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Master thesis

Gastrointestinal parasites in moose (*Alces alces*)

- which ones and what consequences?

Nematode parasites found on abomasum wall (Licina, 2013)

Master in Applied Ecology

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Date   Place   Signature

ABSTRACT

Even though the moose in Norway represents such an important game species, this is one of the first studies of their gastrointestinal parasites. The moose population density in Hedmark county remains at a high level, and because of a decrease in forage availability per moose, reduced slaughter weights have been recorded in all classes during recent years. In this study I aimed to find out which species of GI parasites could be found in moose in Hedmark county, to quantify their prevalence and intensity of infection and to correlate prevalence, probability and intensity of infection with the individual`s sex, age and physical condition. I also aimed to find out what the presence of parasites told us about the general health status of the moose population. Intestinal and other samples from 49 moose were collected between 25.9.2013 and 1.11.2013 in three municipalities Stor- Elvdal, Åmot and Tynset. Analyses of faeces revealed that calves had higher prevalence of *Monizia* sp. and "Dorsal spine larvae" compared to adults, which could indicate that they have not yet acquired immunity against them. I also find "Strongylidae type" eggs, *Strongyloides papillosus* and *Eimeria* sp eggs in faeces. The prevalence of adult abomasal parasites was high in this population and high parasite burdens were associated with poorer body condition. Intensity of infection was also correlated with host age and gender, higher parasite burdens were found in older animals and even though the median abomasal infection intensity was similar between males and females, three animals with highest parasite burdens were females. The most common abomasal nematodes found were *Ostertagia antipini* and *Spiculopteragia alcis*. This study provides vital baseline data for future research, that should be standardized and repeated at a regulars interval and across seasons.

Keywords: *Alces alces*, moose, gastrointestinal parasites, abomasal nematodes, overabundance

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1 INTRODUCTION

Moose (*Alces alces*) are the largest living deer, found in the boreal forests of northern hemisphere from central Europe, across northern Asia and in North America (Geist, 1998; Franzzman et al., 2007). In Scandinavia, moose represent an important natural-economic resource and are a notable tourist attraction (Storaas et al., 2001). Density of moose population in Fennoscandia today, is high. The population size increased in numbers and range, especially since the 1970s, because of changes in hunting management (sex and age-specific harvesting), introduction of clear-cut systems in forestry and the demise of large predators as the most important explanatory factors for this increase (Jaren, 1992; Geist, 1998; Danielsen, 2001; Lavsund et al., 2003; Appolonio et al., 2010). Moose, as with all wildlife, host many species of parasites (Sinclair, 2007), and like other wild ruminants, have a high abundance of gastrointestinal (GI) parasites (Hoberg et al., 2001). Even though the moose in Norway represent an important game species, not a lot of research has been done on the prevalence and intensity of gastrointestinal (GI) parasites infection in them until now.

1.1   Parasites and ecosystems

Parasites play an important role in every ecosystem, as one of the regulating mechanisms of population dynamics for species within that system (Hudson et al., 2001; Tompkins et al., 2002; Begon, 2007; Sinclair, 2007). Biotrophic parasites are defined as organisms that obtain their nutrients from a living host individual (Begon, 2007), they can either live on (ectoparasite) or within (endoparasites) the body of the host (Hendrix & Robinson, 2006). Parasites have evolved and developed with many of their hosts (Foreyt, 2001), and usually are host-specific or at least have a limited range of hosts (Begon, 2007). Endemic parasites are parasites adapted to their hosts, and the hosts are adapted to the presence of them. They cause chronic impacts - low-level, persistent, non-lethal debilities or diseases in contrast with non-endemic ones which cause epizootic disease, clearly harmful to the host (Sinclair, 2007). Endemic parasites reduce host population numbers, by influencing host fecundity and survival, mostly when interacting with other factors such as food availability and predation (Gulland, 1992; Halvorsen et al., 1999; Sinclair, 2007). Parasites are classified into two major groups according to their size and visibility, microparasites - parasites of microscopic size for example viruses, protozoa and bacteria and macroparasites - parasites visible to the naked eye, such as arthropods, nematodes, trematodes and cestodes (Begon, 2007; Sinclair, 2007;

Lawrence, 2008). Parasites can be transmitted directly from one host to another or indirectly, requiring a vector or intermediate host (Begon, 2007).

