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Master Thesis

The presence of the pathogen *Fusobacterium necrophorum* in semi-domesticated reindeer – increased impact associated with intensified feeding

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Abstract

Reindeer (*Rangifer tarandus*) hold a significant cultural and historical importance in Norway, particularly within the Sami community, and continue to play a significant role in the country's landscape and economy. This study aimed to assess the presence and prevalence of *Fusobacterium necrophorum*, a bacterium linked to necrobacillosis, a disease affecting semi-domesticated reindeer populations in Norway. Furthermore, the study aimed to evaluate the relationship between intensified feeding practices and *F. necrophorum* presence. We analyzed 129 fecal samples from female reindeer, aged two years or younger, across three semi-domesticated reindeer herds with differing management practices located in Tana, Mo i Rana, and Røros. Through quantitative PCR (qPCR), one positive case of *F. necrophorum* was detected, a result that diverges from previous studies suggesting its common presence in semi-domesticated reindeer gastrointestinal tracts. The findings raise questions about the bacterium's prevalence in semi-domesticated reindeer and whether it is less prevalent in their gastrointestinal tract than previously assumed. Furthermore, the relationship between *F. necrophorum* and intensified feeding practices remains inconclusive due to limited findings and needs further research.

Keywords: Feeding practices, *Fusobacterium necrophorum*, Norway, *Rangifer tarandus*, Semi-domesticated reindeer

Contents

Introduction

Reindeer (*Rangifer tarandus*) have a rich history in Norway, with their arrival dated to about 10,000 to 14,000 years ago, when the first ice began to recede from the Norwegian coast (Selsing, 2012). This predates the first signs of human presence in Norway, as humans arrived in Norway around 10 000 years ago (Bang-Andersen, 2012). Therefore, when the first humans arrived, reindeer, in addition to fish along the coast, were crucial for their survival. This early period of Norwegian history was a time when humans were part of a hunter-gatherer society, and the concept of reindeer pastoralism was still thousands of years away from being introduced.

When the transition from wild reindeer hunting to pastoralism happened, remains controversial. Some hypothesize it occurred in the late Iron Age, while others propose it happened in the 17th century, which is considered the more accepted hypothesis (Sommerseth, 2011). Regardless of when this shift occurred, it is evident that the utilization of reindeer, for food, clothing, and more, has been a significant part of Norwegian and especially Sami culture for thousands of years (Williams, 2003). From hunting to pastoralism, the importance of reindeer within this cultural landscape has been consistent and strong.

Today, reindeer is still a big part of the Norwegian landscape and society. Norway hosting big populations of wild reindeer (Visitor Centre Carnivore, 2024) and semi-domesticated reindeer being a big part of the sami culture in pre-dominantly northern parts of the country (*Figure 1*). Both wild and semi-domesticated reindeer face numerous challenges. These range from predators such as wolves (*Canis lupus*), bears (*Ursus arctus*), lynx (*Lynx lynx*), eagles, and wolverines (*Gulo gulo*) (Löffler, & Pape, 2012) to environmental challenges like loss of grazing habitats and the impacts of climate change (Riseth, 2009). In addition, Norway's wild reindeer populations are classified by the Norwegian Environmental Agency's quality standard to be in a mainly "bad" condition (Klima- og miljødepartementet, 2021). The quality standard consists of three substandards, where each substandard consists of several parameters to determine the condition of the reindeer populations. Medium condition is considered an acceptable condition where the parameters conclude that management measures might not be needed. However, bad condition are populations where management measures must be taken, and the condition of the population is facing major challenges. No reindeer populations are classified as being in good condition. Furthermore, the conflict with predators has significant

economic implications for the reindeer herding industry (Löffler, & Pape, 2012), as humans are feeling the economic costs when dealing with loss of reindeer. In addition, the reindeer herding industry's dependence on vast grazing lands for semi-domesticated reindeer is threatened by the construction of infrastructure, cabins and human disturbances, which leads to the loss and fragmentation of these grazing areas (Skarin, & Åhman, 2014; Skarin et al., 2015).

