UiT The Arctic University of Norway

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Gastrointestinal nematodes in Icelandic reindeer (Rangifer tarandus tarandus)

Prevalence and factors influencing fecal and abomasum nematodes in an isolated population

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Abstract

Reindeer (*Rangifer tarandus*) are host to gastrointestinal-tract nematodes (GINs) throughout the world. Some populations of reindeer exist in almost complete isolation from others, and this can have a key influence on the species and infection rates of GINs. An example of an isolated reindeer population occurs on Iceland.

In this study, I have investigated the prevalence of GINs in Icelandic reindeer in a range of age gender and area classes. In total, I checked for the presence of GINs in fecal and abomasum samples of 115 reindeer, including adult females (n= 72) and males (n= 29), as well as 14 calves. The fecal samples were analyzed based on McMaster and Baermann method. The reindeer samples analyzed in this study were from individuals shot during August–September 2018 in eight hunting management areas of eastern Iceland. Analysis of fecal samples for parasite eggs revealed an overall mean prevalence for Trichostrongylidae and *Capillaria* sp. of 35.1% and 22.5%, respectively.

A total of four species of nematode parasites were found in the abomasum: *Telodorsagia circumcincta* and its minor morphotype *T. trifurcata, Spiculopteragia spiculoptera, Ostertagia gruehneri* and *Ostertagia* sp. Interestingly, overall male individuals showed higher prevalence and probability of nematode infection than females, based on egg counts.

My study also revealed *Ostertagia gruehneri* in one adult male which was not reported in previous studies conducted in Iceland. Probability of *Capillaria* sp. infections was significantly different in the GLM model with animals from the west central areas having significantly lower infection prevalence than the other areas. I conclude that, my study suggests the Icelandic reindeer shows healthy status in the country with an overall lower parasite prevalence than detailed in earlier studies.

However, low prevalence of GINs in this study might be associated with the lack of 'freshness' of the samples. However, no study has evaluated parasite detection with samples that have been frozen for some time, nevertheless laboratory tests at the site right after collection could improve future studies.

1. Introduction

The type of parasites and their prevalence varies between reindeer (*Rangifer tarandus*) populations. An isolated population of Eurasian tundra reindeer (*Rangifer tarandus tarandus*) exist in Iceland, and these individuals show a lower parasite prevalence than mainland populations. This isolated Icelandic reindeer population represents an interesting case, because the only mammals they encounter are Arctic fox *(Vulpes lagopus)* and domestic sheep (*Ovis aries*) (Thorisson,1984) on their natural pastures. Therefore, the sources of mammalian parasites on the reindeer pastures in Iceland are limited. The parasite prevalence in Icelandic reindeer has received some attention; however, the gastrointestinal parasites of this population was last described 14 years ago (Gudmundsdottir and Skirnisson 2006). Contact with new parasites and changes in parasite ecology could have occurred in the last 15 years, and this study will provide updated information on parasite prevalence and examine how it may vary with age, sex, and geographical location within Iceland. Subclinical infection rate with gastrointestinal parasites were common in calves.

Parasites are abundant in natural habitats, and they rely on appropriate hosts for their life cycle. Some parasites specialize on a single host species, while others are dependent on a wide range of species. Reindeer have regular contact with domestic livestock such as sheep, cattle, and horses, but the extent of contact depends on their geographic range. Therefore, it is highly important for the parasites to select their hosts so they can continue their life cycle.

1.1 Reindeer

The reindeer is a wild and semidomesticated ruminant mammal that occurs in Eurasia, as well as in North America, where it is known as the caribou. It is a species in the order of Artiodactyla, family Cervidae and genus *Rangifer*. Their range extends across much of the northern Boreal zone. Their habitat is forests and some grasslands, and they occur in Canada, Finland, Greenland, Mongolia, Norway, Russia, Sweden, and the USA. Reindeer have also been introduced to some islands, most notably Iceland in the northern hemisphere, and in the southern hemisphere some subantarctic islands. In their native range the indigenous people have used them for centuries as a source of food, hides and antlers.

The species was categorized as vulnerable on The Red List of Threatened Species (IUCN) at the last assessment, dated 24 December 2015. The estimated total world population of reindeer is 2 890 400 (IUCN), but the population trend is decreasing due to unknown factors. Understanding the exact causes of this decline is important for conserving them. It is likely that the declines are linked to many factors, including environmental change causing food shortages, increasing insect abundance, warmer winters with changing rainfall, and anthropogenic factors such as energy production, mining, habitat disturbance, forestry, and livestock farming. Livestock farming brings the reindeer into closer contact with other domestic ruminants, which could cause increased disease or parasite transfer. They are known to be an important prey species for predators and blood sucking insects.

1.1.1 Reindeer in Iceland

The Icelandic reindeer population stems from a single introduction. A total of 35 individuals (30 cows and 5 bucks were gathered in Kautokeino, northern Norway, and released in north-eastern Iceland in 1784 (Thorisson,1984). The source Norwegian reindeer were semi-domesticated; however, since their release in Iceland, the population has always been feral. Importantly, this reindeer population has been isolated from other reindeer populations since its introduction (Gudmundsdottir and Skirnisson 2005). Therefore, studying this population gives unique insight compared to other extant reindeer populations.

In Iceland, the reindeer population is restricted to the eastern part of the island. Their distribution extends about 120 km inland from the east coast. More recently the population has been managed, mainly via hunting, which is conducted annually from 1 August until 20 September for females and from 15 July until 15 September for males (Þórisson, 2018). About 4000 individuals kept stable by this hunting management and it has increased to 6000 by 2008, and annual counts now total about 6400 animals in summer (Þórisson 1993 and Þórisson 2018). The reindeer population is divided into nine hunting areas (Fig 1), which are based on hunting management areas. Annual hunting targets are set by the East Iceland Natural Research Centre. Their goal in setting the annual hunting quotas is to ensure a sustainable population with a density less than one animal per km^2 of land that is vegetated in winter. They also aim to

maintain a sex ratio of six males to ten females (Þórisson, 2018). As a result, all hunting is done with guides, who are obliged to record the date, location, sex, carcass weight and estimated age (based on eruption and wear of teeth) of hunted reindeer (Þórisson, 2018).

The conditions in Iceland are particularly favorable for reindeer. The climate is maritime, with two climate types that feature cool summers and mild winters: temperate rainy climate with cool, short summers in the south and west; and snowy climate in the north, east and highlands. There is a general lack of predators; the only predator is the Arctic fox. There is also an absence of insects like warble flies *(Hypoderma tarandi* and *Cephenemyia trompe*) and mosquitoes (*Aedes nigripes*), which harass reindeer in their native range (Þórisson 2018). In addition, Icelandic reindeer have limited contact with other ruminants. In the reindeer pastures in eastern Iceland, the only other mammalian grazers are sheep and a few horses. There are approximate 416 000 sheep in Iceland, but this population is about 50% of what it was in 1980 and less than 100 000 sheep grazes in Eastern part of the island (O'Donnell 2020 and Hagstofa Íslands 2018).

1.2 Parasites

1.2.1 Parasites in reindeer

Reindeer host a wide variety of parasites, including those in the gastrointestinal system (Jokelainen et al., 2019). Over 45 species of helminth, protozoan and arthropod parasites have been reported in wild and semidomesticated *Rangifer* species throughout the world, and they can negatively affect host health and can cause mortalities (Stien et al 2002). Parasites can also influence the dynamics of wildlife populations (Robert 1998; Gunn et al.,2003; Hughes et al.,2009& Preston et., 2010; Rollinson et al., 2012) by having a negative effect on individual performance and growth rate (Hrabok 2006; Oksanen 1999), delaying the age of first reproduction and potentially reducing the pregnancy rate (Albon et al., 2002).

The impact of parasites on the host is usually a function of the number of parasites infecting the animal, with more parasites causing more damage and ultimately either mortality or reduced

fecundity (Kutz et al.,2018). Therefore, gaining an understanding of the prevalence of parasites in different populations is important for improving reindeer husbandry practices.

Reindeer are susceptible to a range of nematode parasites from other ruminants (sheep and cattle), for which they are not the primary host (Hrabok et al., 2006). The diversity of parasites that have been documented in reindeer includes roundworms (nematodes), flatworms (trematodes), tapeworms (cestodes) and tongueworms (*Linguatula arctica*), gastrointestinal worms e.g. *Ostertagia gruehneri*, *Nematodirus tarandi*, *Nematodirella longissimespiculata*, lungworm *Dictyocaulus eckerti*, brainworm
Elaphostrongylus rangiferi), pentastomids (sinusworm *Linguatula arctica*), and insects (e.g. the throat bot fly *Cephenemyia trompe* and warble fly *Hypoderma tarandi*) and protozoa that are associated with reindeer (*Besnoitia tarandi*, *Sarcocystis* spp., *Eimeria* spp.) and cestodes (tapeworms; e.g. *Moniezia* spp.) (Jackie, 2006) They depend on reindeer for different life stages, parasitizing virtually every organ system, and their impact can vary from undetectable to severe disease and death. Three orders (or suborders) of nematodes are found in the gastrointestinal tract of *Rangifer*: the Strongylida, Oxyurida and Trichocephalida (Kutz et al., 2019).

1.2.2 Parasites in Icelandic reindeer

Due to the population's isolation for 230 years, the parasite status of Icelandic reindeer differs from that of other populations. The last study that was conducted on reindeer parasites in Iceland identified five helminth species (Guðmundsdóttir, 2006). She investigated fecal samples from 192 calves in the summer of 2003 from three distinct areas and examined 58 samples from hunted reindeer which was collected during 2003–2005 for parasites. Altogether they documented 17 parasitic species: eight protozoans, one cestode and eight nematodes. The protozoans *Entamoeba* sp., *Giardia duodenalis*, *Eimeria mayeri*, *Eimeria rangiferis* and *Eimeria hreindyria* were found in fecal samples. The nematodes *Ostertagia ostertagi*, *Teladorsagia circumcincta* and *Trichostrongylus axei* were found in the abomasum. *Eimeria rangiferis* and *Eimeria hreindyria* were previously unknown and were described as new species in that study (Guðmundsdóttir et al., 2005). Interestingly, *Eimeria* and the *Sarcocystis* species are host specific whilst *Capillaria bovis* has never been found in other ruminants in Iceland. It

is assumed that these parasites must have already been present in the animals when they were introduced from Norway(Guðmundsdóttir , 2006).

1.3 Parasite identification

Parasites are generally identified based on morphological and molecular similarity. The use of polymerase chain reaction (PCR) techniques sequencing of key gene regions is a valuable tool that can be used to better define parasite diversity. However, this method is still limited, since it cannot be used to quantify larval abundance in mixed infections or to identify rare species, because it is based on the sequencing of the PCR-amplified DNA of individual larvae from a sample. Traditional microscopy-based parasite identification is therefore still commonly used, and it is a useful technique because it can be performed with minimal equipment and lab expertise (Kafle et al., 2017).

The presence of nematode eggs in feces is useful for diagnosing infections, since they can be identified and counted in fecal samples. In ruminants, an excess of 1000 eggs per gram is generally considered indicative of heavy infection, and over 500 indicates a moderate level of infection. However, a low egg count does not necessarily indicate a low level of infection, since patency may just have been established, or the egg count may have been affected by the development of immunity.

Nematodes have separate sexes. The posterior end of the female tapers to a blunt point, which is rarely of diagnostic value. The male tale, in contrast, often has accessory sexual structures that are useful for identification (Jacobs et al., 2016).

