostrongylus rangiferi, also referred to as brainworm or meningeal worm, is a nematode of the Protostrongylidae family and is a common and widespread parasite in reindeer in Fennoscandia (Josefsen & Handeland, 2014; Kutz et al., 2019).

1.2.1 Lifecycle

The parasite has an indirect lifecycle which uses gastropods (snails and slugs) as intermediate hosts. First stage larvae (L_1, larvae at first developmental moult stage) are shed by reindeer in faeces, whereafter the L_1 penetrate their intermediate host and develop into their infective L_3 stage. Infections in reindeer – the final host – occur when reindeer consume these gastropods containing infective larvae during grazing. After reaching the gastrointestinal tract, the L_3 larvae penetrate the venules of the abomasa wall and migrate through the body via the vascular system. They eventually enter the lungs via the pulmonary artery. There the larvae enter pulmonary venules with which they get carried to the heart, and subsequently from there to all tissues through general blood circulation (Handeland, 1994).

Larvae that reach the central nervous system can develop further to adults, which usually takes 48 to 90 days post infection (Hemmingsen et al., 1993). Between 90 and 196 days post infection the majority of adults migrate via the spinal nerves to the skeletal muscles, where they reproduce. However, exceptions in which worms stay in the central nervous system during the reproductive phase have been observed (Hemmingsen et al., 1993; Handeland, 1994). Female parasites deposit eggs in the veins, through which the eggs are transported to the lungs. In the lungs the L_1 hatch, are coughed up and swallowed again, and are excreted via faeces (Handeland, 1994). These L_1 larvae can be detected in the faeces from approximately 4 months post infection (Handeland et al., 1994).

An experimental study by Handeland (1994), in which reindeer calves were infected with *Elaphostrongylus rangiferi*, reported the complete migratory life cycle of the parasite in reindeer. During the migration of the L_3 through the vascular system, the larvae caused focal inflammation and necrosis, bleeding and infarcts. In the central nervous system the nematodes caused inflammation and degeneration in all structures, and in the skeletal muscles inflammatory oedema was observed. Hatching L_1 in the lungs caused a chronic pneumonia, which persisted from day 103 post infection (Handeland, 1994).

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1.2.2 Clinical signs

Clinical signs can start to show from four weeks post infection, with ataxia, partial paraparesis of the hindlimbs and not completely raised tails during walking (Handeland et al., 1994). Other symptoms include abnormal posture, lameness, stiff neck, general weakness, stumbling and falling, and reduced vision. In severe cases, complete paralysis of the hindquarters or in all four legs may occur. In calves, reduced growth may be a result (Handeland et al., 1994; Josefsen & Handeland, 2014). It can take up to 5 months before clinical signs start to show, which will likely negatively impact survival chances of infected animals (Handeland et al., 1994; Davidson et al., 2020)

1.2.3 Diagnosis

The diagnosis of a brainworm infection cannot, with absolute certainty, be made for living animals. If several animals in the same herd are affected, it strengthens the suspicion of the diagnosis. Brainworm larvae found in faeces do not provide a good diagnostic tool for disease, as the larvae generally do not appear in the faeces until long after the clinical symptoms are visible. In addition, a positive detection of brainworm in faeces is also common in clinically healthy animals (Josefsen & Handeland, 2014). However, faecal analysis can give an overview of the prevalence and abundance of brainworm larvae in a herd, particularly over time. Larvae can be detected in faeces using the Baermann technique (see 2.3.1). *Elaphostrongylus rangiferi* larvae can be recognized by their s-shaped tail (Fig. 1).

Figure 1. Living *Elaphostrongylus rangiferi* larvae at first developmental stage L1. Note the s-shaped tail and the dorsal spike (arrow). Photos: Mattanja Stuut

1.2.4 Population dynamics of *E. rangiferi*

The output of *E. rangiferi* larvae in the faeces of infected reindeer has been demonstrated to follow a seasonal cycle, which is dependent on the sex of the reindeer. For both female and male reindeer, a minimum larval output was seen during mid-summer. Males showed a peak output in autumn/early winter and females showed a peak output during late winter/spring (Halvorsen et al., 1985). Gaudernack et al. (1984) suggested that this seasonal larval output is related to the immune status and condition of the reindeer. Reindeer are in their best condition during late summer and here the suppression of larval output is strongest (i.e. larval output is lowest). This suppression is weakest (i.e. larval output is highest) when reindeer are under high environmental stress and are in their poorest condition, which is for males during the rutting season (autumn) and for females during late winter/spring due to the pregnancy and calving period (Gaudernack et al., 1984). Halvorsen and Andersen (1982) estimated the life span of adult *E. rangiferi* to be about three years and suggested that an initially established infrapopulation of the parasite is long lived in reindeer. This suggestion was supported by the results of a later study, in which the output of larvae remained on the same level from year to year in samples of individual reindeer (Halvorsen et al., 1985).

Halvorsen (1986) tested the hypothesis that dominant reindeer have a higher risk of ingesting parasites that are transmitted in the food chain. Larger reindeer are dominant in the herd and are therefore presumed to eat more of the preferred calcium rich forbs, which are also incidentally preferred by the intermediate host (gastropods) of *E. rangiferi* (Andersen & Halvorsen, 1984). Halvorsen (1986) found that the heaviest calves were the most infected ones. Infections with *E. rangiferi* occurred most often in the largest male calves, but also infected female calves were on average heavier than the uninfected ones (Halvorsen, 1986).

1.2.5 *Elaphostrongylus* in other animal species

Reindeer are not the only species that can be affected by brainworm. There are separate species of brainworm in deer (*Elaphostrongylus cervi*) and moose (*Elaphostrongylus alces*) (Josefsen & Handeland, 2014). Stéen et al. (1997) showed that cross-infection with *Elaphostrongylus rangiferi* and *Elaphostrongylus alces* between reindeer and moose is also possible. However, the ability of the parasite to reproduce is reduced in a different host species (Stéen et al., 1997).

Sheep and goats can also get infected with *E. rangiferi*, but the parasite is not able to complete its lifecycle in these species. The migration of the parasite seems to end in the central nervous

system and the larvae do not develop further to adults (Handeland et al., 1993; Handeland & Skorping, 1993). However, the parasite can still harm its host. Symptoms in sheep and goats are mostly similar to those in reindeer, with ataxia, lameness, posterior paresis, abnormal posture and, additionally, pruritus causing hair loss and skin wounds (Handeland & Skorping, 1993; Handeland et al., 1993; Handeland & Sparboe, 1991).

1.3 Intermediate host: gastropods

After the L_1 larvae are shed in faeces by the reindeer and are out in the open, they will penetrate the foot of snails or slugs that they encounter in the environment. The larvae most commonly penetrate their host via superficial furrows on the sole of the snail or slug body (Rezác et al., 1994).

1.3.1 Development and temperature

Various gastropod species have been demonstrated to be suitable intermediate hosts for *E. rangiferi* (Mitskevich, 1964; Skorping & Halvorsen, 1980), but the speed of larval development varies among different gastropod species and at different temperatures. Halvorsen & Skorping (1982) found the developmental time of *E. rangiferi* from L_1 to L_3 of approximately 12 days at 28°C to 2 months at 12°C in gastropod species *Arianta arbustorum* and *Euconulus fulvus*. At or below 8°C development of *E. rangiferi* did not occur (Halvorsen & Skorping, 1982). Skorping & Halvorsen (1980) studied the development of *E. rangiferi* in several intermediate host species and found that in gastropod species *Discus ruderatus*, *Arion silvaticus*, *Deroceras leave*, *Euconulus fulvus* and *Trichia hispida* the development to L_3 took approximately 20 days at 20°C. This development took longer in the gastropod species *Succinea pfeifferi*, *Deroceras reticulatum* and *Arianta arbustorum*. In other species, such as *Arion subfuscus* and *Arion hortensis*, the authors found only a small number of larvae that managed to develop and in *Vertigo lilljeborgi* and *Punctum pygmaeum* no larval development was seen (Skorping & Halvorsen, 1980).

Based on previous studies on larval development in various gastropod hosts and on temperature data, Rose Vineer et al. (2021) developed a degree-day model to predict spatio-temporal thermal suitability for *E. rangiferi* transmission. This model predicted an overall increase in thermal suitability for *E. rangiferi*, leading to an increase in intensity of infections and a potential change from a 2-year transmission cycle to a 1-year transmission cycle of *E. rangiferi* (Rose Vineer et al., 2021).

1.3.2 Environmental factors

Various studies have shown that soil moisture has a significant effect on gastropod abundance and that many terrestrial gastropod species are strongly dependent on moisture for feeding, reproduction and survival (Glen & Moens, 2002; Friedlander, 2017). Also, the extent to which habitats are able to fulfil the nutritional requirements of gastropods are of great importance for gastropod abundance (Andersen & Halvorsen, 1984). Several studies reported a correlation between gastropod abundance and the calcium content of the soil (Valovirta, 1968; Waldén, 1981; Andersen & Halvorsen, 1984). Calcium-rich fens and bogs and tall herb birch forests in particular are mentioned as habitats that are rich in gastropods and with a greater infection risk (Andersen & Halvorsen, 1984; Handeland et al., 2019). In a study on other *Strongylus* parasite species with gastropod intermediate hosts, hardwood or mixed hardwood forest were registered as most common areas to find infected gastropods (Cebra & Gemensky-Metzler, 2014).

Several studies reported that gastropods occur mainly below the treeline (Halvorsen et al., 1980; Handeland et al., 2019). Andersen & Halvorsen (1984) suggest that the poor quality of soils may be the reason for the absence of gastropods above the treeline. This phenomenon was also shown more specifically for *Elaphostrongylus* spp., of which the prevalence decreased with increasing summer grazing altitude (Handeland et al., 2019). Since reindeer usually move to higher pastures after the snow has melted due to insect harassment, there is a potential for higher grazing pressure – and therefore a higher infection pressure – at summer pastures at higher altitude (Halvorsen et al., 1980). However, grazing areas in the summer range, in general, have a poorer gastropod fauna than areas along the migration routes of the reindeer, which contain sites such as lake shores, bogs and mires, etc. where the environmental conditions are more suitable for gastropods. Sites with a high ground water level and readily available minerals for plants result in the richest gastropod faunas (Andersen & Halvorsen, 1984).

1.4 About the project

This study is conducted as part of the project 'Klimasyk rein', which was started in 2020 and is directed by the Norwegian Veterinary Institute in collaboration with the Norwegian Institute for Nature Research (NINA), Inland Norway University of Applied Sciences (INN), the University of Liverpool in Great Britain and Sami reindeer herders in central Norway, Trøndelag county. The project arose by the request of reindeer herders for advice on treatment

and on alternative strategies to prevent future outbreaks of brainworm in an ever-warming climate. The end goal of this project is to create a mathematical model incorporating weather parameters and parasite biology to estimate the exposure risk in selected herds in Trøndelag, Norway. These results could be used to allow herders to implement targeted treatment or other measures such as moving to new grazing areas to avoid intense infection prior to disease outbreaks.