The degree of parasite load in the host depends on many extrinsic and intrinsic factors that can influence the dynamic of the host parasite relationship (Foreyt, 2001; Hudson et al., 2001). The degree of infection is a complex interplay between host factors such as gender, age and physiological condition - nutrition status, immune system as well as genetic differences in susceptibility to infection, host behavior and on individual variation in their exposure to parasites (Demarais et al., 1983; Zuk & Mckean, 1996; Coop & Kyriazakis, 2001; Foreyt, 2001; Hoberg et al., 2001; Hudson et al., 2001; Gunn & Irvine, 2003; Ezenwa, 2004; Body et al., 2011; Milner et al. 2013) in addition to parasite factors. To reduce their exposure or infection by fecal-oral transmitted parasites, animals have evolved different antiparasitic behaviours for example selective defecation and selective foraging (Van der Wal et al., 2000; Gunn & Irvine, 2003; Ezenwa, 2004b).

Parasite prevalence in a host population can increase directly or indirectly, interacting with other factors such as weather condition, quantity and quality of forage or absence of large predators to name a few (Hoberg et al., 2001; Sinclair, 2007; Body et al., 2011). One direct reason for an increase in parasite prevalence is increased host density. The later can lead either to increased indirect (e.g. with faeces) - or direct - interference - contact rates of the host (Body et al., 2011). Regardless of the main underlying reason, an increase in prevalence is a consequence of multiple factors that benefit the survival, reproduction and transmission of parasites.

1.2 Gastrointestinal parasites

The gastrointestinal tract is divided into different anatomical sections, with different species of parasites parasitizing the different sections of the tract. A ruminant gastrointestinal tract is divided into four stomachs (reticulum, rumen, omasum and abomasum) in addition to the small (duodenum, jejunum and ileum) and large (caecum, colon) intestine (Dyce et al. 1987). This study has chosen to focus on GI nematodes (Roundworms; belong to group macroparasites (Sinclair, 2007)) in particular those found in the abomasum and caecum. Previous studies in other host species have indicated that abomasal and caecum parasites can have an effect on host at subclinical levels (Gulland, 1992; Hudson et al., 1992a; Stien et al., 2002a; Hughes et al., 2009).

Gastrointestinal (GI) parasites are found in the host's digestive tract (stomach and intestines) and rarely cause mortality of the host (Gunn & Irvine, 2003; Irvine et al., 2006; Sinclair, 2007). Usually they can cause malnutrition because of reduced appetite (Houtert & Sykes, 1996; Arneberg et al., 1996; Gunn and Irvine, 2003), disruption of metabolic functions (Foreyt, 2001) and food assimilation (Houtert & Sykes, 1996; Coop & Kyriazakis, 2001), which has negative consequences for growth, reproduction success (Foreyt, 2001; Hoberg et al., 2001; Albon et al., 2002; Stien et al., 2002), competitive ability and can increase susceptibility to other pathogens (Schmitz & Nudds, 1994). GI nematodes can cause parasitic gastroenteritis, with the most common clinical signs including diarrhea and weight loss or poor weight gains, bleeding, anemia, anorexia, poor pelage and among others deficiency of important microelements – calcium, magnesium, phosphorus etc. - and protein deficiency (Brglez, 1990; Coop & Kyriazakis, 2001; Hoberg et al., 2001; Gunn & Irvine, 2003; Taylor et al., 2007). Not all parasites have the same pathogenic potential and in general, gastroenteritis develops in non-malnourished animals if they harbor a high parasite burden. In macroparasites, host mortality and morbidity tends to be dose-dependent. Nevertheless, because of synergistic effects between different parasites species, morbidity may result at lower level of infection intensity (Parkins & Holmes, 1989; Houtert & Sykes, 1996; Coop & Kyriazakis, 2001; Hoberg et al., 2001; Wilson et al., 2002). Gastrointestinal nematodes can cause pathological changes to the gastrointestinal mucosa, and level of damage to abomasal mucosa can be diagnosed by the increase in the concentration of serum pepsinogen (Parkins & Holmes, 1989; Gunn & Irvine, 2003). The amount of parasite burden in connection with environmental conditions, physiological and nutritional status of the host, contributes to development of either subclinical diseases - reducing the probability of a host reproducing or surviving or clinical disease - defined as signs, including death (Gunn & Irvine, 2003). Even though parasites present in the host are potentially pathogenic, the host doesn't need to exhibit outward clinical signs or diseases (Hendrix & Robinson, 2006).