In the identification of gastrointestinal-tract nematodes (GINs) in ruminants, there are certain morphological features to consider, such as the male spicules, bursal lobes, and the dorsal ray of the bursa in the tail parts. In *Ostertagia* and *Teladorsagia* species, major and minor morphotypes appear in the population. Although males look very different to each other even though DNA analysis of selected genes cannot tell them apart. However, genetic isolation between *Teladorsagia circumcincta* and *T. trifurcata* has not been found (Dróždž, 1995).

1.4. Aim of the study

My study aimed to provide updated information on parasite prevalence in reindeer in Iceland. I will examine how parasite prevalence may change with age and gender class and location (hunting region) within Iceland. To understand potential impacts , it is important to have an accurate estimate of parasite burdens; overall therefore, my study would provide valuable information about the current health status of the local Icelandic reindeer population in terms of gastrointestinal nematodes. My approach was based on examining freshly collected fecal samples which kept in the freezer including abomasum sample from over 100 reindeer from Eastern Iceland during the annual hunting which provides opportunities to collect samples for researchers in a variety of fields. The reason behind of the collecting fecal and abomasum sample are, egg counts are limited value in estimating parasite abundance due to various factors affect egg production, including variation in the number of eggs produced by the species of parasite, individual host immunity and stage of infection (Anne & Gary, 2012). Also, identification to the species level from the eggs can be very challenging since it is not possible to identify some of the trichostrongylidae eggs into species based on morphology alone (Milner et al., 2013). To obtain an accurate estimate of the GINs prevalence Milner et al. (2013) recommended identification of adult nematodes from the abomasum to species level.

My principal aims in this project were:

1. To assess GIN prevalence and abundance in the Icelandic reindeer population.
2. To investigate the abundance of parasites across age, hunting areas and sex classes, and to correlate parasite load with individual reindeer.

2. Material and methods

2.1 Ethic statement

The reindeer sampled were hunted subject to regional regulations in their natural mountain or coastal pastures, and their carcasses were taken to a slaughterhouse, where the carcasses were trimmed and the samples were collected.

2.2 Study area and sample collection

Obtaining data on the impact of parasite on host populations often requires the live capture of animals or terminal sampling, and is extremely costly (Kutz et al.,2019). For this cross-sectional study, I used reindeer that were shot subject to the annual hunting regulations, since samples obtained from dead animals are still useful for evaluating the prevalence of infectious agents at the population level. To understand the potential effects of the parasites, it is important to have accurate parasite burden estimates. To estimate infection intensity, I used indirect measures such as counting the juvenile parasite stages in the feces as well as abomasum nematode counts.

A total of 117 reindeer were hunted and shot during August and September 2018 in eight hunting areas in Iceland (1-Hellisöxl, 2-Stóröxl, 3- Beinageit Innri Skagi, 4- Eskifjörður, 5-Hálíðarhorn/Svartafjall, 6-Flatarheiði, 7- Steinártungur, 9-Flateyjaraurar; see Figure 1) in eastern Iceland. The carcasses of hunted individuals were taken to slaughter-houses where a range of samples were collected between 24 August 2018 and 16 September 2018. Due to a low number of hunted/sampled reindeer from some of the hunting areas, and thus skewness of data, I pooled individuals into three main areas. For my analyses, I categorized: 1-Hellisöxl called “West-Central” (n = 29), 2-Stóröxl called “Central” (n = 44), and pooled data for the six small hunting district 3 – 7 and 9 called “East Coast” (n = 40, see Figure 1 and figure 2). Four individuals did not have hunting area information provided. This provided more balanced spatial distribution of samples within existing geographical areas and these three areas were used in all summary statistics and analyses.

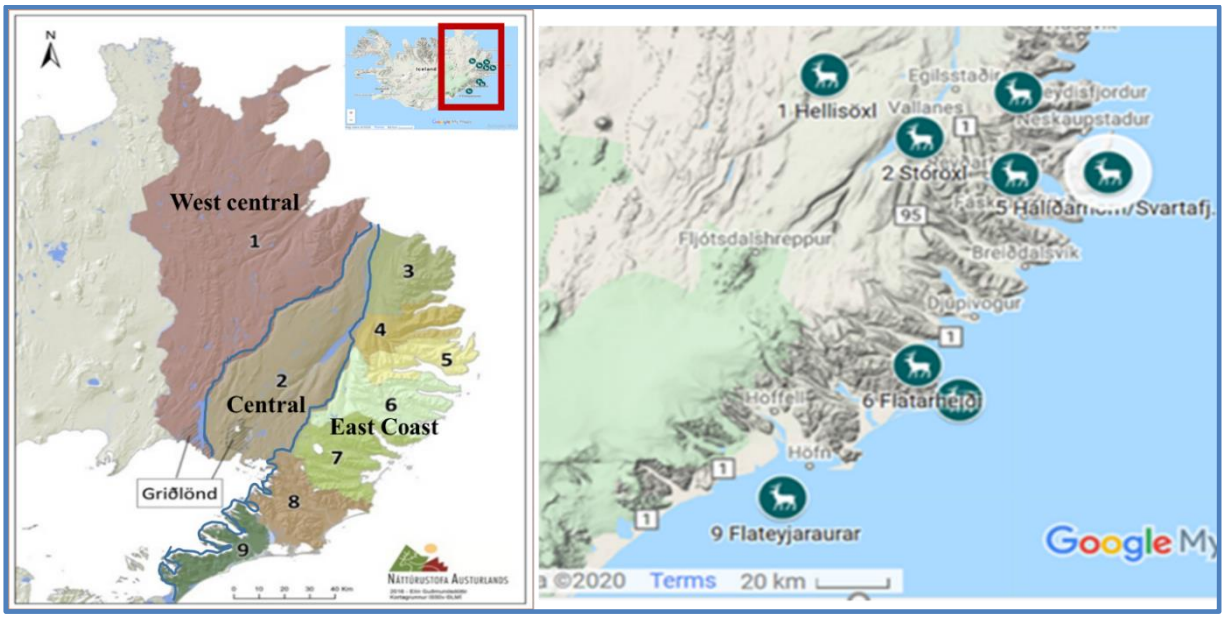


Figure 2*. Reindeer samples were obtained from eight hunting areas in eastern Iceland in August and September 2018. Map of reindeer hunting areas reproduced with permission of Þórisson and Þórarinsdóttir (Þórisson 2018) and modified to highlight the three study areas. The more detailed map shows the coordinates provided for the reindeer hunted during the same hunting period (24 August to 14 September 2018) plotted in Google Maps.*

West-central: 1. Hreksstaðakvísl, Skjaldklofa, Hjarðarhagaheiði, Rjúkandi, Bjallkolla, Grunnavatn, Ánavatn, Kollseyra, Southern Súlendur, Hnjúksvatn, Þórfell, Gestreiðarstaðakvísl.

Central: 2. Stóröxl, Þuríðarstaðaá, Þuríðarstaðadalur, Urgur, Hallormsstaðaháls, Southern Fjallkoll, Þórfell, Miðheiðargrjót, Kofaalda, Þrælaháls, Eyvindarfjall, Snæfell, Vesturöræfi, Hálslón, Vesturöræfi, Þrímelar, Bessastaðaá by Brú, Þórisstaðakvísl, Þrælaháls, Vestaradrag, Sauðafell, Northern Tregla, Miðheiðarháls, Bessastaðaá, Sauðabanalækur, Vegaskarð, Fellaheiði.

East Coast: 3. Botnsdalur, Hólalandsdalur, Beinageit, Beinageit Innri Skagi, Upsir; 4. Southern Eskifjörður, Eskifjarðarheiði; 5. Hálíðarhorn/Svartafjall, Lambeyrardalur; 6. Flatarheiði, Grílutindur; 7. Geithelladalur, Bótahnjúkar, Steinártungur, Berufjarðarskarð, Langagilsfjall, Hellisfjörður, Merkjahryggur, Northern Hvannavellir, Geithelladalur; 9. Flatey, Flateyjaraurar, Flateyjaröldur, Flateyjaröldur, Kálfafellsdalur, v/Nautastigsgil-Kálfafellsdal, Fláajökull.

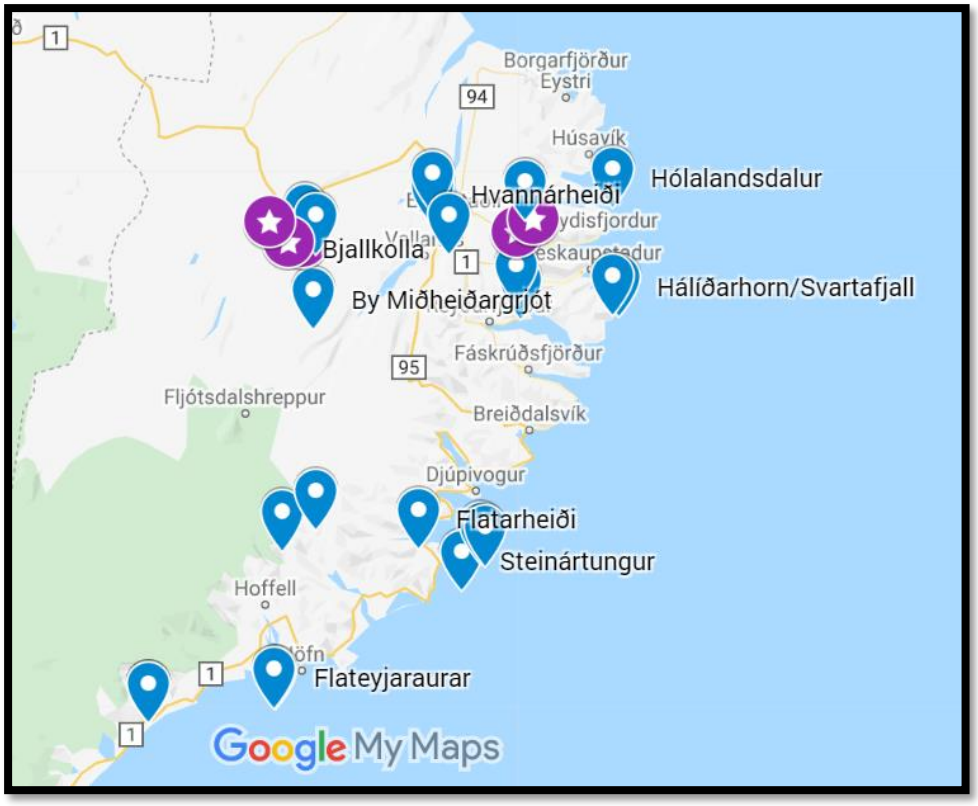


Figure 2. *Map showing the locations at which adults (blue points) and calves (purple points) were hunted.*

2.3 Individuals sampled and sampling details

The individuals were sexed visually by the hunters, and the dataset contains 76 (65%) females, 38 (32.5%) males, and three of the calves did not have their sex recorded. Carcass weight was recorded for 106 of the 117 individuals. The carcass weight was the weight of the animal after the internal organs, the blood, the skin, distal part of the legs and head had been removed since the hunters weighed the animals to estimate the weight of meat available for consumption. In reindeer, carcass weight is usually measured this way. There were no possibility of weighing the animals in the field.

In some animals, the tissue area damaged by the shot was also cut out before the carcass was weighed. Nevertheless, the masses should be broadly comparable.

Age was evaluated during sampling by examining the animals' teeth and body size and asking the hunters. I received age data in five classes: a) calves, 4–6 months old (<1), which were born in May and shot in August (n = 17); b) yearlings, 16–20 months old (1 year old), which were born the previous year (n = 9); c) two-year-old, 20–27 months old (n = 13); d) younger adults27 months to 5 years old (>2-5 years old), (n = 57); and e) older adults, > 6 years old (n = 6). Another 15 individuals were missing data regarding age. Due to the small numbers of individuals in some of the age categories, and skewed age distribution between categories, I pooled individuals into three age classes for my analyses: 1) calves (n = 17); 2) immatures (yearlings and two-year-old, n = 22); and 3) adults (n = 63).

