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Giardia duodenalis in sympatric wild reindeer and domestic sheep in Norway

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ABSTRACT

Wild and semi-domesticated reindeer graze freely on natural pastures in Norway, often sharing these with domestic sheep and other domestic and wild ruminants. In this study, faecal samples from wild reindeer and domestic sheep were collected from two areas in southern Norway and analysed to assess the occurrence and assemblage of *Giardia duodenalis*. Among 162 wild reindeer samples, 25 (15%) were positive for *Giardia*, showing high infection intensities, with most of the samples belonging to sub-assemblage AI, which has zoonotic potential. Interestingly, this study did not detect subassemblage AIII, known to be found in wild ruminants. Among 45 sheep samples, 13 (29%) were *Giardia*-positive, with most belonging to assemblage E.

The finding of predominantly assemblage AI in the reindeer was surprising, particularly given the large proportion of sheep shedding assemblage E *Giardia* cysts. As the number of sheep on these natural pastures far outnumbers the wild reindeer, it is intriguing that they do not seem to share *Giardia* assemblages.

1. Introduction

The intestinal protozoan parasite *Giardia duodenalis* has the potential to infect many mammal species. Infectious cysts are shed, often in large numbers, with the infected host's faeces. Transmission may be direct via the faecal-oral route or by contaminated drinking water or food. The infective dose is considered to be low in humans (Adam, 2003).

Eight different subtypes or genotypes (named assemblages A to H) have been recognised in *Giardia*, and these differ in their host-specificity. *Giardia duodenalis* assemblages A and B have the lowest host specificity, can cause infection in several mammal species, and are particularly associated with human infection. Assemblage A is further divided into sub-assemblages AI, AII and AIII. Subtype AI is predominantly found in livestock and pets, AII is most common in human infections, and AIII seems adapted to wild hoofed ungulates (Sprong et al., 2009).

Assemblage B is the most prevalent genotype in humans and primates but may infect a wide range of species (Sprong et al., 2009). Assemblage B can be divided into sub-assemblages BI - BIV, although in contrast to assemblage A, no distinct host-adaptation has been described for the sub-assemblages (Sprong et al., 2009). This may be due to low phylogenetic resolution at standard loci for this assemblage with high sequence diversity (Seabolt et al., 2021). Despite the long-held inference of zoonotic transmission of *Giardia*, substantiated cases are rare, and it is becoming increasingly accepted that animal-to-human transmission occurs relatively rarely (Cai et al., 2021; Dixon, 2021).

Based on reports of the isolation of this parasite from different species, wild animals are often suspected as potential sources of *G. duodenalis* infection in humans. However, detection of even potentially zoonotic assemblages of *G. duodenalis* in wildlife does not provide evidence for wildlife acting as a reservoir of a given isolate. Conversely, it may just as well be that humans are sources of infection for wildlife. As early as 1978, Davies and Hibler stated that humans are the most critical component in the epidemiology of giardiosis and suggested that the increased use of wilderness by the public is at least partly responsible for the apparent rise of *Giardia* occurrence in wildlife (Kutz et al., 2009; Davies and Hibler, 1978). Since then, human encroachment on and human activity in wildlife habitats has expanded, probably increasing the likelihood of spillover from humans and domestic animals (Daszak

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et al., 2001). In addition, land-use changes and interventions such as pasture fragmentation due to, e.g. tourism, hiking paths, road and cabin constructions, cause aggregation and high functional population densities which exacerbate the situation by facilitating intra- and interspecies transmission of pathogens.

Wild cervids are widely distributed in Norway, but wild reindeer avoid human infrastructure and activity, and are consequently confined to relatively small areas. The winter population of wild reindeer in Norway is about 25,000 animals, and these are found in 24 distinct wild reindeer management areas in the southern half of the country (Gjerde, 2022). These areas are also used by other wild animals, such as red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and moose (*Alces alces*), as well as being seasonally grazed by domestic sheep. The areas are also popular recreational areas for locals, tourists, and hunters.