The current study 'Investigating the effect of herd and landscape factors on brainworm (*Elaphostrongylus rangiferi*) prevalence and infection intensity in a semi-domesticated reindeer (*Rangifer tarandus tarandus*) herd' is part of this project and focuses on the effects of landscape use and sex and age of reindeer on the prevalence, intensity and development of infections with *Elaphostrongylus rangiferi* in semi-domesticated reindeer. Parts of this study are designed and executed in cooperation with the study by Closset (2021), which is also part of the project 'Klimasyk rein' and contains a parallel study in wild reindeer.

1.4.1 Research question and aims of the thesis

The research question that I target in my thesis is:

How does landscape use during the grazing season in addition to host age and sex affect the prevalence and intensity of *Elaphostrongylus rangiferi* infections in semi-domesticated reindeer?

The specific aims were as follows:

- Quantify prevalence and intensity of brainworm infections in semi-domesticated reindeer during the study period;
- Correlate the prevalence and intensity of brainworm infections with reindeer sex and age;
- Analyse the landscape use of semi-domesticated reindeer during the summer grazing season;
- Correlate the landscape use of semi-domesticated reindeer with the prevalence and intensity of brainworm infections in semi-domesticated reindeer.

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2. Materials and methods

2.1 Study subject and area

Two semi-domesticated reindeer herds from central Norway, Trøndelag county, were to be studied over the course of 10 months from spring 2020 to winter 2021. Through a collaborative agreement with NINA it was possible to build upon an ongoing study that had been monitoring one of the herds using GPS, since 2018, and carrying out annual winter faecal sampling since 2019. NINA shared their GPS data in return for the analysis of faecal samples collected in winter 2019, 2020 and 2021. Consent to share this additional data was obtained from the herders.

One herd, from the North Trøndelag reindeer district, was lost to follow up. Faecal samples were obtained in spring and in summer 2020 when collars were placed on 12 individuals in this herd. However, it proved impossible to gather the herd in the following autumn. Weather and other logistical factors also resulted in the herd not being gathered during the winter sampling period. The GPS data was made available to the project which allowed for gastropod sampling. However, the results from spring and summer faecal samples in this herd were ultimately excluded from the analyses given that all faecal sample data was missing after activation of the GPS collars.

The second herd belonged to the South Trøndelag/Hedmark reindeer district. This district consists of four sijte and has a total of 13,757 animals (each sijte with roughly 2000-5000 animals) with an average distribution of sexes in the district as 78% adult females, 4% adult males and 18% calves (Hætta, 2020). Unfortunately the GPS data only became available in winter 2021 which meant that targeted gastropod sampling during summer 2020 was not possible for this herd.
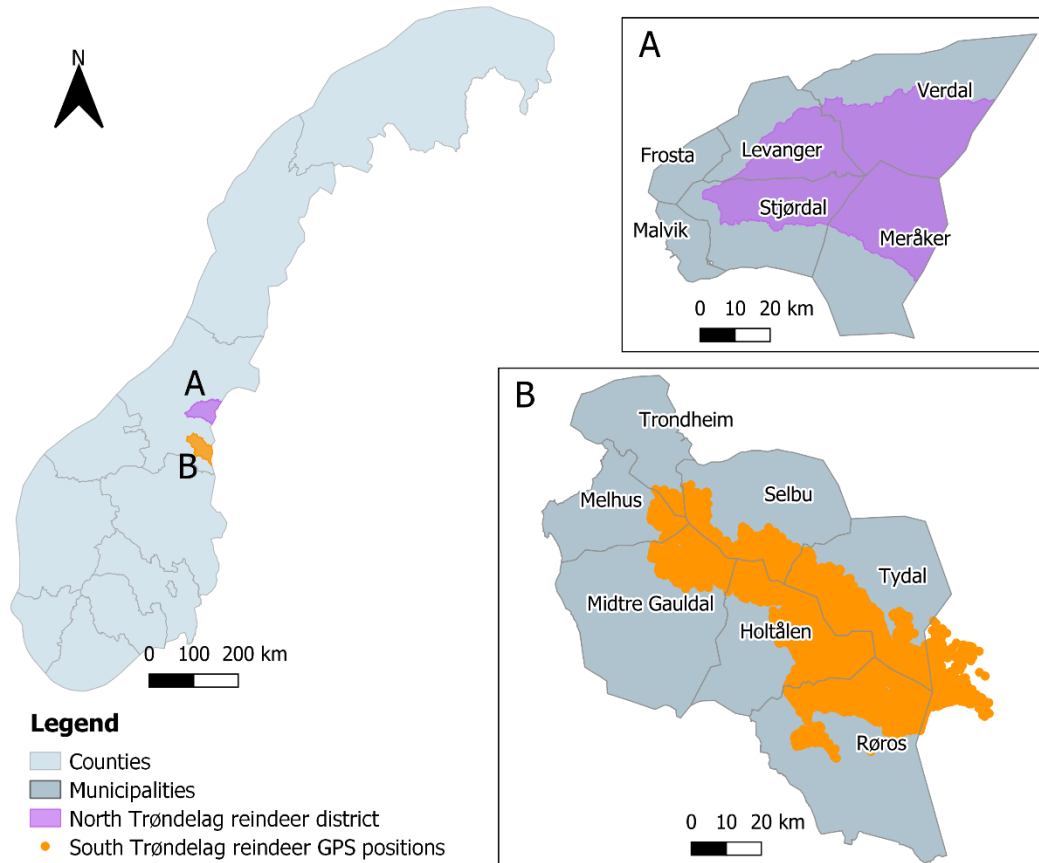
Figure 2. Map of Norwegian county borders (2020) with the two study areas of the two reindeer herds. The grazing district of the North Trøndelag reindeer herd is shown in box A (purple). The South Trøndelag reindeer district is shown in box B, with GPS positions of 54 GPS-collared reindeer in the studied herd (orange dots), recorded in the grazing seasons (May-September) of 2018-2020. The orange dots that are outside the map area represent animals that have grazed in Sweden. Names and grey lines in the cut-out are outlines of Norwegian municipalities and their borders. Maps were constructed using QGIS version 3.4.15 with GRASS 7.8.2. Reindeer district maps were collected from GeoNorge (Norwegian Agriculture Agency, 2020) and reindeer GPS data was retrieved from NINA.

2.1.1 Ethical consideration

The leader of the sijte was involved in the planning of the study and is an active project partner in Klimasyk rein. The project obtained written consent from the sijte to carry out faecal sampling and to allow the GPS collar data to be shared with the project partners for the purposes of modelling landscape use, parasite prevalence and brainworm infection intensity. The leader of the sijte can withdraw this consent at any time during the course of the project without negative consequences. Published research data from the herd is anonymised so that individual animals and herders cannot be identified. Publications resulting from this work are to be shared in advance of publication with the reindeer herder project partner to allow for comment and revision prior to final publication.

This thesis was sent to the reindeer herders for comment on the 28th of May 2021. This thesis will be temporarily classified as project restricted until such time as the reindeer herders provide written consent for publication.

The data has been collected in accordance with national GDPR regulations.

2.2 Data collection

Data collection was conducted at three time periods during the study: spring/early summer (July 2020), autumn (October 2020) and mid-winter (February 2021). Additional faecal data from winter 2019 to winter 2020 and GPS data from summer 2018 to winter 2021 was provided by NINA and the Norwegian Veterinary Institute.

2.2.1 Faecal sampling

Faecal sampling took place while the reindeer were gathered in the enclosure by the herders. Faeces were taken rectally while the reindeer were being handled by the herders. At each sampling period, faeces from roughly 30 animals of both sexes and different age groups (calf, yearling, adult) were collected in plastic bags, which were marked with all available information about the individual reindeer (e.g. sex, age group, owner, collar ID, colour/appearance, name). The aim was to get an equal division between the three age groups and the two sexes, but this was not always possible. Samples were stored in a freezer ($\pm$ -20°C) until further analysis.

2.2.2 Gastropod sampling

Gastropod sampling took place in autumn 2020 for both herds. For the selection of the gastropod sampling locations, a vegetation map (retrieved from NIBIO, 2020) of the reindeer area with GPS positions of the collared reindeer was made using QGIS version 3.4.15 with GRASS 7.8.2 for the North Trøndelag herd. To investigate the differences in presence/absence of gastropods between different vegetation types, I selected sampling locations inside each vegetation type on the map (NIBIO, 2020) in the area and used the reindeer GPS positions to make sure that the samples were located inside areas that were visited by the reindeer. Selection of the sampling locations was based primarily on vegetation type and secondarily on variety in altitude and on proximity to trails or roads. Since no GPS positions were available for the South Trøndelag herd, the sampling area for this herd was defined based on directions with road and valley names that were communicated by the herder to the samplers in a

telephone conversation. QGIS was used to select sampling locations inside different vegetation types in this area.

Inside each vegetation type, I defined three 1 m^2 quadrants which were located at least 10 meters away from each other. Each quadrant was visually observed for 10 minutes by two observers who hand-picked all visible snails, shells and slugs on the soil surface, vegetation and in the topsoil layer. The collected gastropods were temporarily stored in plastic bags for further species determination. The pH and moisture level of each quadrant was measured using a Vistefly 3 Way Soil Meter and the GPS-location and altitude were recorded with a Garmin GPSMAP Astro® 320. Other information about the vegetation and microhabitat in each plot was noted down.

The collected gastropods were measured and visually examined, and the online Terrestrial Mollusc Tool (White-McLean, 2011) was followed to determine the species of each individual. All specimens were closely photographed to save this data.

2.3 Analysis

2.3.1 Faecal analysis

The faecal samples were analysed using the Baermann method, which is suitable for larval count (Taylor et al., 2015).

The frozen faecal samples were transferred to a refrigerator to defrost at least 24 hours before starting the Baermann analysis. A parcel with 5-10 grams of faeces was made and wrapped in double layered gauze and tied off with a tie wrap. A short stick was carefully pushed through the gauze at the height of the tie wrap and excess gauze was cut off. The parcel was suspended at the top of a lukewarm water filled plastic champagne glass. The faeces were left in the water for at least 12 hours.