My study focused on fecal and abomasum samples collected from individuals (Figure 3 and Figure 4). The fecal samples were taken from the distal part of the rectum. The abomasum, the final stomach compartment in ruminants, were removed from the animals. Fecal samples were refrigerated and abomasum samples were frozen soon after collection and stored this way until they were transported to Norway for analysis. The feces was kept refrigerated prior to sending and was kept refrigerated at the Norwegian Veterinary Institute (NVI) until analysis. The abomasum sub samples were frozen prior to shipping, thawed enroute and refrozen upon arrival. They were stored at −20 ºC until analysis at the NVI in Tromsø, Norway. The shipment of the samples was not under refrigerated conditions so some thawing of the materials may have occurred before they were refrozen at the institute.

***Figure 3**. Reindeer feces packaged and labeled for transport to the laboratory from Iceland (2018)*

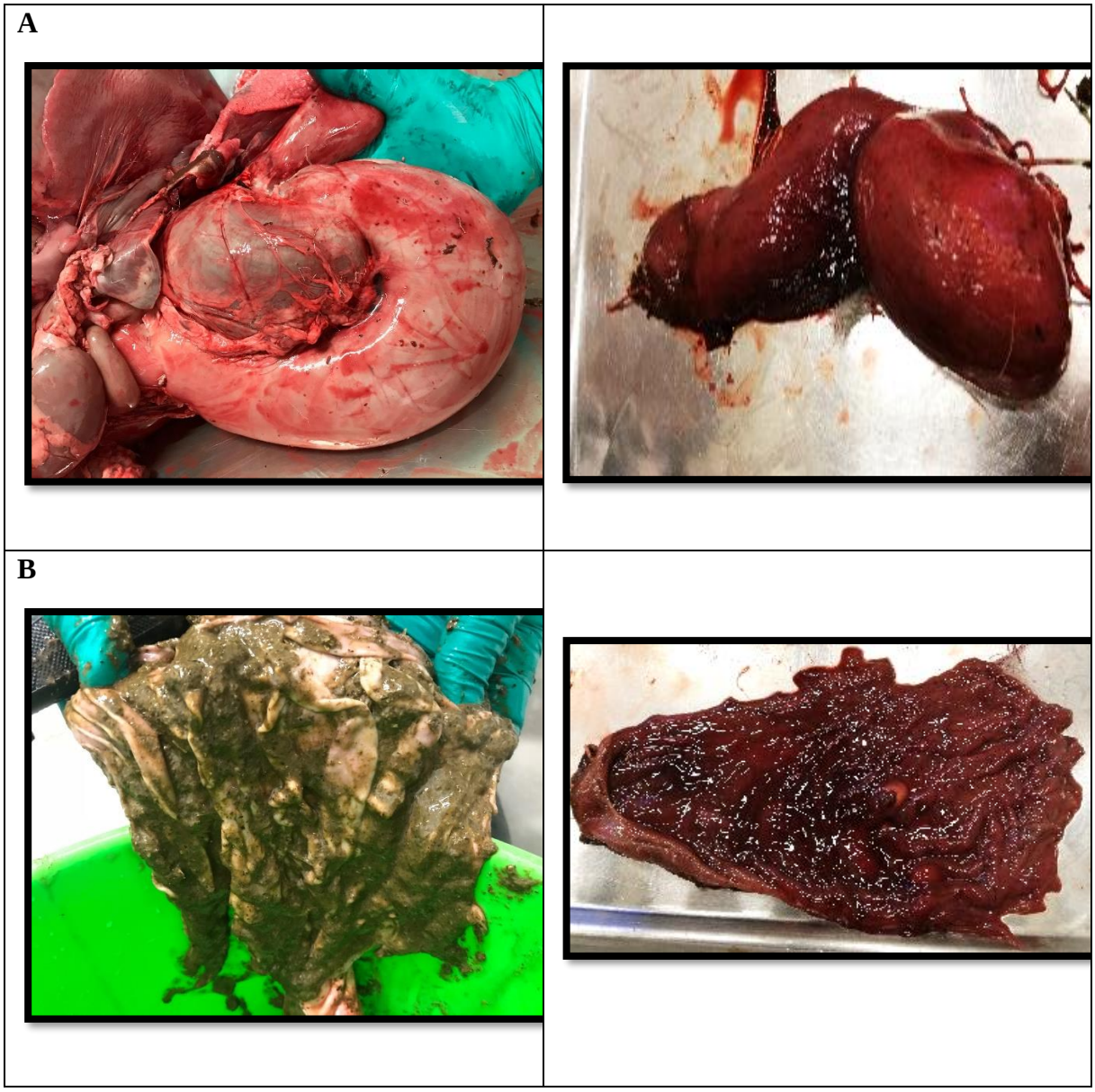


Photo A and B to the left: Javier Sánchez Romano

Figure 4. *(A). Omasum and the abomasum before the latter is opened. The reindeer abomasum was removed from the gastrointestinal tract and then opened along the greater curvature prior to washing into a bucket . The abomasum lining with contents, and the green is the bucket. Abomasum displayed after been washed (B).* A total of 111 individuals were sampled for feces (Table 1), and a total of 81 individuals had an abomasum sample taken (Table 2). Both types of samples were taken from 76 individuals, leaving 35 with only fecal samples and five with only abomasum samples. I analyzed the samples using two commonly performed techniques: the McMaster egg flotation method and the Baermann larvae method (Taylor et al., 2015).

Table 5. *Number of Icelandic reindeer from which fecal samples were collected in 2018, categorized according to sex, age class and hunting region.*

Age class	Sex	Hunting region				
		West-central	Central	East Coast*	Unknown	**Total (N)**
Calves (n = 17)	male	1	4	2	2	9
	female		4	1		5
	unknown			2	1	3
Immatures* (n = 21)	male	1	3	1		5
	female	5	7	4		16
Adults (n = 59)	male	7	1	14		22
	female	10	19	8		37
Unknown (n = 14)	male				1	1
	female	4	3	6		13
	Total (N)	28	41	38	4	**111**

*Comprises six hunting districts along the east coast combined

*immatures (yearlings and two-year-olds)

Table 6. *Number of Icelandic reindeer from which abomasum samples were collected in 2018, categorized according to sex, age class (calves >1; immatures 1-2 year olds; adults >2 years old)and hunting region.*

Age class	Sex	Hunting region				
		West-central	Central	East Coast*	Unknown	**Total (N)**
Calves (n = 12)	male	1	3	2	1	7
	female		3	1		4
	unknown			1		1
Immatures* (n = 19)	male	1	2	2		5
	female	5	7	2		14
Adults (n = 43)	male	7		7		14
	female	8	18	3		29
Unknown (n = 7)	male					
	female	4	2	1		7
	Total (N)	26	35	19		**81**

* Comprises six hunting districts along the east coast combined

*immatures (yearlings and two-year-olds)

2.4 Laboratory analysis

2.4.1 Fecal sample analysis: egg counting by flotation

To estimate parasite burden by counting the number of eggs per gram of feces, I used a modified version of the McMaster quantitative method based on Taylor et al.,(2015). I mixed 3.0 g of feces with 57 ml of cold water and poured the resulting suspension through a wet sieve (mesh size: 250 μm). I collected the suspension from the sieve and poured it into a 14 ml sample tube, which I centrifuged at 1100 G for 3 min (Figure 5).

To detect the eggs, I subjected the suspension to flotation in a NaCl-$ZnCl_2$ (specific gravity 1.3) in two chambers of a McMaster slide (Whitlock Universal four-chamber worm egg counting slide, 4 × 0.5 ml, JA Whitlock & Co, Eastwood, Australia) and placed it under a Leica (DC750) microscope at 100 x magnification (images of the parasites were taken on the microscope using the camera module Leica ICC50). For this study, I used two of the chambers, giving a volume of 2 × 0.5 ml. This method, using the dilutions described, has a sensitivity of 20 eggs per gram (EPG) / oocysts per gram (OPG). The eggs were counted, and were identified to genus or suborder level where possible.

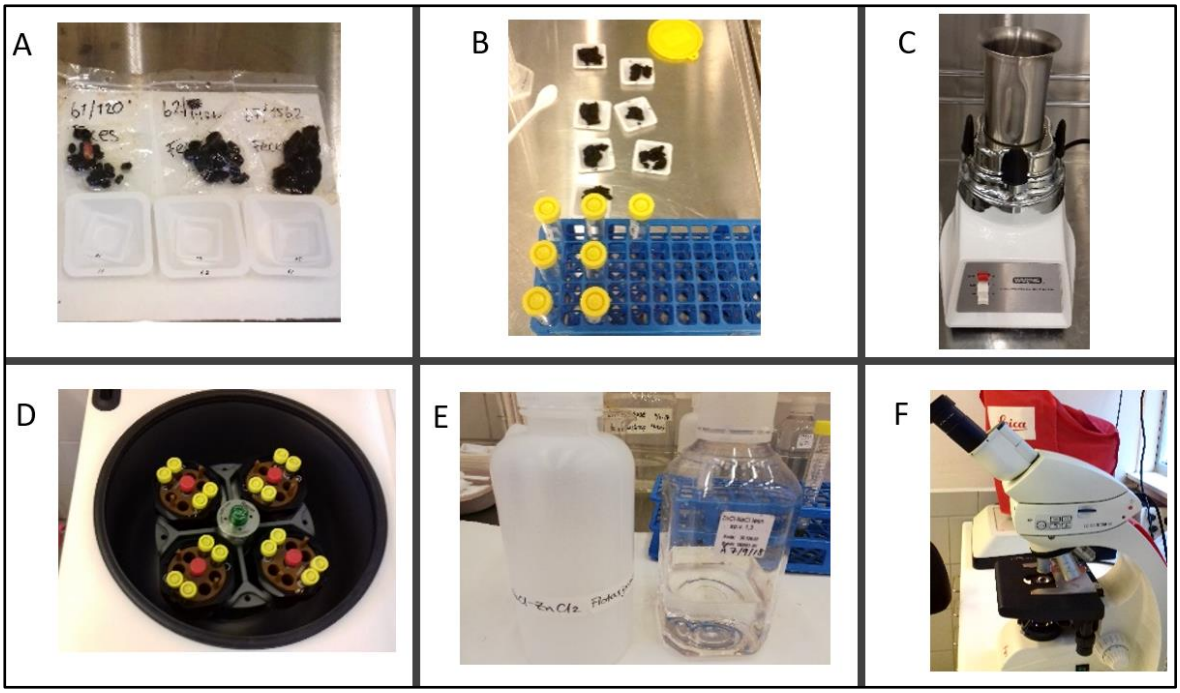


Figure 5. *Schematic details of the sample preparation and tools used for the McMaster analysis. The process started with weighing fecal samples (A), and preparing the tubes containing 3 grams fecal samples (B), which were mixed with water solution and sieved (C), before centrifugation of the samples (D), with the suspension floated in NaCl-$ZnCl_2$ solution (E) and finally placed in McMaster slides and analyzed and counted under a microscope.*

2.4.2 Fecal sample analysis: larval recovery

The larval recovery analysis was based on a modified version of the Baermann method based on Taylor et al., (2015). I weighed 5.0 g of feces and wrapped each sample in a double layer of gauze tied off with a plastic string. These samples were suspended in a plastic conical cup filled with tepid water (37–40 ºC) for 12 h at room temperature. During this time, the larvae emerged from the feces and sank to the bottom of the cup (Figure 6). After 12 h, I removed the feces and the upper 90% of the supernatant. The remaining 5–10 ml suspension was transferred to a 15 ml conical tube and centrifuged for 3 min at 1100 G After the centrifuging, the supernatant was removed without disturbing the bottom 1 ml of sediment. I mixed the remaining sediment thoroughly and used a micropipette to extract a 100 µl subsample. For those in which there were no larvae in first 100 µl subsample, I took a second 100 µl sample from the sediment

(without further mixing) for analysis. I identified the larvae by examining the subsamples under the microscope at 100× magnification. This method has a theoretical sensitivity of two larvae per gram (LPG) given the weight of fecal sample and volume of sample analyzed (10%).