1.2.1   GI nematode lifecycles

In the Nematoda the sexes are separate with the males generally smaller than the females (Taylor et al., 2007). Gastrointestinal nematodes have a direct life cycle - eggs produced by females (Taylor et al., 2007) are spread in the environment by faeces of infected animals (Hendrix & Robinson, 2006). The survival potential of the egg outside of the host body varies and it depends to the thickness of the shell - parasites whose infective form is the larvated egg usually have very thick-shelled eggs which can survive for years on the ground (Taylor et al.,

2007). Eggs may hatch outside the host (controlled by temperature (optimal: 18-26°C) and humidity (optimal 100%) and partly by larvae itself) or after ingestion. If the latter, it is very important that the hatching occurs in appropriate regions of the gut (Taylor et al., 2007). The stimuli for hatching differs among GI nematodes, depending on their final destination. It is believed that dissolved carbon dioxide is a constant essential (Taylor et al., 2007).

The complete life cycle of nematodes is composed from four moults - four successive larvae stages (L_1, L_2, L_3, L_4) and L_5 which is the immature adult (Taylor et al., 2007). In the common form of direct life cycle the free-living L_1 larvae after hatching from the egg undergo two moults before becoming the infective third stage (L_3), which is also the stage that is ingested by the definitive host (Taylor et al., 2007). The first three larval stages can develop in the external environment or within the intermediate host. After infection of definitive host, two further moults take place to produce L_5 stage - immature, or preadult nematode which eventually develops into the sexually mature adult stage. Development to L_5 larvae may take place entirely in the gut lumen or with only limited movement into the mucosa (Hendrix & Robinson, 2006; Taylor et al., 2007). Prepatent period is the time span between infection (ingestion of L3 larvae) and production of eggs by mature adult parasites (Taylor et al., 2007).

1.2.2   Identification of adult nematodes

The classification of adult nematodes from *Ostertagia* genus is based mostly on the morphology of the tail part of males - structure of bursa, genital cone and spicules and esophageal valve dimensions (Dróždž, 1965; Hoberg et al., 2001). In some species from subfamily Trichostrongyloidea - many *Ostertagia* and *Telodorsagia* species - polymorphisem among adult males have been recognized - pairs of species, or male morphotypes, always occur together, with one constituting a major proportion (major morphotype) and another a minor proportion (minor morphotype) of the population. Even though the morphotypes differ in their morphological features, genetical analyses showed they are genetICaly the same (Lichtenfels & Hoberg, 1993; Dróždž, 1995).

1.2.3   Nematode transmission strategies

There are two phenomena present in the life cycle of some nematode species: arrested larval development and periparturient rise in faecal egg counts (Taylor et al., 2007). Arrested larval development, hypbiosis, can be defined as the temporary cessation of parasite development in the host - remaining sexually immature until more favourable environmental conditions

return. The onset of hypobiosis in the northern hemisphere coincides with cold autumn/winter conditions. Arrested development over the winter effectively maintains the parasites, in a hypobiotic state in the host, during extended periods of poor environmental conditions which conceivably minimizes the energetic costs to the host during periods of possible nutritional stress (Kutz et al., 2012). Arrested development can also occur as a result of both age and acquired immunity in the host (Taylor et al., 2007). The continued parasite development in female hosts, is linked with the breeding cycle and occurs at or around parturition when they are immunologically compromised (Taylor et al. 2007). With males, relaxation in parasite immunity coincide with the increase in their testosteron level, for example during the mating season (Klein, 2000). The importance of immunity for activation of arrested development is secondary compared to environmental condition (Almería et al., 1996). In ruminants, hypobiosis has been reported for example in the abomasal nematodes such as *Ostertagia* sp (Taylor et al., 2007).

Periparturient rise (PPR) refers to an increase in the numbers of nematode eggs in the faeces around parturition because of the decrease of host immunity which enables maturation of arrested larvae as well as increased fecundity of the existing adult worm population to name two of the mechanisms behind PPR. The importance of PPR is that it occurs in a time when the numbers of new susceptible hosts are increasing and so ensures survival and propagation of the worm species (Taylor et al., 2007).

