

# MASTER'S THESIS

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Gastrointestinal nematode quantities in salt lick soils and transmission potential to wild reindeer (*Rangifer tarandus tarandus*).

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## **Abstract**

Norway is home to the only remaining population of wild reindeer in Europe, which is dispersed across southern Norway in different sub populations. Among these, ten areas are categorized as ‘national reindeer area’. Knutshø and Forollhogna are two such national reindeer areas, where in recent decades, the wild reindeer population in Knutshø has decreased in number and body condition without any apparent cause. The discovery of Chronic Wasting Disease (CWD) in reindeer in Nordfjella in 2016, has drawn attention to the use of saltlicks and their role as a potential transmission hotspot, and saltlicks have been suggested to increase the risk for pathogen transmission between animals visiting the salt licks.

The objective of this master thesis was to investigate the occurrence and abundance of parasite eggs and oocysts in soil around saltlicks and control areas to assess the potential role of saltlicks as transmission hotspots for gastrointestinal parasites. Additionally, the thesis also aimed to assess burden and species composition of endoparasites found in reindeer’s abomasum and faeces. Soil samples were collected from Knutshø and Forollhogna in summer and autumn 2020-2021. Samples from abomasum and faeces were collected in collaboration with wild reindeer hunters and wildlife officials during autumn 2021.

The results from the soil samples revealed significantly higher concentrations of parasites eggs and oocysts in the soil surrounding saltlicks compared to soil from control areas. The parasite numbers were also higher in Knutshø than Forollhogna, and higher during autumn compared to summer. The dominant species identified was the invasive species *Nematodirus battus*, along with *Moniezia* and *Eimeria* spp. Analysis of the reindeers` abomasum for endoparasites identified *Teladorsagia circumcincta* as the predominant parasite in all samples, which is a well-known parasite of sheep. Faecal samples from reindeer revealed findings of several genera of gastrointestinal parasites, including *N. battus*.

This study demonstrates that saltlicks serve as a hotspot for parasite accumulation, which may increase the risk of parasite spillover between different species. The findings from this study can contribute to the future management of natural pastures shared by sympatric sheep and wild reindeer.

Key words: *Wild reindeer, sheep, saltlicks, gastrointestinal parasites, Nematodirus battus, Teladorsagia Circumcincta, Ostertagia gruehneri, national reindeer areas*

## Sammendrag

Norge har den siste gjenværende villreinpopulasjonen i Europa. Populasjonen er spredt over flere områder i Sør-Norge, hvorav ti av disse områdene er nasjonale villreinområder. To av disse områdene er Knutshø og Forollhogna, og de siste tiårene har villreinpopulasjonen i Knutshø blitt redusert i antall og størrelse uten noen tydelig årsakssammenheng. Etter oppdagelsen av skrantesyken (CWD) i Nordfjella i 2016, har bruken av saltsteiner og deres rolle som samlingspunkt for ulike arter fått mer oppmerksomhet, da saltsteiner har blitt foreslått som en mulig risikofaktor for overføring av patogener mellom dyr som besøker saltsteinene.

Denne masteroppgaven undersøkte jorden rundt saltsteiner for å se på forekomsten av egg og oocyster fra endoparasitter, med tilhørende kontrollsteder for å vurdere om saltsteiner fungerer som overføringspunkter for gastrointestinale parasitter. Oppgaven undersøkte også belastningen og artssammensetningen av endoparasitter som ble funnet i villreinens løype og avføringsprøver. Jordprøver ble samlet inn fra Knutshø og Forollhogna sommeren og høsten 2020-2021. Prøver fra løype og avføring ble samlet inn i samarbeid med villreinjegere og oppsynsmenn høsten 2021.

Resultatene fra jordprøvene viste en signifikant høyere konsentrasjon av egg og oocyster rundt saltsteiner, sammenlignet kontrollområdene. Antallet parasitter var også høyere i Knutshø enn i Forollhogna, og var høyere om høsten enn om sommeren. Den dominerende parasitten fra begge områdene var *Nematodirus battus*, etterfulgt av *Moniezia* and *Eimeria* spp. Etter å ha undersøkt villreinens løype for endoparasitter viste resultatet at *Teladorsagia circumcincta*, en parasitt som er utbredt hos sau, dominerte i alle prøver. I avføringsprøvene ble det påvist flere typer gastrointestinale parasitter, inkludert egg av *N. battus*.

Studien indikerer at saltsteiner fører til akkumulering av parasitter i jordsmonnet, og kan dermed gi en økt risiko for overføring av parasitter mellom forskjellige arter. Resultatene fra denne studien kan bli brukt i framtidig forvaltning av utmarksbeiter for både sau og villrein.

Nøkkelord: *Villrein, sau, saltsteiner, gastrointestinale parasitter, Nematodirus battus, Teladorsagia Circumcincta, Ostertagia gruehneri, nasjonalt villreinområde*

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# Table of content

<b>ABSTRACT .....</b>	<b>I</b>
<b>ACKNOWLEDGMENTS.....</b>	<b>III</b>
<b>1.INTRODUCTION .....</b>	<b>1</b>
<b>2. METHODS.....</b>	<b>8</b>
2.1 STUDY AREAS .....	8
2.1.1 <i>Knutshø</i> .....	9
2.1.2 <i>Forollhogna</i> .....	9
2.3 DATA COLLECTION .....	11
2.3.1 <i>Sample collection</i> .....	11
2.3.2 <i>Parasitological procedures in lab</i> .....	12
2.4. DATA ANALYSIS .....	15
<b>3. RESULTS.....</b>	<b>16</b>
3.1. DESCRIPTIVE STATISTICS .....	16
3.1.1 <i>Eggs detected in soil around saltlicks in Knutshø</i> .....	16
3.1.2 <i>Eggs detected in soil around saltlicks in Forollhogna</i> .....	17
3.1.3 <i>Adult nematodes in the abomasum</i> .....	18
3.1.4 <i>Faecal eggs and oocysts</i> .....	19
3.1.5 <i>Saltlick, area, and seasonal effects on the parasite load in soil samples</i> .....	20
3.1.6 <i>Difference among species in soil samples</i> .....	21
3.1.7 <i>Comparison between summer and autumn saltlicks</i> .....	24
<b>4. DISCUSSION.....</b>	<b>25</b>
4.1 SOIL SAMPLES .....	25
4.2 ABOMASUM SAMPLES .....	29
4.3 FAECAL SAMPLES .....	31
4.4 NEMATODIRUS BATTUS .....	32
4.5 EIMERIA SPP.....	33
<b>5. MANAGEMENT IMPLICATIONS.....</b>	<b>35</b>
<b>6. CONCLUSIONS.....</b>	<b>37</b>
<b>7. REFERENCES .....</b>	<b>38</b>
<b>8. APPENDIX .....</b>	<b>45</b>

## 1. Introduction

During the last 2.5 million years, wild reindeer have lived through several ice ages and warm periods. Wild reindeer (*Rangifer tarandus tarandus*) have existed in Europe for more than 40000 years and has been hunted by Norwegians for over 10000 years. They played a crucial role for human subsistence (Punsvik et al., 2016, pp. 7-11), and they have a substantial, historical, ecological, and cultural importance (Kaltenborn et al., 2015). In modern times, the wild reindeer population in Norway has become fragmented into 23 different sub populations, and 10 out of these are categorized as “national reindeer areas” (Rolandsen et al., 2022). The classification of national reindeer areas is based on their ability to provide a suitable habitat with sufficient space and good quality resources to sustain wild reindeer populations over an extended period. This classification aims to support the long-term preservation of wild reindeer in Norway (Bjørnøy, n.d.).

Reindeer belongs to the cervid family and their diet varies between seasons and the nutrition value of forage in the pasture, and can consist of lichen, mushrooms, shrubs, and different types of herbs and grasses (Heggberget et al., 2002; Langvatn, 2023). Reindeer can be divided into three main groups: Artic reindeer, Forest reindeer and Tundra reindeer (Punsvik et al., 2016, p. 11). The wild reindeer in Norway belongs to the Alpine Tundra reindeer category, which live in large herds on the tundra and have different grazing areas throughout the year (Andersen & Hustad, 2004). The development to Nomadic herding of reindeer is believed to have happened around 15-16<sup>th</sup> century in Fennoscandia, due to strong decrease in the wild reindeer population because of climate change and the need to secure food (Andersen & Hustad, 2004; Holand, 2003, p. 24). Reindeer husbandry has its origins in the Sami population, and there are approximately 250 000 semi-domesticated reindeer in Norway today (Landbruks og matdepartementet, 2023).

Today, Norway is the only remaining country with a population of wild reindeer, and hence has the responsibility to protect the species and its habitat. The total winter population currently consists of about 25000 individuals. The population size in each reindeer management area is decided locally and regulated through organized hunting (Miljødirektoratet, 2021). The hunting period in Norway are set by the Directorate of nature administration (DN) to certain periods in August and September, and the quota of allocated wild reindeer for hunting may differ between years and areas, based on the expected growth from the previous year and the number of total animals (Forollhogna villreinutvalg, u.å.; Knutshø villreinutvalg, 2018; Miljødirektoratet, 2021). Despite Norway’s responsibility to protect the wild reindeer, the recent assessment of

the ten national reindeer areas after the ‘Environmental quality standard for wild reindeer’ describes that the population in national reindeer areas are not doing very well (Rolandsen et al., 2022). The national reindeer areas and populations were all classified as either “good”, “medium”, or “poor”, and parameters like population conditions (genetical variation, population numbers slaughter weight on calves etc.), lichen availability, human impact, and habitat (draft passage, land utilization) were used in the assessment. Knutshø and Forollhogna are two of the national reindeer areas, and are classified as ‘average’ and ‘poor’, respectively (Rolandsen et al., 2022).

During the last two decades there has been a negative development in the wild reindeer population performance in Knutshø. Both the relative number of reindeer calves in the population and their slaughter weight is decreasing. The slaughter weight of adults seems to be stable, however, which may suggest that the problem is not related to poor grazing conditions or a too high population density. In the recent quality assessment, the authors pointed out that high parasite load can be one of the possible causes of declining slaughter weights in calves and calf production (Rolandsen et al., 2022).

There has been an increase in the number of free ranging sheep in Knutshø during the last few decades, and parasites from sheep can have a spillover effect on sympatric reindeer (Utaaker et al., 2023). In the context of infectious diseases, “spillover” refers to the transfer of a disease or pathogen from one species to another. This occurs when a pathogen, typically associated with one species, crosses over to a different species and causes illness. The increase in sheep numbers is also probably accompanied by increased use of saltlicks in the area. Saltlicks are commonly used by sheep farmers to avoid mineral deficiencies (Animalia, 2018) and ease supervision and herding of the sheep at pasture. Since the discovery of Chronic Wasting Disease (CWD) in reindeer in Nordfjella in 2016, saltlicks have received more focus as a possible hotspot for pathogen exchange (Hansen et al., 2017). The saltlick stone attract several wild and domestic ungulates, where they lick on the same saltlicks, and often defecate and urinate in the immediate vicinity of the saltlick, which facilitates pathogen accumulation and transmission (O’Connor et al., 2006). Several members of the cervid family are also known to eat the soil around the saltlick (Hansen et al., 2017). Camera traps installed around saltlicks in Knutshø and Nordfjella showed that large groups of reindeer and sheep gather around the saltlick area and eat the soil around it, which contains salts that seeped from the saltlick itself (Johansson, 2021; Strand et al., 2023). Thus, pathogen spill over from sheep has been suggested to be one plausible

cause for the negative development in reindeer calves, and saltlicks could be a potential hotspot of pathogen transmission.

Understanding the effect of salt licks as transmission hotspots, could serve useful for monitoring and assessing parasite burdens of wild reindeer in Knutshø and Forollhogna, and may explain some of the negative development of wild reindeer production parameters in these areas. Bye (1987) studied abomasal parasites in wild Norwegian reindeer in 1980s, and did not find any evidence of parasite spillover between sympatric sheep and reindeer. Robertsen (2020) performed a similar study in these areas more than thirty years later, and found strong evidence of parasite spill over, with a higher number of parasites commonly found in sheep in the reindeer's abomasum than species of parasites associated with reindeer. This suggests that a spill over in the direction from sheep to reindeer has occurred since the 1980s.

Free ranging livestock husbandry has long traditions in Norway, and a significant part of the feed units in sheep production are gathered from pastures on rangeland (Wam & Herfindal, 2020). In 2004, it was estimated that pasture on rangeland contributed with around 300 million feed units, which is equivalent to 1 million hectares of cultivated pasture (Asheim & Hegrenes, 2006). Managing livestock this way is financially beneficial for the farmer and important for the maintenance of rangeland and its biodiversity (Strand et al., 2021). In March 2022, about one million winter-fed sheep were registered in Norway (Statistics Norway, 2022). During the last few decades, there has been an increase in the number of sheep grazing on the natural pastures in Forollhogna and Knutshø. From 2000 to 2018, the number of sheep has increased from approximately 37.000 to over 46.000 and 36.600 to over 40.000 sheep in Knutshø and Forollhogna, respectively (Utaaker et al., 2023).

Saltlicks have been used in Norway since early last century and are now spread all over the country where livestock are kept on natural pastures. Anecdotal evidence indicates that the number of salt licks has increased considerably the last three decades (Ytrehus et al., In press). Saltlicks are used as a preventative measure against mineral deficiencies in sheep, and are considered important supplements as the mineral content on pastures vary (Animalia, 2018). The use of saltlicks is also practical for the farmer, as they facilitate the compulsory monitoring of the sheep on pasture by being a point of attraction for the animals (Animalia, 2018).