Figure 6. *Baermann sample preparation: fecal samples packed in gauze and suspended in water-filled conical glasses.*

2.4.3 Abomasum sample analysis

Abomasum samples were obtained by emptying out the abomasum contents into a bucket and washing the internal abomasum wall into the same bucket up to the 2 l mark in the bucket, a modified version of the method described by Eysker and Kooyman (1993). A subsample of the entire washed contents was collected at the time of sampling in Iceland. Samples were collected in 100 ml tubes and 130 ml tubes stored in a freezer at -20C prior to analysis. I defrosted the samples by putting them in front of the heater, under running water, or leaving them at room temperature until they had thawed. Once the samples had defrosted, I removed the excess water by pipetting. I placed the remaining contents in a petri dish to separate out male and female nematodes, which were identified based on their reproductive organs. The nematodes were separated under low-level magnification (up to 40×) using a Leica stereomicroscope. I put the males in a small dish with ethanol to prepare them for the next stage: species identification, based on the morphology of adult males (Umur et al., 2011; Fruetel et al., 1989 and Dróżdż, 1965 and 1995) (Figure 7). The identification method is based on their spicule form, length,

dorsal ray pattern, and bursa morphology after clearing in polyvinyl lactophenol (Waldeck, Münster, Germany) for a few minutes. The species were identified for minor and major morphotypes (Figure 8).

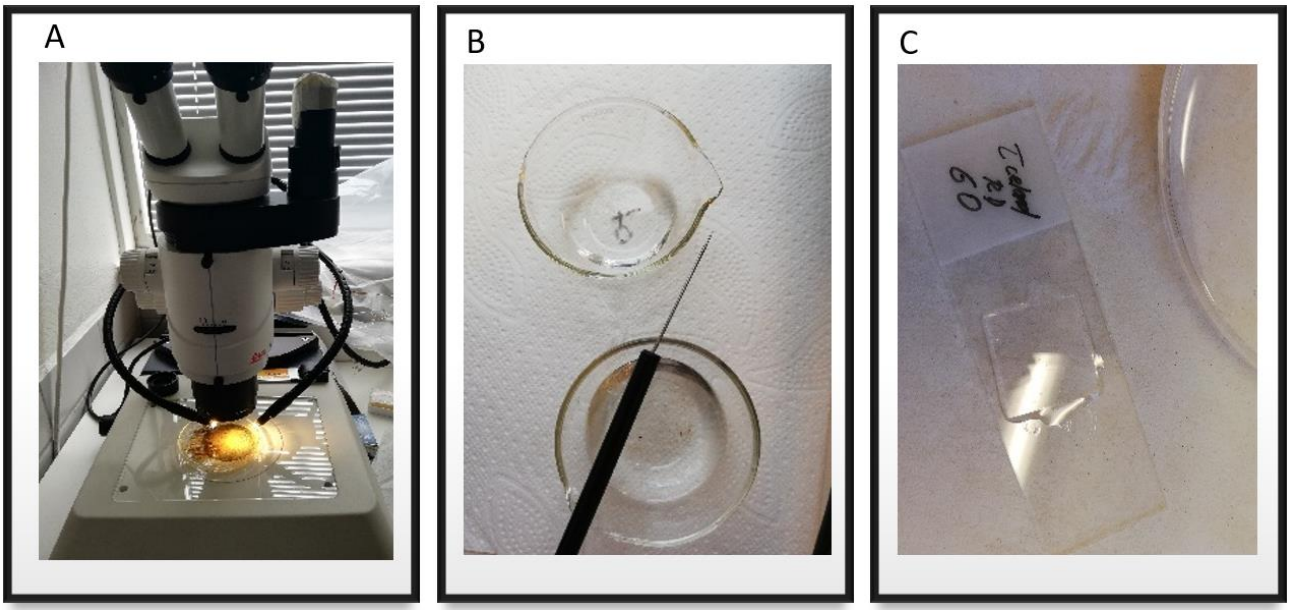


***Figure 7**. Preparation and analysis of abomasum content, started with nematode separation by gender under low-level magnification (A), separated male nematodes ready for the species identification (B) and lastly male nematodes were secured in polyvinyl lactophenol glass slides.*

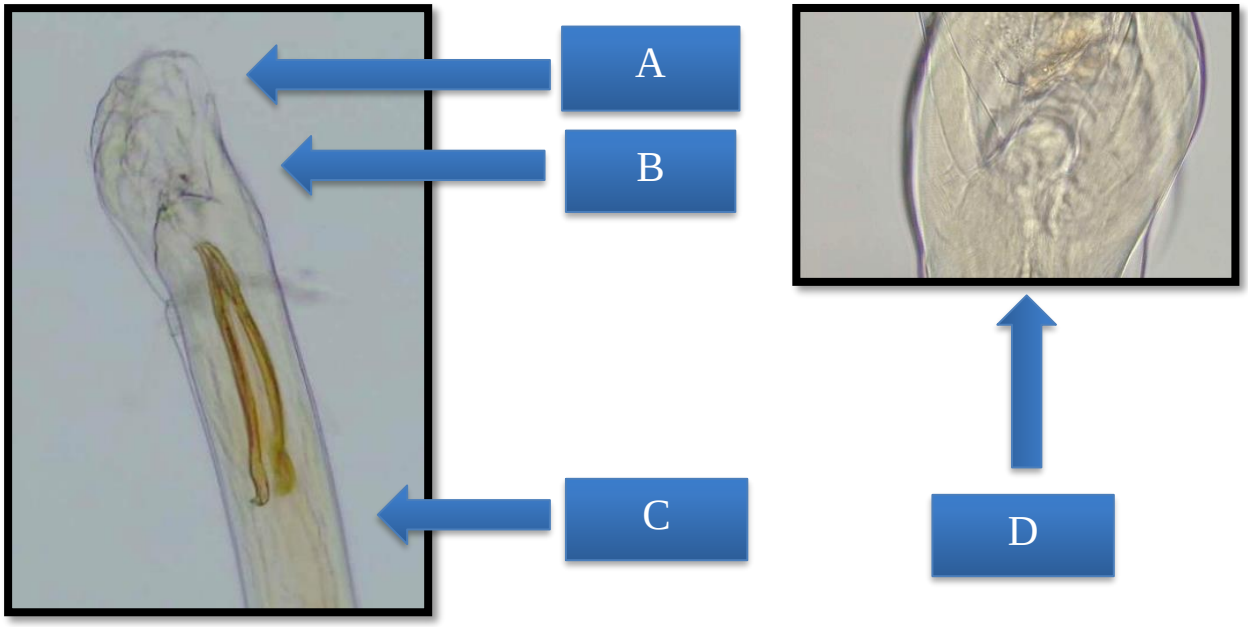


Figure 8. *The identification method is based on their, dorsal ray pattern (A), bursa morphology(B) spicule form, length (C) and genital cone (D)*

2.5 Data analysis

Data were analyzed and visualized using the software R, v. 3.4.0. (R Core Team 2017). Summary tables were produced using pivot tables in Excel (Microsoft Windows 10, Microsoft 2019). A statistical significance level of p = 0.05 was selected for all analyses. Summary statistics and 95% confidence intervals (CIs) were calculated using JMP 14.0.0 (JMP Statistical Discovery, SAS).

2.5.1 Parasite prevalence

Summary statistics concerning parasite prevalence were calculated for sex, age, and area groups of reindeer. The calculation of parasite prevalence (confidence interval for proportion) was based on the calculation available at https://www.sample-size.net/confidence-interval-proportion/.

2.5.2 Individual probability of infection

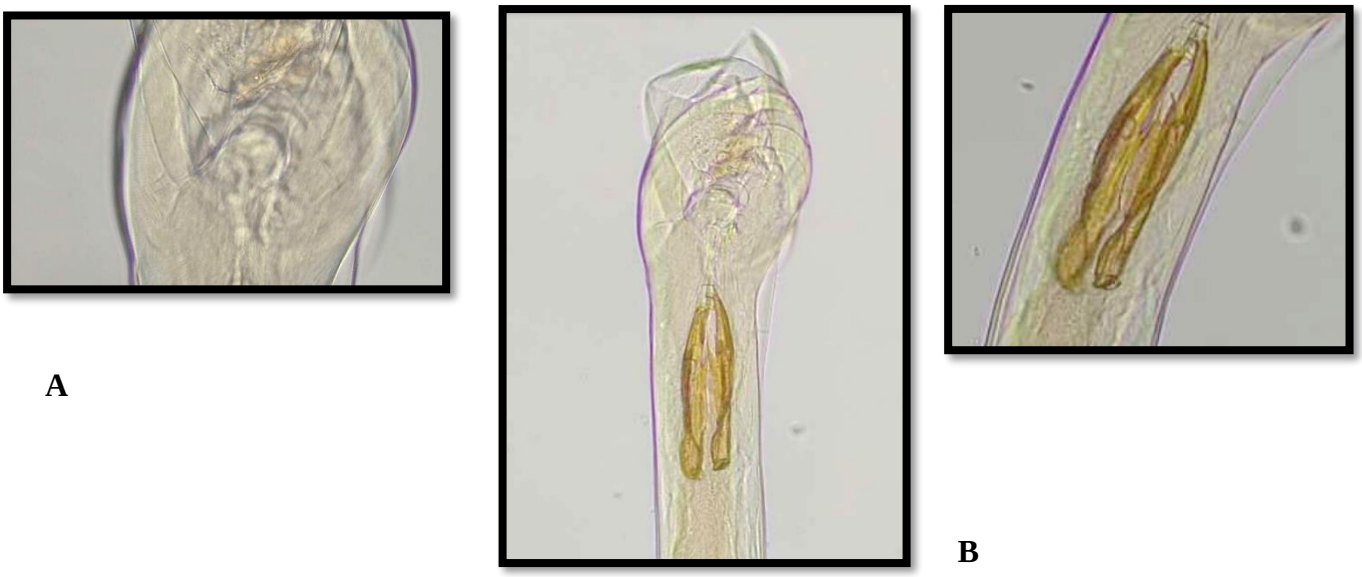
I investigated how individual-level factors influenced the probability of an individual being infected with different nematode species. The parasite categories for which I had enough detections for analysis were Strongylida-type eggs and *Capillaria* spp. eggs from the fecal samples and adult nematodes (all species pooled) in the abomasum samples. I used generalized linear models in which the detection of each parasite category (0 – not detected, 1 – detected) was used as the response variable. I therefore used models with a binomial distribution and logit-link function, to account for these binary response variables. In each model (for a specific parasite), I entered three individual-level explanatory factors: sex (two classes), age (three classes), and area (three classes). I also included a sex–age interaction term to test whether infection probability in different age classes differed between the sexes. I did not include individual carcass weight in the models because carcass weights differed by sex (see Results).

The full models including the three main terms of interest were used as the final models. I used backward model simplification to remove only the interaction term (sex*age) as this did not explain a significant amount of variation in the response variable (i.e., $p > 0.05$). I used the lsmeans function to conduct Tukey's *post hoc* tests to investigate differences between the levels of significant model terms.