Many of these challenges can be linked to health-related issues, with semi-domesticated reindeer herds facing a range of infectious diseases that can pose serious risks to their overall health. Such diseases can be influenced by environmental factors, management practices, and interactions with other animal species (Tryland, 2013). Understanding the dynamics of these diseases is essential for implementing effective control and prevention strategies. One such disease that has historically affected reindeer herds is digital necrobacillosis, characterized by severe lesions in the distal parts of the legs (Handeland et al., 2010). In the past, this disease was often seen among semi-domesticated reindeer kept in corrals with poor hygienic conditions, often associated with feeding and milking practices (Tryland et al., 2020).

Over the years, changes in herding practices, particularly the shift from milking in the 1950s, which involved corralling smaller groups of reindeer into tighter spaces, to a more traditional large-scale herding practice, have led to a significant disappearance of digital necrobacillosis in semi-domesticated reindeer herds (Handeland et al., 2010). However, new challenges have emerged, with a different form of the disease, oral necrobacillosis, becoming an increasingly prominent concern (Tryland et al., 2019). This disease is often associated with feeding practices in semi-domesticated reindeer (Tryland, et al., 2019). As reindeer herding practices continue to evolve, transitioning from traditional herding methods to more intensive herding approaches (Axelsson-Linkowski et al., 2020), it is important to investigate the presence and implications of specific pathogens in semi-domesticated reindeer herds.

Fusobacterium necrophorum, a bacterium assumed to be prevalent in the gastrointestinal tract of reindeer, including semi-domesticated herds (Aagnes et al., 1995), is a notable pathogen of interest. While typically a commensal organism, *F. necrophorum* can become an opportunistic pathogen under certain conditions (Tryland et al., 2019). This pathogen has been implicated in the development of both digital and oral necrobacillosis in reindeer (Riseth et al., 2020; Tryland et al., 2019). Investigating the presence, prevalence, and factors influencing the transmission

and persistence of *F. necrophorum* in semi-domesticated reindeer populations is crucial for understanding the disease dynamics and implementing appropriate management measures.

By studying the dynamics of *F. necrophorum* in semi-domesticated reindeer herds, we can gain insights into the current occurrence of the bacterium and its associated diseases. Even though this bacterium has been found in the gastrointestinal tract of reindeer before (Aagnes et al., 1995), it is still of importance to discover how commonly it is present. According to a study done by Aagnes et al. (1995) *Fusobacterium necrophorum* is a common bacterium in reindeer. They studied the ruminal microbial digestion in four different categories of reindeer: Free-Living, captive lichen-fed and starved reindeer for 1 day and 4 days. In total their sample size consisted of eight reindeer, four free-living, and two of each in the remaining categories. In their results they found that four of the eight reindeer showed a presence of *Fusobacterium spp*. in the rumen fluids of the reindeer. Two of the free-living reindeer and two in the starved reindeer category. Furthermore, this study provides an opportunity to investigate the impact of changing herding practices, such as intensified feeding strategies and the transition toward more non-traditional herding methods, on the prevalence and severity of possible diseases. This knowledge could help develop targeted strategies to mitigate the impact of *F. necrophorum* and associated diseases in semi-domesticated reindeer.

The main objective of this study was to assess the presence and prevalence of *F. necrophorum* in the gastrointestinal tract of semi-domesticated reindeer, by analyzing fecal samples collected from semi-domesticated reindeer. This is done since fecal samples provide a source of bacterial DNA for analysis (Zielińska et al, 2016). The samples were collected from three different herds (Tana, Mo i Rana and Røros) representing different management and feeding practices.