To avoid accumulation of pathogens in the soil, it is important that the saltlicks are placed on a spot that the sheep only visit temporarily, and preferably on bedrock (Landbrukets film- og billedkontor, 1967). When salt licks are used in the mountains, its common to use a salt stone



‘vending machine’, where salt stones of 10 kg are placed upon each other (Figure 1). Snowmobiles are often used to place them out in late winter, which allows farmers to cover large areas and reach high altitudes (Ytrehus et al., In press). There is no registry on the number of saltlicks in Knutshø and Forollhogna, although, since the number of sheep in these areas are increasing, it is likely that the number of saltlick sites also have increased.

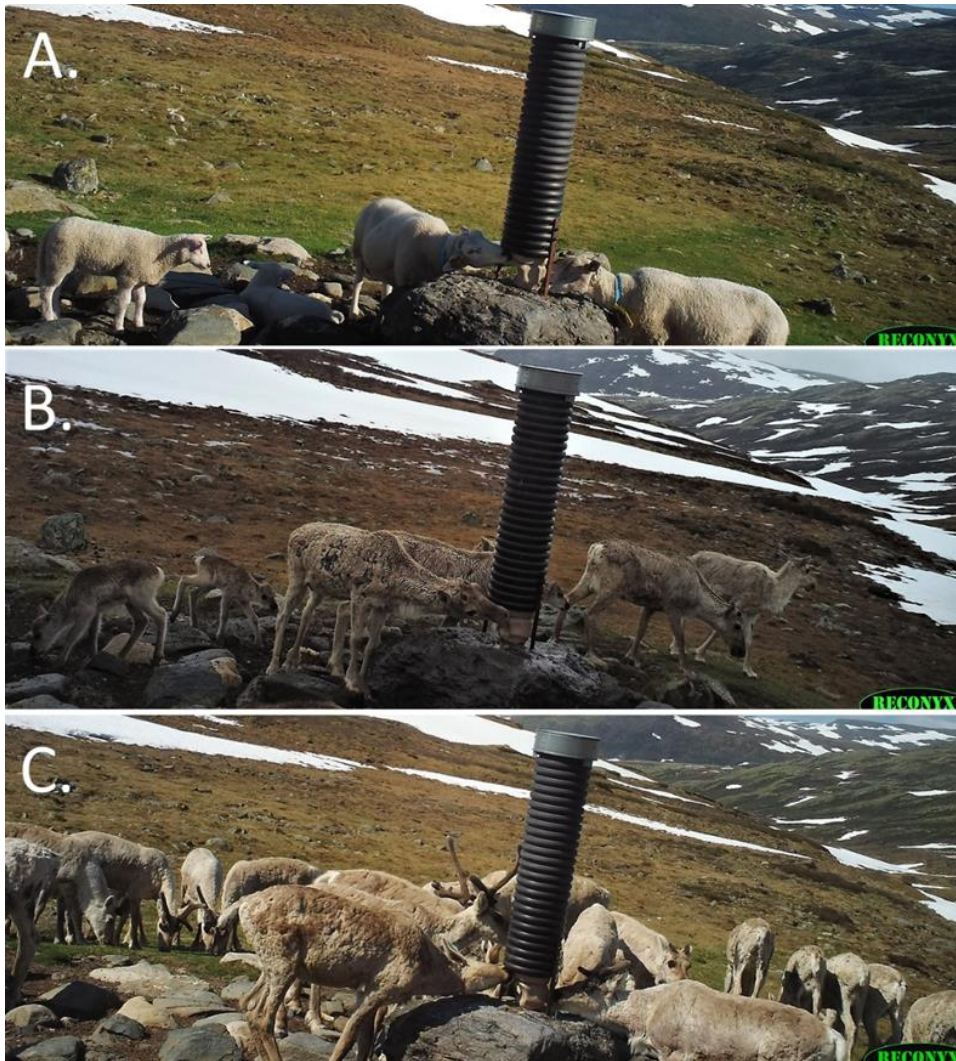


Figure 1: Camera trap images from a single saltlick location in Knutshø from 6 – 18 June 2020. A. Sheep at saltlick stone, B. Reindeer using a saltlick and a calf (left in the image) eating soil, and C. several reindeer grazing vegetation around the saltlick (Utaaker et al., 2023).

Parasites are organisms that infect a host, to whom it inflicts some damage while itself benefits in some way from the host (Loker & Hofkin, 2022, p. 535). Based on their location on or in the host, they are either endoparasites (live inside the host) or ectoparasites (live on the host). Parasites can either have a direct lifecycle, i.e. that the life cycle of the parasite only involves the host in which the reproductive stage of the parasite lives, or an indirect lifecycle where one or more stages of the parasite infect one or more intermediate hosts before they reach the main host and complete their lifecycle (Gjerde, 2015; Josefsen et al., 2014). Gastrointestinal parasites are well known in livestock farming and their impact on ruminants can result in reduced animal health and welfare (Scott, 2015, pp. 347-375). The clinical signs of the host will vary based on the parasite species and burden, but the general condition of the animal gets reduced. This can manifest as reduced appetite, weight loss, dehydration, and reproduction problems (Tryland & Kutz, 2019, p. 178). Some species can cause rather severe damages when present in high numbers, such as severe diarrhea and anemia (Gjerde, 2015).

This study will focus on occurrence of gastrointestinal nematodes found in the abomasum and faeces of reindeer and eggs and oocysts from similar nematode species in soil around saltlicks and corresponding control sites. The most common nematode species in reindeer is typically *Ostertagia gruehneri* and can be found in the abomasum together with *Trichostrongylus* spp and *Teladorsagia boreoarcticus*, and *Marshallagia marshalli*. In the intestine, species like *Nematodirus* and *Nematodirella* are most common (Tryland & Kutz, 2019, p. 180). The most common gastrointestinal nematodes in Norwegian sheep are *Teladorsagia circumcincta* and *Haemonchus contortus*, both of which infect the abomasum of their hosts, and *Nematodirus* spp, which are found in the small intestine (Domke et al., 2013).

Three parasites are especially relevant for sheep husbandry and wild reindeer health (Gjerde, 2011; Josefsen et al., 2014; Tryland & Kutz, 2019, p. 182): *T. circumcincta*, *Nematodirus battus* and *O. gruehneri*. *T. circumcincta* belongs to the Strongylida order which includes several families (Hrabok, 2006; Roeber et al., 2013). Strongylide eggs are not very robust against the environment, except for *T. circumcincta* eggs which has a protective shell and can develop in low temperatures. The infective larvae stage 3 (L3) can overwinter in the pastures, which can result in an accumulation of eggs and larvae in the pastures (Gjerde, 2011). The development from larva stage one (L1) to L3 in the environmental conditions (such as moisture, humidity, and temperature), depends on the species, temperature, and weather conditions (Hrabok, 2006; Roeber et al., 2013). After ingestion of an infective larvae, the parasite goes into hypobiosis in the host, where they remain inactive before they complete their lifecycle. This happens in the

mucous membrane of the abomasum or small intestine in the host. After several months, they get reactivated and complete their development into adult nematodes. The temperature during fall can affect some species to go straight to hypobiosis after getting in a host, and in late winter/spring, they get reactivated and complete their lifecycle (Gjerde, 2011). *T. circumcincta* is found in sheep abomasum, and immunity is developed slowly against this nematode. Infections with this parasite can cause reduced appetite and poor digestion of feed, which leads to weight loss. High burdens may cause diarrhea (Gjerde, 2011; Taylor et al., 2015).

Another Strongylid, *N. battus*, belongs to the family *Thrichostrongylidae*, and is an highly pathogenic parasite (Nikbin et al., 2022). *N. battus* is mostly associated with sheep and can be found in the small intestine. The eggs of *N. battus* are very resistant to the environment and can survive through the winter (Gjerde, 2011; Nikbin et al., 2022). The larvae develops inside the egg, and after a chilling period and a temperature range between 11-17°C, it hatches synchronously as infective larvae (van Dijk & Morgan, 2008). It was unintentionally imported to Norway in 1951 with infected sheep from Great Britain and has now spread all over the country (Helle, 1969; Vatn et al., 2009, p. 201). Due to developed immunity after initial infection, the nematode mainly affects younger animals. *N. battus* infection can cause reduced appetite, severe diarrhea, dehydration and in severe cases, mortality (Gjerde, 2011).

*O. gruehneri* is an abomasal nematode mostly associated with a reindeer host. It has a high prevalence in both semi-domesticated and wild reindeer (Hrabok et al., 2006b; Kutz et al., 2012). It is distributed on more northern latitudes (Hoberg et al., 2001) which could be due to the distribution of its natural host, although it has been found in domestic sheep sharing pasture with reindeer (Manninen et al., 2014). As most strongyles, it has a direct life cycle with three free living stages which are influenced by environmental conditions. Its larval stages are robust to temperature fluctuations when compared to strongyles found in temperate and tropical regions, and can overwinter on pastures (Hoar, 2012; van der Wal et al., 2000) *O. gruehneri* infection can cause reduced food intake, weight loss and poor condition and reproduction (Tryland & Kutz, 2019, p. 182) . In contrast to strongyle infections in domestic livestock, no apparent immunity against this nematode is developed in reindeer (Irvine et al., 2000).

In summary, all strongyles described above have robust developmental and transmission stages which survives the winter, and the host is infected by ingesting infective larvae from faeces.

*Eimeria* is a family that consists of several different *Eimeria* species that can cause coccidiosis in livestock, which can result in reduced milk production, diarrhea, and weight loss. (Gjerde,

2011; Mat Yusof & Md Isa, 2016; Paul et al., 2020). It is a protozoan parasite, and they are highly host specific. After excretion the oocysts will sporulate and depending on the temperature, it will become infective after a few days or weeks, and can survive in moist conditions as sporulate infektive oocysts for up to a year (Gjerde, 2011).

Since reindeer and sheep often share natural pastures, there is a risk of pathogen spillover, especially when their population density increases. This risk may be exacerbated by an increased functional density at the salt licks. In addition, the animals ingest and/or lick on the salt laden soil, which may increase the exposure to nematode eggs and larvae. A seasonal effect is included in the analysis as we expect to see a higher parasite burden during autumn than summer, which we believe that visitation from sheep at saltlicks through the summer leads to an increase in the number of parasite transmission stages in the soil, and due to high survival rate of some species it will lead to accumulation of parasites in soil.

This study had two main aims:

1. To investigate the occurrence and abundance of quantify parasite eggs and oocysts in soil around saltlicks and control areas, to assess the potential of salt licks as transmission hotspots for gastrointestinal parasites.
2. To assess burden and species composition of endoparasites found in reindeer's abomasum and faeces.

I hypothesize that (H1) parasite loads will be higher in soils around saltlicks compared to soils at random locations, (H2) particularly in Knutshø and (H3) during autumn. Furthermore, I hypothesize that parasite loads in the (H4) abomasum and faeces will be higher in reindeer from Knutshø compared to reindeer in Forollhogna.



## 2. Methods

### 2.1 Study areas

Sampling was done in Knutshø and Forollhogna national reindeer area, Norway, during summer and autumn (June – September) 2020 and 2021.

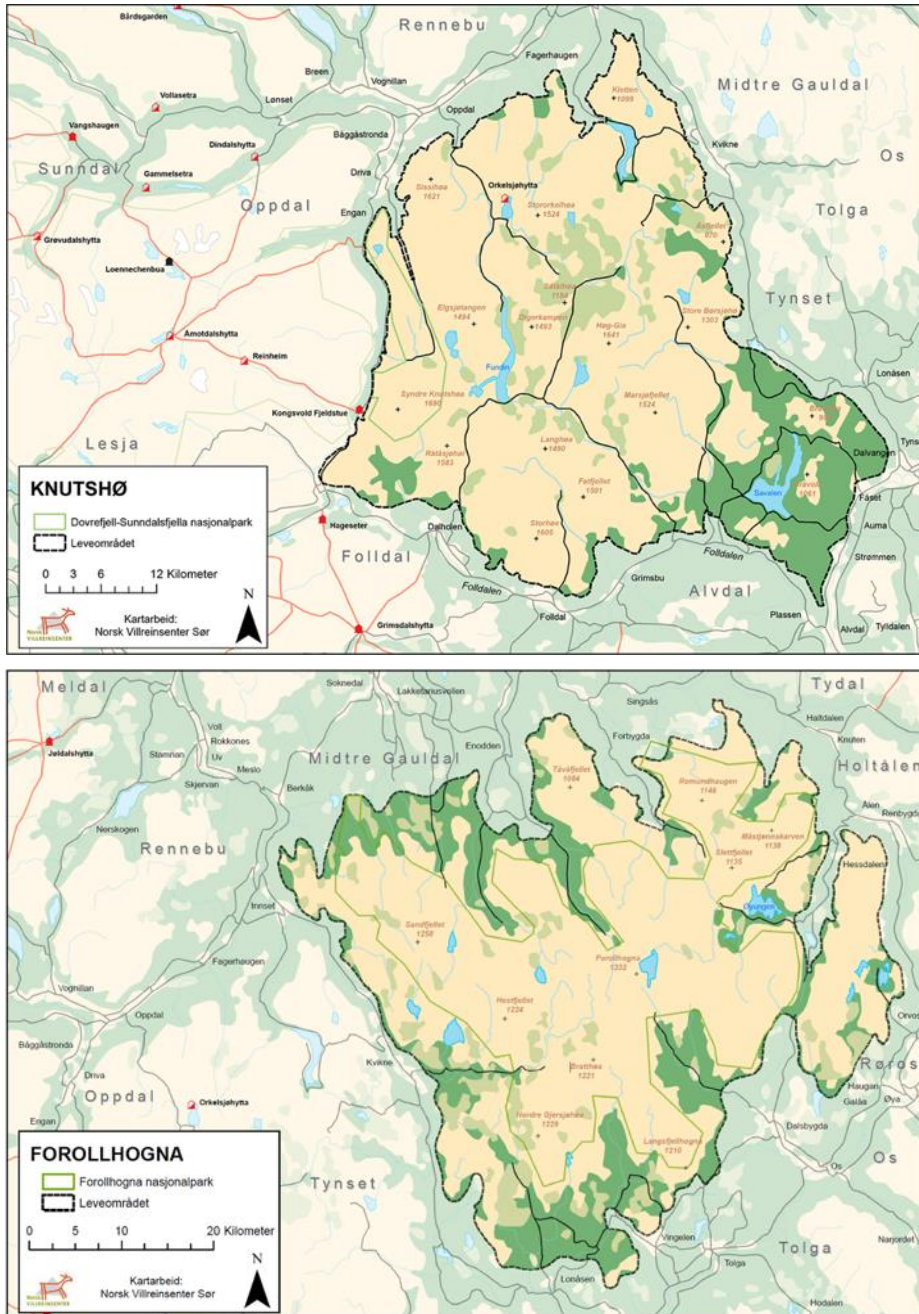


Figure 2 : Map over Knutshø (top) (Norsk villreinsenter, n.d.-b) and Forollhogna (bottom) (Norsk villreinsenter, n.d.-a).