Semi-domesticated reindeer are owned, but always kept outdoors, mostly as free-ranging animals. They are usually gathered a few times a year for transport between natural pastures, marking, and slaughter. These animals are mainly kept in Sápmi; northern Norway, Sweden, Finland, and the Kola Peninsula (Ricci et al., 2017; Berg-Nordlie and Gaski, 2005–2007). Herding of reindeer crosses the border between Norway and Sweden as many herders have pasture rights in both countries. In spring 2015, the number of semi-domesticated reindeer in Norway, Sweden, and Finland was around 657,000, of which approximately 212,000 were in Norway (Ricci et al., 2017).

A previous study on *Giardia* in wild cervids in Norway (Hamnes et al., 2006) concluded that the parasite was widespread geographically, but only present at relatively low occurrences, being found in less than 2% of red deer, 7% of reindeer and up to around 12 and 15% in moose and roe deer samples, respectively. Hamnes et al. (2006) applied sucrose flotation in addition to immunofluorescence staining, theoretically giving a higher recovery rate of *Giardia* cysts than this study. Molecular analysis (Robertson et al., 2007) of the samples collected by Hamnes et al. (2006) also revealed the potentially zoonotic assemblage A in 6 reindeer (although whether this was AI, AII or AIII was complicated by differing results being obtained at different genes), with an additional report of one reindeer shedding assemblage B *Giardia* cysts (Robertson et al., 2007). However, for that particular sample, it is unknown where in Norway it originated and whether the reindeer was wild or semi-domesticated.

Elevated gastrointestinal parasite burdens have been linked to increased animal density and lower body condition scores in wild reindeer (Bye, 1987). Spillover of gastrointestinal nematodes from domestic sheep to wild reindeer through the common use of artificial salt licks has been suggested as one of the reasons for an observed decline in calving rate and body condition of wild reindeer in Knutshø, a wild reindeer area in Norway (Utaaker et al., 2023). *Giardia* infections are also known to impede nutrition uptake and have been linked to decreased weight gain in ruminants (Geurden et al., 2010; Aloisio et al., 2006). A longitudinal study of naturally acquired *Giardia* infections in sheep found reduced meat productivity amplified by increased intensities of faecal shedding of *Giardia* cysts (Jacobson et al., 2016). The effect of *Giardia* infection on weight gain has also been shown in experimentally infected calves, where the group receiving treatment had a significantly higher weight gain than the untreated group, though no differences in the general health between the two groups were recorded (Geurden et al., 2010).

This study aimed to assess the occurrence, intensity, and molecular diversity of *G. duodenalis* shed by sympatric wild reindeer and sheep, with an emphasis on the wild reindeer population in Knutshø.

2. Material and methods

2.1. Map created using data from

Norwegian Environment Agency. ***Wild Reindeer areas***.[map] 1:1000–1:50000. Oslo: Norwegian Environment Agency, 2024.

Norwegian Agriculture Agency. ***Reindeer husbandry***. [map] 1:50000. Oslo: Norwegian Agriculture Agence, 2024.

The Sami Parliament of Sweden. ***Grazing areas of the Sami villages***. [map] Scale not given. Kiruna: Sami Parliament of Sweden.

2.2. Study sites and reindeer populations

Innlandet is a landlocked county located in southeast Norway, bordering Sweden. This county also constitutes, wholly or partly, five areas of wild reindeer populations, including the neighbouring areas Knutshø and Forollhogna (See Fig. 1).

A recent assessment by an expert group (Rolandsen et al., 2022) of the ten largest wild reindeer areas concluded that they were all of medium to poor quality. The classification was based on criteria such as slaughter weight of calves, pasture quality, health status, functional migration passages, and anthropogenic interventions; Knutshø and Forollhogna were classified as “poor” and “medium”, respectively (Rolandsen et al., 2022).

Knutshø is an area of 2106 km^2, with a population of 1500–1900 wild reindeer. It was formerly regarded as a very productive area for wild reindeer (Solberg et al., 2015), but during the last 20 years, the number of wild reindeer calves per female and the body condition of calves shot during hunting have declined. During the summer season, domestic sheep also graze in Knutshø, and their number has increased by 27% to46,000 animals over the last two decades (Utaaker et al., 2023). In addition, increased human activities, such as the development of infrastructure and recreational areas, have also fragmented the natural pastures and increased the functional density of wild and domestic ruminants on these natural pastures (Rolandsen et al., 2022).