3. Results

In the 111 individuals examined, I identified two types of nematode eggs (and *Capillaria* spp.) in the fecal samples and five species of abomasal nematodes (*Teladorsagia circumcincta* and its minor morphotype *T. trifurcata, Spiculopteragia spiculoptera, Ostertagia gruehneri,* and *Ostertagia* spp.) (Appendix 1). I also recorded *Eimaria*, a genus of Coccidia parasite, although only in two individuals, both of which were adult males. Carcass weight differed significantly between the sexes ($t = -4.86$, df = 34.4, $p < 0.001$; Figure 9): males were significantly heavier than females.

Ostertagia gruehneri was identified from adult male based on its genital cone (prominent proconus) (A) and separated distal branches of spicules.(B)



A

B

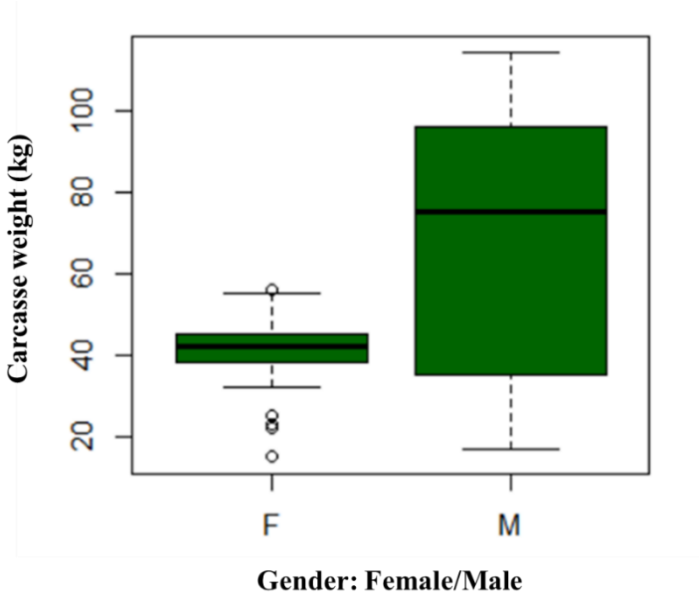


Figure 9*. Carcass weight of the reindeer according to sex. The mean is indicated by the solid black line, the green area indicates the interquartile ranges, and SD is indicated by the whiskers.*

3.1 Parasite prevalence

The McMaster analysis revealed two types of nematode egg in the fecal samples taken from the Icelandic reindeer (Table 3). The majority of sampled individuals had no parasite eggs in their feces (57/111), 42 individuals had single-species infections, and 12 had eggs of both *Capillaria* spp. and Trichostrongylidae in their feces.

***Table 7**. Prevalence (%) of nematode eggs in Icelandic reindeer feces by reindeer age class and sex, with mean, median, and range of egg counts.*

Age class	Sex	N	Trichostrongylidae egg prevalence (%) [95% CI][a]	Mean EPG[b] [95% CI] *Median (range)* Trichostrongylidae	*Capillaria* sp. egg prevalence (%) [95% CI]	Mean EPG *Median (range)* *Capillaria* spp.
Calf	**male**	9	55.6 [37.8–72.2]*	17.7 [1.5–33.8] *20 [0–60]*	11.1 [6.3–19.7]	2.2 [−2.8–7.2] *0 (0–20)*
	female	5	20 [9.7–36.5]	7.9 [−13.9–29.6] *0 (0–39)*	0	0
	unknown	3	33.3 [12.6–61.3]	6.6 [−21.7–34.9] *0 (0–20)*	0	0
	class total	17	41.2 [21.6–64.0]	12.8 [3.3–22.3] *0 (0–60)*	5.8 [1.0–27.0]	1.2 [−1.2–3.6] *0 (0–20)*
Immature	**male**	5	40 [21.8–61.0]	15.8 [−16.3–47.8] *0 (0–59)*	60 [32.7–83.1]*	15.9 [−4.7–36.5] *20 (0–42)*

Age class	Sex	N	Trichostrongylidae egg prevalence (%) [95% CI][a]	Mean EPG[b] [95% CI] *Median (range)* Trichostrongylidae	*Capillaria* sp. egg prevalence (%) [95% CI]	Mean EPG *Median (range)* *Capillaria* spp.
	female	16	18.8 [14.3–25.0]	3.9 [−0.6–8.5] *0 (0–24)*	25 [19.3–32.2]	6.5 [−0.2–13.3] *0 (0–42)*
	class total	21	23.8 [10.6–45.1]	6.8 [0.1–13.4] *0 (0–59)*	33.3 [17.2–54.6]	8.8 [2.5–15.1] *0 (0–42)*
Adult	**male**	22	59.1 [48.0–69.4]*	21.4 [11.2–31.7] *20 (0–74)*	27.3 [22.3–33.3]	9.3 [1.4–17.2] *0 (0–65)*
	female	37	24.3 [21.1–28.1]	10.2 [–0.04–20.5] *0 (0–181)*	21.6 [18.8–25.1]	9.7 [1.9–17.4] *0 (0–100)*
	class total	59	37.3 [26.1–50.0]	14.4 [7.0–21.8] *0 (0–181)*	23.7 [14.7–36.0]	9.5 [4.0–15.1] *0 (0–100)*
Unknown	**male**	1	100	25 EPG	100	49 EPG
	female	13	30.8 [22.9–40.3]	6.1 [0.03–11.8] *0 (0–20)*	15.4 [10.9–22.2]	3.0 [−1.4–7.5] *0 (0–20)*

Age class	Sex	N	Trichostrongylidae egg prevalence (%) [95% CI][a]	Mean EPG[b] [95% CI] *Median (range)* Trichostrongylidae	*Capillaria* sp. egg prevalence (%) [95% CI]	Mean EPG *Median (range)* *Capillaria* spp.
	class total	14	35.7 [16.3–61.2]	7.4 [1.4–13.4] *0 (0–25)*	21.4 [7.5–47.6]	6.3 [−1.9–14.5] *0 (0–49)*
Total	**All**	111	35.1 [26.9–44.4]	11.8 [7.5–16.2] *0 (0–181)*	22.5 [15.7–31.1]	7.7 [4.4–11.0] *0 (0–100)*

[a] 95% CI: 95% confidence intervals for either prevalence or mean EPG.

[b] EPG: eggs per gram.

* significant difference in prevalence between sexes in age class.

Trichostrongylidae eggs

There were no significant differences in the prevalence of Trichostrongylidae eggs between sexes or age classes, with the exception of male adults and calves, which had a significantly higher prevalence of Trichostrongylidae eggs than females in the same age class (Table 3). This pattern of higher prevalence in males was also apparent in the immature age class, but the difference was not significant. The mean prevalence across all age classes was 35% and the median EPG was zero. The maximum EPGs were also low (< 100) for nearly all age classes; the only exception was adult females, in which the maximum was 181 EPG.

***Capillaria* sp. eggs**

There were no significant differences in the prevalence of *Capillaria* sp. eggs between sexes or age classes, except that immature males had a significantly higher prevalence than immature females (Table 3). The mean prevalence across all age classes was 22% and the median EPG was zero.

3.2 Abomasal parasites

Abomasal nematodes were identified in 24 of the 81 individuals investigated (prevalence 29.6% [CI20.8%–40.3]). Since only 5% of total abomasal content was investigated, the number of parasites counted was multiplied by 20 to provide an estimate of total abomasal parasite burden. The mean total burden was 49.1 nematodes per abomasum (95% CI: 16.2–82.1; median: 0; range: 0–1000; Table 4). *Teladorsagia circumcincta* (and its minor morphotype *T. trifurcata*) was predominant and was found alone or in mixed infections in 13 individuals. It was not possible to identify the species involved in 10 individuals given the lack of male nematodes in the sample, or in two of these cases due to loss or degradation of the nematode. Three individuals also had low numbers of *Ostertagia* spp. and one had *Spiculopteragia spiculoptera* (Table 4; Figure 10).

***Table 8**. Species of nematode identified in the abomasa of Icelandic reindeer. The mean is given when more than one animal was found to be infected.*

Abomasal nematodes identified	**Reindeer infected (n)**
Not suitable for morphological identification	10
Ostertagia spp.	1
T. circumcincta	10
T. circumcincta/T. trifurcata and *O. gruehneri*	1
T. circumcincta/T. trifurcata and *S. spiculoptera*	1
T. *trifurcata* and *Ostertagia* spp.	1
Total	**24**

Diversity of the abomasal nematode was highest in the East coast and West coast region with 4 species each with slightly differ. However, least diversity was in the Central region with 2 species (Figure 10 and Figure 11)

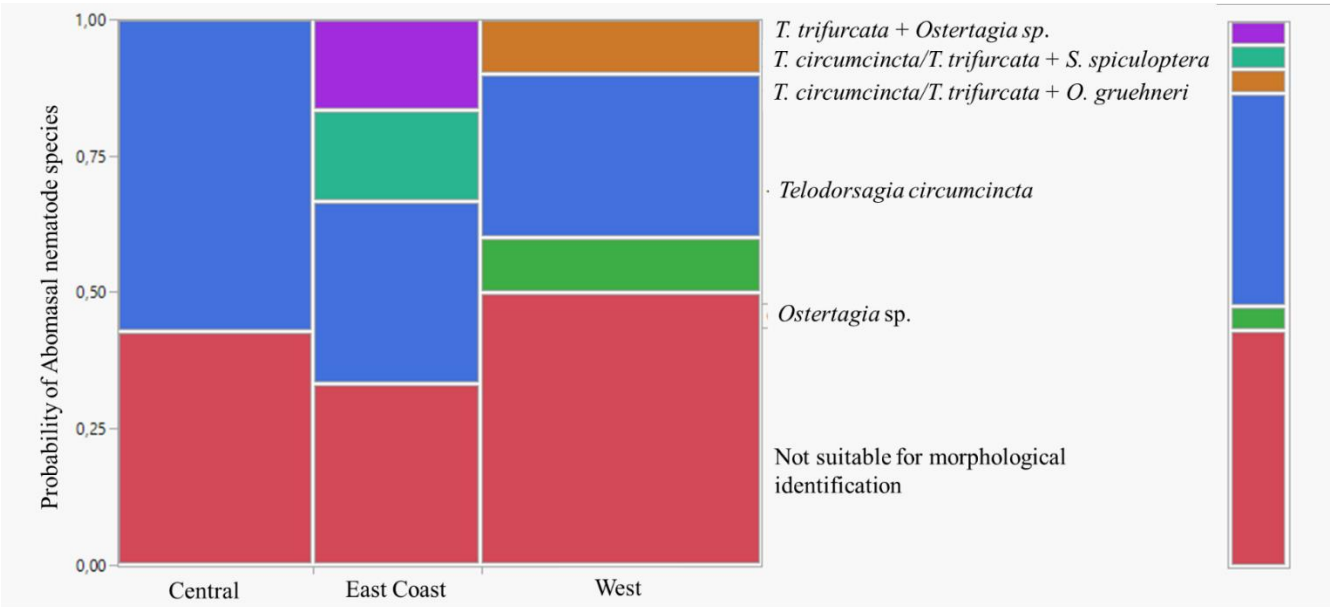


Figure 10*. Mosaic plot showing the probability of abomasal nematode species detected in Icelandic reindeer by area.* Ostertagia *spp. were detected in individuals from the West and East Coast regions,* Teladorsagia circumcincta/T. trifurcata *was detected in all three regions, and* Spiculopteragia spiculoptera *was only detected in one individual from the East Coast.*