### **2.1.1 Knutshø**

Knutshø is a mountain area in Trøndelag County, and the reindeer rangeland encompasses 1776 km<sup>2</sup>. It borders to other mountain areas, namely Forollhogna in northeast, Snøhetta in the West and Rondane in the South (Andersen & Hustad, 2004). The area is dominated by wide valleys, with mountains covered in vegetation. The highest mountain in Knutshø is 1690m above sea level. The area is categorized as a good pasture for the wild reindeer, and lichens covers 40% of the total area. The reindeer in Knutshø were originally part of a bigger herd that lived in Dovrefjell. During the middle of the 20<sup>th</sup> century, reindeer almost disappeared from Knutshø due to an increase in human activities in this area (Punsvik et al., 2016, pp. 355-360).

Due to migration of wild reindeer from Snøhetta, the population became re-established around 1960 - 1970 and the population size of the wild reindeer has increased, but with large variation from year to year (Punsvik et al., 2016, p. 359). In 2022, Knutshø was classified as one of six reindeer areas of poor quality due to its bad population condition, even though it was classified as a “good” lichen pasture and “medium” on the human impact and living area (Rolandsen et al., 2022). The population size goal is set to 1500 reindeer in this area (Punsvik et al., 2016, p. 355).

### **2.1.2 Forollhogna**

Forollhogna is a mountain area in Trøndelag County, and the reindeer rangeland encompasses 1843.2 km<sup>2</sup>. The wild reindeer in this area represents the northernmost population of wild reindeer, as reindeer herds north of this all are semi-domesticated reindeer. The area borders to Knutshø in the southwest (Andersen & Hustad, 2004). The area consists of several high mountains, but most of the area lies in the low alpine region. The highest mountain is ‘Forollhognatoppen’ which is 1332 m above sea level. Compared to other wild reindeer areas, Forollhogna is known to have the best distribution of the seasonal grazing pastures (Punsvik et al., 2016, p. 343).

Wild reindeer have a long history in Forollhogna, as they have inhabited this area since the end of the last ice age (Punsvik et al., 2016, p. 347). Documents from 1784 show that the wild reindeer and semi-domestic reindeer shared the area, and in 1890 counted over 10000 animals. Due to conflict between reindeer herders and farmers, semi-domestic reindeer herding was prohibited in Forollhogna in 1901 (Punsvik et al., 2016, p. 348). The number of wild reindeer in Forollhogna has shown to be stable for the last 20 years and are known to have a high calf

production and relatively high slaughter weights. Forollhogna fulfils the requirements as a wild reindeer area of “medium” quality, having a “medium” score at every parameter (Rolandsen et al., 2022). The population size goal is set to 2000 animals. (Punsvik et al., 2016, p. 343).

## **2.3 Data collection**

### **2.3.1 Sample collection**

Soil samples from randomly selected saltlicks were collected during summer and autumn (June – September) in 2020 and 2021 according to the following procedure: A two-meter ruler was laid on the ground with one end against the pole holding the saltlick and positioned in the runoff direction. Two other rulers were placed in a 45 ° angle to this first ruler so that they were 90° degrees to each other with the first in the middle. Loose faeces, dirt and debris were removed from the soil surface before the upper 2-5 cm of soil was sampled using a tablespoon. A spoon of soil was collected every 20 cm along the rulers, starting 20 cm from the saltlick, and stopping at the 2-meter mark. The subsamples were gathered in a cylindrical plastic container, making up approximately 500 ml of soil. The container was closed with an airtight lid and thoroughly shaken to mix the material. A control site was chosen 300 – 500 m from each sampled saltlick. The control sites lay on the same altitude and had similar topography, slope and vegetation as the saltlick, but without visible signs of frequent animal activity.

Intestinal samples were collected from already eviscerated carcasses in collaboration with wild reindeer hunters and wildlife officials in autumn 2021. Samples were collected ad hoc and not later than 12 hours after evisceration. The abomasum was secured with cable ties and disconnected from the gastrointestinal tract using scissors, and the viscera was placed into marked plastic bags. Faeces were collected from the rectum of the gastrointestinal tract and placed into marked containers.

The samples were transported from the sampling site and stored at -12°C until further shipping to Nord University for analysis. Samples were packed with a cooling block and sent overnight. The faecal samples were analysed promptly after arrival, and the viscera were stored at -12°C and thawed overnight at 4°C before analysis.



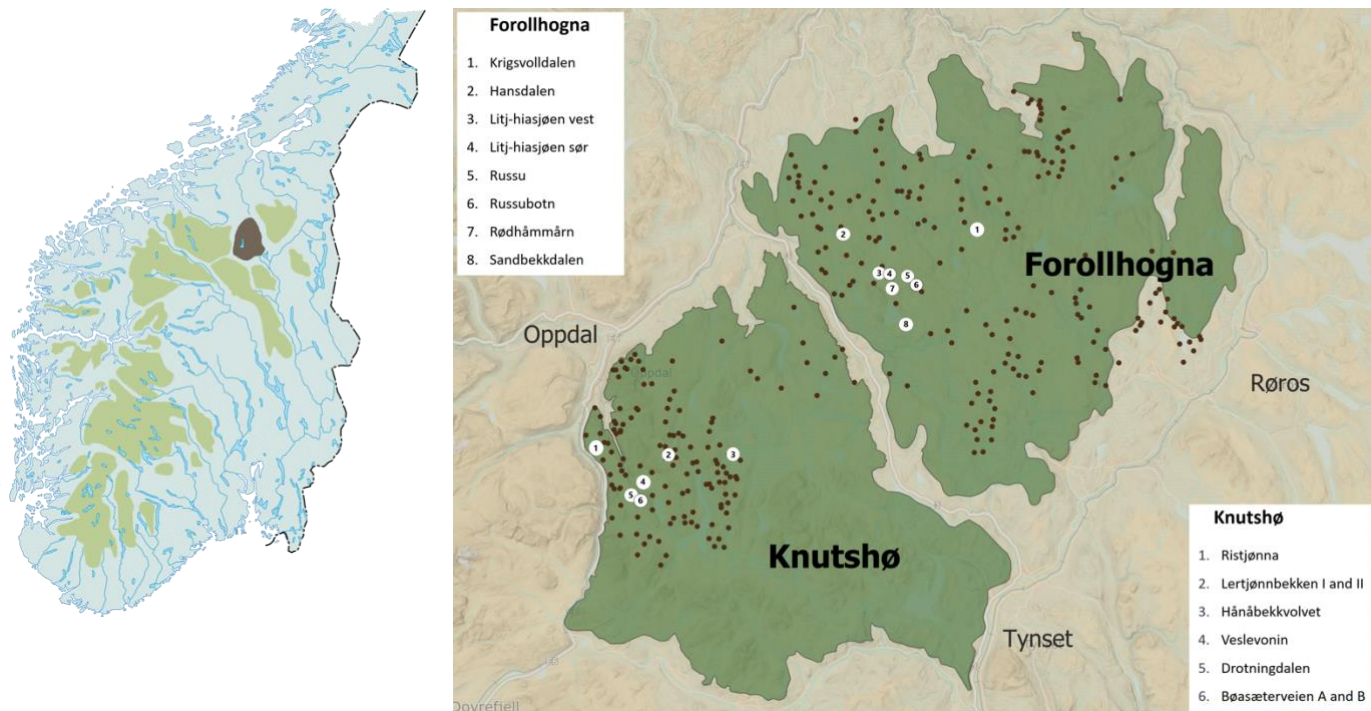


Figure 3: Location of Knutshø and Forollhogna in Norway (left) (Norsk villreinsenter, n.d.-b). Overview of sampled saltlicks (right). Sampled saltlicks are marked with number and brown pins are registered saltlicks.

## 2.3.2 Parasitological procedures in lab

### 2.3.2.1 Soil samples

After thawing, the samples were sifted through a sieve with pore diameter of 2 mm. The sifted soil was thoroughly mixed before further processing. For isolation of parasite eggs and larvae, sifted soil was prepared according to Steinbaum et al. (2017) albeit with slight modifications: 15 g of soil was transferred to a 50 ml centrifugation tube and filled with 35 ml 1% 7X detergent. The tube was shaken by hand for 2 minutes and filled with detergent till 45 ml and left overnight. Next, the tube was shaken for 1 minute and the contents sifted through a 500  $\mu\text{m}$  pore diameter sieve into a beaker. The sieve and tube were washed with detergent into the beaker till 150 ml was reached. After sedimentation for 1 h, sedimented soil was divided into two aliquots in 50 ml centrifugation tubes and filled with detergent up to 40 ml. The tubes were centrifuged for 10 min at 1000 relative centrifugal force (rcf) and the supernatants discarded.

Subsequently, 5 ml of NaCl/ZnCl<sub>2</sub> solution (Specific gravity 1.3 g) was added to the remaining pellet of soil after decanting the supernatant, and the centrifuge tube was vortexed for 30 seconds and topped with the salt solution up to 40 ml. The tubes were then centrifuged for 5

minutes at 1000 rcf, and the supernatant was sieved through a 20 µm mesh. The mesh was thoroughly washed with detergent and distilled water, and the suspension from the washing step was transferred to a 50 ml centrifuge tube. This step was repeated twice.

In the final step, the tubes containing the soil pellets were centrifuged at 1000 rcf for 10 minutes, and the pellets were combined until one centrifuge tube per soil sample remained. The supernatant was carefully discarded until 1 ml pellet remained, which was pipetted on a Sedgwick rafter slide and read at 40X and 100X magnification for detection and quantification of parasite eggs.

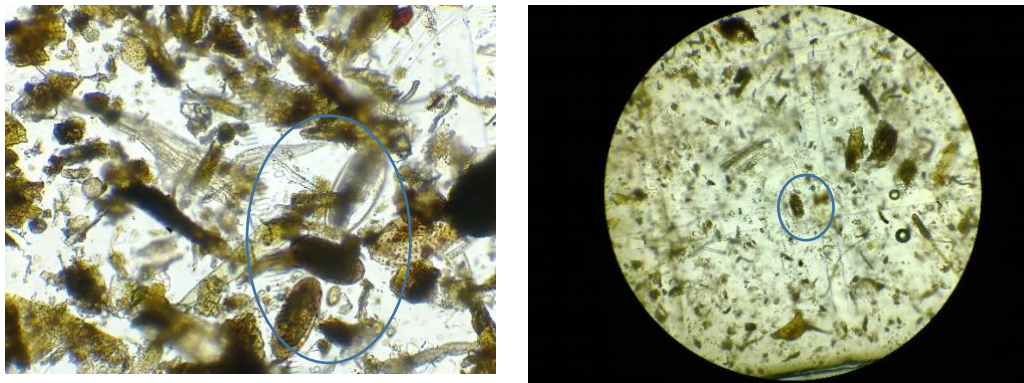


Figure 4: Picture of parasite eggs, *N. battus* eggs (brownish in colour with parallel sides) and *Nematodirinae* egg (thin shell, colourless, oval shape), 100x (left) and *N. battus* egg 40x (right) magnification.

### 2.3.2.2 *Abomasum*

After thawing, the content of the abomasa was poured into a bucket and filled with tap water until the total volume was about 2 L. Samples were taken with four 50 ml tubes (4 x 2.5% aliquots), while stirring the suspension. The subsamples that were not examined the same day, were stored in a refrigerator at 4°C and analysed the following day.

One aliquot was poured into a 500ml beaker at time and to clear the suspension, tap water was added up to 300 ml was reached. The nematodes were picked out visually and counted using a stereomicroscope (Leica M50 with Lecia cold light source CLS 100X) and stored in Petri dishes with 70 % ethanol. This was repeated until the number of nematodes reached >100 or 2.5% (one aliquots) of the abomasal content was analysed. The males and females were then counted separately, using the male's bursal organs to identify sex. Species identification of the nematodes was only conducted for the males, by comparing the structures observed with those described in the literature (Drozdz, 1966; Fruetel & Lankester, 1989; Lichtenfels et al., 1990; Taylor et al., 2015). The species of each male was identified using a microscope. A drop of

water was added over the nematode, which was placed on a microscope slide, together with a coverslip. If the nematode was difficult to identify to species level, a drop of polyvinyl lactophenol under the cover was used. The nematodes were discarded after species identification.