After at least 12 hours, the parcel with faeces was removed from the glass. A pipette was used to remove the fluid from the top of the glass – without disturbing the sediment – until 5 ml was left. The suspension at the bottom was transferred, with a pipette, into a labelled 14 ml tube. A new ~5 ml of water was used to rinse the glass, and this suspension was also transferred into the same 14 ml tube. The tube was centrifuged at 1700 G for 3 minutes. After centrifuging, the supernatant was removed with a pipette until 1 ml left. The remaining sediment was mixed using a precision pipette with 100 µl tip and a 100 µl sub-sample was taken out and placed on

a microscope slide, covered with a cover slide and examined using a microscope at 100x magnification. If no larvae were found in the first sub-sample, then a second 100 µl sub-sample was taken from the sediment and the larvae were counted. *Elaphostrongylus* larvae were identified by their S-shaped tail and body size (length: 381-490 µm, width 17-24 µm (Davidson et al., 2020) and counted. Larvae per gram faeces (LPG) were calculated with the formula:

$$LPG = \left(larvae\ counted \times \frac{volume\ water\ (\mu l\ in\ tube)}{volume\ examined\ (\mu l\ in\ pipette)} \right) \div weight\ faeces\ (g)$$

2.3.2 Landscape use analysis

GPS data from 54 collared adult female reindeer in the South Trøndelag herd was provided by collaborators at NINA. Three reindeer were removed from the dataset, because no faecal data was available for these animals. GPS positions were set to be recorded every six hours from June 2018 to March 2021, with varying regularity between animals. Additional spatial data was retrieved from different Norwegian databases, including Vegetation – Habitats (NIBIO, 2020), Height DTM10 (Kartverket, 2021), N50 Map Data (Norwegian Mapping Authority, 2017). The acquired data was processed and analysed using QGIS version 3.4.15 with GRASS 7.8.2. A digital elevation model of the study area was created using the height data and this digital elevation model was joined with the GPS data to evaluate at what altitude each GPS positions was located. Average altitudes were calculated from the GPS positions for each reindeer per year's grazing season (May-September).

Landscape use of the GPS collared reindeer during the grazing season was analysed in two different ways: (1) by examining the use of vegetation types at the real GPS positions and (2) by creating seasonal home ranges around the GPS positions and examining the available landscape types inside each reindeer's home range.

Landscape use analysis from GPS positions

In QGIS, the vegetation data was joined with the GPS positions that were recorded during the grazing seasons (May-September) of 2018-2020 to see in which vegetation type each GPS position was located. In Excel, the 15 vegetation types in the dataset Vegetation – Habitats (NIBIO, 2020) were divided into 5 groups, based on the main nature type of these vegetation types (Table 1). Proportions of these vegetation groups were calculated for each reindeer per

year's grazing season (if faecal data of the following winter was available). Further analyses were conducted in R.

Landscape use analysis from home ranges

Home ranges with a 90% fixed Kernel density estimator were calculated for each reindeer's grazing season (May-September) per year (if faecal data of the following winter was available) in R version 4.0.4 (R Core Team, 2021), using packages *sp* (Pebesma & Bivand, 2005) and *adehabitatHR* (Calenge, 2006). The vegetation data was joined with the seasonal home ranges in QGIS to see which vegetation types were present inside the grazing season home ranges of the reindeer. In Excel, proportions of the 5 vegetation groups (Table 1) inside the seasonal home ranges were calculated for each reindeer. Further analyses were conducted in R.

Table 1. Division of the 15 vegetation types in the study area into 5 vegetation groups. Vegetation types taken from the dataset Vegetation – Habitats (NIBIO, 2020).

Agriculture	Bogs	Forest	Open areas	Other
Agriculture	Bogs forested	Forest, high prod.	Open areas, vigorous veg.	Fresh water
Fenced pastures	Bogs open	Forest, intermediate prod.	Open areas, intermediate veg.	Bare rocks and boulder fields
		Forest, low prod.	Open areas, sparse veg.	Populated or infrastructure
		Forest, unproductive		
		Forest, unregistered		

2.3.3 Statistical analysis

Statistical analysis was conducted in R version 4.0.4 (R Core Team, 2021) Data exploration was conducted following the protocol described by (Zuur et al., 2010). Packages *tidyverse* (Wickham et al., 2019) and *lubridate* (Grolemund & Wickham, 2011) in R were used for data preparation and *ggplot2* (Wickham, 2016) was used for visualising data and making graphs. Statistical significance was indicated using the *p*-value, with $p < 0.05$ indicating statistical significance.

Prevalence of infection

The prevalence of infection was expressed as a percentage of the faecal samples (i.e. reindeer individuals) containing larvae. This percentage was obtained by dividing the number of infected reindeer by the total number of examined reindeer.

Probability of infection

The probability of a reindeer being infected with brainworm and the influencing factors on this probability were examined in a binomial logistic regression using generalized linear models. The detection of brainworm larvae in the faeces was used as binary response variable,

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with value 1 for infected and 0 for uninfected. Explanatory variables that were included in the model were sex, age class, body weight, season and interactions between these variables. Mann-Whitney U tests were used to compare differences between groups. The package *DHARMa* (Hartig, 2020) was used to assess the fit of the models. Models were compared using the AICc of the models, which is an AIC (Akaike information criterion) estimate with a correction for small sample sizes (Akaike, 1974). AICc, ΔAICc, Akaike's weights and log-likelihood were calculated using the package *AICcmodavg* (Mazerolle, 2020). A top ranked model was considered as significantly better than other models when ΔAICc of the next ranked model was more than two.

Infection intensity

The infection intensity was expressed as the number of brainworm larvae per gram faeces (LPG). Infection intensity only included the positive samples. There was an overdispersion due to the fact that most positive samples contained a small number of larvae. Therefore, a generalized linear model with negative binomial distribution was computed using the package *MASS* (Venables & Ripley, 2002) in R. The number of larvae in the positive samples (LPG) was used as response variable. Explanatory variables were sex, age class, body weight, season and interactions between these variables. The package *DHARMa* (Hartig, 2020) was used to assess the fit of the models. Model selection was based on the AICc of the models. AICc, ΔAICc, Akaike's weights and log-likelihood were calculated using the package *AICcmodavg* (Mazerolle, 2020).

Landscape use

The effects of the proportions of the vegetation groups and altitude on the LPG in all samples were examined using negative binomial generalized linear models, using the package *MASS* (Venables & Ripley, 2002) in R. The number of larvae in the positive samples (LPG) was used as response variable. The vegetation groups "Agriculture", "Bogs", "Forest", "Open areas" and "Other" were used as explanatory variables. The package *DHARMa* (Hartig, 2020) was used to assess the fit of the models.

3. Results

A total of 319 faecal samples were included in the analysis, of which 223 contained brainworm larvae. A total of 293 samples were taken from females and 26 were taken from males. Most of the samples were taken during winter in 2019, 2020 and 2021 (n=265). The remaining samples were taken in summer 2020 (n=28) and autumn 2020 (n=26). Sampled reindeer were divided into four age classes: calf (<1 years old, n=55), yearling (1-2 years old, n=30), 2 year (2-3 years old, n=28) and adult (>3 years old, n=206). The body weight of the reindeer was recorded for 264 samples.

Out of the 67,621 6-hourly GPS positions that were recorded during the grazing seasons (May-September 2018, 2019 and 2020), 65 seasonal home ranges were created (2018 n=30; 2019 n=25; 2020 n=10). The variety in the number of seasonal home ranges between years is due to the fact that not all reindeer were sampled in all three years – seasonal home ranges were only created for reindeer of which faecal data of the following winter was available.

3.1 Prevalence of infection

The prevalence of faecal samples containing brainworm larvae varied from 0 to 100%, depending on the season of sampling, age class and sex of the sampled reindeer.

Females showed an overall higher prevalence of positive samples (72.0%; 95% CI 66.9-77.2%) than males (46.2%; 95% CI 26.6-65.7%). Adults had the highest prevalence (80.6%; 95% CI 75.2-86.0%) of the four age classes and calves had the lowest prevalence (23.6%; 95% CI 12.3-35.0%). Male calves (33.3%; 95% CI 12.7-54.0%) and male yearlings (100%) showed a higher prevalence than females in the same age classes (resp. 17.6%; 95% CI 4.6-30.7% and 72.0%; 95% CI 54.0-90.0%).

Table 2. Prevalence of infection with *Elaphostrongylus rangiferi* and parasite abundance per sex and age class in the South Trøndelag reindeer district, average numbers of three years together (2019-2021).

Age class	Sex	n	Prevalence %	95% CI	Prevalence % for age class	95% CI	LPG mean	LPG median	LPG min	LPG max
Calf	Female	34	17.6%	4.6-30.7%	23.6%	12.3-35.0%	50	0	0	648
	Male	21	33.3%	12.7-54.0%			83	0	0	1091
Yearling	Female	25	72.0%	54.0-90.0%	76.7%	61.3-92.1%	191	81	0	1324
	Male	5	100.0%				168	39	3	419
2year	Female	28	75.0%	58.7-91.3%	75.0%	58.7-91.3%	148	94	0	878
Adult	Female	206	80.6%	75.2-86.0%	80.6%	75.2-86.0%	113	43	0	1057
Total		**319**	**69.9%**	**64.9-74.9%**			**114**	**35**	**0**	**1324**

When putting all samples and age classes together, the prevalence of positive samples was highest in winter samples in general (72.8%; 95% CI 67.5-78.2%) and specifically for winter 2020 (79.2%; 95% CI 71.0-87.3%). Autumn was the season with the lowest prevalence of positive samples (53.8%; 95% CI 34.3-73.4%). Infections in calves were only seen during winter and their infection prevalence during this season was lowest of all four age classes (36.1%; 95% CI 20.2-52.0%).

3.2 Probability of infection

The model with explanatory variables sex, age and season, with sex as an independent variable and an interaction between age and season (M12p, *{Infection ~ Sex + Age * Season}*, see Table A1 in Appendix A) was selected as the best model in predicting the probability of infection, because the AICc was smallest and stood out from the second best model with ΔAICc more than 2. The three top models all included an interaction between age and season and clustered within ΔAICc less than 4.

An ANOVA test of the top ranked model (M12p) showed that the levels of explanatory variables sex (p=0.008), age (p<0.000) and the interaction between age and season (p=0.002) were associated with a significant difference in the probability of infection. The latter interaction indicates that the relationship between season and infection depends on the age of the reindeer.

In the top ranked model (M12p), males were associated with a significantly increased probability of infection with brainworm (logit slope ± SE = 1.423 ± 0.687, p=0.038). The positive estimates in this model for the separate variables for age classes indicated that the probability of being infected increases with an increase in age class. This association was strongest, but not significant, for adults (logit slope ± SE = 20.685 ± 1193.464, p=0.986) and yearlings (logit slope ± SE = 19.351 ± 1193.464, p=0.987). For reindeer classed as 2 years old the effect was smaller, but significant (logit slope ± SE = 2.342 ± 0.662, p<0.000).

The probabilities of infection per season, sex and age class, as predicted by the top ranked model, are presented in Table 3. For calves, the predicted probabilities of being infected were zero for summer and autumn. For all seasons and age classes, the probability of being infected was higher for males than for females.

Table 3. Probability of infection with *Elaphostrongylus rangiferi* by season and age class in the South Trøndelag reindeer district predicted by model M12p {Infection ~ Sex + Age * Season}.