Forollhogna, with an area of 2354 km^2 and a wild reindeer population of 2000 animals, have relatively stable calf recruitment rates and slaughter weights, though with a negative trend. Domestic sheep also graze in Forollhogna in summer, and their numbers have risen by around 8% to 40,000 animals during the last two decades. Although increasing, human activities in this area are more scattered, resulting in a lower degree of habit fragmentation than in Knutshø (Rolandsen et al., 2022).

2.3. Ethical statement

The current study does not involve experiments or animal handling. Faecal samples were collected directly after observing resting wild reindeer herds, i.e. after the herds had moved on, or collection of faecal samples from already dead animals who were shot during ordinary wild reindeer hunting. Faecal samples were collected from the rectum of sheep already slaughtered at an approved slaughterhouse and were not required to apply for ethical approval according to institutional policy or Norwegian legislation.

2.4. Sample collection

Altogether, 207 faecal samples were collected, 162 reindeer samples and 45 sheep samples.

Among these, 54 faecal samples were collected from wild reindeer in Knutshø and 44 from wild reindeer in Forollhogna during the autumn hunting seasons (from 20th of August to 30th of September) in 2018 and 2019. The samples were collected directly from the intestines of shot animals. During 2018 and 2019, in the same season, 20 samples were collected from sheep that had been on natural pasture in Knutshø and 25 samples from sheep that had been grazing in Forollhogna. These samples were collected from a local abattoir where the animals were slaughtered shortly after their return from grazing. The samples from sheep were collected together with parts of the gastrointestinal tract. Thus, the faeces were squeezed from the rectum and had not been in contact with the ground. The consistency of these samples was either pelleted or formed.