### ***2.3.2.3 Faecal samples***

A 3 g faecal sample was homogenized in 57 ml water and sieved through a sieve with pore diameter of ~1000 µm. The suspension was divided into two 10 ml tubes and centrifuged at 1500 rcf for 3 minutes, after which the supernatant was discarded. The concentrated pellet from one of the tubes was subsequently resuspended in Curavet flotation solution. McMaster counting chambers (Whitlock Universal, Australia) were filled with 2.5 ml of this suspension and the whole slide was examined at 10 – 20 x magnification for detection and quantification of helminth eggs and *Eimeria* oocysts. Eggs were identified based on morphological features to genus or species level if possible. Some eggs could only be identified to category of parasites (i.e., Strongylide) (Gibbons et al., n.d.). Results were reported as eggs per gram (EPG) for each genus, species or category and oocysts per gram faeces (OPG) for *Eimeria* spp.

## 2.4. Data analysis

First, the total abundance of parasites observed in soil samples was investigated in relation to area, treatment (saltlick vs control), and season. A non-parametric multivariate analysis of variance test ('permanova') was used for this, with the parasite community matrix as the response variable and area, season, and treatment as interactive predictors. The permanova test was specified to have 9999 permutations the methods was set as 'Euclidean'. Interactions that were not significant were removed for simplicity.

Secondly, a negative binomial regression model was used to assess the impact of area and treatment on the abundance of parasites per species. Because of data deficiency and singularities, the model only included an additive 'area + treatment' effect, which did not consider season as a covariate, or site as a random effect. A negative binomial model was chosen, as initially fitted Poisson regression models were overdispersed (dispersion parameters generally >1.4). Akaike's Information Criterion (AIC) metrics were used to select the most parsimonious model out of two candidate models, i.e., a model with treatment and area as additive effects, and a null model. The candidate model with the lowest AIC value was considered as the most parsimonious model. Model validation was done with QQ-plots and zero inflation and overdispersion tests were conducted using the 'dHarma' package in R (R Core Team, n.d.).

Thirdly, to see whether parasites were more abundant in autumn compared to summer, the data was subsetted to only include saltlicks. Sub-setting was necessary to compare saltlicks sites to each other (a seasonal effect at random sites was not expected). Because of missing data from Forollhogna in autumn, four sites were removed from this analysis. To assess a seasonal effect on parasite abundance at saltlicks (i.e., parasite load in summer vs. parasite load in autumn), a paired Wilcoxon Signed-Rank Test was used. Analysis of the data of soil samples was performed in R (version 4.2.2) (R Core Team, n.d.). The significance level of  $\alpha = 0.05$  was used for all statistical analyses.

### 3. Results

#### 3.1. Descriptive statistics

A total of 50 soil samples in 2020 and 30 in 2021 were collected from saltlick and control areas in Knutshø and Forollhogna. In addition, 10 gastrointestinal samples from Knutshø (8 calves and 2 adult) and 2 samples from Forollhogna (2 calves), and their faeces were included in the study. A total of six different species of parasites occurred in the soil samples, three different abomasal nematode species were detected, and four different types and species occurred in faecal samples.

##### 3.1.1 Eggs detected in soil around saltlicks in Knutshø

Six different categories of parasites egg/oocysts were detected from the soil samples in Knutshø, one species (*N. battus*) and five other categories. Eggs and oocysts were found in all 28 saltlick soil samples, while parasites were present in only 4 of the 24 control areas. During summer, 492 parasite eggs and oocysts were detected in 28 saltlick soil samples and 1 egg from one control area. During autumn, 847 parasite eggs and oocysts were detected at saltlicks, whereas only 17 parasite eggs and oocysts were found in control site samples.

Table 1. Overview of the occurrence and abundance of parasite eggs and oocysts documented in soil samples collected at saltlicks and control sites in Knutshø, divided into six different categories of species. The total amount of eggs/oocysts were higher at saltlick sites than at control sites, and higher during autumn than summer at both sites. Eggs of *N. battus* was the dominant species found in soil around saltlicks at both seasons and had the largest seasonal variation. *Moniezia* and *Strongylid* spp. had also a large increase between seasons. Every sample contained 15g of soil.

	<b>Total saltlick (N=28)</b>	<b>Total control (N=24)</b>	<b>Total summer saltlick</b>	<b>Total summer control</b>	<b>Total autumn saltlick</b>	<b>Total autumn control</b>	<b>Total saltlick</b>	<b>Total control</b>
<i>N. battus</i>	27/28	1/24	270	0	429	7	699	7
<i>Nematodirinae</i>	6/28	0/24	3	0	4	0	7	0
<i>Moniezia</i>	20/28	0/24	60	0	154	0	214	0
<i>Eimeria</i>	24/28	1/24	126	0	135	2	263	2
<i>Trichuris</i>	18/28	0/24	23	0	31	0	54	0
<i>Strongylid</i>	15/28	2/24	10	1	94	8	125	9
Total	28/28	4/24	492	1	847	17	1362	18
egg/oocysts								
Median			42	0	115	1	170	1
Mean			82	1	141	3	227	3

### 3.1.2 Eggs detected in soil around saltlicks in Forollhogna

Five different categories of parasite egg/oocysts were detected from the soil samples in Forollhogna. Eggs and oocysts were found in all 14 saltlick soil samples, and only in 1 sample from the control areas. During summer, 19 parasite eggs and oocysts were detected in 14 saltlick soil samples and 1 egg at from one control area. During autumn, 61 parasite eggs and oocysts were detected at saltlicks, and one parasite egg and oocyst were found in control site samples.

Table 2. Overview of the occurrence and abundance of parasite eggs and oocysts documented in soil samples collected at saltlicks and control sites in Forollhogna, divided into five different categories of species. The total amount of eggs/oocysts were higher at saltlick sites than at control sites, and higher autumn saltlicks than at summer saltlicks. No eggs were found in control sites during autumn, but one egg was found at control site at summer saltlicks. Eggs of *N. battus* was the dominant species found in soil around saltlicks at both seasons and had the largest seasonal variation, along with *Moniezia*. Every sample contained 15g of soil.

	<b>Total saltlicks</b>	<b>Total control</b>	<b>Total summer saltlicks</b>	<b>Total summer control</b>	<b>Total autumn saltlicks</b>	<b>Total autumn control</b>	<b>Total saltlick</b>	<b>Total control</b>
<i>N. battus</i>	6/14	0/14	9	0	34	0	43	0
<i>Moniezia</i>	6/14	0/14	4	0	16	0	20	0
<i>Eimeria</i>	4/14	0/14	4	0	6	0	10	0
<i>Trichuris</i>	3/14	0/14	2	0	3	0	5	0
<i>Strongylid</i>	2/14	1/14	0	1	2	0	2	1
Total	14/14	1/12	19	1	61	0	80	1
egg/oocysts								
Median			4	0	6	0	10	0
Mean			4	1	12	0	16	1

### 3.1.3 Adult nematodes in the abomasum

Three different gastrointestinal nematode species from abomasal samples (N=10) in calves (N=8) and adults (N=2) from Knutshø were found. *T. circumcincta* was found in 100% in all samples.

*Teladorsagia trifurcata* was found in 75% of the samples in calves, and 50% of the samples from adult. *O. gruehneri* was detected in 25% of the samples from calves, and in all samples from adults.

Two different abomasal nematode species from abomasa samples in calves (N=2) were detected in Forollhogna. The occurrence of both *T. circumcincta* and *T. trifurcata* was 100%.

Table 3. Prevalence of adult nematodes species detected in abomasum samples from 12 calves/adults in wild reindeer in Knutshø and Forollhogna. *T. circumcincta*, typical for sheep was found in every sample in both areas, with the largest mean.

	<u>Calves</u>				<u>Adults</u>			
	n-positive/ n-total	Mean	Median	Min - max	n- positive n- total	Mean	Median	Min - max
<b>Knutshø</b>								
<i>T. circumcincta</i>	8/8	1481	1256	560- 2436	2/2	1175	1176	467- 1884
<i>T. trifurcata</i>	6/8	91	74	64-148	1/2	94	94	94
<i>O. gruehneri</i>	2/8	97	97	64-129	2/2	968	968	942- 993
<b>Forollhogna</b>								
	<u>Calves</u>							
<i>T. circumcincta</i>	2/2	814	814	747-880				
<i>T. trifurcata</i>	2/2	67	67	40-93				

### 3.1.4 Faecal eggs and oocysts

Four categories of nematodes were found in faecal samples from Knutshø. Parasites eggs were found in all 10 samples. No parasite eggs were detected in faecal samples from Forollhogna (N=2).

Table 4. Prevalence of parasite eggs and oocysts in wild reindeer faeces collected in Knutshø and Forollhogna. Mean, median and range are calculated based on the parasite intensity (eggs and oocysts per gram faeces).

<b><u>Calves</u></b>				
	<b>n- total/ n-positive</b>	<b>Mean (EPG)</b>	<b>Med Ian (EPG)</b>	<b>Range (EPG)</b>
<b><u>Knutshø</u></b>				
<i>N. battus</i>	4/10	37,5	45	10-50
<i>Nematodirinae sp.</i>	5/10	30	10	10-80
<i>Moniezia sp.</i>	1/10	-	-	-
Strongylida	3/10	40	50	10-60
<b><u>Adults</u></b>				
<b><u>Forollhogna</u></b>				
<i>N. battus</i>	0/2			
<i>Nematodirinae sp.</i>	0/2			
<i>Moniezia sp.</i>	0/2			
Strongylida	0/2			



### 3.1.5 Saltlick, area, and seasonal effects on the parasite load in soil samples

The permanova test revealed that the main effect of treatment (i.e., saltlick vs. control) explained most (about 22%) of the variation in the parasite community structure in soil samples abundance ( $p < 0.001$ ), followed by area (explained variance  $R^2 = 7.3\%$ ,  $p = 0.001$ ) and season ( $R^2 = 3.6\%$ ,  $p = 0.02$ ). There was a moderate significant interaction between treatment and season ( $R^2 = 2.8\%$ ,  $p = 0.040$ ), whereas the treatment \* area interaction was highly significant ( $R^2 = 6.6\%$ ,  $p < 0.001$ ). All variables combined explained about 42 % of the variation in the observed parasites community structure in soil samples.

Table 5. Results from permanova analyses to investigate how treatment (saltlick vs. control), season (summer vs. autumn), area (Knutshø vs. Forollhogna), and interactions between those affect the parasite community structure in soil samples. *df* = degree of freedom, *R*<sup>2</sup> = amount of explained variation in response variable, *Pseudo-F* = test statistics (non-parametric approximation) *P* = probability of that the null hypothesis is true given the data. \* = *p* – value < 0.05, \* = *p*-value < 0.01, \*\* = *p*-value < 0.001.

	<b>df</b>	<b>R<sup>2</sup></b>	<b>Pseudo-F</b>	<b>P</b>
Treatment	1	0.21856	27.9629	0.0001 ***
Season	1	0.03560	4.5549	0.0216 *
Area	1	0.07282	9.3171	0.0010 ***
Treatment*Season	1	0.02855	3.6525	0.0408 *
Treatment*Area	1	0.06608	8.4539	0.0014 **
Residual	74	0.57839		

### 3.1.6 Difference among species in soil samples

The lack of variation in factor levels or combinations of factor levels resulted in singularity issues and prevented running a formal statistical regression-based test on three of the six species. For Nematodirinae eggs, were only eggs detected in Knutshø at saltlicks (n=6). For *Moniezia* spp. eggs, very few eggs were detected at Forollhogna (n=20) compared to Knutshø (n=211), and only at saltlicks. *Trichuris* spp. eggs were only found at saltlicks, in both Forollhogna (n=5) and Knutshø (n=54), and not at the controls.

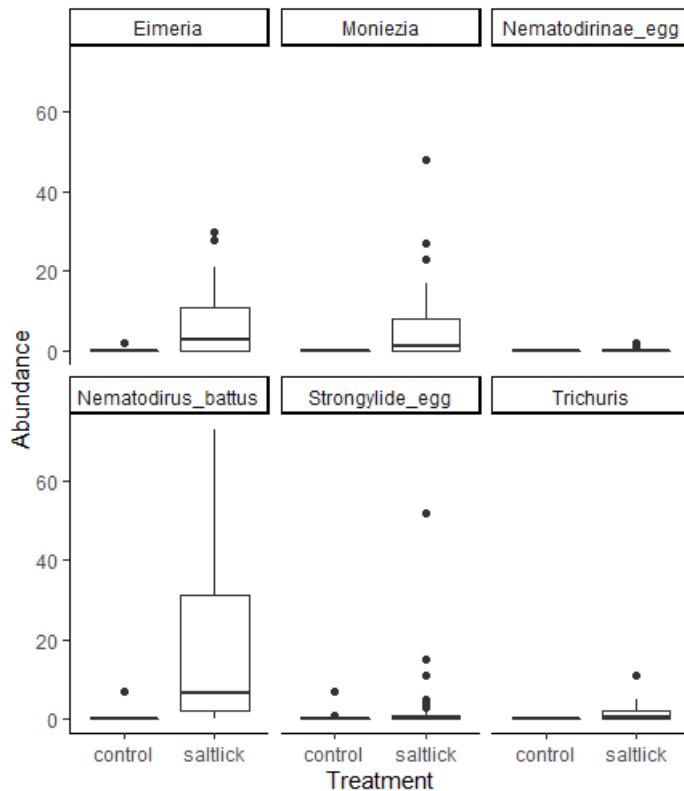


Figure 5: Overview of the different species and their abundance observed in soil samples collected at saltlicks and control sites in Knutshø and Forollhogna in summer and autumn in 2021. All the species show higher abundance in saltlicks than control, especially *N. battus*, *Eimeria* and *Moniezia* from eggs and oocysts counts. Abundance represents number of parasites per 15 grams of soil samples.