Additionally, we wanted to assess the relation between feeding practices and the presence of *Fusobacterium necrophorum*. This objective aims to explore the relationship between feeding practices in semi-domesticated reindeer herds and the presence of *F. necrophorum*. By analyzing the prevalence of the bacterium in relation to differing feeding practices, valuable insights can be gained into the potential risk factors associated with the bacterium's associated diseases. By comparing the prevalence of *F. necrophorum* in reindeer herds with varying herding practices, the study aims to understand the implications of these changes.

Finally, we wanted to provide recommendations for the management and control of potential related diseases. Based on the findings in this study and potential prior studies, the objective is to generate recommendations for the management and increased control of related diseases in semi-domesticated reindeer herds. In addition, we aim to add possible useful knowledge to a question that has limited prior answers. These recommendations can aid in the development of targeted strategies to mitigate the impact of *F. necrophorum* associated diseases.

Material and Methods

Study area

The study area consists of semi domesticated reindeer herds at three selected sites: Tana, Mo i Rana and Røros (*Figure 1*). The Tana/ Rákkonjárga reindeer herding area practices seasonal grazing. This is the most northern area where fieldwork is being completed in this study. During summer, animals graze along the coast and in winter, they graze near the Finnish border. Sometimes, supplemental feeding is provided to a limited extent, particularly when natural food is scarce or to help guide the animals. Usually, this extra feed is taken to the animals in their usual grazing areas. However, if some animals are weak or young, they may be moved to specific fencing areas for feeding. This way, animals can get the nutrition they need throughout the year (Anonymous, 2011).

Positioned between the northernmost study region in Tana and the southernmost one in Røros, we find the Mo i Rana/Ildgruben herding area. This area is primarily coastal. Compared to Tana/Rákkonjárga, this area features a more systematic practice of supplemental feeding. The supplemental feeding primarily takes place in or near the fenced area around Tverrvatnet, though it can also occur elsewhere. It's worth noting that during periods of supplemental feeding, animals tend to remain within these areas for extended periods of time (Anonymous, 2017).

Primarily for the Røros/Gåebrie sitje reindeer, the Femunden reindeer herding district serves as the winter grazing areas. The calving areas, as well as the summer grazing areas, are located in the mountainous regions at the center of the reindeer herding district. The entire district is situated along the border to Sweden. There is minimal to no supplemental feeding utilized here (Anonymous, 2020).