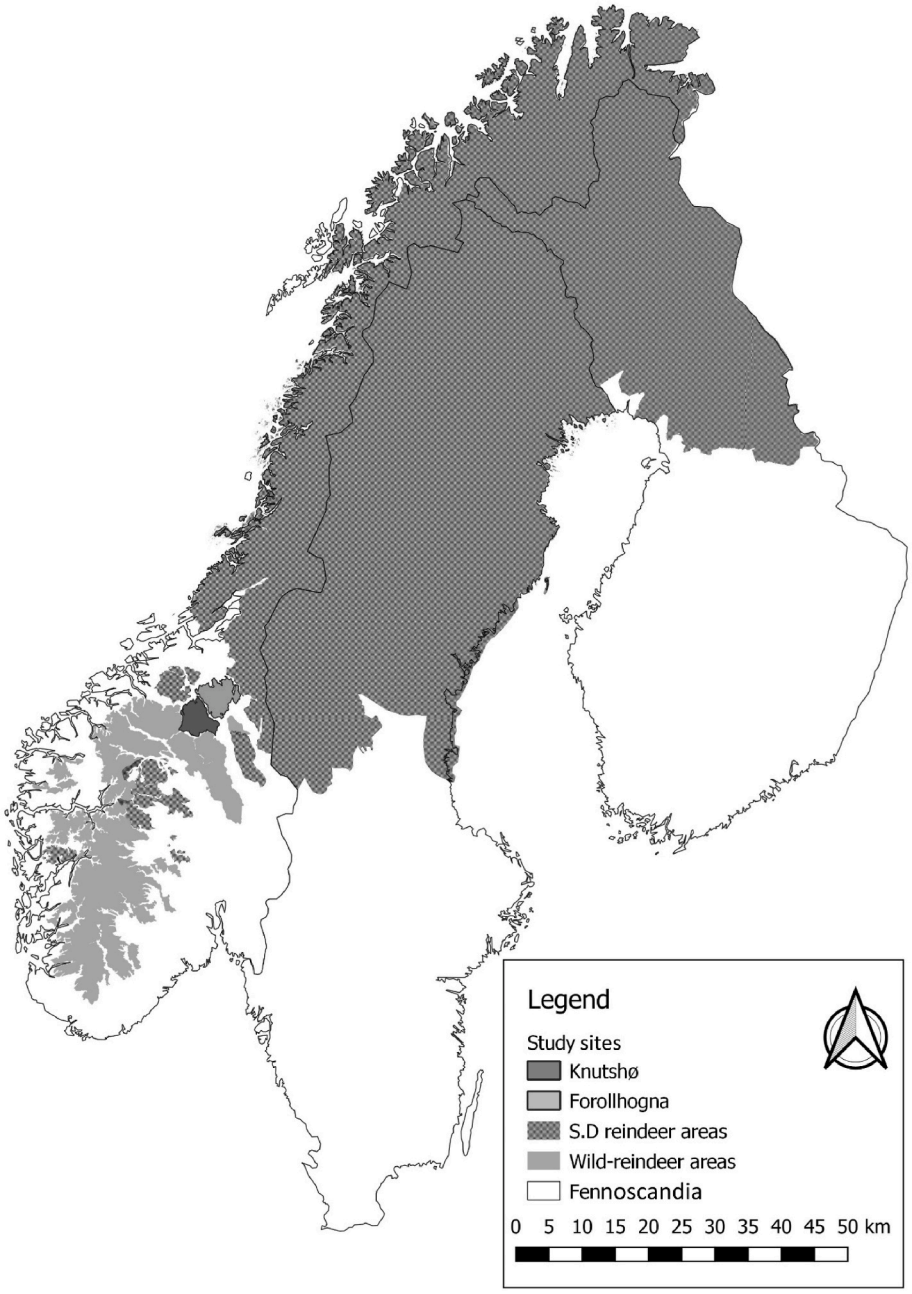

Fig. 1. Map showing Fennoscandia and semi-domesticated reindeer areas, with Knutshø and Forollhogna wild reindeer management area marked with dark highlights.

In addition, during the reindeer calf censuses in Knutshø during the summers of 2020 and 2021, mountain rangers collected 64 samples from the ground or snow directly after observing resting wild reindeer herds, i.e. after the herds had moved on. The samples collected were also either pelleted or formed. Some animals were observed to have diarrhoea, but these samples were impossible to retrieve in the snow. The relative size of the pellets was used to determine whether the samples belonged to a calf or an older animal. In total, 64 wild reindeer faecal samples were collected from the ground on July 16th, 2020 and July 2nd, 2021.

Wild reindeer and sheep samples collected during 2018 and 2019 were transported to the laboratory within 12 h of collection and stored at 4 °C for a maximum of a week before processing and analysis. The viscera and faeces from this study were analysed for presence of macroparasites, helminth eggs and L1 larvae, and the results from this study can be found in Robertsen (2020).

Faecal samples from wild reindeer sampled from 2020 to 2021 were collected in the field and frozen at −12 °C within 8 h of collection. Samples were stored for up to 3 months before shipment overnight with a cooling block to Nord University, where they were stored at 4 °C for maximum a week before processing and IFAT analysis. Positive samples were transported to the Parasitology lab at the Norwegian University of Life Sciences where DNA extraction and PCR was performed.

2.5. Sample analysis

Standard methods were used to analyse the faecal samples for *Giardia* cysts. In brief, 3 g of faeces were mixed with 57 ml of tap water and sieved (aperture of approx. 500 μm). The suspension was poured into two 15 ml tubes and centrifuged at 1500 relative centrifugal force for 3 min. The supernatant was discarded, and the remaining pellet was

retained for parasitological examinations.

Samples were analysed qualitatively and semi-quantitatively using an immunofluorescent antibody test (IFAT) for the presence of *Giardia* spp. cysts on direct faecal smears. Homogenised and sieved faeces were placed on a microscope slide using an inoculating loop with a 10 µl size. Slides were air dried before fixation by applying a drop of methanol and then staining with 15 µl of FITC-labelled monoclonal antibodies (Mab) against *Cryptosporidium* and *Giardia* (oo)cysts walls (Aqua-Glo; Waterborne INC., NO, USA). Samples were incubated at 37 °C in a humid chamber for 30–45 min, and excess Mab was removed using distilled water. A cover slip was placed on the faecal smear, and the stained smears were screened using fluorescence microscopy using a standard FITC filter. Samples were graded by counting the number of cysts per field view at ×200 magnification; 1–9 cysts were graded as G +, 10–50 cysts as G++, 51–99 as G+++ and counts >100 as G++++