1.3 Moose parasites in Norway

In a recent pilot study of GI nematode parasites in moose in Hedmark, Milner et al. (2013a) found that 75% (N = 68) of individuals were infected with at least one GI parasite, with a 65% prevalence of *Trichostrongylidae* and 25% of *Nematodirus* spp. respectively. They quantified nematode parasite prevalence by analyzing parasite eggs in the faeces. Egg counts are of limited value in estimating parasite abundance and making judgments about the clinical condition of individual hosts because many factors affect egg production, including variation in the number of eggs produced by the species of parasite, individual host immunity, and stage of infection (Hudson et al., 1992b; Zajac & Conboy, 2012). In addition, identification to the species level from the eggs can be very difficult (Milner et al., 2013a). To obtain an accurate estimate of the GI nematode prevalence Milner et al. (2013a) recommended identification of adult nematodes from the abomasum to species level - which became one of the aims of this thesis.

1.4   Study aims

In Norway there is a strong tradition of recreational hunting, and most game management is aimed at keeping populations highly productive without causing unacceptable damage to agriculture or forestry (Geist, 1999). When wildlife management strategies lead to increased game species density and aggregation, it is important to generate systematic and updated information on the game animal population health status (Geist, 1998), especially because increasing population density can lead to increased pathogen transmission, including parasites, which can, in turn, lead to reduction in the potential yield of harvested species (Sinclair, 2007).

In this study I aimed to:

- find out, which species of GI parasites could be found in moose in Hedmark county
- quantify prevalence and intensity of infection of GI nematodes in moose
- correlate prevalence, probability and intensity of infection with the individual`s sex, age and physical (short term or long term body condition, carcass weight) condition
- find out what does the presence of parasites reveal us about the general health status of the moose population

2 MATERIALS AND METHODS

2.1 Study area

Our samples were collected in three municipalities in southeast Norway - Stor-Elvdal, Åmot and Tynset in Hedmark county (Figure 1).
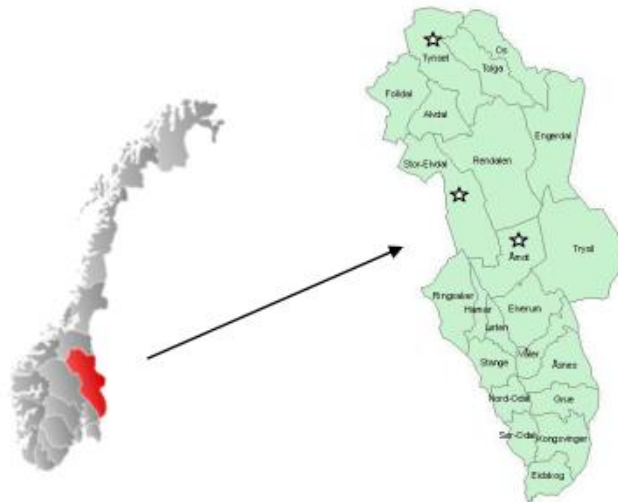
Figure 1: Study areas where we collected samples between 25.09.2013 and 1.11.2013, areas are marked with a star ☆ (Source:http://upload.wikimedia.org/wikipedia/ commons/1/16/Hedmarkskommuner.jpg)

Hedmark county is a boreal forest zone with Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) as the dominant tree species. Minor stands of deciduous forest are also present forming mixed conifer-decidious woods. Hedmark forests account for 20 percent of Norway's commercial forest resources. Next to forestry, the area is a leading farming county, with 10 percent of Norway's total agricultural land present in this area (Agriculture & Forestry in Hedmark County, Norway; County Deparnt of Agriculture and Forestry), primarily used for raising livestock. The climate of the Hedmark county is continental with 30 year mean summer (May - September) and winter (October - April) temperatures of 10.6 °C and -5.8 °C, respectively. The 30 year mean annual precipitation was 628 mm and the mean snow depth (October - April) was 39 cm (Mathisen et al. 2014).

The winter density of moose in Hedmark is high, around 1.3 moose per km^2, and has been largely stable for the last 15 years (Milner et al., 2013a). Even though large predators are present in the county, the main cause of moose mortality remains hunting. During the 2012/2013 hunting season, 7111 (77% of all the hunting licences issued) moose were culled in Hedmark county. This makes Hedmark the "leading moose hunting county" in Norway (Table 1). High mortality is also caused by traffic accidents. During the 2012/2013 hunting

year a total of 1724 moose were killed by collisions with cars or trains in Norway as a whole, and of these 446 were killed in Hedmark county (Statistics Norway: http://www.ssb.no).