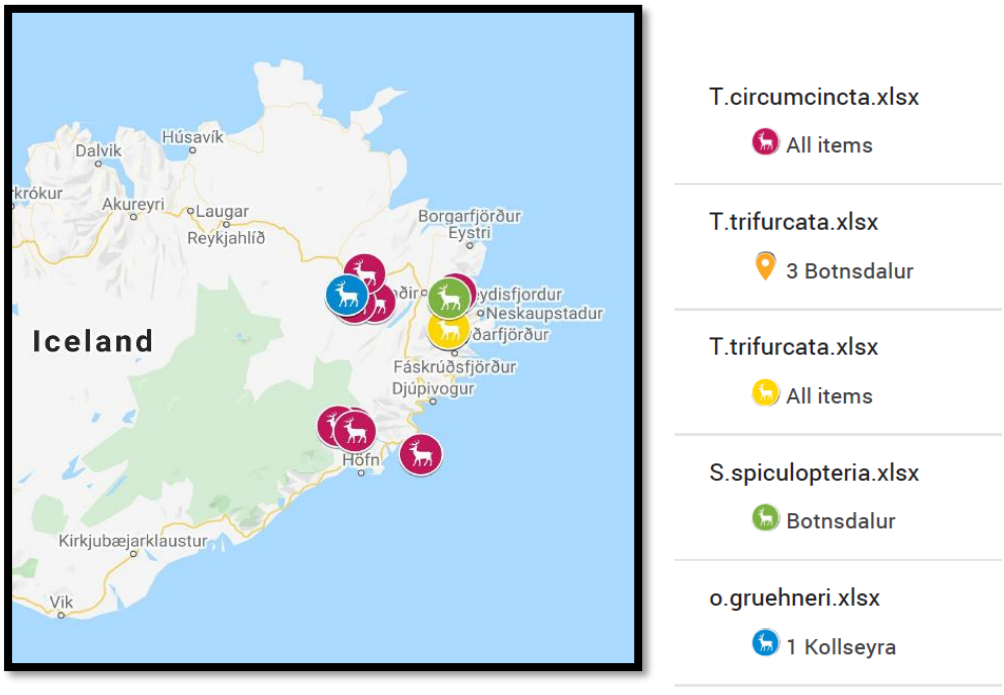


***Figure 11**. Areas shows with positive cases of abomasal nematode species.*

3.3 Probability of nematode infection

The results of the full generalized linear models (GLM) are shown in Table 5.

***Table 5**. Model summaries for generalized linear models investigating factors explaining the detection probability of nematode infection in individual reindeer.*

Response variable	Explanatory variable	χ^2 test statistic	df	P
a. Strongylida-type eggs	**Sex**	**7.15**	**1**	**0.008**
	Age	0.53	2	0.767
(binomial, 0/1)	Area	0.54	2	0.764
Denominator df: 86				
b. *Capillaria* eggs	Sex	1.14	1	0.286
(binomial, 0/1)	Age	4.63	2	0.099
Denominator df: 86	**Area**	**6.72**	**2**	**0.035**
c. Abomasal nematodes	Sex	1.27	1	0.259
(binomial, 0/1)	Age	1.04	2	0.594
Denominator df: 64	Area	1.67	2	0.434
	Sex*Age	**7.83**	**2**	**0.02**

Table 6. Prevalence (%) in Icelandic reindeer age and sex classes of nematode parasite species found as adults in abomasum samples.

Age class	Sex	N	*Ostertagia gruehneri*	*Ostertagia* spp.	*Teladorsagia circumcincta*	*Teladorsagia trifurcata*	*Spiculopteragia spiculoptera*
Calf	Male	7	0	0	1	0	0
	Female	4	0	0	50	0	0
	unknown [a]	1					
Immature	Male	5	0	0	20	0	0
	Female	14	0	0	35.7	0	0
Adult	Male	14	7.1	7.1	28.5	7.1	7.1
	Female	29	0	3.44	13.7	3.44	0
Unknown	Female	7	0	0	14.2	0	0

[the] sample of this calf individual unknown was not analyzable due to labelling.

The probability of an individual being infected with Strongylida-type eggs was significantly associated with the sex of the individual (Table 5a), with males overrepresented (Figure 12). The age class and area from which the individual was hunted did not explain the prevalence of Strongylida-type eggs. Male reindeer had a significantly higher probability of infection with Strongylida-type eggs than females (males: 0.52; 95% CI: 0.34–0.70; females: 0.23; 95% CI: 0.12–0.39; Figure 12).

***Table 9**. Model summaries for generalized linear models investigating factors explaining the detection probability of nematode infection in individual reindeer.*

Response variable	Explanatory variable	χ^2 test statistic	df	P
a. Strongylida-type eggs	**Sex**	**7.15**	**1**	**0.008**
	Age	0.53	2	0.767
(binomial, 0/1)	Area	0.54	2	0.764
Denominator df: 86				
b. *Capillaria* eggs	Sex	1.14	1	0.286
(binomial, 0/1)	Age	4.63	2	0.099
Denominator df: 86	**Area**	**6.72**	**2**	**0.035**
c. Abomasal nematodes	Sex	1.27	1	0.259
	Age	1.04	2	0.594
(binomial, 0/1)	Area	1.67	2	0.434
Denominator df: 64	**Sex*Age**	**7.83**	**2**	**0.02**

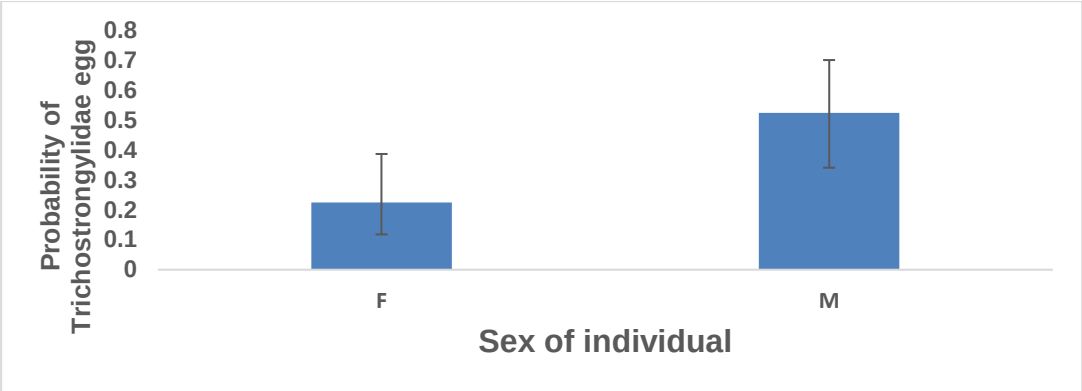


***Figure 12**. Probability of patent infections with Trichostrongylidae eggs by sex. Males had a significantly higher probability of having a patent infection than females. No such trend was seen for age class or hunting areas.*

The probability of an individual being infected with *Capillaria* eggs was significantly explained by the area in which the individual was hunted (Table 5b). Age class and sex did not explain *Capillaria* egg prevalence (Table 5b). Reindeer from the West-central region had the lowest probability of being infected by *Capillaria* (0.06; 95% CI: 0.01–0.24); that in the Central (0.22; 95% CI: 0.1–0.43) and East Coast (0.29; 95% CI: 0.13–0.54) regions was substantially higher (Figure 13). However, Tukey's *post hoc* test suggested that this difference was not strictly significant ($z > 1.77$; $p > 0.06$).

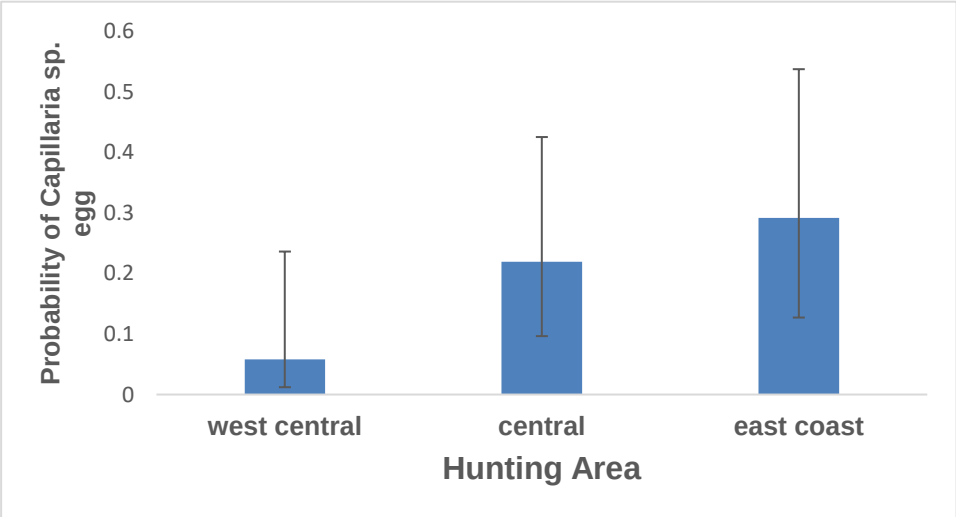


***Figure 13**. Probability of* Capillaria *sp. infections was significantly different in the GLM model with animals from the west central areas having significantly lower infection prevalence than the other areas. Subsequent Tukey's post-hoc testing found a similar trend but the significance level was not reached.*

The probability of an individual having abomasal nematodes was significantly explained by the interaction between sex and age (Table 5c , Figure 14). The main difference between the sexes is that female calves show a significantly (or near-significantly) higher abomasal nematode probability than females classed as immatures or adults. This pattern was not seen in males.

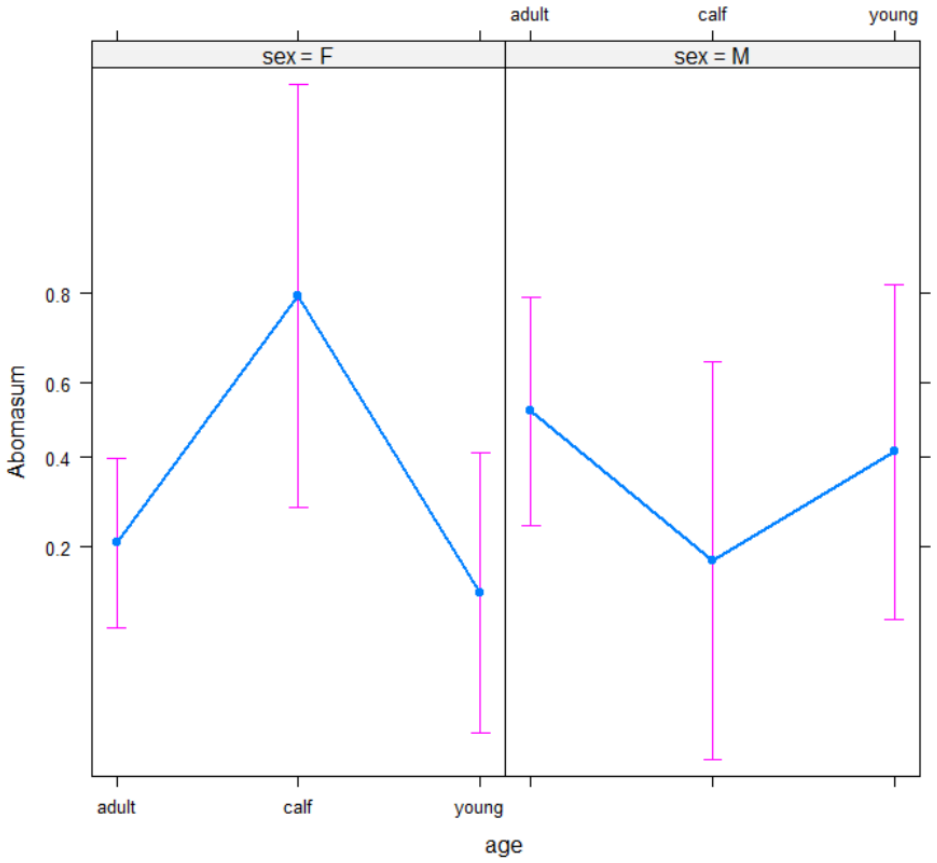


Figure 14. *Probability of presence of abomasal nematodes by sex and age class (adult: > 2 years; immature: 1–2 years; calf: < 1 year). The red lines indicate the 95% confidence intervals for the probabilities.*

4. DISCUSSION

The study provides updated prevalence of the gastrointestinal tract nematodes for the reindeer population in Iceland 2018, which revealed prevalence rates slightly lower than earlier work (Guðmundsdóttir, 2006). My study also reveals the male reindeer have a higher prevalence of parasites overall.