2.6. DNA isolation

DNA extraction was performed from all *Giardia*-positive samples using the DNeasy PowerSoil Kit protocol (Qiagen, Oslo, Norway) with minor modifications. In the PowerBead Tubes, the faecal pellet (250 µl) was mixed with 60 µl of the lysis solution (solution C1). Bead beating was then applied to release the DNA by breaking the walls of the cysts in two cycles of 4 m/s for 60 s with a pause of 45 s between each cycle using a FastPrep-24 5G (MP Biomedicals). The DNA was then eluted in 50 µl of elution solution (solution C6) and stored at −20 °C overnight.

2.7. PCR, electrophoresis, purification of PCR product, and sequencing

Three genes were targeted for genotyping investigations of the *Giardia*-positive samples: glutamate dehydrogenase (*gdh*; Read et al., 2004), beta-giardin (*bg*; Lalle et al., 2007), and DNA repair and recombination protein RHP 26 (Ankarklev, 2018). Primers and target amplicon size are described in Table 1.

2.8. Polymerase chain reaction and sequencing

The reaction mixture included 8.3 µL PCR water, 12.5 µL of 2 × DreamTaq Green PCR Master Mix (Thermo Scientific), 1 µL reverse and 1 µL forward primers (at a final concentration of 0.1 mM), 0.2 µL BSA (20 mg/L) and 2 µL template DNA were used in the primary PCR. BSA was omitted for DNA repair and recombination protein RHP 26, but replaced with 0.2 µl of PCR water.

Positive controls (P101, *G. duodenalis* cysts, human isolate H-3, assemblage B, Waterborne Inc, LA, USA) and negative controls (lab-grade purified water) were included for each PCR. SYBR™ Safe DNA Gel Stain (Life Technologies, CA, USA) was used to visualize PCR products after electrophoresis on 2% agarose gel. For positive results, the DNA amplicons were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermofisher Scientific) and sequenced in both directions. Purified products were sent to Eurofins Genomics in Germany for sequencing. Sequences were examined, assembled and manually corrected by analyzing the chromatograms using Geneious Prime® 2022.0.2 software (New Zealand). Sequence comparisons were conducted using the National Center for Biotechnology Information Basic Local Alignment Tool (NCBI BLAST, MD, USA). Sequences were submitted to GenBank® and assigned Accession Numbers (see supplementary data).

2.9. Statistics

A database of the results was created in Microsoft Excel. Each sample was given a unique ID number, and the season and year of sampling were included in the database, together with the results from the IFAT analysis. Occurrence proportions from the two seasons of reindeer sampling in Knutshø were compared by contingency table analysis (Chi square).

3. Results

3.1. Occurrence and cyst shedding intensity

Cryptosporidium oocysts were not detected in the samples.

Giardia cysts were detected in 25 of the 162 reindeer samples (approximately 15%). Of these, 18 (of 41 samples) were collected from the field in Knutshø during a single day in July 2021. 18 of the 25 (72%) positive samples had a high intensity of cysts.

Among the 45 sheep samples, 13 (29%) were positive for *Giardia* cysts. See Table 2 for details.

Table 2
Occurrence and cyst intensity in wild reindeer and sheep samples in Knutshø and Forollhogna.

Location	Season	Animals (N)	Occurrence n (%)	intensity (n)
Knutshø	Summer	Reindeer (64)	21 (32.8%)	2 G+ 3 G++ 7 G+++ 9 G++++
	Autumn	Reindeer (54)	0	
		Sheep (20)	7 (35%)	5 G+ 2 G++
Forollhogna	Autumn	Reindeer (44)	4 (9.1%)	2 G+ 2 G+++
		Sheep (25)	6 (24%)	5 G+ 1 G++

Table 1
Primers that were used to amplify the targeted *Giardia* genes by PCR.