Table 1: The number of moose hunted (age class and gender) in hunting season 2012/2013 in the whole country of Norway and in Hedmark county, (source - Statistics Norway: http://www.ssb.no)

		calf		yearling		adult	
		male	**female**	**male**	**female**	**male**	**female**
The whole country	34 611	5 786	5 442	5 649	4 179	7 704	5 851
Hedmark county	7 111	1 266	1 247	1 118	787	1 446	1 247

2.2 Data collection

Data were collected during the autumn moose hunting season 2013/2014 in three areas Stor-Elvdal (N=22), Åmot (N=23) and Tynset (N=4) municipalities (Table 2, Appendix 2). Intestinal (abomasum, caecum, faeces) and other samples (blood, milk, ovaries/uterus, jaw, hunter protocol) were collected between 25.9.2013 and 1.11.2013, with the majority collected in the first 11 days (N=38) after the start of the hunting season. In September we collected samples from 29 animals, in October from 18 and in November from 2 animals only. We cooperated with 21 hunting teams, in Stor - Elvdal with nine teams, in Åmot with eleven teams and in Tynset with one team.

In total, we collected intestines (abomasum, caecum) from 49 animals (Table 2) and faeces from 45 animals. We also collected 42 EDTA and 45 NORMAL blood tube samples, 1 milk sample, 3 ovaries/uterus, 46 jaws and 50 hunter protocols. The blood and milk samples were not analysed as part of this study and have been stored frozen at -18 °C for future analysis.

Table 2: Number of collected gastrointestinal samples by gender, age and study area

Age class	Gender	N	ÅMOT (N=23)	STOR-ELVDAL (N=22)	TYNSET (N=4)
calf	Female	5	3	1	1
(N=10)	Male	5	4	1	0
1.5	Female	3	0	2	1
(N=10)	Male	7	3	3	1
2.5 or older	Female	12	6	5	1
(N=29)	Male	17	7	10	0

Before the hunting season started we prepared "hunting bags" that contained hunting protocols (Appendix 1), labels for labelling guts and heads, strings to close the rectum and three tubes, one for milk and two for blood (EDTA tube and a normal blood tube). Hunters were asked to fill in the protocol form in which they had to estimate the amount of fat around the kidneys and heart (Appendix 1). Later this data was used as the basis of a short term body

condition estimate (poor, normal, good) of culled animals. Hunters were also asked to mark the guts with a unique identifier number, close the rectum with a plastic string - to prevent contamination with ground free-living nematodes - and to take a GPS positioning read at the site of the kill.

After receiving GPS coordinates we went to the site within two to twelve hours post notification. The abomasum, caecum (we closed them with a string) and faeces from the rectum were collected. All organs and faeces were properly marked and stored in plastic bags. After taking the organs we went to the hunters to retrieve the jaw, the completed protocols, as well as the blood and milk samples. Faeces, blood and milk samples were stored in a transportable fridge.

2.3   Preparation and laboratory analyses of samples

2.3.1   Faeces

Faecal samples were collected directly from the animals' rectum, to avoid contamination with free-living soil nematode fauna, and stored in the plastic disposable glove with which we had retrieved them. Before storing the faeces we noted their condition. We noted the colour, presence of blood, mucus, consistency and presence of parasites - tapeworm segments (Hendrix & Robinson, 2006). Four animals had no faeces in their rectum and in four other cases the amount of faecal material was very small. When the amount of faeces was small we conducted just McMaster egg flotation method and not Baerman larvae examination (see below). The decision was based on our main interest - GI parasite estimation count.

Because of work load at the beginning of data collection Baerman larvae examination was done immediately after the field work and McMaster was done later - maximum two weeks after the data field collection. Faeces were kept in the fridge until examination at 4 °C.

2.3.1.1   Modified McMaster flotation method

We mixed 3 grams +/- 0.1 grams of faecal material and 75 ml of cold water, and we poured the obtained suspension over a wet sieve (200mm*25mm, 250 micro) to collect the suspension. We poured this suspension into two 14 ml tubes and centrifuged them at 3000 G for 5 minutes. The supernatant was discarded and the sediment pellet was resuspended in flotation fluid: $ZnCl_2$-NaCl (specific gravity 1.3) up to the 14ml mark. After carefully mixing, we pipetted the solution into both chambers of a dry, clean McMaster slide. The entire chamber had to be filled without any air bubbles occuring. If they occurred, the chambers had

to be refilled before counting could take place. Before examination we left the solution to sit for at least 5 minutes to allow the flotation process to occur. The slides were examined using a microscope with 100x magnification, the focus was on the top layer - at this layer the lines of the grid are in focus. We counted eggs in each line in every chamber and identified them based on morphological features either to genus level (*Moniezia* sp., *Trichuris* sp*., Nematodirus* sp*., Eimeria* sp*.),* species level (*Strongyloides papillosus*) or as a category of parasites ("Strongylidae type" eggs) (Table 3, Appendix 3).