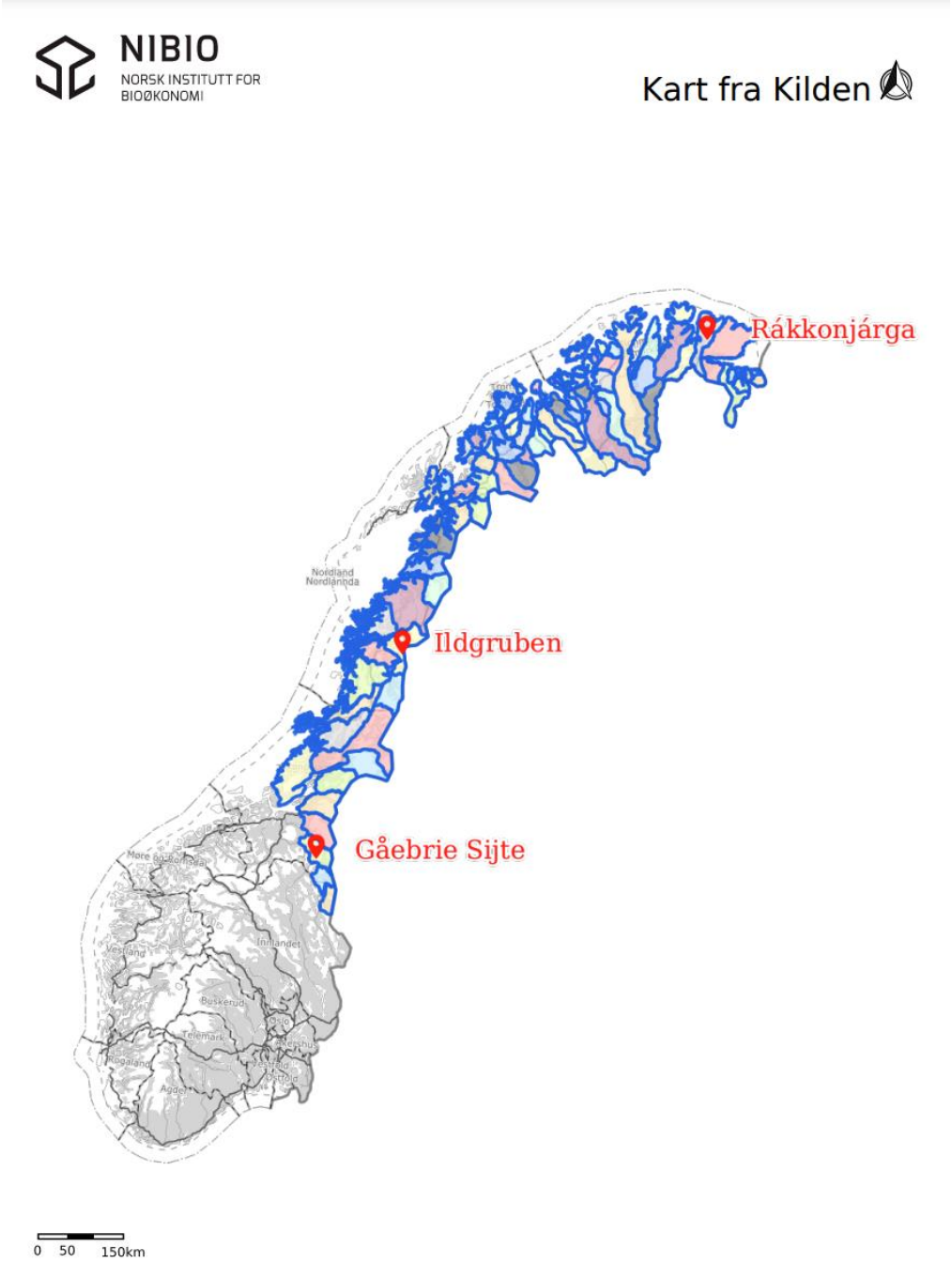

Figure 1. Map of reindeer herding districts in Norway (Landbruksdirektoratet, 2024) with points showing each of the study areas

Sample collection

Fecal samples were collected from semi-domesticated reindeer at three selected reindeer herds during summer and winter gathering in the corrals. These gatherings occur during calf marking (summer) or sorting for slaughter (winter). All samples were collected from female reindeer born in spring 2022. We gathered feces by gloved hand directly from the rectum while the reindeer was restrained. It was then placed in plastic bags and stored in -20ºC until analysis was performed. In total, 129 fecal samples were collected (*Table 1*) from 72 reindeer included in the study. 18 samples did not have sufficient feces for analysis (encompassing the period from December 2022 until November 2023).

Table 1. Number of samples collected for presence of Fusobacterium necrophorum in three different study areas in different years and seasons. N = number of samples collected

	2022/23 December-February	July 2023	September-November 2023	
	N	N	N	Total
Tana	28	0	37	65
Mo i Rana	16	0	13	29
Røros	19	16	0	35
Total	63	16	50	129

Sample preparation and analysis

DNA extraction was conducted with a Qiagen Power fecal kit (bead beating protocol with Fastprep homogenizer) on a semi-automatic platform (Qiagen QIAqube) at the Norwegian Veterinary Institute. Any samples above 60 ng/ul were diluted prior to the PCR reaction.