Primer name		*Giardia* primers			
		Primer sequences 5′- 3′	Target	Size	Reference
First amplification	G7	AAGCCCGACGACCTCACCCGCAGTG	beta giardin *(bg)*	753 bp	
	G759	GAGGCCGCCCTGGATCTTCGAGACGAC			
Second amplification	βGiarF	GAACGAGATCGAGGTCCG		511 bp	Lalle, 2005
	βGiarR	CTCGACGAGCTTCGTGTT			
First amplification	GDH1	TTCCGTRTYCAGTACAACTC	glutamate dehydrogenase *(gdh)*	755 bp	
	GDH2	ACCTCGTTCTGRGTGGCGCA			
Second amplification	GDH3	ATGACYGAGCTYCAGAGGCACGT		530 bp	Cacciò et al. (2008)
	GDH4	GTGGCGCARGGCATGATGCA			
First amplification	RHP 26F	GGTCTAGGGCTCAACCTTACTGCT	DNA repair and recombination protein *(RHP 26)*	864 bp	
	RHP 26R	CTCCAACAGCGTGTGTGTCTGTAG			
Second amplification	RHP 26F	GACAACGCCTCCGTCACTTC		557 bp	Ankarklev, 2018
	RHP 26R	GACTCCTTGATGGCATACAACG			

3.2. Molecular analyses

In the molecular characterisation, sequences were obtained from 27 samples using the *gdh* primer set and 4 samples using the *bg* primers. Subtyping using the *RHP26* primer was performed on 4 positive samples from July 2021. An overview of the sequencing results is presented in Table 3.

3.3. Statistics

A chi-square test comparing the results from wild reindeer samples showed a significant association between season and positive samples ($p = < 0.00001$), with samples more likely to be positive in the summer.

4. Discussion

The main finding of this study is that *Giardia* infection circulates among wild reindeer in Knutshø, with a generally low occurrence, and an apparent absence of *Cryptosporidium*. The constant presence of *Giardia* in the environment may result in infection rate rising when animals are more susceptible, for example during early summer, when the calves are young and immunologically naïve, and any protection from maternal antibodies has decreased. This is also the period when wild reindeer most frequently visit salt lick locations and lick/eat moist soil loaded with salt, and also contaminated with faeces from both reindeer and sheep (Utaaker et al., 2023). Genotyping revealed the potentially zoonotic sub-assemblage AI, which has also been reported from other studies on cervids (Solarczyk et al., 2012; Idland et al., 2021; Lalle et al., 2007; Cui et al., 2022), though sub-assemblage AIII, associated with wild cervids, was not detected in this or other studies from Norway (Idland et al., 2021; Eira 2022, Robertson et al., 2007). Assemblage B was found in one sample, which may indicate a human source of infection. Additionally, although these animals are sympatric with domestic sheep that far outnumber them on pasture, our results indicate that they do not share *Giardia* assemblages. Assemblage AI has also been found among muskoxen sharing natural pastures with domestic sheep in Norway (Davidson et al., 2014), which also contrasts with domestic ruminants such as cattle, sheep and goats where assemblage E is the most commonly occurring genotype (Santin, 2020).

Little is known on the pathogenic effect of *Giardia* in reindeer, but studies on domestic ruminants (sheep and cattle), have linked *Giardia* infection with diarrhoea and weight loss as well as reduced feed efficiency, contributing to decreased herd performance (Aloisio et al., 2006; Geurden et al., 2010; Jacobson et al., 2016; Olson et al., 1995). Infection is primarily found in young animals, and production losses have been identified in intensive and extensive farming systems (Aloisio et al., 2006; Olson et al., 1995; Olson et al., 2004; Sweeny et al., 2011).

Infections of wild cervids with *G. duodenalis* have been frequently reported, with varying prevalences, which may reflect study-specific factors such as the age of the study population and sampling season, or simply variation in occurrence. Location-specific factors that facilitate high functional population density and faecal-oral transmission, such as salt licks for example, may also affect infection prevalence. As *Giardia* cysts are shed intermittently from infected domestic ruminants (Ralston et al., 2003; Gómez-Muñoz et al., 2009), the same pattern likely occurs in wild cervids. Thus, most reports may be an underestimation of the true prevalence, particularly as studies of wild animals' faeces are usually restricted to one sample per animal.

The finding of a high occurrence in the samples collected from the snow where a single herd had rested on one single occasion indicates that this herd may have experienced an upsurge in infection at that time. As watery faeces are hard to identify and collect from the ground, the true prevalence and mean infection intensity may actually have been higher than observed.