Table 3: Identified parasite eggs found in the faeces (Foreyt, 2001, Taylor at al., 2007, Zajac & Conboy, 2012, http://www.rvc.ac.uk/review/parasitology/RuminantEggs/Common.htm)

	Moniezia sp.	*Trichuris* sp.	*Nematodirus* sp.	*Eimeria* sp.	*Strongyloides papillosus*	*Strongylidae type* *
class	Cestoda	Nematoda	Nematoda	Protozoa	Nematoda	Nematoda
superfamily	Anaplocephalidae	Trichuroidea	Trichostrongyloidea	Eimeriidae	Rhabditoidea	for example Trichostrongyloidea
life cycle, infection by	intermediate host: free-living pasture mites	direct L_1	direct L_3	typically coccidian	both parasitic (partenogenesis) and free-living reproductive cycles	direct L_3
location	small intestine	cecum & colum	small intestine	small intestine	small intestine	abomasum small intestine
importance, clinical signs	relatively nonpathogenic, anecdotal reports that heavy infection may cause reduced growth in young animals only tapeworms of ruminants in many countries of western Europe	rarely pathogenic, overwhelming infection may cause a diphtheritic inflammation of the caecal mucosa or a fetal hemorrhage into cecum, diarrea associated with heavy infection	do not usually cause clinical disease, acute diarrhea in young animals	some pathogenic species cause clinical coccidiosis. Young animals: bloody diarrhea, death. Adults: decreased production, diarrhea	usually no clinical significance, heavy infection in young animals can cause severe diarrhea, anorexia, loss of weight or reduced growth rate, transmammary transmission possible	* for example *Ostertagia*, *Trichostrongylus*... - common infection, asymptomatic infections - young, nonimmune animals are most susceptible to subclinical and clinical disease (diarrhoea, anaemia, hypoproteinaemia, reduced growth...)
main morphological features	Thick shell Irregular shape, tri- or quadrangular	Thick-walled Lemon-shaped with polar plugs	Thin shell Ellipse 2-8 large cells surrounded by wide fluid-filled cavity	Thin wall Oval or round	Thin shell Broad ellipse Embryonated, L_1 larva present	Thin-shelled Oval contain morula
size	Size 80 - 90 μm	Length 70-80 μm Width 30-42 μm	Length 150 - 260 μm Width 67- 120 μm	Length 12 - 45 μm	Length 40 - 60 μm Width 32 - 40 μm	Length approximately 65-100 μm Width 34 -50 μm
presence in livestock	cattle sheep	cattle sheep	cattle sheep	cattle sheep	cattle sheep	cattle sheep

2.3.1.2   Baermann sedimentation method

We took 10 grams +/- 0.1grams of faeces and wrapped them in a piece of gauze tied off with a plastic string. We suspended our sample over a plastic champagne glass with a hollow stem, filled with tepid water (37 - 40º C). The faecal parcel, wrapped in gauze, was submerged in the water. We placed a plastic bag over the glass to decrease the evaporation overnight. We left the faeces in the water for at least 12 h at room temperature.

After minimum12 h, we removed the faeces and almost all of the water from the top of the glass, ensuring not to disturb the bottom 5-10ml of the suspension. We kept the bottom suspension which we poured into a 14 ml tube and centrifuged at 1500 G for 5 minutes. After centrifuging we removed the supernatant to the 1 ml mark, then we mixed the remaining sediment and with a micropipette, we took out a 100 µl sub-sample. This sub-sample was placed on a microscope slide and examined using a microscope at 100x magnification. We counted and identified larvae based on their morphological features either to species level (*Dictyocaulus viviparus*) or just as a category of larvae (S-Shaped "dorsal-spine larvae" (*Varestrongylus* spp*., Elaphostrongylus* spp), or GI-hatched larvae) (Table 4, Appendix 3). After identification, we disposed of the microscope slide.