Age class	Summer		Autumn		Winter	
	Female	Male	Female	Male	Female	Male
Calf	0.0000	0.0000	0.0000	0.0000	0.2426	0.5707
Yearling	0.7434	0.9232	0.6981	0.9056	0.7500	0.9256
2year	0.0000	0.0000	0.5000	0.8058	0.7692	0.9326
Adult	0.9167	0.9786	1.0000	1.0000	0.7914	0.9403

Body weight

The Pearson correlation coefficient showed a high correlation between age and body weight (r = 0.84). Therefore, I split the dataset for the different age classes. Because of the small datasets that remained for yearlings (n=16) and 2 year olds (n=26), I only conducted analyses with body weight for calves (n=37) and adults (n=185).

The best model for investigating the relationship between body weight and probability of infection for calves (M3pwc, *{Infection ~ Weight * Sex}*, see Table A1 in Appendix A) included an interaction between weight and sex. An ANOVA test for this model indicated that the interaction between weight and sex (p=0.003) was associated with a significant difference in the probability of infection in calves. The model M3pwc showed a decreasing probability of infection with increasing weight (logit slope $\pm$ SE = -0.7299 $\pm$ 0.3369, p=0.0303) and this effect was significant.
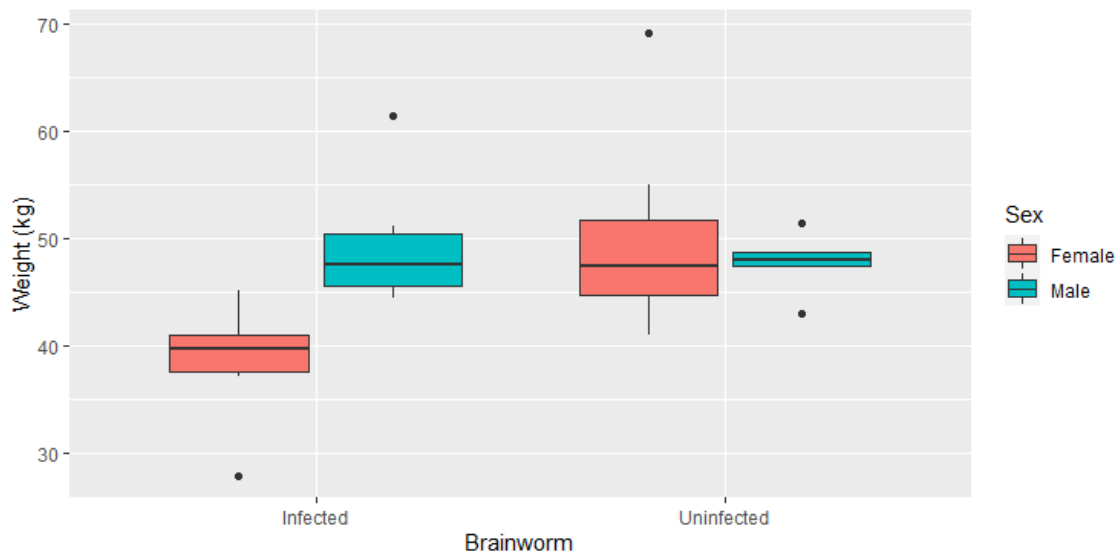

Figure 3. Boxplot of body weight (kg) of infected and uninfected calves in the South Trøndelag reindeer herding district at winter weighing in 2019-2021. Average weight of infected calves (n=13) was 44.4 kg (95% CI 39.6-49.2). Infected female calves (n=6): 38.5 kg (95% CI 32.4-44.6); infected male calves (n=7): 49.4 kg (95% CI 44.0-54.8). Average weight of uninfected calves (n=21) was 48.7 (95% CI 46.0-51.4). Uninfected female calves

(n=15): 49.1 kg (95% CI 45.3-52.8); uninfected male calves (n=6): 47.8 kg (95% CI 44.9-50.6). The difference in weight between infected and uninfected female calves was significant (p=0.003).

Mann-Whitney U-test showed that there was a significant difference in weight between infected and uninfected female calves (W=83, p=0.003). Therefore, uninfected female calves were generally heavier than infected female calves (Fig. 3). For males, this difference was not significant (W=20.5, p=1).

For adults, only one model was created to investigate the relationship between body weight and probability of infection, because only adult females and no males were weighed so including the variable sex would be meaningless. Therefore, model selection was not needed here. The model *{Infection ~ Weight}* for adult females showed a decreasing probability of infection with increasing weight (logit slope $\pm$ SE = -0.057 $\pm$ 0.027, p=0.036). This effect was significant.

Mann-Whitney U-test showed that there was a significant difference in weight between infected and uninfected adult females (W=2201.5, p=0.045). Uninfected adult females were generally heavier than infected adult females (Fig. 4).
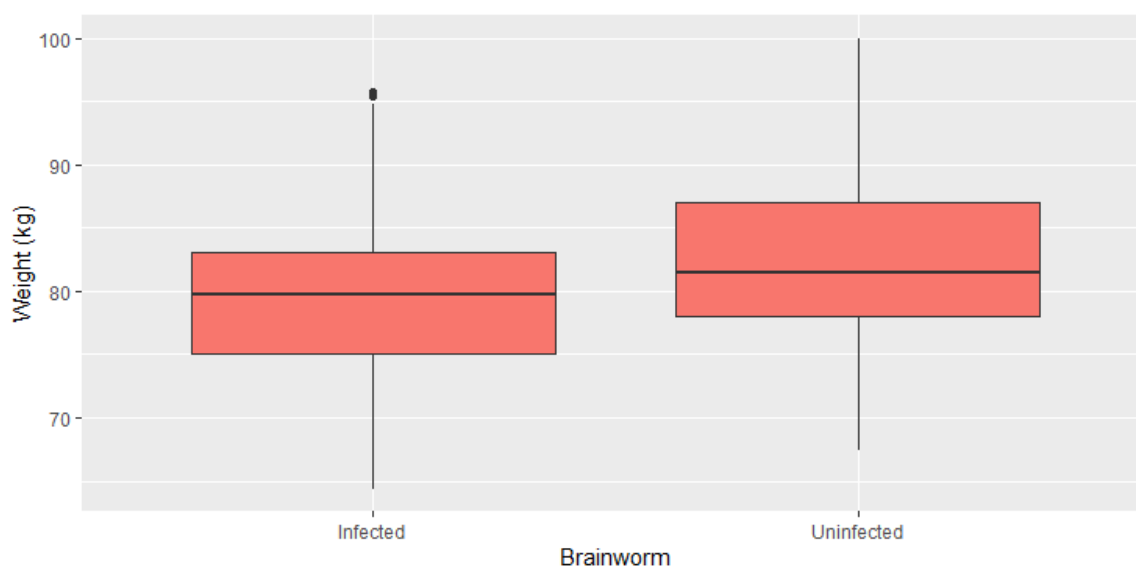

Figure 4. Boxplot of body weight (kg) of infected and uninfected adult females in the South Trøndelag reindeer herding district at winter weighing in 2019-2021. Average weight of infected adult females (n=147) was 79.5 kg (95% CI78.4-80.6) and average weight of uninfected adult females (n=38) was 82.1 (95% CI 79.7-84.6). The difference between infected and uninfected adult females was significant (p=0.045).

3.3 Infection intensity

The main effects model with the number of larvae (LPG) in the positive samples as response variable and age and season as explanatory variables (M7b, *{Intensity ~ Age + Season}*, see Table A2 in Appendix A) was selected as the best model for predicting the infection intensity. This model stood out from the second best model (M11b) that included the variable sex, with ΔAICc more than 2. An ANOVA test for the top ranked model (M7b) showed that the levels of explanatory variables season ($p<0.000$) and age ($p=0.004$) were associated with a significant difference in infection intensity.
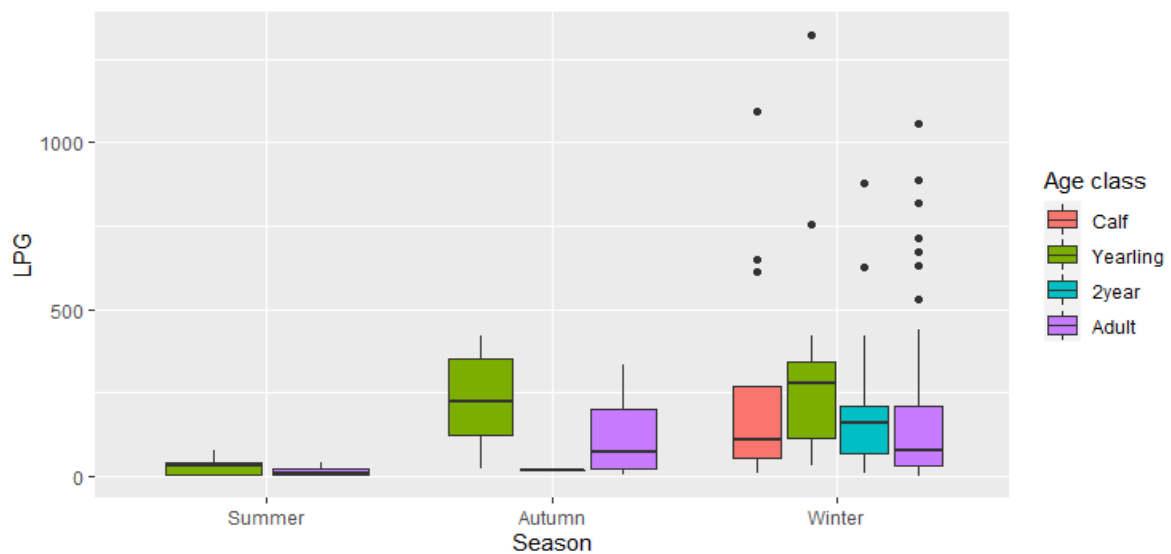
Figure 5. Boxplot of *Elaphostrongylus rangiferi* infection intensity (LPG) per season per age class in the South Trøndelag reindeer district, data of three years together: summer 2020 (n=16), autumn 2020 (n=14), winter 2019 (n=75), winter 2020 (n=76) and winter 2021 (n=42).

The top ranked model (M7b) showed a significant increase in infection intensity in both autumn (logit slope ± SE = 2.015 ± 0.396, $p<0.000$) and winter samples (logit slope ± SE = 2.370 ± 0.292, $p<0.000$) relative to the prior season. Yearlings showed a slight increase in infection intensity relative to calves and 2 year old reindeer and adults both showed a slight decrease in infection intensity relative to the prior age class, but these effects were not significant.

Body weight

The relationship between body weight and infection intensity was only investigated in calves and adult females, due to the too small sample sizes for the other age classes and the lack of weight data for adult males.

For calves, the main effects model (M1bwc, *{Intensity ~ Weight}*, see Table A2 in Appendix A) was selected as the best model for examining the relationship between body weight and infection intensity. This model showed a decreasing infection intensity with increasing weight (logit slope $\pm$ SE = -0.033 $\pm$ 0.040, p=0.409). This effect was not significant.