A qPCR assay was used to detect DNA from F. necrophorum, based on a previous study by Jensen et al (2007). The qPCR was done with a 25µl reaction, including 12.5µL SsoAdvanced Universal Probes Supermix, 2.5µL of 100 nM Taq Man probe, 2.5µL of 300 and 400 nM forward and reverse primer and 5µL of template DNA. Primers were based on the 277-bp fragment of the RNA polymerase β-subunit (rpoB) gene, including RPO-forward 5'-TCTCTACGTATGCCTCACGGATC-3' and RPO-reverse 5'-CGATATTCATCCGAGAGGGTCTC-3'.

The thermal cycling was done on a CFX Opus 96 Real-Time PCR System, consisting of 10 min at 95°C followed by 55 cycles of 30 s at 95°C and 60 s at 64°C. The last step of the cycles was modified from 60°C to 64°C following completion of an initial temperature gradient trial to identify the best annealing temperatures for the primers on our PCR machine. Positive and negative controls were included in each run. The positive controls consisted of gBlock gene fragments created from the F. necrophorum subsp. necrophorum RNA polymerase beta subunit gene (GenBank Accession AF527637.1), along with positive control DNA extracted from F. necrophorum isolated from a wild reindeer (*R. t. tarandus*) with digital necrobacillosis (Ref. no.: 2016-4-11473, Norwegian Veterinary Institute, Oslo). Negative controls included negative extraction controls and no template controls.

Though the positive and negative controls worked for each qPCR run, all samples from extracted feces were not possible to read on the qPCR machine (amplification curves were 'messy' with many peaks). This may have been due to inhibitory factors in the samples that could have interfered with fluorescence from the probe. To eliminate the possibility of primers and probes not functioning, an aliquot of each was sent to UiT (The Arctic University of Norway) to be run on a separate qPCR machine. Primers and probe worked appropriately. Due to this limitation, a 2% agarose gel was used to visualize any positive bands (created with SYBR safe DNA gel stain and read on a transilluminator).

Positive amplicons on the gel were prepared for sequencing using a Qiagen PCR Purification Kit. Purified samples were sent to Eurofins Genomics (Germany) for sequencing.

Results

The study managed to identify one PCR-positive reindeer (WelFed code WKT23-8) in Tana. This animal was sampled in November 2023.

Discussion

Given the growing concerns regarding reindeer health and future populations because of challenges such as infrastructure development, cabin construction, and human disturbances leading to habitat fragmentation, research on the health and status of reindeer has become increasingly important (Skarin, & Åhman, 2014; Skarin et al., 2015). Norway's reindeer populations are classified by the Norwegian Environmental Agency's quality standard and shows a concerning trend where wild populations are primarily in "bad" or "medium" conditions, with none meeting the criteria for "good" condition (Klima- og miljødepartementet, 2021). Although this standard focuses on wild populations, similar challenges such as climate change, habitat fragmentation, and grazing area loss, are comparably concerning for semi-domesticated reindeer as well. The same pressures could make semi-domesticated reindeer more vulnerable to poor health conditions and disease (Tryland, 2013; Mysterud et al., 2023).

Necrobacillosis, in both oral and digital, represents a significant concern for reindeer herders and the reindeer herding industry, primarily due to the economic losses associated with these diseases (Riseth et al., 2020) and the bacterium *Fusobacterium necrophorum* is recognized as the primary pathogen responsible for necrobacillosis. There are very few studies that tell us how prevalent this bacterium is in reindeer specifically and to this date, the only study that has been conducted on this topic, to my knowledge is from Aagnes et al (1995). This study has suggested that *F. necrophorum* is common or normal in reindeer's rumen. However, the conclusion that *F. necrophorum* is normal in reindeer, is based only on a small sample size of eight animals, where only half of this sample size showed signs of *F. necrophorum.* This is insufficient to definitively state that this bacterium is a regular part of reindeer gut flora. In contrast, this study, which sampled 72 reindeer for fecal matter, identified only one positive case of *F. necrophorum*. This larger sample size further questions the idea that *F. necrophorum* is a normal part of reindeer gut flora, suggesting that *F. necrophorum* may be less common in the gastrointestinal tract in reindeer, than previously thought. At least the study could indicate that's the case in females of two years old or younger. Perhaps in older reindeer the case might be different as the reindeer have been exposed to possible contaminants for a longer period compared to younger animals. Considering in the study by Aagnes et al (1995) they only looked at adult reindeer, for future studies it could be interesting to see if there is a difference when it comes to age. It's important to note that all the reindeer in this study were females sampled

when they were two years old or younger and therefore, we cannot generalize this finding to all reindeer groups.