Table 4: Identified parasite larvae found in the faeces (Foreyt, 2001; Franzzman & Schwartz, 2007; Taylor at al., 2007; Zajac & Conboy, 2012)

	Dictyocaulus spp.	*Varestrongylus* spp. (*S-shape*)	*Elaphostrongylus* spp. (*S-shape*)
class	Nematoda	Nematoda	Nematoda
superfamily	Trichostrongyloidea	Metastrongyloidea	Metastrongyloidea
life cycle, infection by	direct L_3	indirect intermediate host: mollusc	indirect intermediate host: mollusc
location	lungs (trachea, bronchi and bronchioles)	lungs	spinal cord, brain, muscle
importance	heavy infections may cause severe respiratory signs Disease seen in young animals, before immunity develops	pulmonary oedema, emphysema and inflammation of the lungs, secondary bacterial infection can lead to pneumonia, emaciation and death	Nonpathogenic in many hosts, but may cause neurological diseases (meningitis and CNS disease)
morphological features	Round and chubby tail Length 300-580 µm	bend or crook near the end of the tail	bend or crook near the end of the tail
presence in livestock	*D.viviparus*: cattle *D. filaria*: sheep and goats		(sheep and goats)[1]

[1]*Elaphostrongylus* spp. do not complete their lifecycle in sheep and goats but infection can cause significant clinical disease (neurological symptoms) in these aberrant hosts (Handeland 2002)

2.3.2   Postmortem examination

Necropsy is an important method for diagnosing parasitism of the digestive tract (Hendrix & Robinson, 2006). In our case we decided to examine the abomasum and caecum using a sieving method. Both parts were collected on the field but washed in the laboratory directly after the field work. We washed both parts in separate buckets. After washing we estimated the number of worms present by counting an aliquot (a known percentage of the total volume) of solution (content plus water). Worms found were preserved for identification in 75% ethanol.

2.3.2.1   Abomasum

We washed the abomasal content and the internal wall thoroughly with running water until reaching a volume of 2 L. If the volume after washing was larger than 2 L, we waited at least 30 minutes to allow the content to sediment. This enabled us to withdraw the sediment back to the 2 L. Six adult females (from 3.5 till 8.5 years old) had very large abomasa with their content itself exceeding 2 L. In this case, we used as much water as necessary to wash the abomasal wall until it was clean (between 2.5 L and 5 L). In all abomasa we noted the final volume of solution (between 2 L and 5 L), and this volume was later used to calculate the final adult abomasal nematode count. In all abomasa we, after washing, stirred the content to get a homogeneous suspension and then we, in all cases neglecting the final volume, quickly took a subsample (100 ml) from the middle of the content. If the final volume was 2 L, 5 percent of aliquot was taken (100ml of 2L), if the final volume was larger (max 5L), percent of aliguot taken was smaller (2% if 100ml of max 5L). Nevertheless, we always used the final volume to calculate the final abomasal count.

After taking the subsample (100 ml), we poured it into two 50 ml tubes, first tube was marked A, the second one was marked B. The tubes were allowed to stand and sediment for 30 minutes prior to pipetting off the excess water, taking care not to disturb the sediment. The tube was then refilled with 75% ethanol. The subsamples were stored in the freezer until examination. We also stored the washed abomasums in the freezer, at -18 °C for future analysis (arrested larvae in the mucosa).

The 100 ml subsamples were carefully examined under a magnifying lamp with 40x magnification. We searched for adult nematodes which we counted - the final total estimate (count) was based on an examined subsample representing from 2% till 5% of total abomasal

content - and divided the genders based on their sexual organs. Male adult nematodes were later used for species identification. We identified only 50 males from tube A, if present or all males present if there were fewer than 50. Adult males were identified by placing male nematodes in polyvinyl lactophenol (Chemi-Teknik AS, Oslo, Norway) for 2-5 minutes to clear the parasite and allow the relevant morphological features to be identified using microscopy.

The following moose abomasal parasites were anticipated (based on unpublished data of Giulio Guidi and Rebecca K. Davidson) with the major morphotype listed first followed by the minor (Drózdz,1995):

- *Ostertagia leptospicularis / O. kolchida*
- *Spiculopteragia alcis / S. dagestanica*
- *Ostertagia antipini / O. lyrataeformis*
- *Spiculopteragia boehmi / S. mathevossiani*
- *Teladorsagia circumcincta / T. trifurcata / T. davtiani*
- *Trichostrongylus axei*

Description of the most prevalent species with drawings are given in Appendix 3.

2.3.2.2 Caecum

The procedure of washing the caecum was the same as washing the abomasum - the caecum was washed thoroughly with water until 2 L of volume was reached. Because parasites in the caecum can be seen without the use of a magnifier we poured the subsample of 100 ml through a sieve (200mm*50mm, 1mm) and we scanned the precipitate for *Trichuris sp.* (Table 3, Appendix 3). Any *Trichuris* sp. found were stored in 75% alcohol.