Because only adult females were weighed, sex as explanatory variable was not considered to be included in a model to investigate the relationship between body weight and infection intensity. Accordingly, only one model was built and no model selection was needed. The model *{Intensity ~ Weight}* for adult females showed a decreasing infection intensity with increasing weight (logit slope $\pm$ SE = -0.038 $\pm$ 0.013, p=0.005; Fig. 6). This effect was significant.
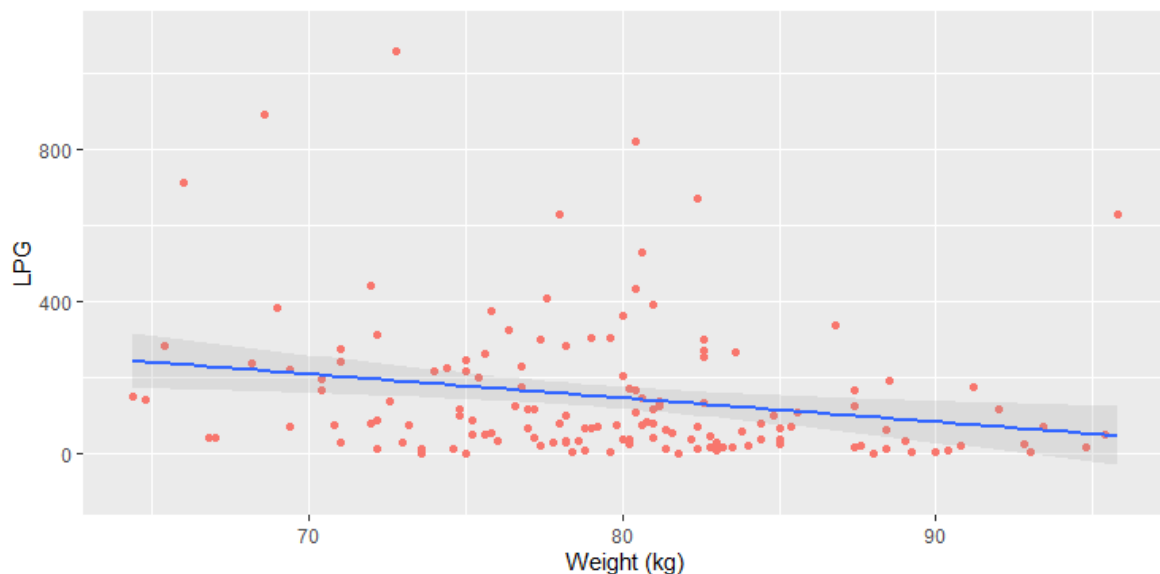

Figure 6. Relationship between body weight and *Elaphostrongylus rangiferi* infection intensity (LPG) of infected adult females (n=147) in the South Trøndelag reindeer district. The females were weighed in winter 2019 (n=55), winter 2020 (n=61) and winter 2021 (n=31). There is a decrease in infection intensity with increasing weight, this effect was significant (p=0.005). The grey zone surrounding the blue line represents the 95% confidence interval.

3.4 Landscape use

Landscape use in GPS positions

The vegetation group that was most used on average in the GPS positions (Fig. 7) was "Open areas" (63.8%), followed by "Forest" (19.1%), "Bogs" (15.0%), "Agriculture" (1.1%) and "Other" (1.0%).
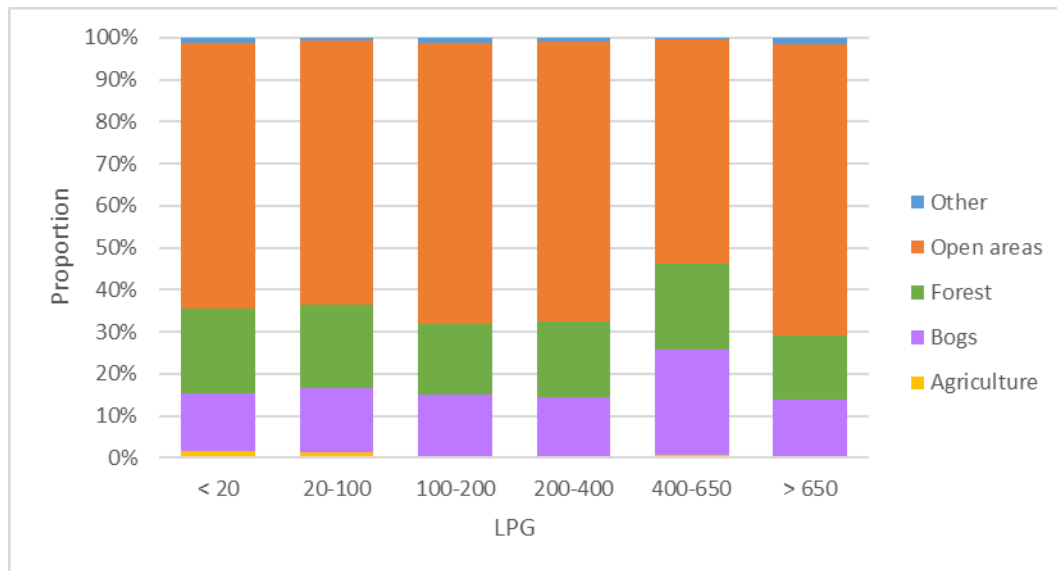

Figure 7. Average proportions of used vegetation types of 6-hourly GPS positions of collared reindeer (n=51) in the South Trøndelag reindeer district during summer grazing seasons of 2018-2020 by LPG category in the subsequent winter samples from 2019-2021. No obvious patterns were seen between landscape use and subsequent infection intensity.

The model {*Intensity ~ Agriculture + Bogs + Forest + Open.areas + Other*} was built using the proportions of vegetation types at the used GPS positions (see Landscape use analysis from GPS positions). Because the vegetation types were expressed as proportional data, with all five vegetation types together adding up to 100%, I included them together in one model and no model selection was carried out. This model showed a positive effect of bogs (logit slope $\pm$ SE = 5.259 $\pm$ 33.687, p=0.876) and a negative effect of forest (logit slope $\pm$ SE = -1.459 $\pm$ 33.341, p=0.965) and agriculture (logit slope $\pm$ SE = -14.976 $\pm$ 34.415, p=0.663) on the infection intensity in winter samples. Vegetation type "Open areas" also showed a small negative effect whilst "Other" showed a small positive effect on the infection intensity. However, none of these effects were significant. An ANOVA test revealed that none of the predictor variables added significantly to the model.

Landscape use in Kernel density home ranges

Inside the home ranges, "Bogs" was on average the most present vegetation group (51.5%), followed by "Forest" (25.5%), "Other" (11.2%), "Open areas" (10.1%), and "Agriculture" (1.7%).
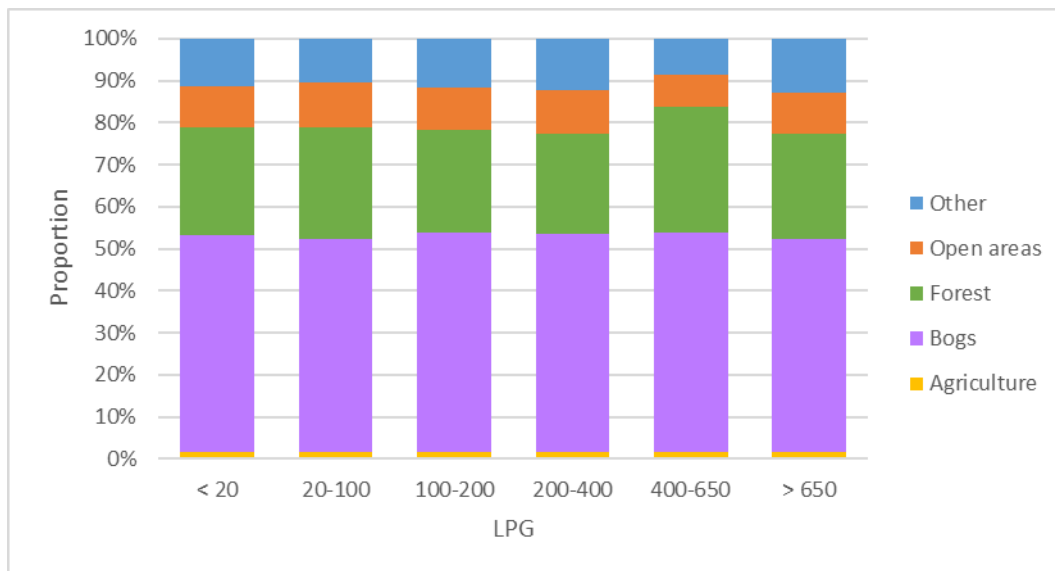

Figure 8. Average proportions of available vegetation types in summer grazing season home ranges of 2018-2020 of collared reindeer (n=51) in the South Trøndelag reindeer district by LPG category in the subsequent winter samples from 2019-2021. No obvious patterns were seen between landscape use and subsequent infection intensity.

The model {*Intensity ~ Agriculture + Bogs + Forest + Open.areas + Other*} was built using the proportions of vegetation types in the reindeer seasonal home ranges (see Landscape use analysis from home ranges). Because the vegetation types were expressed as proportional data, with all five vegetation types together adding up to 100%, I included them together in one model and no model selection was carried out. This model showed a positive effect of bogs (logit slope $\pm$ SE = 6.676 $\pm$ 34.205, p=0.845) and forest (logit slope $\pm$ SE = 12.396 $\pm$ 32.312, p=0.701) on the infection intensity in winter samples. Vegetation types "Open areas" and "Other" also showed a small positive effect whilst "Agriculture" showed a small negative effect on the infection intensity. However, none of these effects were significant. An ANOVA test revealed that none of the predictor variables added significantly to the model.

Altitude

The altitude of the positions during the summer grazing seasons of 2018-2020 varied from 214 to 1491 meters (mean=842, 95% CI 841-843). The average altitude during the summer grazing seasons inside vegetation group "Agriculture" was 670 meters (95% CI 662-678), in "Bogs" it was 737 meters (95% CI 735-740), in "Forest" it was 741 meters (95% CI 739-742), in "Open areas" it was 899 meters (95% CI 898-900) and in "Other" the average altitude was 887 meters (95% CI 872-902).