For *N. battus*, the full model by far outperformed the null model ( $\Delta\text{AICc} = 58.96$ ). *N. battus* were significantly more abundant in Knutshø than in Forollhogna ( $\beta=2.1820$ ,  $\text{se} = 0.4518$ ,  $p < 0.001$ ) Furthermore, they were more prevalent at saltlicks compared to control sites ( $\beta= 4.5650$ ,  $\text{se}= 0.5176$ ,  $p < 0.001$ ). Model validation showed no clear violation of the model assumptions (appendix).

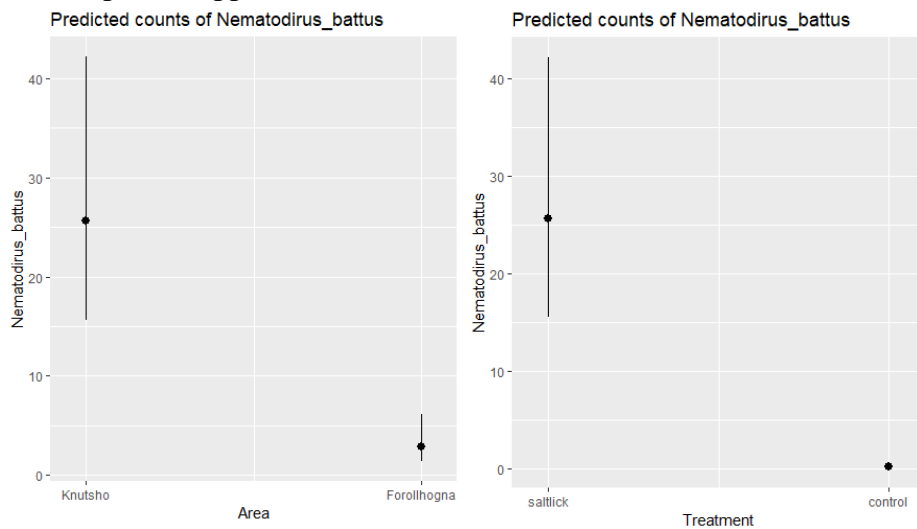


Figure 6: Plots of predicted counts of *N. battus* eggs in soil (15g soil sample) show that they were substantially higher in Knutshø than Forollhogna (left), and at saltlicks vs. control (right).

For *Eimeria*, the full model also outperformed the null model (delta  $\Delta\text{AICc} = 61.41$ ). They were significantly more abundant in Knutshø compared to Forollhogna ( $\beta = 2.5804$ ,  $\text{se} = 0.4772$ ,  $p < 0.001$ ). The prevalence of *Eimeria*, was also significantly higher at saltlicks compared to control sites ( $\beta = 4.7603$ ,  $\text{se} = 0.7676$ ,  $p < 0.001$ ). Model validation showed no clear violation of the model assumptions (appendix).

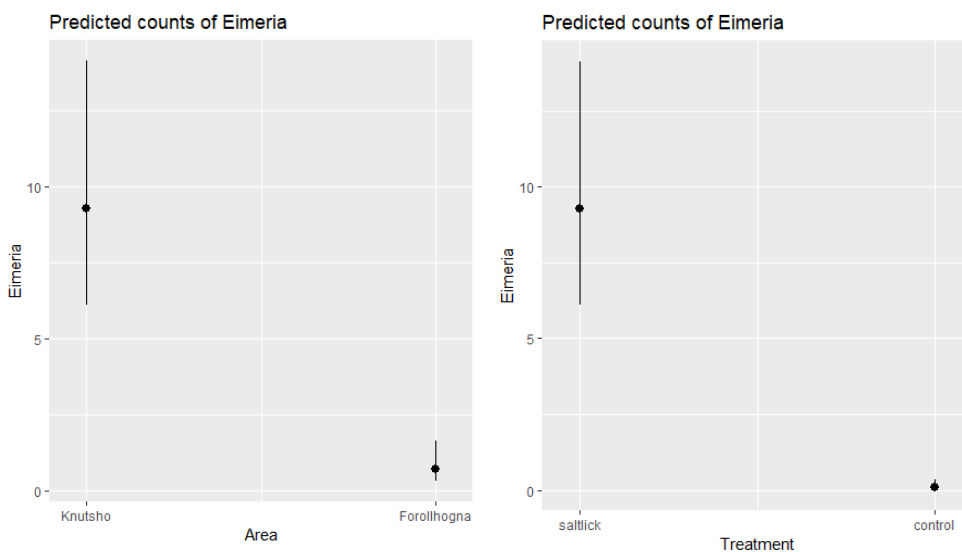


Figure 7: Plots of predicted counts of *Eimeria* eggs in soil (15g soil sample) show that they were substantially higher in Knutshø than Forollhogna (left), and at saltlicks vs. control (right).

The full model for *Strongylide* eggs outperformed the null model ( $\Delta AIC = 13.78$ ). They were significantly more abundant in Knutshø than in Forollhogna ( $\beta = 2.7578$ ,  $se = 0.8116$ ,  $p < 0.001$ ). *Strongylide* eggs were significantly more prevalent at saltlicks compared to controls ( $\beta = 1.9414$ ,  $se = 0.6364$ ,  $p < 0.002$ ). Model validation showed no clear violation of the model assumptions (appendix).

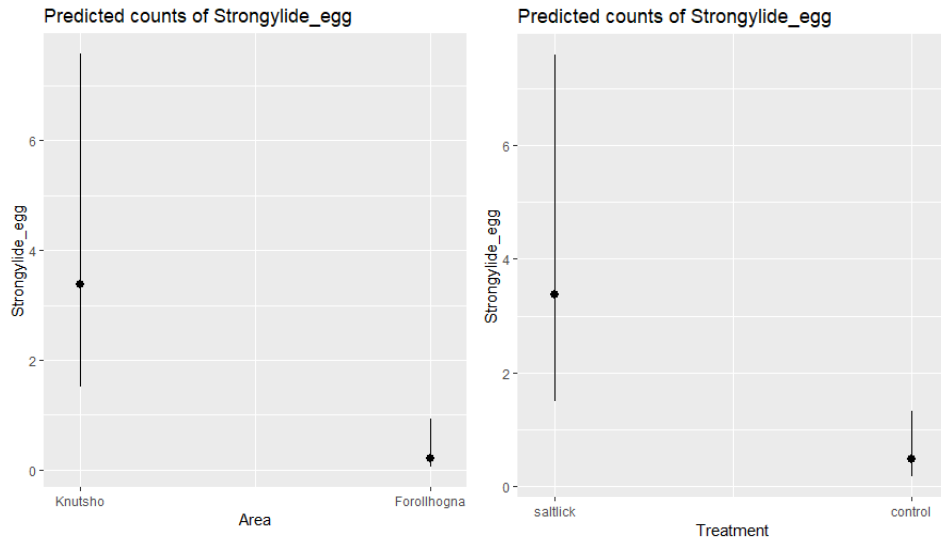


Figure 8: Plots of predicted counts of *Strongylide* eggs in soil (15g soil sample) show that they were substantially higher in Knutshø than Forollhogna (left), and at saltlicks vs. control (right).

### 3.1.7 Comparison between summer and autumn saltlicks

A Wilcoxon Signed-Ranks Test showed that the parasites *N. battus* (min = 0, med = 20, max = 73), *Moniezia* (min = 0, med = 8, max = 48) and *Strongylide* egg (min = 0, med = 1, max = 52) were significantly more abundant at saltlicks during autumn compared to summer ( $p < 0.05$ ).

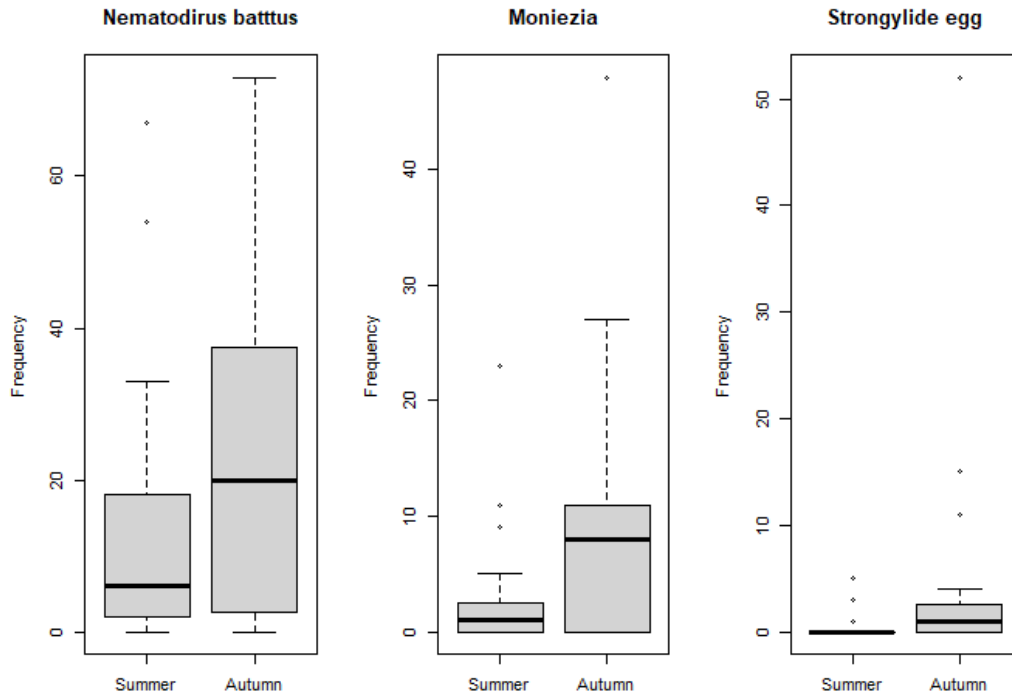


Figure 9: Difference in the observed parasite burden in soil (frequency, y-axis) between summer at autumn saltlicks (x-axis) for different parasite species. All species showed a higher prevalence during autumn compared to summer. The dots represent 15 grams of soil sample.

## 4. Discussion

This study is novel in regards of assessing a broad species range of parasite presence and quantification in the soil around saltlicks and controls, as opposed to a qualitative molecular study with a narrow species sensitivity, no quantitative assessment nor any observation of the parasite transmission stage itself. This study is also supplemented with parasitological examinations of abomasum and faeces from wild reindeer in Knutshø and Forollhogna.

The parasite loads in soil near saltlicks were significantly higher compared to soil samples from control sites. In addition, the parasite load was higher at saltlicks in the study area with high sheep density compared to the area with low sheep density. There was also a seasonal difference between autumn and summer, with a higher parasite burden during autumn.

Considering this, it was not a surprise to find adult sheep parasites in the abomasum of wild reindeer. Due to small sample sizes, however, it is not possible to draw conclusions based on these results, but there appears to be a trend of calves having a higher number of parasites than adults, which may be explained by developed immunity in adults. There was also a higher number of parasites associated with sheep infection rather than parasites commonly found in reindeer. Faecal samples of wild reindeer also revealed other species than those found in the abomasum, such as the invasive species *N. battus*, as have been found in the same areas in 2020 (Robertsen, 2020).

### 4.1 Soil samples

The study found clear evidence that the occurrence of parasite eggs and oocysts is higher in soils around saltlicks than at control sites. The parasites with the highest prevalence in both areas were *N. battus*, *Eimeria* spp. and *Moniezia* spp., which are all parasites commonly found in sheep, and can overwinter as eggs/oocysts (Gjerde, 2011).

*N. battus* was the dominant species found in both Knutshø and Forollhogna. As earlier described, this parasite is highly pathogenic, very resistant to the environment and can survive the winter (Gjerde, 2011; Nikbin et al., 2022). The environmental conditions in Knutshø and Forollhogna are quite suitable for the development and survival of *N. battus*, as it must go through a period of chilling and have a temperature range between 11-17°C before hatching (van Dijk & Morgan, 2008). The hatching will cease if the temperature is outside this range and continue when the temperature is restored, which in theory means that it can develop in several areas if the temperature rises over 10°C and under 25°C (van Dijk & Morgan, 2008).

*Eimeria* oocysts could potentially survive in moist conditions for up to a year (Gjerde, 2011), and the condition around saltlicks are very suitable for this parasite to survive and accumulate, since it gets frequently visited from animals where they trample the ground, and leave faeces and urine, which makes the soil moisty.

*Moniezia* is a tapeworm that excretes out proglottids containing eggs, and one proglottid can contain 15000 eggs. Up to 140 proglottids can be excreted per day per individual (Stoll, 1936). The eggs are resistant to climate and can survive for several months, and they can survive as infective cysticeroid in the intermediate host (earth mite) for up to 2 years (Gjerde, 2011). The accumulation of these eggs can therefore become quite large, something that the results also showed, and the number of eggs were also significantly higher in autumn compared to summer. A study done in Russia, showed that none of the eggs survived the winter, but they could survive up to nine days in sheep faeces, and over 50% of the eggs that were still in the proglottids survived the night (Kuznetsov, 1959). Light and moderate infection of *Moniezia* is assumed to have low clinical importance in lambs, but growth, anemia and diarrhea can occur with stronger infections (Josefsen et al., 2014). There are few studies of *Moniezia* in reindeer, (Idland et al., 2021; Josefsen et al., 2014). However, it is believed that infection with these tapeworms does not have any significant impact on reindeer health (Josefsen et al., 2014).