I found that fecal samples revealed overall mean prevalence for Trichostrongylidae and *Capillaria* sp. of 35.1% and 22.5% respectively. The intensity of infections were overall low, with majority of individuals being uninfected or with low intensity and only few individuals showing high intensity infections. I identified two types of nematode eggs, *Capillaria* sp. and Trichostrongylidae, in the fecal samples. *Capillaria* sp. is previously known to be one of the most abundant parasites in egg counts in calves and adult reindeer in Fennoscandia (Hrabok, 2009).

Low numbers of adult nematodes were found in the abomasal samples too. Further, I have updated nematode faunas in Iceland by five species, *Telodorsagia circumcincta* and its minor morphotype *T. trifurcata, Spiculopteragia spiculoptera, Ostertagia gruehneri* and *Ostertagia* sp., from abomasal samples. These species all have been recovered from adult males and females. Interestingly, male individuals showed higher prevalence and probability of nematode infection than females, and age was not important in explaining these differences. According to the prior and most recent parasite study that covered 192 calves in 2003 and 58 reindeer in 2003-2005 from Iceland, *Ostertagia ostertagi, Teladorsagia circumcincta* and *Trichostrongylus axei* were found in the abomasum samples (Guðmundsdóttir ,2006).

My study revealed *Ostertagia gruehneri* which was not discovered in previous studies in Iceland.
I have identified it based on its morphological figures shape of genital cone which separated the species. However, *O. gruehneri* was reported in pastures where reindeer shares pasture with sheep in Finland and known to be the dominating parasite in Svalbard reindeer (Manninen et al., 2014; van der Wal .,2000& Irvene et al., 2000). *O. gruehneri* was also documented in caribous from Dolphin-Union, Canada, shown >50% in 80% of abomasal samples (Hughes et al., 2009). This is the most common gastrointestinal nematode of reindeer/caribou and the

development in the reindeer host is arrested, but infectious larvae may be ingested from pasture all year round. (Kutz et al., 2012, 2017 and Mehlhorn, 2015).

In my study *O. gruehneri* was found from one adult male reindeer recorded as 3-5 years old, 105 kg carcass weight, and shot on 3rd September 2018. This individual was identified as being host of two nematode species but major and minor morphotypes of the same species(*T. circumcincta and T.trifurcata.)* According to the studies of Svalbard reindeer, abundance of nematode increased for reindeer aged 3-5 years old and they do not seem to develop an efficient immune response towards infection as shown abomasal nematode infection in cattle occurs during the end of their first grazing season. (Larsson et al., 2006).

There were no significant differences in prevalence between the different age classes and gender with the exception of adult males and male calves that had a significantly higher Trichostrongylidae egg prevalence than females in the same age classes. This trend of higher prevalence in males was also seen in the young age class but the significance level was not reached. This finding could be explained as microparasite distribution is often aggregated. This has important implications for estimations of infection in a population and for study design and data analysis.

Rut stress and increased testosterone concentrations in the autumn, may depress acquired immunity for male reindeer. This relaxation of immunity can result in increased parasite survival and increased larval or egg output (Bradley 1979; Folstad et al.,1989; Gaudernack et al. 1984; Halvorsen et al. 1986; Nieminen 1980)Even if the distribution of the infective stages were random, small differences in feeding rate due to body size, dominance status and differences in immunological status, behavior or genetic susceptibility interact with spatial distribution of infective stages amplify the degree of aggregation observed in the host population.

This degree of aggregation tends to increase with age, thus youngest age class sampled may have a distribution that is almost random, but as the individuals age, small differences between them amplify the differences in susceptibility to give more aggregated distributions.

My study also shows lower number of fecal eggs counts in the fecal samples of female reindeer as compared to males which could be associated with intraspecific competition. If estimates of worm burden are based purely on counts of parasite eggs in feces, they will tend to underestimate degree of aggregation. This primarily occurs if the fecundity of female worms decreases in heavily infected hosts, as this may lead to heavily infected hosts producing similar number of eggs in the hosts feces as hosts with lower worm burdens (Anderson et al., 1978).

My study investigated a quite large sample of individuals that were all collected by hunting, which may introduce bias in the selection of individuals. Two sources of non-random bias from hunting may be introduced; firstly, hunters often select the largest animals as a trophy rather than random sampling meaning these individuals may be over-represented in the sample. However, given the fact that the reindeer hunting represents population culling, this non-random sampling may not be a biologically relevant issue because the as per protocol the hunting guide selects the animals and has a strict quota regarding males and females. Second, hunters may find it easier to hunt slower individuals in poorer condition, meaning these individuals may be over-represented in the samples investigated. Although, mean carcass weight of female reindeer corresponds to the previous study in 2 years old reindeer from area 1 was 41.1 kg. However, the male carcass weight in my study was lower than previous study that shows 2 years old males weighing 91.9kg. (Skarphéðinn, 2018).This may increase estimates of parasite prevalence in this study as parasites are known to negatively impact individual condition (Kutz et al.,2019). Given the low prevalence of GINs revealed by my study, this potential makes my conclusions even more robust.
However, there are certain factors needs to be considered such as number of eggs and larvae from the parasite detected is dependent on season, host condition, parasite and host species and adult parasite fecundity (Kutz et al.,2019).

My study also provides new prevalence estimates, updating the information of the last study from about 15 years ago (Gudmundsdottir & Skirnisson 2005, 2006). My study suggests that the Icelandic reindeer shows healthy status in the country; parasite prevalence is overall lower than those detailed in earlier studies (Gudmundsdottir & Skirnisson 2005, Skirnisson 2006).

Low number of presences of GINs parasite might be associated with the ‘freshness ‘of the samples. According to studies of Jagła et al., 2013, storage and preservation in strongylidae nematode egg in the horse shows, short-lasting, three-day freezing resulted in a decrease of

detectability from 100% to 94.1%, with an equally significant decrease in the mean number of eggs per one gram of feces, from 1,238.97 to 983.82. Extending the freezing period from 3 to 14 days had a highly significant effect on the prevalence, which was then 85.3%. Considering dates on fecal egg and abomasum sampling for the current study (adults were shot between 24th August 2018 and 12th September 2018 and calves shot between 1st September and 13th September) and samples were prepared 1-2 days prior to laboratory analyses. Fecal egg McMaster analyses, I performed 6th September – 4th October 2018; for Baermann analysis 6th September - 5th December 2018; finally for abomasum analysis 7th January - 10th October 2019. These dates show the time lag between sample collection and analyses was longer than recommended and thus, laboratory tests at the site right after collection could improve future studies.

The lower intensity of infection of parasite prevalence could also have been associated with timing of the sample collection since it was conducted end of August.
In a study on fecal egg counts, prevalence of eggs in animals can vary with the period of sampling. By late June the fecal egg count was 20% and increased to 80% towards end of July. The egg counts reduced by the late August and September and reached only 10% by mid-October (Hughes et al., 2009) The previous reindeer study in Iceland, calves sample was collected at the calving sites between 7th June and 13th August 2003 and analyzed within 2 days (Gudmundsdottir, 2006).

There were no differences in parasite prevalence for any species in different reindeer age classes. This result contrasts with the reviews of studies provided by Jokelainen and colleagues (2019) that showed changing parasite infection rates with age. This review however did not include Icelandic populations and it seems plausible that due to the isolation of the Icelandic reindeer population that parasite dynamics may be different due to absence of parasitic insects and natural predator.

Host-parasite interactions are predicted to change significantly under warming temperatures (Rohr et al., 2011) with some parasites at risk or extinction and others predicted to increase.
And it is documented that accelerated and continuing climate change in the Arctic is transforming ecological boundaries, parasite faunal diversity and host–parasite interactions (Kutz et al.,2006; 2014).

The climate of most parts of Iceland is characterized by strong winds, frequent precipitation, mild winters, and cool summers. Mean temperatures are typically close to 0˚C in the winter and not far from 10˚C in the summer. The mean annual precipitation is about one thousand millimeters in the south of Iceland, but less in the north (Haraldur et al., 2007). However, Iceland has experienced climate change resulting land rise, melting glaciers due to warm ocean water which alters their ecosystem (ref?). Thus, studying parasite prevalence correlating with climate change could be the future study considering timing of sample collection and freshness.

I hope that my study could be the baseline for future GIT parasite research. There are especially a limited number of studies on parasite loads in Iceland mammal populations, but Iceland is an environment that is under rapidly changing conditions from the climate change perspective.

This study shows that in general Icelandic reindeer population are in good condition at good numbers and seem to have less parasite burdens compared to other Rangifer populations.

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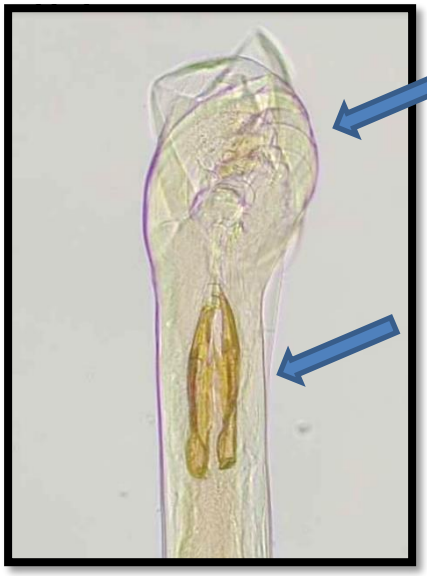
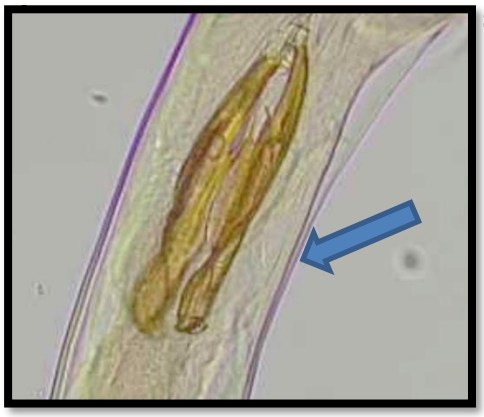
6. APPENDIX