One model was built to examine the relationship between infection intensity and average used altitude during the grazing season. The model {*Intensity ~ Altitude*} showed a decreasing infection intensity with increasing altitude (logit slope ± SE = -0.001 ± 0.003, p=0.626). This effect was not significant.
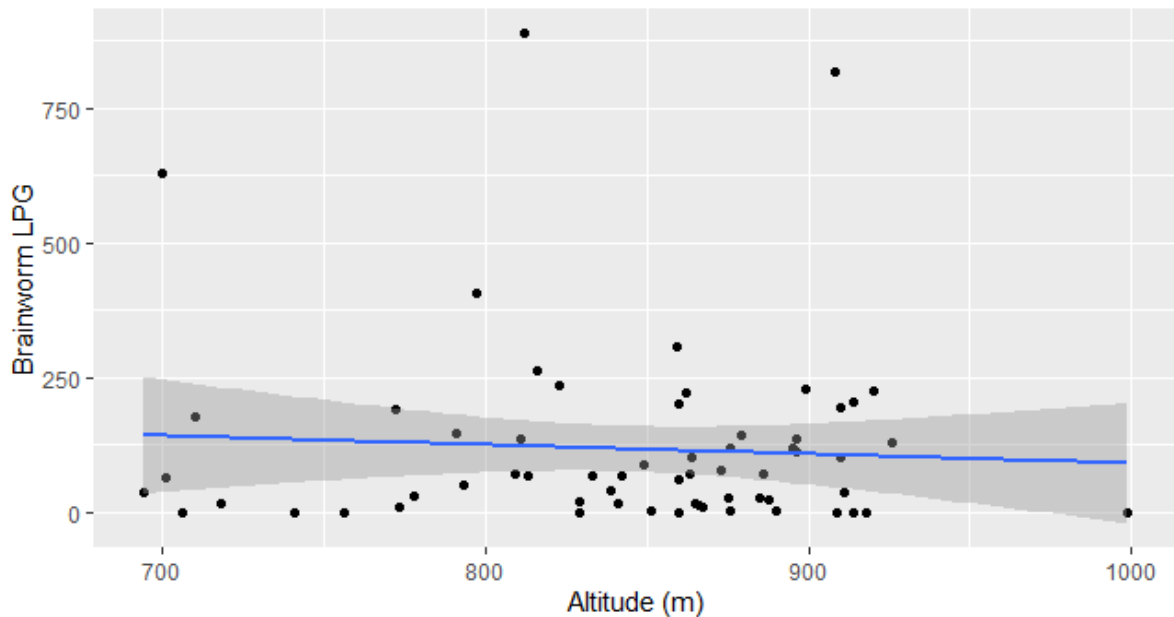

Figure 9. Relationship between average altitude of recorded positions of collared reindeer (n=51) in the South Trøndelag reindeer district during the summer grazing seasons 2018-2020 and infection intensity in subsequent winter samples of 2019-2021. There is a decrease in infection intensity with increasing altitude, but the significance level for this trend was not reached (p=0.626). The grey zone surrounding the blue line represents the 95% confidence interval.

3.5 Gastropod sampling

Gastropod sampling was conducted at 34 locations, of which 26 plots were located inside the area of the North Trøndelag reindeer herd and 8 plots were located inside the grazing area of the studied South Trøndelag herd. The altitudes of the chosen plots ranged between 441-745 meters. The average altitude in North Trøndelag was 515 meters (95% CI 481-549) and in South Trøndelag it was 732 meters (95% CI 726-737). The pH of the soil inside the plots varied from 6-7.5, with an average pH of 6.8 (95% CI 6.7-7.0) in North Trøndelag and 7.1 (95% CI 6.9-7.2) in South Trøndelag. The moisture level (range 1-10: 1-3= dry, 4-7= neutral, 8-10= wet; provided by manufacturer of 3 Way Soil Meter, see 2.2.2) in the plots ranged from 2-8, with an average moisture level of 4.6 (95% CI 4.0-5.3) in North Trøndelag and 5.3 (95% CI 4.1-6.4) in South Trøndelag.

I found a total of 49 gastropods, of which 37 gastropods (75.5%) were found in South Trøndelag. Table 4 and Fig. 10 show which gastropod species were found in North and South Trøndelag respectively and in which vegetation types. Seven out of the 49 collected gastropods could not be identified due to too small size, damage or loss of specimens.

Table 4. Recorded gastropod species in North and South Trøndelag reindeer herding districts.

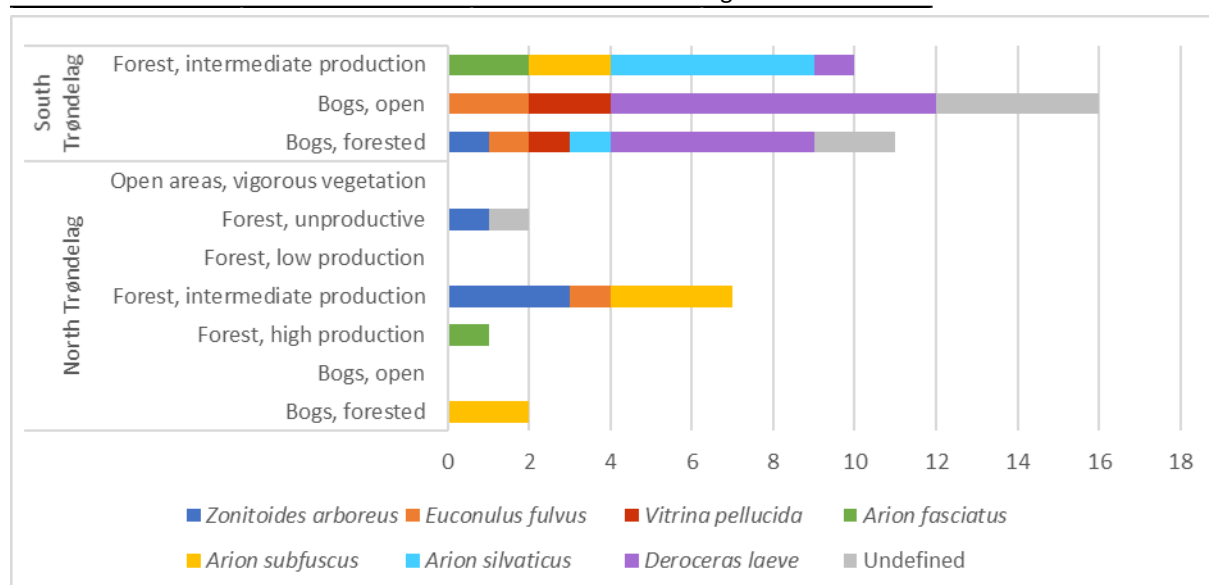| Snail species	Family	Slug species	Family
Euconulus fulvus	Euconulidae	*Arion fasciatus*	Arionidae
Vitrina pellucida	Vitrinidae	*Arion silvaticus*	Arionidae
Zonitoides arboreus	Gastrodontidae	*Arion subfuscus*	Arionidae
		Deroceras laeve	Agriolimacidae

Figure 10. Count of the gastropod species found during gastropod sampling in the North Trøndelag reindeer herding district (sampling carried out 4-6 September 2020) and in the South Trøndelag reindeer herding district (sampled on 23 September 2020) by the vegetation types in which the sampling plots were located.

In South Trøndelag, most gastropods were found in open bogs (16/37, 43.2%). *Deroceras leave* was the most present species in South Trøndelag (14/37, 37.8%), followed by *Arion silvaticus* (6/37, 16.2%). I found more slugs (24/31, 77.4%, undefined gastropods excluded) than snails (7/31, 22.6%). The average number of gastropods was higher in plots with a moisture level above 4.5 (Table 5). Also, the average number of gastropods was more than twice as high in the plot with a pH of 7.5 compared to the plots with pH 7. However, because of the small sample size and small variety in pH between the plots, no statements with statistical substantiation can be made about the effect of pH on the presence of gastropods. The same limitation applies to altitude. Visually, no patterns were seen in the effect of altitude on the presence of gastropods.

In North Trøndelag, most gastropods were found in forests with intermediate production (7/12, 58.3%). The number of found snails (5/11, 45.5%, undefined gastropods excluded) was almost equal to the number of found slugs (6/11, 54.5%). *Arion subfuscus* was the most found species in North Trøndelag (5/12, 41.7%). Most plots in North Trøndelag had a pH of 7, and this is also the pH in which the average number of gastropods was highest (Table 5). Visually, no patterns were seen in the average number of found gastropods in relation to moisture level and altitude.

Table 5. Average number of gastropod individuals found in 1m^2 plots with different moisture and pH levels in North and South Trøndelag reindeer herding districts.

		North Trøndelag			South Trøndelag		
		n plots	mean # gastropods	SD	n plots	mean # gastropods	SD
moist	<3.0	5	0.8	1.2	1	0.0	0.0
	3.0-4.4	6	0.7	0.5	1	1.0	0.0
	4.5-5.9	7	0.1	0.3	3	6.7	3.3
	6.0-7.4	7	0.3	0.5	2	5.0	4.0
	≥7.5	1	1.0	0.0	1	6.0	0.0
pH	6	3	0.0	0.0			
	6.5	3	0.0	0.0			
	7	20	0.6	0.7	7	4.0	3.6
	7.5				1	9.0	0.0

4. Discussion

Although reindeer have been commonly infected with the brainworm parasite *Elaphostrongylus rangiferi* for decades, there is concern that this parasite is currently increasing. Climate change causes the development of the parasite inside its intermediate host to accelerate, increasing the risk of large severe outbreaks of elaphostrongylosis in reindeer. With the late appearance of symptoms and the lack of treatment, this disease often results in death, posing a great threat to traditional reindeer herding practices in Norway. An alternative strategy to prevent future outbreaks is urgently needed.

In this thesis, the prevalence and intensity of infections with *E. rangiferi* in a semi-domesticated reindeer herd have been quantified over a period of three years and correlated with reindeer sex and age. In addition, I analysed the landscape use of this reindeer herd, with the aim to discover how landscape use affects the intensity of infections with brainworm in semi-domesticated reindeer. This thesis presents an updated study on the current brainworm situation, which can later be incorporated into a mathematical model to predict the exposure risk to brainworm, allowing reindeer herders to better manage future outbreaks.

4.1 Data collection and analysis

Faecal sampling was performed in collaboration and consultation with the reindeer herders. The uneven proportion of males and females in the herd (see section Study subject and area) resulted in a sampling bias, with the vast majority of samples taken from females. The same applies to the different age classes, where the majority of samples were taken from adults. This should be taken into account when interpreting the results. As described by Halvorsen et al. (1985) and Gaudernack et al. (1984), the larval output of *E. rangiferi* in infected reindeer shows a seasonal cycle that is sex-dependent. During summer, the larval output is at its minimum for both males and females. Males show a peak in larval output during autumn/early winter, while females show a peak in larval output during late winter/spring (Halvorsen et al., 1985). The sampling bias in this thesis may have affected and altered the results, causing patterns – such as the latter – to be different than expected from the literature. Because of the size of the herd, it was impossible to sample the same reindeer at each sampling period and follow the development of infection inside an animal. Yet, the random selection of reindeer with a sample size of 319 faecal samples in this thesis should give a good representation of the whole herd and therefore reliable results.