Hrabok et al. (2006) demonstrated that reindeer are highly suitable hosts for ovine infective nematode larvae, among others *T. circumcincta*. The parasite goes into hypobiosis in the reindeer host in the same manner as in the original host and may as such be expected to reemerge in the periparturient period when the pregnant female is immunosuppressed (Gjerde, 2011; Hrabok et al., 2006). As this parasite survives on frozen and snow-covered pasture for months, it may be ingested by a wild ruminant host sharing natural pastures with domestic sheep. Due to its shown ability to infect reindeer, and the repeated presence around salt licks, this parasite is likely to establish in the wild reindeer population (Ytrehus et al., In press). It is therefore not surprising that *T. Circumcincta* was one of the prominent species in the soil samples.

The results also show that the soil around saltlicks in Knutshø contained significantly more parasites than the soil around saltlicks in Forollhogna. Since Knutshø has higher sheep numbers and density than Forollhogna, and had a larger increase of sheep numbers during the last decades (Ytrehus et al., In press), this may indicate that higher sheep numbers and densities are associated with higher parasite burden in the context of contamination of parasites around

saltlicks. This is interesting as it has been pointed out that the general status of the wild reindeer population is worse in Knutshø than in Forollhogna (Rolandsen et al., 2022). This report also hypothesized that high parasite burdens could be one of the causes of the negative development of reindeer, and this study can help substantiating this claim by presenting quantitative data from suspected hotspots of parasite transmission and paired controls.

In addition to higher numbers of sheep in Knutshø, the frequency of sheep visiting the saltlicks is higher in this area compared to Forollhogna, according to a recent bachelor thesis (Johansson, 2021). Pictures from camera traps around saltlicks in Knutshø and Forollhogna were analyzed and showed that the saltlicks were frequently visited by both sheep and reindeer. During a period from 2018-2020 in Knutshø, Johansson (2021) showed that the highest number of registered sheep from one camera was over 60000, and the highest number from one camera in Forollhogna from a period from 2016-2020, showed under 1000 pictures of sheep. Since my results show that the parasite burden in soil around saltlicks in Knutshø are significantly higher than in Forollhogna, this can probably be explained by the higher visitation rate of sheep. In 2019, three cameras in Knutshø also registered that reindeer had their head sunk down (suggesting foraging) around the saltlick in over 50 % of the time. The total amount of visited animals was also higher in Knutshø than in Forollhogna, which was dominated by sheep (Johansson, 2021). This may explain the high occurrence of parasites in salt lick soil in Knutshø and may indicate that the negative development in the wild reindeer population in Knutshø could be caused by higher number of sympatric sheep. The findings here strengthen the hypothesis that negative development in wild reindeer population in Knutshø but not in Forollhogna could be caused by a higher sheep density and parasite spillover.

Seasonal variation in parasite loads in soil samples was apparent, since the parasite number during autumn was significantly higher than in summer. When comparing just the saltlicks in summer and autumn, *N. battus*, *Moniezia* and *Strongylida* eggs were significantly more abundant in autumn compared to summer, and which are, as earlier described, all species that can survive the winter and are resistant against harsh environmental conditions. *N. battus*, *Moniezia*, and *Strongylida* were by far more common at saltlicks compared to controls, in Knutshø compared to Forollhogna, and during autumns compared to summer.

A higher parasite count during autumn than summer, suggests that visitation of sheep at saltlicks may affect the parasite burden in soil, and the parasites' ability to survive leads to accumulation of parasites in soil, which makes the parasite accumulation at its highest in autumn. Lower



parasite counts during summer also indicates that some of the parasites die during winter, but due to high survival rate by some species they are also present during summer. During summer, the conditions are favorable for parasite survival, and with an increase of sheep visiting the saltlicks throughout the summer, the parasite eggs are probably accumulating.

The seasonal parasite burden in Knutshø and Forollhogna are quite different, and as shown by Johansson (2021) the sheep in Knutshø also visited the saltlicks more frequently than the sheep in Forollhogna, and Knutshø has a higher sheep density overall. *N. battus* is clearly the dominant species with the largest seasonal variation in both areas. In Knutshø, *Moniezia* had a two-fold increase from summer to autumn, and as stated earlier one proglottid of *Moniezia* could contain up to 15000 eggs (Stoll, 1936), so the total amount of eggs from this parasite are potentially quite large. After the sheep are collected down from the mountains in late autumn, the wild reindeer can also seek out the saltlicks without competition and has therefore the potential to ingest contaminated soil when the parasite numbers in the soil is at its highest.

Because of developed immunity in the hosts, some of these parasites mainly affect younger lambs and give symptoms like loss of appetite, diarrhea, dehydration, and in severe cases, death (Gjerde, 2011). It is therefore plausible that the same symptoms may be found in reindeer as well, and increased parasite loads could be one reason for the reported negative development of reindeer calf body condition (Rolandsen et al., 2022).

## 4.2 Abomasum samples

Based on the findings of high parasite numbers near saltlicks and cameras that show reindeers eating the soil (Johansson, 2021; Utaaker et al., 2023), it was not a surprise to find sheep parasites in the reindeer's abomasum. Two species were found, *T. circumcincta*, its minor morph *T. trifucata*, and the reindeer nematode *O. gruehneri*. Due to the small sample size of abomasa, and that most of the samples were from calves in Knutshø, my results are too weak to be compared and concluded upon. Nevertheless, these results may give an indication of the prevalent nematode species in the wild reindeer's abomasum. As immunity can be developed against several of the identified species, samples from calves are the most relevant age group to investigate. The total infection intensity was considered as a light to moderate parasite burden (Hansen & Perry, 1994), with a range from 560 to 2220 adult nematodes. It's important to emphasize that since the samples were collected during autumn, some nematode species may have gone into hypobiosis, and would therefore affect the number of detected nematodes (Nazarbeigy et al., 2021).

The species that was found most of was *T. circumcincta*, in both calves and adults, and in both areas. Considering that this species also goes in hypobiosis in autumn/winter, it should be taken into consideration that these numbers could have been higher (Nazarbeigy et al., 2021). There was also a clear indication that calves had higher burdens of this parasite than adults, and that the adults had higher burdens of the reindeer nematode *O. gruehneri*. A study done on reindeer in Svalbard, suggest that reindeer don't develop immunity response against *O. gruehneri* (Irvine et al., 2000), and is therefore likely to find higher concentrations in adults than calves.

*O. gruehneri* was the parasite that was found in the lowest number, and it was only detected in Knutshø. This parasite is the most common reindeer nematode and is known to occur in high prevalence in reindeer populations (Josefsen et al., 2014), suggesting a shift in the abomasal parasite fauna in Knutshø.

When Bye did his study in 1986, he found that *O. gruehneri* was the most prevalent species in both Forollhogna and Knutshø, and that nematodes from sheep have limited transmission to reindeer (Bye, 1987). The authors found that the prevalence of *T. circumcincta* was higher in the areas which also had the highest sheep density. In 1984, this was Forollhogna with 35000 grazing sheep when Knutshø had only about 15-20000. Since only adult animals were examined from Knutshø and Forollhogna, it is possible that they had developed immunity against these

parasites. Data from Hardangervidda however, showed that *O. gruehneri* and *T. circumcincta* occurred in calf abomasa (Bye, 1987).

If we compare the results from Bye (1987) and Robertsen (2020), we can see that there has been a change of nematode fauna in wild reindeer in Knutshø and Forollhogna. Bye (1987) found a higher prevalence of *T. circumcincta* and *O. gruehneri* in Forollhogna than Knutshø, and over 30 years later Robertsen (2020) found a higher prevalence of *T. circumcincta* in Knutshø than Forollhogna. The results reported here can also support the change of nematode fauna in wild reindeer calves, although more extensive studies in term of sample size and further investigation on occurrence of larva in the abomasal mucosa of wild reindeer calves are warranted.

Robertsen (2020) found fewer abomasal parasites in reindeer calves from Knutshø than this study. However, both studies combined, do indicate that there might be a spillover in Knutshø, where more sheep than reindeer parasites in calves occurred. Robertsen (2020) also found a higher prevalence of *T. circumcincta* than *O. gruehneri* in calves in both areas, and that *O. gruehneri* was lower in Knutshø than Forollhogna. This can indicate that *T. circumcincta* could have outcompeted the other species or have different seasonal patterns. For example, it has been proposed that *O. gruehneri* enters obligate hypobiosis upon entry of a host, regardless of the season of ingestion (Hoar et al., 2012), which may also give *T. circumcincta* the advantage as it can get an earlier start on its lifecycle and contaminate the environment with eggs one season earlier than *O. gruehneri*.

### 4.3 Faecal samples

Faecal samples collected from Knutshø contained eggs of *N. battus*, Nematodirinae, *Moniezia* spp. and *Strongylida*. There were no findings of eggs in faecal samples from Forollhogna, but only two faecal samples were taken from that area. Thus, due to small sample size and a low egg number, these results remain inconclusive. It is, however, important to note that a low egg count doesn't necessarily mean that an animal has only a light infection of parasites. The low egg counts may have been caused by several factors; the samples were frozen before examination (Van Wyk & Van Wyk, 2002), the time lag from collection to analysis was long (Sengupta et al., 2016), and seasonal effects could occur (Höglund et al., 2021). Samples were taken in the autumn, and since some of the parasites go into hypobiosis during autumn and winter (Gjerde, 2011), it is expected to find less of them during this time.

Even though it remains inconclusive, these few samples were important as other species of sheep parasites in wild reindeer were confirmed. This applies especially to *N. battus*, as its eggs are quite distinguishable, thus making it possible to confirm the presence of this parasite by faecal flotation.

Since samples used in this study were frozen and stored before examination, it could have affected the results and makes it difficult to compare results reported here with other studies. Robertsen (2020) collected samples from Knutshø during the same season as my samples were collected, which can give a good basis for comparison, even though this study and Robertsen (2020) included relatively few samples. The species that is most comparable with (Utaaker et al., 2023). Robertsen (2020) in terms of parasite load is *N. battus*, which showed a median of 50 EPG, while my results showed 45 EPG. Although the sample sizes are small, these two studies indicate that this parasite species in wild reindeer calves might be common.

#### **4.4 *Nematodirus battus***

The occurrence of *N. battus* in this study was high in samples from both soil and faeces. The species was discovered in Norway for the first time in 1961 in imported sheep from the UK (Helle, 1969), and have since been found in several species all over the country such as cattle (*Bos taurus*), muskox (*Ovibos moschatus*), moose (*Alces alces*) and reindeer today (Hamnes et al., 2018). It is categorized as a species with a potential high risk and has a great impact on the farm economy and animal welfare in the UK, where it is estimated to cause mortality of and kills several thousand sheep each year (van Dijk & Morgan, 2008). Since older animals develop immunity, its often the younger animals which have the strongest infections (Gjerde, 2011; McCoy et al., 2004).

It remains unclear what effect this parasite may have on reindeer, but it's likely to think that it could have similar pathogenesis as it has in sheep. Since we found significantly more of these parasite eggs in soil around saltlicks and not in the control samples, it seems plausible that saltlicks sites function as a hotspot for this and other parasites, and the climate in the mountain areas of Knutshø and Forollhogna are suitable for the parasite eggs to survive and accumulate. The difference in egg counts between the two study areas are also interesting, and potential consequences of higher egg counts in soil should be explored further. These results may aid in understanding the declining population parameters of reindeer in Knutshø compared to Forollhogna, although other factors such as human disturbance and encroachment and available pasture should, among others, also be evaluated.

#### **4.5 *Eimeria* spp.**

*Eimeria* oocysts were only found in soil samples, and mostly around saltlicks. Studies show that *Eimeria* oocysts can accumulate in soil and are quite resistant against environmental conditions like heat and desiccation. Additionally, *Eimeria* oocysts can survive the winter, and faeces can help to protect them against changing seasons and environments (Lassen et al., 2013; Lassen et al., 2014). Parasites of the genus *Eimeria* can cause coccidiosis in several ruminants and affects mostly young animals, but because of strict host specificity of this parasite, no cross infections have yet been reported (Chartier & Paraud, 2012). Little is known about coccidiosis infection in reindeer, but it is assumed to have low importance under normal pasture conditions (Josefsen et al., 2014). To have numerous saltlicks in the reindeer's habitat, however, cannot be considered as normal pasture conditions for this species. These saltlicks may thus have unknown consequences for wild reindeer, as they frequently visit these areas of accumulation of *Eimeria* spp. In domestic animal livestock management, coccidiosis has a great importance as infection causes decreased animal welfare as well as economic losses due to diarrhea and weight loss (Chartier & Paraud, 2012).

Due to findings of more *Eimeria* oocysts around saltlicks than at control sites, there is reason to believe that saltlick sites function as hotspots for this parasite group as well. Since the *Eimeria* oocysts are known to be highly host specific, and due to lack of studies on the effects of *Eimeria* in reindeer, it's unclear if this parasite affects reindeer health.

It is challenging to interpret these results in terms of how these findings affect the uptake of viable transmission stages of parasites at salt licks by wild reindeer and domestic sheep. As shown by Johansson (2021), animals visiting the salt licks do scatter around the lick and keep their head bent to the ground, indicating interest in the soil itself. In addition, camera traps around salt licks have documented ingestion of soil by ungulates (Utaaker et al., 2023). Quantification of ingested soil is currently not measured in these animals, but it has been shown that wild ungulates do ingest soil containing minerals; (Ayotte et al., 2006) found that the inorganic material in faecal samples collected from different wild ungulates close to dry salt licks contained significantly higher levels of inorganic material than samples collected at control areas, indicating a higher intake of soil in these areas. In addition, the sodium level in forage plants for these species were too low to meet the estimated requirements, underscoring the need for mineral supplements outside of forage for these animals. As these needs are determined by factors such as life stage and physiological state of the grazing animals, a set value for intake is challenging to determine. Nonetheless, salt-seeking behaviour has been

documented in both wild and domestic ruminants (Lavelle et al., 2014; Pedernera et al., 2020), so the potential for ingesting soil around salt licks, and thus potentially parasite transmission stages, is present. In addition, it should be noted that only 15 grams of soil were analyzed per sample in this study, which can be roughly estimated to a handful. Some of these samples contained over 140 parasite eggs and oocysts (appendix), which is just under 10 parasites per gram sample. It does seem plausible that ingestion of soil around these salt licks may also result in unwanted guests in the gastrointestinal system, but the quantification of ingestion of both soil and parasite transmission stages remains to be elucidated.