Limitations

There are some limitations to my study. First, this study can be described as a pilot study for further research. Since the findings in the study of Aagnes et al. (1995) there hasn't been any or enough research looking into the prevalence of *Fusobacterium necrophorum* in the gastrointestinal tract of reindeer specifically. However, other studies have found that it is quite common to isolate this bacterium from other species and ruminant species in the gastrointestinal tract, but also in abscesses and respiratory tracts (Jang, & Hirsh, 1994; Nagaraja et al., 2005). This study could be a gateway into further research expanding on this topic in reindeer. This study is by no means debunking previous research, considering the timespan and findings is limited in this study, and the use of statistical analysis tools was not possible and therefore is not able to create a broader understanding of the topic. Future studies could include a broader sample across various age groups and both genders, male and female, to evaluate if findings hold across a more representative demographic. The age composition of this study is limited to only young animals two years or younger. It could be interesting to see how with time and age, the results might vary. In addition, by integrating long-term climate and weather data with bacterium prevalence, we could also gain insights into how environmental factors influence the findings into how this bacterium reacts to different factors.

DNA sequencing is another limitation of this study. The positive sample was sent in for sequencing to Eurofin Genomics, unfortunately, likely because of shipment delays leading to a deterioration of the sample between our campus in Evenstad, Norway, and Germany, this was not successful. There are at the time of this study being taken steps to isolate additional DNA from the PCR positive animal for sequencing. This could be beneficial for further studies. DNA sequencing presents verification of the bacterium, which further provides a tool for phylogeny. Phylogeny can help show how *F. necrophorum* is related to other *Fusobacterium* species, classifying the bacterium and placing it into a group. This could be useful for investigating shared traits between bacterium species and spot genes associated with pathogenicity, such as the leukotoxin gene, which aids in the bacterium's ability to infect animals. (Zhang, F. et al. 2005; Nagaraja et al., 2005)

Conclusion

This study reveals that *F. necrophorum* may be present in the feces of semi-domesticated reindeer, even though further verification through DNA sequencing is needed. The finding of only one positive case, however, is unexpected, as previous research indicates that *F. necrophorum* is a common part of the gastrointestinal tract and other sites in different species and other ruminants. This low detection rate aids in creating questions about its prevalence within reindeer feces.

As for the relationship between intensified feeding practices and the presence of *F. necrophorum*, this study does not provide any significant insights. While the only positive sample came from an area with limited feeding practices, this result does not show how or if increased feeding might influence the presence of this bacterium in reindeer. We could hypothesize a higher prevalence in areas with intensified feeding, considering it could create conditions for the bacterium to spread (Handeland et al., 2010). However, with only one positive result, our findings are limited, and we cannot draw any concrete conclusions about the impact of feeding habits on the presence of this bacterium.

These limited findings set the basis for the need for further research. Continuing research into the prevalence of *F. necrophorum* in reindeer populations, especially in relation to feeding practices, age groups and other environmental factors, could provide insights that could aid our understanding of the disease necrobacillosis. This disease can have major impacts on reindeer, potentially leading to higher mortality within reindeer populations (Mysterud et al., 2023), and subsequently also impact the reindeer herding industry. Given the impact of necrobacillosis when it comes to health and welfare, a better understanding of how specific management practices affect disease prevalence could create a more proactive and effective disease management strategy, which would benefit both reindeer populations and herding communities.

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