4.2 Seasonality and population dynamics of *E. rangiferi*

The prevalence of infection and the infection intensity differed strongly between seasons, with an overall highest prevalence during winter. The average and maximum infection intensity was also highest during winter for all age classes and both sexes. This can be explained by the developmental time of the parasite inside the reindeer body, as described by Handeland et al. (1994), and by the period during which reindeer get infected. Infections with *E. rangiferi* mainly occur during the summer months, as snow coverage during the rest of the year limits the possibility for reindeer to graze as well as the accessibility and intake of gastropods. Since L_1 larvae can be detected in the faeces approximately 4 months post infection (Handeland et al., 1994), infection prevalence and intensity will be highest during the winter months. The result that infections in calves were only observed during winter can be explained by the fact that calves are usually born around the end of the spring/beginning of summer and mainly feed on milk during the first period of their lives. Calves are only old enough to start grazing towards the end of the summer, and will therefore first show infections in winter faeces and with a lower prevalence and intensity compared to reindeer in the other age classes, who have been grazing and getting infected during the whole grazing season.

There was also a difference in infection prevalence and intensity between males and females. In all seasons, females had a higher prevalence than males. However, the predicted probabilities of being infected (3.2, Table 3) showed the opposite, with males having a higher predicted probability of being infected than females, in all seasons and age classes. This table also showed a higher predicted probability for adults during summer and autumn compared to winter, which is in contrast with the observed prevalence of infection that was highest during winter. Though, this may be influenced by the sampling bias of an uneven proportion of sampled males and females, and the uneven number of winter samples (3 years) and summer and autumn samples (both 1 year).

The average and maximum LPG was also higher in females than in males, except for in autumn. Males showed a higher average and maximum LPG than females in autumn samples. This is in line with the study of Halvorsen et al. (1985), in which the maximum density of larvae was highest for males in autumn/early winter, and for females in late winter/spring. The suggestion of Gaudernack et al. (1984) stating that larval output can be related to host stress and condition is also applicable to the results of this study, with males undergoing most stress and having the highest larval output during the rutting season (autumn) and females being in

their poorest condition and having the highest larval output during the pregnancy and calving period in late winter/spring (Gaudernack et al., 1984).

The probability of being infected and prevalence of infections was highest for adults and lowest for calves, which could be expected by the previous results where calves – in contrast to other age classes – only showed infections during winter. In the older age classes, infection could have happened during an earlier grazing season than the previous one, as suggested by Halvorsen and Andersen (1982) who estimated the life span of adult *E. rangiferi* to be up to three years. This would explain why adults show the highest infection probability, which supports the suggestion by the latter study of a long lived infrapopulation in reindeer.

I noticed that, in adults, the infection intensity often remained around the same level from year to year, which corresponds to the results of Halvorsen et al. (1985) and also supports this suggestion of a long lived infrapopulation. Remarkable was the increase in infection intensity for yearlings relative to calves, and a decrease in infection intensity for 2 year old and adult reindeer relative to the prior age class. A possible explanation for the decrease in infection intensity in the older age classes could be that partial immunity to brainworm develops as the reindeer get older, slightly lowering – but not resolving – the larval output. Previous studies have shown that nematode infection intensities may be limited by the immunity of older ungulates, as described by Armour (1989), and Ploeger (2011) showed that cattle are capable of rapidly developing immunity against *Dictyocaulus* spp. (lungworm), a parasite that is also common in reindeer. Another idea, as suggested by Halvorsen et al. (1985), is that the declining maximum larval output over the years is a reflection of the death rate or reduction of fecundity in the parasite infrapopulations.

4.3 Size does matter

For the relationship between brainworm infection and body weight, a decreasing probability of infection and decreased infection intensity with increasing body weight was shown. There was a significant difference in body weight between infected and uninfected female calves and adults, with uninfected animals being heavier than infected ones. This is in contrast with the results of Halvorsen (1986), who found that the heaviest calves were the most infected ones. However, the maximum LPG in the faecal samples of the calves in the latter study was markedly lower than the maximum LPG of calves in this thesis, namely 447 and 1091 LPG respectively (3.1, Table 2). This higher infection intensity may have had an effect on the body

condition of the calves in the form of reduced growth, which is – as mentioned earlier in the introduction – one of the symptoms of elaphostrongylosis in calves (Handeland et al., 1994; Josefsen & Handeland, 2014). If this is true, the fact that the calves in Halvorsen (1986) did not show this pattern may indicate that there is a lower boundary of LPG from where weight loss occurs. This would suggest that the level of infection found in the herd in this study is having an impact, albeit sub-clinical (no clinical symptoms of elaphostrongylosis), with reduced weight gain or weight loss in the more heavily infected individuals. For calves, this could have a negative impact on winter survival (Nieminen et al., 2011). In females, a reduced condition may decrease fertility, the probability of a successful pregnancy, foetal growth and it may also influence early calf survival (Cameron et al., 1993).

However, the correlation between lower weight and higher infection probability and intensity does not prove causation. It could be that lighter weighing animals are more vulnerable to infections, with poorer immune responses, and therefore show higher infection intensities than heavier animals. However, this is speculative and there may be different reasons for this pattern. The contradictory findings between this study and Halvorsen (1986) could result from multiple different factors, not just brainworm infection probability and intensity. For example, differences in food availability related to an animal's hierarchical position in the herd, different herd locations, climate (change) in the 35 years between the two studies and/or differences in supplementary feeding between the two studies. Longer-term baseline data, within and between herds, is needed, that not only includes infection probability and intensity but also information on the animals' weight and a standardised measurement of their size, to confirm this relationship between brainworm infections and body weight.

4.4 Gastropods

Although most sampling plots (26/34) were located in North Trøndelag, the vast majority of gastropods (37/49, 75.5%) were found in South Trøndelag. The difference in number of gastropods found between North and South Trøndelag may be due to the difference in climate between the two sampling areas. First of all, the sampling area in South Trøndelag was located more inland while the sampling area in North Trøndelag was located closer to the coastline. The proximity to the sea may have an influence on the presence of gastropods through such factors as soil quality, nutrient levels, salinity of water, weather patterns or rainfall. Although this was not tested in this thesis, it should be considered in future studies. The small number of sampling plots in South Trøndelag was due to unforeseen logistical difficulties such as

planned sampling plots inaccessible (private roads), sudden change of weather circumstances that halted sampling and then early snowfall which made gastropod sampling later in autumn impossible.

Several studies reported that gastropods occur mainly below the treeline, which may be due to the poor quality of soils above the treeline (Andersen & Halvorsen, 1984; Halvorsen et al., 1980; Handeland et al., 2019). The same was observed in this thesis. As expected, no gastropods were found in the few plots that were located above the treeline. No further patterns were seen between the number of gastropods found and the altitude of sampling plots below the treeline. The average number of gastropods found was highest in plots with a soil pH above 7, and this pattern corresponds with the literature. Previous studies have found a correlation between gastropod abundance and the calcium content of the soil (Andersen & Halvorsen, 1984; Valovirta, 1968; Waldén, 1981) and calcium-rich fens and bogs were mentioned as habitats that are rich in gastropods (Andersen & Halvorsen, 1984; Handeland et al., 2019). In this thesis, no pattern was seen between the number of gastropods found and moisture level of the soil, while Andersen & Halvorsen (1984) reported that sites with a high ground water level result in the richest gastropod faunas. The absence of this pattern in this thesis may be a result of the small sample size.

With the exception of the gastropod species *Zonitoides arboreus*, in which data is missing, the gastropods species that were found during sampling have been shown to be suitable intermediate hosts for *E. rangiferi* (Halvorsen & Skorping, 1982; Mitskevich, 1964; Skorping & Halvorsen, 1980). Although the direct relationship between gastropod presence and infection with brainworm in reindeer has not been tested in this thesis, the fact that the majority of gastropods found in South Trøndelag have been proven to be suitable intermediate hosts for the parasite may potentially be an indication for the magnitude of the risk of infection in this area. With more gastropod data of this area, a statistical analysis could be performed, which may allow for connecting gastropod presence directly with landscape use and brainworm infections in reindeer.

4.5 Landscape use

The landscape use of the GPS collared reindeer in this thesis was analysed in two different ways: (1) by examining the use of vegetation types and altitude at the real GPS positions and (2) by creating Kernel density home ranges around the GPS positions and examining the

available landscape types inside each reindeer's home range. The use of the real GPS positions gives information about which landscape types were actually used by the reindeer, but this method also brings uncertainty in the form of gaps with missing information between each recorded position. The GPS collars were programmed to record positions only every six hours in order to keep battery use low and thereby extend the period of use. Because of the missing information and possible patterns when using 6-hourly GPS positions, I chose to also analyse the landscape use by looking at the available landscape types inside each reindeer's home range. Although the 90% Kernel density reduces the chance of overestimating the home range, this method still brings uncertainty about which landscape types were actually used by the reindeer. In future studies, the collars could be used for a shorter period (only during the grazing season), which would allow for more frequently recorded positions (for instance every one or two hours) to get a more precise estimate of the landscape use of reindeer during the grazing season. This would eliminate the overestimation that comes with analysing landscape use in home ranges and reduce the gaps with missing information and possible patterns that come with the use of position data.

Remarkable was the difference in the calculated average proportion of vegetation groups during the summer grazing season between method 1 (GPS positions) and method 2 (Kernel density home ranges). Method 1 showed that the vegetation group "Open areas" was most used on average in the GPS positions (63.8%), but method 2 showed that this vegetation group was not the most present one (10.1%) inside the summer home ranges. Method 2 showed that the vegetation group "Bogs" was most present (51.5%) inside the summer home ranges, but method 1 showed that this vegetation group was not the most used by the reindeer (15.0%). This suggests that reindeer have strong preferences for certain vegetation types, even if these vegetation types are scarcer than others. A grazing study by White & Trudell (1980) with reindeer has tested this before, and found that reindeer have a high preference for forbs, deciduous shrubs and lichens. This latter vegetation type was also observed during gastropod sampling to be present in the most used vegetation group "Open areas", and thus may be one of the reasons why this vegetation group was most used during the grazing season.

In both methods for analysing landscape use, no significant relationships were found between infection intensity in winter samples and landscape use during the previous summer. However, in both methods a trend was observed in which availability of or time spent in the vegetation group "Bogs" had a positive effect on the infection intensity. Earlier studies have noted that

soil moisture had a positive effect on gastropod abundance and that calcium-rich bogs and hardwood forests are habitats that are rich in gastropods and therefore would have a greater infection risk (Andersen & Halvorsen, 1984; Handeland et al., 2019; Cebra & Gemensky-Metzler, 2014). Also in my study most gastropods were found in bogs in South Trøndelag and, additionally, the most frequently identified species in this vegetation type, *Deroceras leave*, was among the species in which brainworm larval development is amongst the fastest (Skorping & Halvorsen, 1980). The fact that the vegetation type "Bogs" was, on average, most present in the summer home ranges of the South Trøndelag reindeer suggests that the area potentially has a high infection risk. However, as there were no significant effects, the conclusion that frequent use of bogs by reindeer directly leads to higher brainworm larval densities cannot be made with full certainty. The lack of significant effects may be a result of the uncertainty associated with the two methods for analysing landscape use, but it may also be that other factors than vegetation type are of greater importance in predicting brainworm infection intensity.