The first survey of *Giardia* in wild reindeer in Norway found a prevalence of 7.1%. (Hamnes et al., 2006), in which parts of the samples were collected in the same counties as this study, and the majority (75/155) of samples were collected from calves shot during the ordinary hunting season. Our study's overall prevalence was 15%, i.e. twice as high. A reason for the apparent difference could be that the occurrence of *Giardia* is higher earlier in the season. It should be noted that both these studies have relatively small sample sizes, and that many of the positive samples in our study were collected on a single sampling occasion; this may give a bias in the overall data. If this sampling occasion was excluded, the occurrence would be much lower (7/156; 5.5%).

Interestingly, the majority (9/10) of samples from the study of Hamnes et al. (2006) had a low number of cysts (G+), despite performing sucrose flotation of the samples prior to IFAT. In contrast, in our study, most of the samples (16/25) were graded as either G+++ or G++++, indicating active infection in the animals (Robertson and Debenham, 2022).

The first report of assemblage A in wild cervids was published in 2003 (Trout et al., 2003) from White-Tailed deer in the US. Studies since then broadly report the same finding, and it seems that this assemblage is the most prevalent in wild cervids (Procesi et al., 2022).

The molecular results are interesting, as although the reindeer and sheep were sympatric, they appear not to share *Giardia* infection in the sampling sites of this study to a high degree, although one sample from sheep contained assemblage AI which was identical to the AI found in wild reindeer.

Genotype E, the so-called "hoofed genotype", is the predominant *Giardia* type in farmed ruminants. Considering the high number of sheep in these areas, and the fact that spill over of other gastrointestinal parasites from sheep to reindeer occurs in both Knutshø and Forollhogna (Robertsen, 2020), it is intriguing that apparently none of the *G. duodenalis* in wild reindeer belonged to assemblage E.

Assemblage E was found in sympatric sheep in this study, which is the most common assemblage in sheep in Norway (Robertson et al., 2010), and is the most common genotype found in sheep globally (Robertson 2009). There are also many sheep grazing in these wild reindeer areas, and both the reindeer and sheep use common salt licks. As some studies have found assemblage E in wild cervids (Adriana et al., 2016; Huang et al., 2018; Dashti et al., 2023), we expected this to be the most prevalent assemblage among the sampled reindeer in our study. The finding that assemblage AI was the most common *Giardia* assemblage in reindeer in our study suggests that this genotype has some degree of tropism for reindeer.

The sequences from the *Rhp26* gene were identical to two previously sequenced *Giardia* samples (Ankarklev, 2018), of which one sequence was isolated from a moose, and the other from a human sample. The sequences differed on only one nucleotide (see supplementary section). The human sample belonged to a hunter who had participated in deer slaughter one week prior to symptoms of giardiosis (Ankarklev, 2018). These similarities infer that *G.duodenalis* found in our study have zoonotic potential.

A further interesting finding in our study was the detection of *Giardia* assemblage B in one of the samples collected in summer 2021. Most of the samples collected on this occasion, for which the occurrence was

Table 3
Overview of results from sequencing *Giardia*–positive samples at three genes.

G. duodenalis assemblage	Gene		
	Gdh	Bg	RHP26[a]
A	19 reindeer Subassemblage AI 1 sheep Subassemblage AI	2 reindeer	4 reindeer Subassemblage AI
B	1 reindeer		
E	6 sheep	2 Sheep	

[a] Performed on 4 positive samples from July 2021.

high, were assemblage AI isolates. Assemblage B has previously been reported from a reindeer in Norway, although information associated with that finding is limited (Robertson et al., 2007). In addition, assemblage B has been reported in red deer in Spain (Dashti et al., 2023) and in both red deer and roe deer in Poland by Stojecki et al. (2015), although the latter authors noted that the number of cysts detected in these samples was low and may reflect asymptomatic infection or carriage. This may also be the case in the present study, as interestingly, the sequence results from this particular sample provided different assemblages from different genes, with assemblage B identified at the *gdh* gene, and assemblage A at the *bg* gene, possibly indicative of a mixed infection or infection with one of the isolates and carriage with the other. The *gdh* sequence from *Giardia* from this sample had high congruence with a sequence previously reported from a human sample (GenBank accession number MK982477), further indicating the possibility of a human source. Thus, contamination of the environment with faeces of human origin suggests that zoonanthroponotic infections of wild animals in these areas may occur.