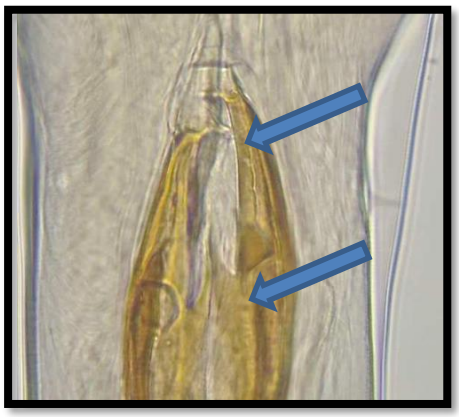


Dorsal ray not paired, bursal ray pattern 2-1-2

Spicules 0.5 mm long

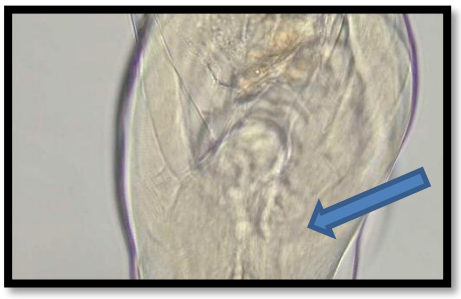


spicules dark yellowish brown, dorsal process of spicule barbed

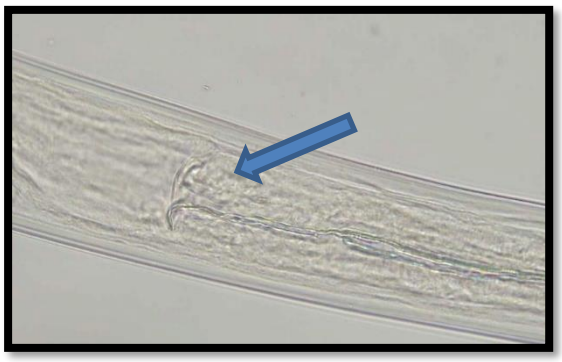


gubernaculum present

tip of spicule with sharp point

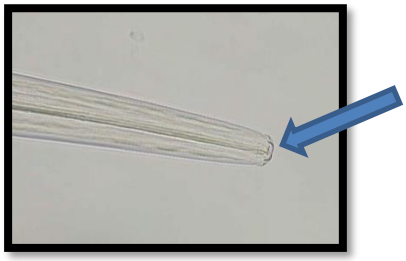


Genital cone and copulatory bursa



Long esophageal valve length 120–131 mm

roughly triple the width



Reduced buccal capsule and square head

Figure 1. Identification of *Ostertagia gruehneri*

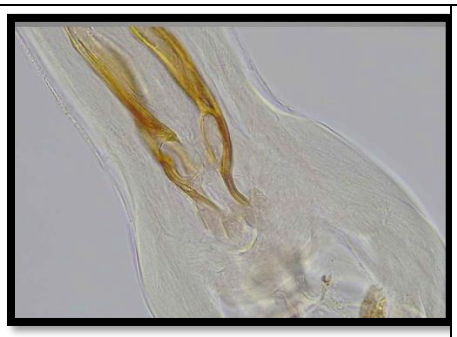
			
Teladorsagia circumcincta long thin spicules	*Teladorsagia trifurcata* thicker spicules	*Spiculopteragia spiculoptera* distinctive fan-shaped ends to the spicules, gubernaculum absent	

Figure 2. *Identification of T.circumcincta,T.trifurcata and S.spiculoptera*

Figure 3. *Ostertagia* spp. (spicules are equal length and shape, tapering towards the distal end)

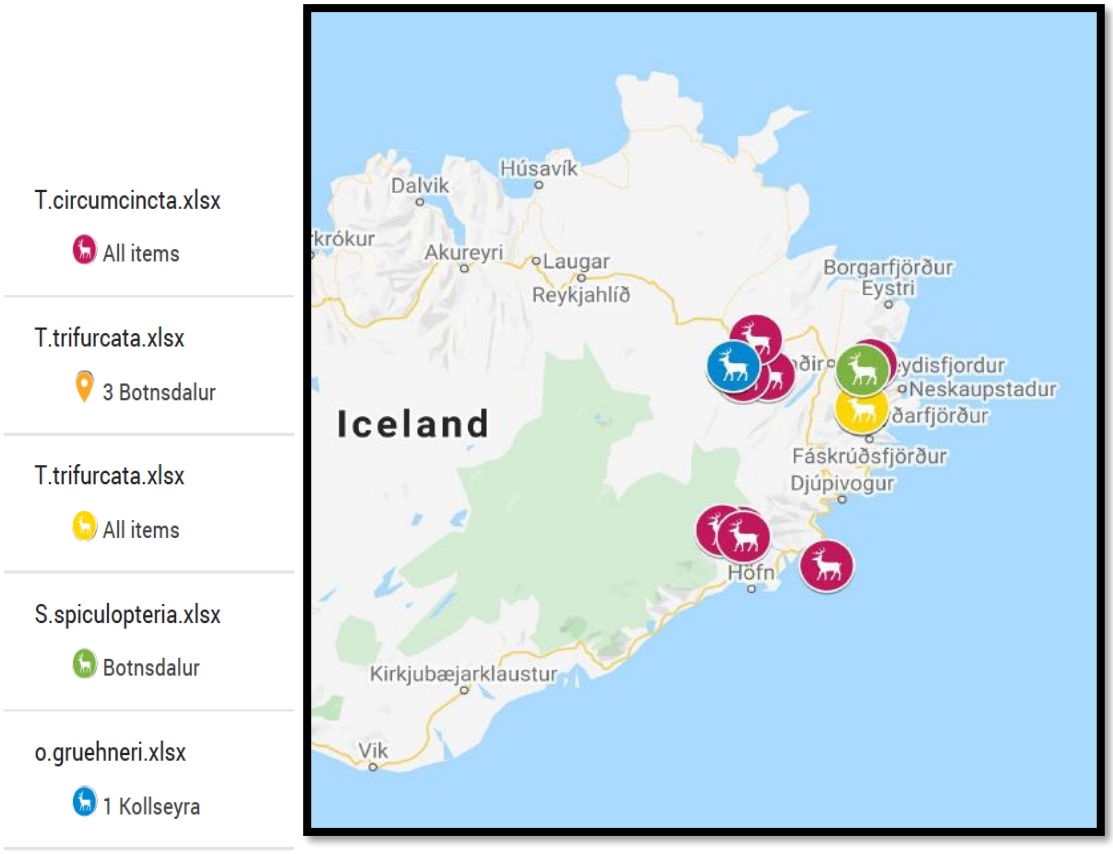


Figure 4. *Areas where positive cases for reindeer with abomasal nematodes were hunted*

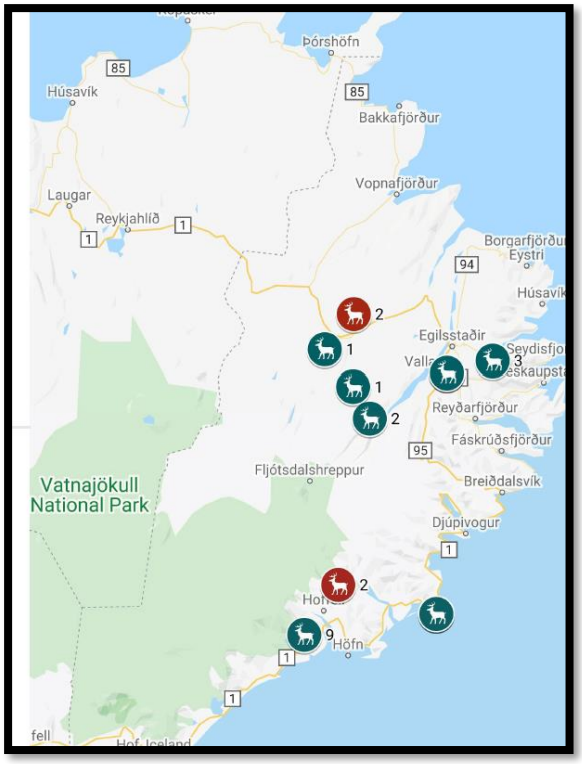


Figure 5. *Locations where calves were hunted. Brown points indicate where calves that were positive for fecal egg counting; green points indicate where calves that were negative for fecal egg counting.*

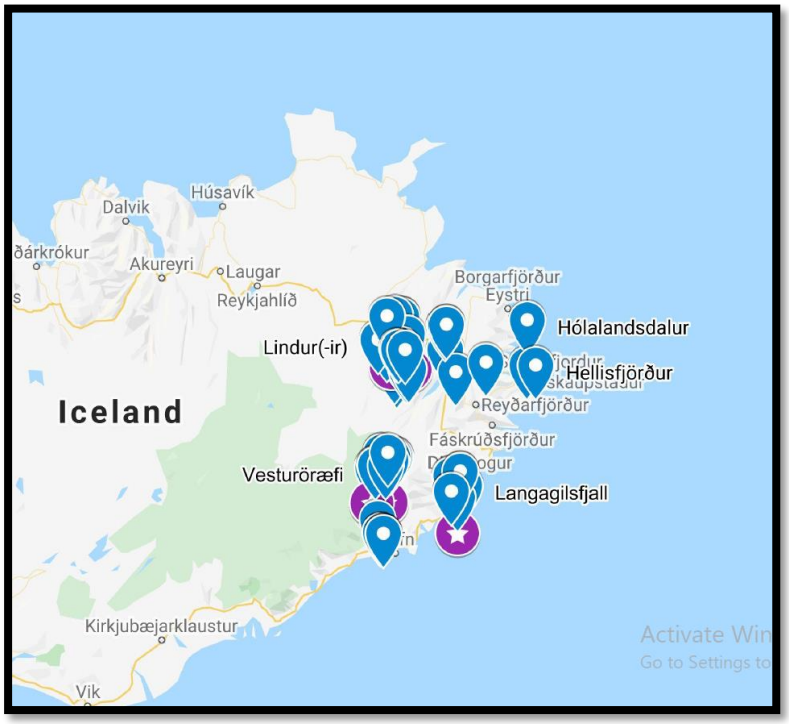


Figure 6*. Locations where females were hunted. Purple points indicate where females that were positive for fecal egg counts; blue points indicate where females that were negative for fecal egg counts.*

Figure 7. *Locations where males were hunted. Purple points indicate where males that were positive for fecal egg counts; blue points indicate where males that were negative for fecal egg counts.*

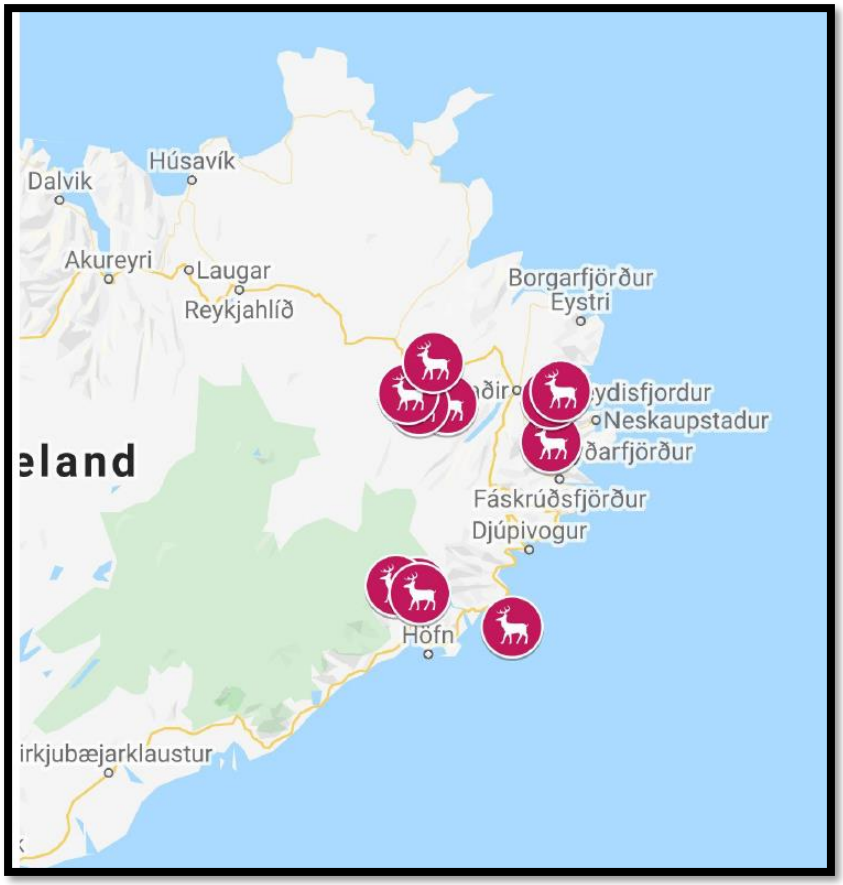


Figure 8*. Hunting locations of cases positive for* T. circumcincta *infection.*