## 5. Management implications

Currently, there is little available user knowledge and information regarding saltlicks placement in rangeland. However, already in 1967 in Norway, it was advertised that it was important to place the saltlicks in a way so pathogens won't accumulate in the surrounding soil (Landbrukets film- og billedkontor, 1967). In addition to saltlicks, congregations of wild animals can also occur at natural salt licks as well as feeding stations.

A study that looked at how supplementary feeding affected endoparasite infection in wild boar, showed that there were more eggs at feeding sites than at control sites and abandoned feeding sites (Oja et al., 2017). There was no evidence that eggs accumulated in soil over time, but supplementary feeding was positively associated with endoparasite infection of wild boars. The study also pointed out that the accumulation and survival rate of parasite eggs and oocysts which are affected by the environment, as well as the parasite species life cycle, will influence the effect of supplementary feeding on wild boar, where parasites with direct life cycles were of most concern (Oja et al., 2017). *N. battus*, *Eimeria* and *Teladorsagia* are parasites with direct life cycles and have robust transmission stages which supports a high survival rate. *Moniezia* spp have an indirect life cycle and can survive in the intermediate host, the earth mite, for years (Gjerde, 2011).

A review that investigated the effects of supplementary feeding of ungulates, showed that it gave more unintended effects than intended effect, and the risk of parasite transmission got significantly enhanced by aggregating wildlife (Milner et al., 2014). The intended effects were among other things increased survival and reproduction rate, as well as increased population size and diversion of animal from traffic arteries. The unintended effects were among other things increased parasitism due to spatial aggregation, increased contact rates and that feed acted as an effective disease fomite (Milner et al., 2014). The risk of disease transmission was found to be significantly enhanced by supplementary feeding, and this was dependent on the parasites survival rate and life cycles (Vicente et al., 2007), where parasites with direct life cycles enhances the risk of transmission (Navarro-Gonzalez et al., 2013). Saltlicks may have several similar unintended, indirect, and unexpected effects as supplementary feeding, and more research about their ecological side effects is warranted.



Research on parasite transmission in relation to management actions that aggregate animals is, however, not unambiguous. A study in semi-domesticated reindeer in Finland and Norway, did not find that supplementary feeding was a significant factor for endoparasites infection (Jokelainen et al., 2019) and neither did Milner (2013) find positive effects of supplementary feeding on gastrointestinal parasite prevalence or infection in moose in Norway. So, why could salt licks, also a point of congregation of free-ranging ungulates, be a hotspot for transmission of gastrointestinal endoparasites?

Ruminants are commonly divided into three feeding-type categories: browsers, grazers, and intermediate feeders (Robbins et al., 1995). Sheep belong to grazers, preferring to eat short grasses and clover, and are thus more in direct contact with the ground. Reindeer belong to the intermediate type, and may both browse and graze, consuming a mixed diet, and they are able to shift their diet from for example forbs and browse (e.g., any part of a woody plant) to grass or roughage (forage rich in plant cell wall) in response to forage quality (Hofmann, 1989). The reindeer will thus browse or graze as preferred. Perhaps when supplementary feeding is offered, the browser is more active, and the most tempting and freshest fodder is eaten, while at a salt lick, the soil itself is tempting enough to “graze” on due to its high salt content (Strand et al., 2023), as was shown through analysis of photos from camera traps at salt licks, where over half of the wild reindeer observed held their heads bent to the ground around the licks, as if to reach the soil with the mouth (Johansson, 2021). Saltlick sites create a common meeting area, where different animals’ visits and leave faeces and urine, which moistens the soil and creates good conditions for parasite eggs and oocysts to develop into the infective stage and remain viable (O’Connor et al., 2006). Conclusively, as many of the studies mentioned above stated, congregation of animals often leads to transmission of diseases.

## 6. Conclusions

This study reveals that there is a high parasite burden around saltlick sites relative to control sites, and that soil around saltlicks in Knutshø is more contaminated than in Forollhogna. The dominant parasite was the invasive species *N. Battus*. Further findings show that the same species found in soil samples, also occurred in reindeer abomasum and faeces. This suggests that through eating soil that is contaminated, the reindeers gets infected and show similar symptoms as those described in sheep. Due to little knowledge and studies, we know little about the clinical effects it may have on reindeer. Since the reindeer population has decreased especially in Knutshø and for no obvious reason, it would be advisable to assume that it can have the same harm in reindeer as in sheep, although more research is warranted. A higher sheep density in Knutshø than in Forollhogna, and a higher parasite burden in autumn than in summer could indicate that a higher sheep density affects the number of parasites in soil and could be one of the causes to why the reindeer population are more stable in Forollhogna than in Knutshø.

Saltlicks are important in sheep husbandry and beneficial for the farmer. But to prevent accumulation of pathogens in the soil and prevent reindeer from eating the soil, the practice of saltlicks must change. Interaction that are meant to be beneficial for farmers and livestock, may have unintended effects on wild animals, such as functioning as hotspots for parasite transmission and accumulates in soil. Since there is no treatment program for the wild reindeer as we have for livestock, Norway has not only a duty to protect the wild reindeer, but a duty to work preventative as well.

The wild reindeer calf and adults should be investigated further with a larger sample size. It would have been interesting to compare findings of abomasal nematodes in reindeer calf and adults, as well as eggs and oocysts from other national reindeer areas to see if the parasite burden is high in other reindeer areas as well. It would also been interesting to see for how long the parasites survive over a longer period in soil around saltlicks.

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## **8. Appendix**

Appendix 1 – Table with an overview of eggs and oocysts found in soil samples in Knutshø and Forollhogna

Appendix 2 – Coordinates of sample sites of saltlicks and controls (UTM).

Appendix 3 – Model validation of *Nematodirus battus*.

Appendix 4 – Model validation of *Eimeria*.

Appendix 5 – Model validation of Strongylide egg.

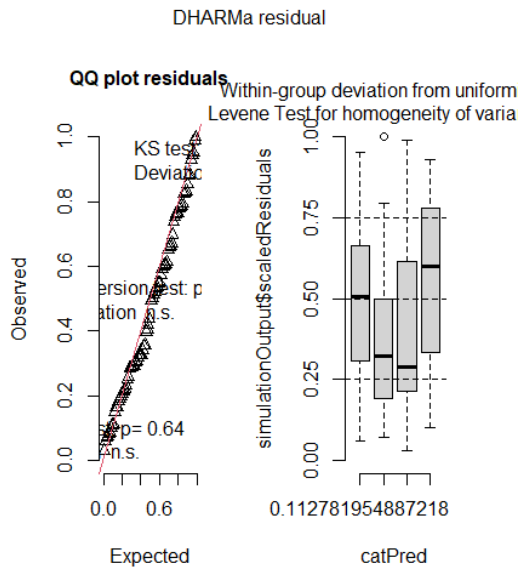
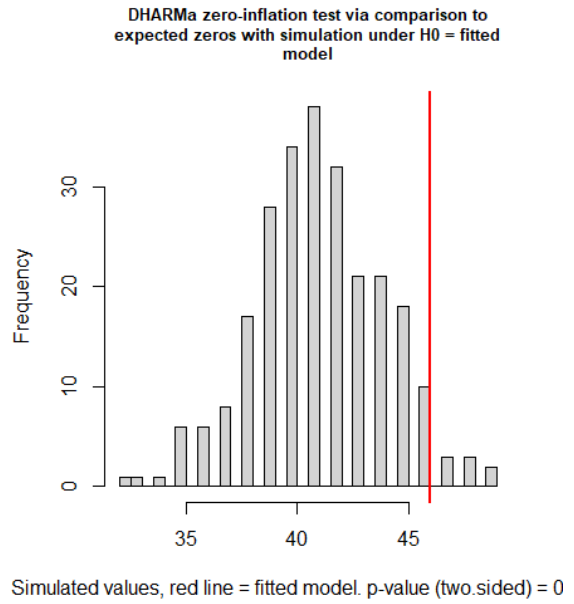
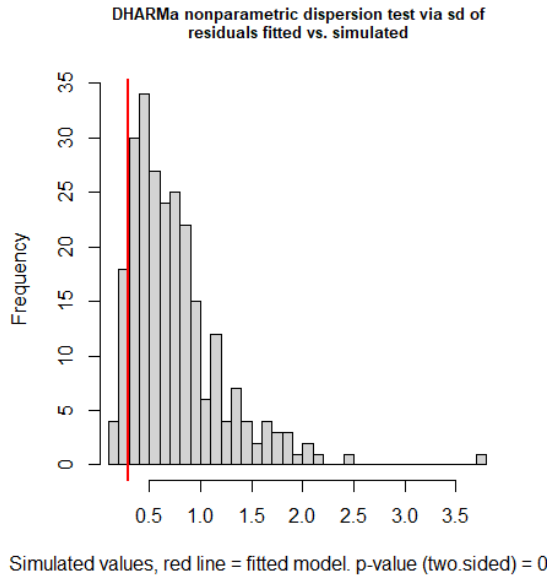
## Appendix 1: Table with an overview of eggs and oocysts found in soil samples in Knutshø and Forollhogna