4.6 Lower risk of infection above treeline

The average altitude in the most used vegetation group (Open areas, 899 meters) was highest of all vegetation groups. Since reindeer usually move to pastures at higher altitudes during summer (Halvorsen et al., 1980), the preference for open areas may actually be a preference for higher altitudes. This may explain the lack of significant effects of vegetation types on infection intensity and brings the expectation that altitude has a bigger effect on infection intensity. Handeland et al. (2019) and Closset (2021) demonstrated a notable reduction in infection intensity of *Elaphostrongylus* infection with increasing altitude. Although in this thesis a trend was observed in which a higher altitude had a decreasing effect on infection intensity, this trend was not significant (p=0.626). Both studies of Handeland et al. (2019) and Closset (2021) were conducted in wild reindeer herds, of which the summer pasture areas are located at, on average, higher altitudes than the semi-domesticated herd in this thesis. Therefore, the wild reindeer will likely spend more time during summer above the treeline where gastropod densities are lower.

Additionally, in contrast to the wild reindeer, the semi-domesticated reindeer are gathered by the herders during summer for the marking of calves, for which they are being moved to fenced areas on lower altitudes where both grazing pressure and infection risk are higher (Andersen & Halvorsen, 1984; Handeland et al., 2019). During the gathering of the herd and returning to

the grazing pastures, the semi-domesticated reindeer will also make more use of migration routes, which have, in general, a richer gastropod fauna – and therefore a greater infection risk – than grazing areas in the summer range (Andersen & Halvorsen, 1984). Because the semi-domesticated reindeer spend more time in areas with a higher infection risk more regularly, it is possible that they already have built up infections – and have a bigger *E. rangiferi* infrapopulation – and that therefore spending the summer months above the treeline has less effect on the infection intensity than it has in wild reindeer. This may be supported by the fact that the average infection intensity of 2020 (winter, summer and autumn) of the semi-domesticated reindeer in this thesis was markedly higher than the average infection intensity of 2020 (winter, summer and autumn) of the wild reindeer in Closset (2021), namely 126 (95% CI 90-162) and 32 LPG respectively. The average infection intensity and the maximum LPG of some animals in the semi-domesticated herd were remarkably high and surprising, as no clinical signs were observed in the herd. These high infection intensities may also pose a threat to co-grazing sheep and goats, since these animals can also get infected with *E. rangiferi* (Handeland & Skorping, 1993; Handeland et al., 1993; Handeland & Sparboe, 1991).

4.7 Limitations of the study and future directions

Data of three years of different animals has been analysed together, providing an overview of the average landscape use and infection prevalence and intensity. However, future studies may want to compare data of each year separate to be able to see the influence of factors such as temperature and precipitation – that can vary per year. These factors have not been included in this thesis, but should be considered in future studies to see in what way climate change affects the development of infections with *E. rangiferi*. This information will also be of importance when predicting future outbreaks. Also, landscape use was in this thesis analysed for all animals together and linked to parasitological data of all animals together. Analysing landscape use per individual and comparing individual reindeer with each other may give more precise insight in the relationship between landscape use and brainworm infections. Including information about the reindeer' daily behaviour may also be useful in examining this relationship. For example, looking at patterns in resting and grazing behaviour in combination with the use of landscape may be useful in determining the exact locations and times where and when the reindeer most likely get infected.

The gastropod study in this thesis was limited to 34 sampling plots. There was a notable difference in the number of found gastropods between North and South Trøndelag, but the

small sample size made me unable to perform a statistical analysis on this part of my study. More gastropod data from South Trøndelag would allow for testing the relationship between gastropod densities and brainworm infections, which may be integrated in a model for predicting brainworm infection risk zones in South Trøndelag reindeer district.

5. Conclusion

The results of my parasitological analyses show that infections with *Elaphostrongylus rangiferi* follow a seasonal cycle that is sex and age dependent. The prevalence and intensity of infections were highest in winter faecal samples. The probability of being infected increased with increasing age, supporting the suggestion by previous studies of long lived infrapopulations of *E. rangiferi* in reindeer. The average infection intensity and maximum LPG in the studied herd were remarkably high and surprising, as no clinical signs were observed. The infection intensity did not linearly increase with increasing age, which may be a sign of reindeer developing partial immunity as they get older. Remarkable was the result of a decreasing probability of infection and infection intensity with increasing body weight for calves and adult females, which is in contrast with a previous study that found that heavier animals were most infected. A possible reason for this may be that that the level of infection found in this herd is having an impact, albeit sub-clinical (no clinical symptoms of elaphostrongylosis), with reduced weight gain or weight loss in the more heavily infected individuals, but longer-term baseline data is needed to confirm this relationship between brainworm infections and body weight.

In the landscape use analysis, a trend was observed in which availability of or time spent in the vegetation group "Bogs" had a positive effect on the infection intensity. This was also the vegetation group where, in South Trøndelag, the majority of gastropods were found during gastropod sampling, which may potentially be an indication for the size of the risk of infection in this area. However, the direct relationship between gastropod presence and infection with brainworm has not been tested in this thesis and to accomplish that, more gastropod sampling has to be done. No significant effects of landscape use on brainworm infection intensity have been found in this thesis. This may either be a result of the uncertainty associated with the two methods that were used for analysing landscape, or it may be that other factors than vegetation type are acting here.

I also observed a trend of a decreasing infection intensity with increasing altitude, which corresponds with results of previous studies. The use of migration routes during gathering of the semi-domesticated herd and the high grazing pressure in fenced areas may pose a greater infection risk to the reindeer. The lack of significance of this trend may be due to a smaller variety of and lower average in altitude and higher average infection intensity in this thesis

compared to previous studies. In future studies, factors such as temperature and precipitation should be included to see in what way landscape use and climate change affects the development of infections with *Elaphostrongylus rangiferi*.

6. Author's contribution

The contribution of MS to this thesis was as follows:

Determined the gastropod sampling method in cooperation with supervisors and thesis colleague. Planned and performed gastropod sampling with some support from thesis colleague. Performed faecal sampling in cooperation with supervisors and with support from thesis colleague. Performed gastropod identification and faecal lab analysis of autumn 2020 samples and winter 2021 samples. Determined and performed method for landscape use analysis. Responsible for data analysis, interpretation and writing the thesis.

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Appendix A

Table A1. The generalized linear models that were built to examine probability of *Elaphostrongylus rangiferi* infection in semi-domesticated reindeer from South Trøndelag, ranked from lowest AICc (top) to highest AICc (bottom). Faecal sampling was carried out in winter 2019-2021 and in summer and autumn 2020. The best ranked and therefore the models chosen for use are indicated in bold.

RV	Model	EV	K	AICc	ΔAICc	WI	LnL
infection binary (1 = infected, 0 = uninfected)	**M12p**	**SEX + AGE * SEASON**	12	329.15	0	0.56	-152.07
	M9p	AGE * SEASON	11	331.6	2.45	0.17	-154.37
	M15p	SEX + AGE * SEASON * SEX	14	332.43	3.28	0.11	-151.52
	M4p	SEX + AGE	5	333.85	4.7	0.05	-161.83
	M5p	SEX * AGE	6	334.62	5.47	0.04	-161.18
	M2p	AGE	4	335.16	6.01	0.03	-163.52
	M14p	SEX * AGE * SEASON	16	336.85	7.69	0.01	-151.52
	M10p	SEX + AGE + SEASON	7	336.95	7.79	0.01	-161.29
	M11p	SEX * AGE + SEASON	8	337.51	8.36	0.01	-160.52
	M13p	AGE + SEX * SEASON	9	337.75	8.6	0.01	-159.58
	M8p	AGE + SEASON	6	338.61	9.46	0	-163.17
	M1p	SEX	2	387.32	58.17	0	-191.64
	M6p	SEX + SEASON	4	387.98	58.83	0	-189.93
	M3p	SEASON	3	390.23	61.07	0	-192.08
	M7p	SEX * SEASON	6	392.06	62.91	0	-189.9
infection binary (1 = infected, 0 = uninfected)	**M3pwc**	**WEIGHT * SEX**	4	37.25	0	0.95	-13.93
	M2pwc	WEIGHT + SEX	3	43.78	6.54	0.04	-18.49
	M1pwc	WEIGHT	2	45.96	8.71	0.01	-20.79

RV= response variable, Model= name of model, EV= explanatory variables, K= number of estimated parameters for the model, ΔAICc= difference between AICc for the selected model compared to the best-ranked model, WI= Akaike's weight, LnL= log-likelihood

Table A2. The generalized linear models that were built to examine *Elaphostrongylus rangiferi* infection intensity in semi-domesticated reindeer from South Trøndelag, ranked from lowest AICc (top) to highest AICc (bottom). Faecal sampling was carried out in winter 2019-2021 and in summer and autumn 2020. The best ranked and therefore the models chosen for use are indicated in bold.

RV	Model	EV	K	AICc	ΔAICc	WI	LnL
intensity (LPG)	**M7b**	**AGE + SEASON**	7	2674.93	0	0.56	-1330.2
	M11b	SEASON + SEX + AGE	8	2677.04	2.11	0.19	-1330.18
	M15b	SEASON + SEX * AGE	9	2679.2	4.28	0.07	-1330.18
	M8b	AGE * SEASON	10	2679.21	4.29	0.07	-1329.09
	M12b	SEASON * SEX + AGE	10	2679.47	4.54	0.06	-1329.21
	M14b	SEASON * AGE + SEX	11	2681.4	6.47	0.02	-1329.07
	M13b	SEASON * SEX * AGE	13	2682.78	7.86	0.01	-1327.52
	M4b	SEASON	4	2683.1	8.18	0.01	-1337.46
	M9b	SEX + SEASON	5	2683.43	8.5	0.01	-1336.57
	M10b	SEX * SEASON	7	2685.76	10.83	0	-1335.62
	M2b	AGE	5	2707.82	32.9	0	-1348.77
	M5b	AGE + SEX	6	2709.46	34.54	0	-1348.54
	M6b	AGE * SEX	7	2711.45	36.52	0	-1348.46
	M3b	SEX	3	2712.36	37.44	0	-1353.13
intensity (LPG)	**M1bwc**	**WEIGHT**	3	178.33	0	0.87	-84.83
	M2bwc	WEIGHT + SEX	4	182.24	3.91	0.12	-84.62
	M3bwc	WEIGHT * SEX	5	187.35	9.02	0.01	-84.39

RV= response variable, Model= name of model, EV= explanatory variables, K= number of estimated parameters for the model, ΔAICc= difference between AICc for the selected model compared to the best-ranked model, WI= Akaike's weight, LnL= log-likelihood