Not all samples gave a positive result from the PCR reactions. This could be due to faecal inhibitors (Schrader et al., 2012), and additionally the samples could have been assessed by 4′-6-diamidino-2-phenylindole (DAPI) staining for integrity of the nuclei prior to PCR isolation (Wilke and Robertson, 2009), which may have indicated the suitability of the samples for DNA amplification prior to DNA isolation.

Although our data may appear to suggest a higher chance of finding *Giardia*-positive samples during the summer than in the autumn, the high occurrence of positive samples in a single sampling occasion in July 2021 could mean that these results are biased. Whether this finding reflects seasonality or was a stochastic event is unclear. Nevertheless, bouts of *Giardia* infection may be more likely to occur during spring/early summer than autumn. Sheep have been reported to shed peak levels of *Giardia* cysts at around parturition (Xiao et al., 1994), and it has been noted that the same may be the case for reindeer (Niine et al., 2017). It has also been demonstrated that chronic *Giardia* infections in cattle calves may last over 7 months (O'Handley et al., 1999), and *Giardia* transmission occurs between infected calves and chronically infected adults (Olson et al., 2004). Semi-domesticated reindeer calves from a research herd in Finland from ages 0–33 days were found to have a cumulative *Giardia* prevalence of 100%, with the most significant sources of *Giardia* cysts being those shed by infected females and other calves (Niine et al., 2017). During July, wild reindeer calves in Norway are usually about 2–3 months old and are probably, as juveniles, more susceptible to parasite infections, as is the case in other ruminants. If *Giardia* cysts are present in the environment, a cycle as suggested by Niine et al. (2017) could also occur in wild reindeer.

The apparent absence of *Cryptosporidium* in wild reindeer is not particularly surprising, as other studies on reindeer in Fennoscandia have reported the same (Hamnes et al., 2006; Idland et al., 2021; Kemper et al., 2006), Although *Cryptosporidium* in reindeer has been detected in a longitudinal study, at low overall prevalence (Niine et al., 2017). It has been speculated that climate changes such as elevated run-off from snowmelt, increased precipitation and increased extreme weather events paired with domestic livestock industries moving northwards may increase the risk of *Cryptosporidium* infections in wild ruminants, though how these changes may interact with parasitic contamination of the environment is difficult to predict (Robertson and Debenham, 2022).

There are some limitations to this study. Watery or loose faeces may be challenging to find and collect under field conditions and such samples may have been overlooked. There is also a possibility that a sample from the same animal was collected more than once.

Also, the number of samples from sheep in this study is probably too low to reach firm conclusions on which *Giardia* assemblages prevail in the sympatric sheep populations and more extensive studies to assess possible *Giardia* spillover between reindeer and sheep, and its direction, may be warranted.

5. Conclusions

The overall results show a relatively low occurrence of *G. duodenalis* infection in wild reindeer in Knutshø and Forollhogna, although on one sampling occasion a high proportion of samples were found to be positive. This occurrence, together with the high numbers of cysts found in positive samples (high intensity), suggest that *Giardia* infections in wild reindeer may be endemic. However, our data do not provide information on the clinical effects of these infections on the individual animals in a herd, and therefore it is not possible to determine from this study whether *Giardia* infections could be a contributing factor to the previously described decline in the population performance of these reindeer.

The findings of both assemblage A and B in wild reindeer, may point to a possible (anthropo)zoonotic potential, with an infection risk both to those enjoying a walk on the wild side, and to the animals living on it.

CRediT authorship contribution statement

Kjersti Selstad Utaaker: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Tsegabirhan Kifleyohannes:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Bjørnar Ytrehus:** Writing – review & editing, Writing – original draft, Resources, Funding acquisition. **Per-Anders Robertsen:** Writing – review & editing, Software, Investigation, Formal analysis, Data curation. **Olav Strand:** Writing – review & editing, Project administration, Funding acquisition. **Lucy J. Robertson:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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