Area	Season	Year	Treatment	Nematodirus	Nematodirin	Moniezia	Eimeria	Trichuris	Strongylide	Total		
Knutshø	summer	2020	saltlick	7	0	0	2	0	1	10	Ristjønna 260 cm	09.06.2020 saltstein
Knutshø	summer	2020	control	0	0	0	0	0	0	0	Ristjønna kontroll	09.06.2020 kontroll
Knutshø	autumn	2020	saltlick	39	0	17	5	5	3	69	Ristjønna 180 cm	06.10.2020 saltstein
Knutshø	autumn	2020	control	7	0	0	2	0	1	10	Ristjønna kontroll	06.10.2020 kontroll
Knutshø	summer	2020	saltlick	2	0	0	0	0	0	2	Veslvonin 220 cm	18.06.2020 saltstein
Knutshø	summer	2020	control	0	0	0	0	0	0	0	Veslvonin ref	18.06.2020 kontroll
Knutshø	autumn	2020	saltlick	2	0	4	0	2	4	12	Veslvonin 140 cm	23.09.2020 saltstein
Knutshø	autumn	2020	control	0	0	0	0	0	0	0	Veslvonin kontroll	23.09.2020 kontroll
Knutshø	summer	2020	saltlick	2	1	0	10	0	0	13	Bøasæterveien A	18.06.2020 saltstein
Knutshø	summer	2020	saltlick	4	0	0	13	2	0	19	Bøasæterveien B	18.06.2020 saltstein
Knutshø	summer	2020	control	0	0	0	0	0	0	0	Bøasæterveien kontroll	18.06.2020 kontroll
Knutshø	autumn	2020	saltlick	32	0	8	5	1	0	46	Bøasæterveien A	23.09.2020 saltstein
Knutshø	autumn	2020	saltlick	3	0	2	14	0	1	20	Bøasæterveien B	23.09.2020 saltstein
Knutshø	autumn	2020	control	0	0	0	0	0	0	0	Bøasæterveien kontroll	23.09.2020 kontroll
Knutshø	summer	2020	saltlick	5	0	1	12	0	0	18	Hånåbekkvolvet I 160cm	23.06.2020 saltstein
Knutshø	summer	2020	control	0	0	0	0	0	0	0	Hånåbekkvolvet II	23.06.2020 kontroll
Knutshø	autumn	2020	saltlick	17	0	0	4	3	2	26	Hånåbekkvolvet I 180 cm	30.09.2020 saltstein
Knutshø	autumn	2020	control	0	0	0	0	0	7	7	Hånåbekkvolvet II 140 cm	27.09.2020 kontroll
Knutshø	summer	2020	saltlick	11	0	1	20	1	0	33	Lertjønnebekken I 140 cm	17.06.2020 saltstein
Knutshø	summer	2020	control	0	0	0	0	0	1	1	Lertjønnebekken kontroll	17.06.2020 kontroll
Knutshø	autumn	2020	saltlick	0	0	0	0	0	1	1	Lertjønnebekken II	25.09.2020 saltstein?
Knutshø	autumn	2020	control	0	0	0	0	0	0	0	Lertjønnebekken II	25.09.2020 kontroll
Knutshø	summer	2020	saltlick	28	0	0	3	1	0	32	Drotningdalen 240 cm	18.06.2020 Saltstein
Knutshø	summer	2020	control	0	0	0	0	0	0	0	Drotningdalen kontroll	18.06.2020 kontroll
Knutshø	autumn	2020	saltlick	43	0	3	2	1	0	49	Drotningdalen 140 cm	23.09.2020 saltstein
Knutshø	autumn	2020	control	0	0	0	0	0	0	0	Drotningdalen kontroll	23.09.2020 kontroll
Knutshø	summer	2021	saltlick	17	0	2	11	1	0	31	Ristjønna 240 cm	09.06.2021 Saltstein
Knutshø	summer	2021	control	0	0	0	0	0	0	0	Ristjønna	09.06.2021 kontroll
Knutshø	autumn	2021	saltlick	20	0	13	9	4	15	61	Ristjønna 180 cm	21.09.2021 Saltstein
Knutshø	autumn	2021	control	0	0	0	0	0	0	0	Ristjønna	21.09.2021 kontroll
Knutshø	summer	2021	saltlick	19	0	11	3	0	3	36	Veslvonin	10.06.2021 Saltstein
Knutshø	summer	2021	control	0	0	0	0	0	0	0	Veslvonin ref 100 cm	10.06.2021 kontroll
Knutshø	autumn	2021	saltlick	59	2	48	30	5	0	144	Veslvonin 120 cm	16.09.2021 Saltstein
Knutshø	autumn	2021	control	0	0	0	0	0	1	1	Veslvonin	16.09.2021 kontroll
Knutshø	summer	2021	saltlick	33	0	23	5	3	0	64	Bøasæterveien A	10.06.2021 Saltstein
Knutshø	summer	2021	saltlick	54	0	3	10	1	0	68	Bøasæterveien B	10.06.2021 Saltstein
Knutshø	summer	2021	control	0	0	0	0	0	0	0	Bøasæterveien kontroll	10.06.2021 kontroll
Knutshø	autumn	2021	saltlick	36	0	11	28	5	2	82	Bøasæterveien A	16.09.2021 Saltstein
Knutshø	autumn	2021	saltlick	56	1	27	21	2	11	118	Bøasæterveien B	16.09.2021 Saltstein
Knutshø	autumn	2021	control	0	0	0	0	0	0	0	Bøasæterveien kontroll	16.09.2021 kontroll
Knutshø	summer	2021	saltlick	15	0	2	5	0	1	23	Hånåbekkvolvet I	11.06.2021 Saltstein
Knutshø	summer	2021	control	0	0	0	0	0	0	0	Hånåbekkvolvet II kontroll	11.06.2021 kontroll
Knutshø	autumn	2021	saltlick	13	0	0	1	0	52	66	Hånåbekkvolvet I 160 cm	20.09.2021 saltstein
Knutshø	autumn	2021	control	0	0	0	0	0	0	0	Hånåbekkvolvet II kontroll	20.09.2021 kontroll
Knutshø	summer	2021	saltlick	6	1	5	13	2	0	27	lertjønnebekken I 180 cm	11.06.2021 saltstein
Knutshø	summer	2021	control	0	0	0	0	0	0	0	Lertjønnebekken kontroll	11.06.2021 kontroll
Knutshø	autumn	2021	saltlick	36	1	10	0	0	2	49	lertjønnebekken I 180 cm	17.09.2021 saltstein
Knutshø	autumn	2021	control	0	0	0	0	0	0	0	Lertjønnebekken II kontroll	17.09.2021 kontroll
Knutshø	summer	2021	saltlick	67	1	9	17	11	5	110	Drotningdalen	10.06.2021 saltstein
Knutshø	summer	2021	control	0	0	0	0	0	0	0	Drotningdalen	10.06.2021 kontroll
Knutshø	autumn	2021	saltlick	73	0	11	16	4	0	104	Drotningdalen 160 cm	16.09.2021 saltstein
Knutshø	autumn	2021	control	0	0	0	0	0	0	0	Drotningdalen	16.09.2021 kontroll
Forollhogna	summer	2020	saltlick	2	0	0	0	0	0	2	Russu	13.07.2020 saltstein
Forollhogna	summer	2020	control	0	0	0	0	0	1	1	Russu kontroll	14.07.2020 kontroll
Forollhogna	autumn	2020	saltlick	0	0	0	0	0	0	0	Russubotn 1,5 m	29.09.2020 saltstein
Forollhogna	autumn	2020	control	0	0	0	0	0	0	0	Russubotn kontroll	29.09.2020 kontroll
Forollhogna	summer	2020	saltlick	2	0	1	0	0	0	3	Vestforlitj-hiasjoen	17.07.2020 saltstein
Forollhogna	summer	2020	control	0	0	0	0	0	0	0	Vestforlitj-hiasjoen 100m	17.07.2020 kontroll
Forollhogna	autumn	2020	saltlick	0	0	0	0	0	1	1	Vestforlitj-hiasjoen	05.10.2020 saltstein
Forollhogna	autumn	2020	control	0	0	0	0	0	0	0	Vestforlitj-hiasjoen kontro	05.10.2020 kontroll
Forollhogna	summer	2020	saltlick	0	0	0	0	0	0	0	Sandbekkdalen	13.07.2020 saltstein
Forollhogna	summer	2020	control	0	0	0	0	0	0	0	Sandbekkdalen	13.07.2020 kontroll
Forollhogna	autumn	2020	saltlick	5	0	8	5	0	1	19	Sandbekkdalen 1,5 m	06.09.2020 saltstein
Forollhogna	autumn	2020	control	0	0	0	0	0	0	0	Sandbekkdalen	06.09.2020 kontroll
Forollhogna	summer	2020	saltlick	0	0	1	0	1	0	2	Sør for litj-hiasjoen	08.07.2020 saltstein
Forollhogna	summer	2020	control	0	0	0	0	0	0	0	Sør for litj-hiasjoen 100m	08.07.2020 kontroll
Forollhogna	summer	2020	saltlick	0	0	0	0	0	0	0	Rødhåmmårn	08.07.2020 saltstein
Forollhogna	summer	2020	control	0	0	0	0	0	0	0	Rødhåmmårn 50 m	08.07.2020 kontroll
Forollhogna	summer	2020	saltlick	3	0	1	1	1	0	6	Hansdalen	30.06.2020 saltstein
Forollhogna	summer	2020	control	0	0	0	0	0	0	0	Hansdalen 50m	30.06.2020 kontroll
Forollhogna	autumn	2020	saltlick	29	0	8	1	3	0	41	Hansdalen	06.10.2020 saltstein
Forollhogna	autumn	2020	control	0	0	0	0	0	0	0	Hansdalen 50m	06.10.2020 kontroll
Forollhogna	summer	2020	saltlick	0	0	0	3	0	0	3	Krigsvollidalen	12.07.2020 saltstein
Forollhogna	summer	2020	control	0	0	0	0	0	0	0	Krigsvollidalen referanse	12.07.2020 kontroll
Forollhogna	autumn	2020	saltlick	0	0	0	0	0	0	0	Krigsvollidalen	09.09.2020 saltstein
Forollhogna	autumn	2020	control	0	0	0	0	0	0	0	Krigsvollidalen referanse	09.09.2020 kontroll
Forollhogna	summer	2021	saltlick	2	0	1	0	0	0	3	Sandbekkdalen 1,5 m	11.06.2021 saltstein
Forollhogna	summer	2021	control	0	0	0	0	0	0	0	Sandbekkdalen	11.06.2021 kontroll
Forollhogna	summer	2021	saltlick	0	0	0	0	0	0	0	Russubotn 1,5 m	10.06.2021 saltstein
Forollhogna	summer	2021	control	0	0	0	0	0	0	0	Russubotn kontroll	10.06.2021 kontroll

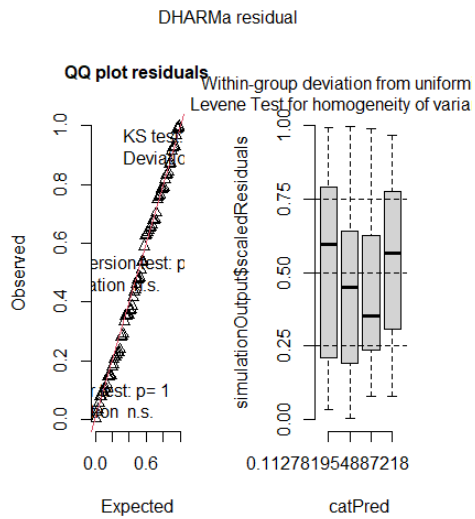
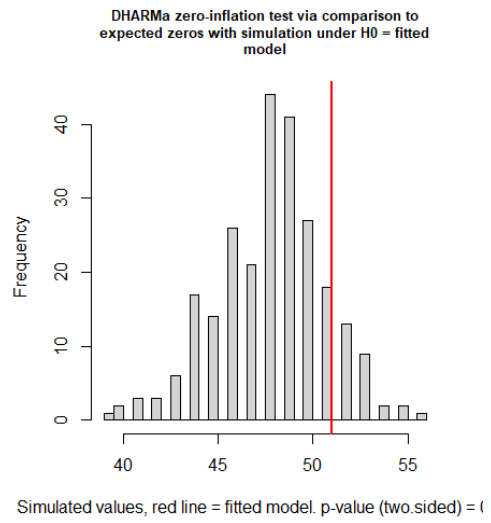
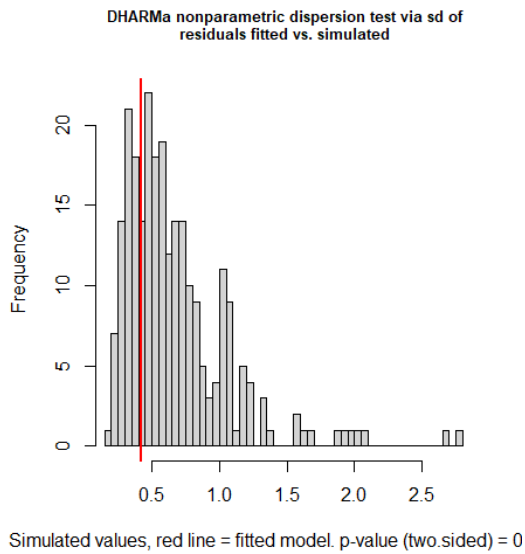
## Appendix 2: Coordinates of sample sites of saltlicks and controls (UTM).

		<b>GPS 32V-Nord</b>	<b>GPS 32V-Øst</b>
Russu		574719	6948163
Russu kontroll		32V0575889	6946944
Russubotn 1,5 m			
Russubotn kontroll			
Vestforlitj-hiasjoeen		32V0570829	6948598
Vestforlitj-hiasjoeen 100m		32V0570835	6948570
Vestforlitj-hiasjoeen			
Vestforlitj-hiasjoeen kontroll			
Sandbekkdalen		32V0574463	6941482
Sandbekkdalen		32V0574472	6941503
Sandbekkdalen 1,5 m			
Sandbekkdalen			
Sør for litj-hiasjøen		32V0572236	6948394
Sør for litj-hiasjøen 100m		32V0572226	6948393
Rødhåmmårn		32V0572576	6946441
Rødhåmmårn 50 m		32V0572588	6946426
Hansdalen			
Hansdalen 50m			
Hansdalen		32V0565848	6953894
Hansdalen 50m		32V0565848	6953894
Krigsvolldalen		584196	6954545
Krigsvolldalen referanse			
Krigsvolldalen			
Krigsvolldalen referanse			
		<b>GPS 32V-Nord</b>	<b>GPS 32V-Øst</b>
Ristjønna 260 cm		32V531983	6924411
Ristjønna kontroll		32V532176	6924818
Ristjønna 180 cm			
Ristjønna kontroll			
Veslvonin 220 cm		32V538462	6919948
Veslvonin ref		32V538587	6919883
Veslvonin 140 cm			
Veslvonin kontroll			
Bøasæterveien A		32V538130	6917438
Bøasæterveien B		32V538144	6917385
Bøasæterveien kontroll		32V0538101	6917523
Bøasæterveien A			
Bøasæterveien B			
Bøasæterveien kontroll			
Hånåbekkvolvet I 160cm		550803	6923757
Hånåbekkvolvet II		550819	6923781
Hånåbekkvolvet I 180 cm			
Hånåbekkvolvet II 140 cm			
Lertjønnbekken I 140 cm		542021	6923664
Lertjønnbekken kontroll		541867	6923579
Lertjønnbekken II		542021	6923664
Lertjønnbekken II			
Drotningdalen 240 cm		537119	6918073
Drotningdalen kontroll		536974	6918086
Drotningdalen 140 cm			
Drotningdalen kontroll			

### Appendix 3: Model validation of *Nematodirus battus*

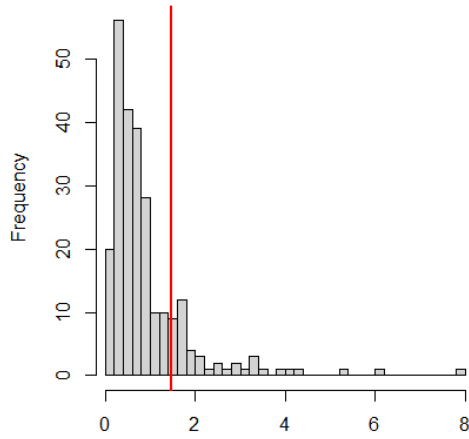


## Appendix 4: Model validation of *Eimeria*



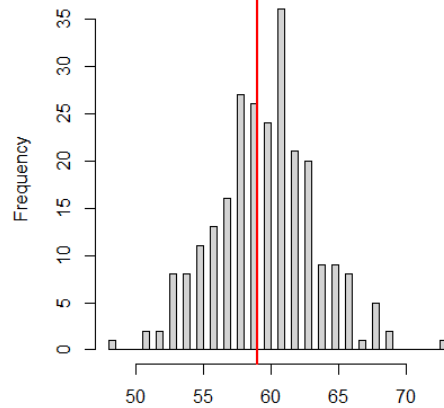
## Appendix 5: Model validation of Stronglyde egg

DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated



Simulated values, red line = fitted model. p-value (two.sided) = 0

DHARMA zero-inflation test via comparison to expected zeros with simulation under H0 = fitted model



Simulated values, red line = fitted model. p-value (two.sided) = 0

DHARMA residual