2.4 Assessement of host physical condition

Physical condition of all culled moose was evaluated. We have measured a "short term" and "long term" body condition. "Short term body condition" (STBC) or an indication of nutritional status in animals (Stephensen et al., 1998) was estimated by hunters - they estimated the amount of body fat around the kidneys and heart and categorized animals accordingly in three condition groups - poor, normal and good (Appendix 1, Appendix 2, Table 5). Because lipids are primary energy stores of the body, estimation of the amount and location of these reserves provide an indication of nutritional status in animals (Stephensen et al., 1998). Assessment of relative fatness of a carcass was based on a field protocol written by Kistner et al. (1980).

"Long term body condition" (LTBC) was estimated from a linear regression model of natural logarithm of slaughter weight against natural logarithm of jaw bone length (logic slope $\pm$ SE= 2.399683 $\pm$ 0.170882) and age and gender interaction included (slope $\pm$ SE = 0.045369 $\pm$ 0.013241). Slaughter weight is weight of carcass without head, skin, lower parts of the legs and viscera and it is believed to be approximately half of the total animal weight (Saether, 1983). Jaw bone length (total length) was measured in the laboratory using a regular wooden measure. After measuring, I took out the – incisor teeth, which were used to determine the age of the animals (by Stig Tronstad Haugen, Hint, Norway; estimation procedure written in Gilbert (1966)). Residuals gained from the best model ($F_{4,38}$=126.1, p-value < 0.001) were used to categorized animals in two categories of long term body condition index - good (above the expected) or bad (under the expected) which was later used in different analyses (Table 5, Appendix 2). There was no significant correlation between the long term body condition and the hunters short term condition estimate (spearman r= 0.14, p= 0.361).

Table 5: Number of animals in different age classes and different gender, with poor, normal and good Short term body condition index (STBC) and good/bad Long term body condition index (LTBC) estimations.

		CALF		YEARLING		ADULT	
		female	male	female	male	female	male
STBC (N=48)							
	poor	1	0	0	0	0	0
	normal	4	5	3	6	8	14
	good	0	0	0	0	4	3
LTBC (N=43)							
	bad	2	3	2	4	4	5
	good	2	2	0	2	6	11

2.5 Statistical analysis

Data were analyed using R x 64 3.0.1 (R Development Core Team, 2013) and Excel programs.

All the models were selected by backwards selection where all the non-significant (p > 0,05) variables were removed. The significance of the models were compared either with "F" test - normal linear regression; or "Chi" test - generalized linear models (e.g. binomial or poisson distribution). In the latter case, where data were overdispersed I used the F-test.

2.5.1   Parasite prevalence

Parasite prevalence is a population level measure of parasitism. It was the number of moose infected / number of moose examined, expressed as a percentage (Margolis et al. 1982). The difference in prevalence between different age and gender groups of host animals was tested using generalized linear model with binomial errors and a logit link function. The response variable was the proportion of infected animals and explanatory variables used were age class, gender of animals, study area and the interaction between gender and age class. We tested the prevalence of eggs of *Moniezia* sp., "Strongylidae type", *Strongyloides papillosus* and *Eimeria* sp.. Prevalence of larvae was presented descriptively because of the high proportion of zeros (uninfected host animals). The prevalence of some of the speciated adult stage abomasal parasites (*Ostertagia antipini, Ostertagia leptospicularis* and *Spiculopteragia alcis*) was also tested statistically using the same approach as for eggs.

2.5.2   Probability of infection

Factors affecting the probability that an individual was infected with a specific parasite were identified using generalized linear modelling with binomial errors and a logit link function (binomial distribution). The analysis was an ANCOVA with a binary response variable - infected (1) or un - infected (0). Explanatory variables used were gender and age of host animals, study area, STBC, LTBC, slaughter weight and interaction between age and gender. The probability of infection was investigated for *Moniezia* sp., *Eimeria* sp., "Strongylidae type" eggs and S-shape larvae.

2.5.3   Intensity of infection

Intensity of infection is an individual measure of parasite abundance. It was the total number of either eggs per gram (EPG) of Strongylidae type eggs or the total number of adult stage abomasal worms found in a particular host individual / total number of host - animals examined (Margolis et al. 1982)

I investigated the distribution frequency of Strongylidae type EPG and adult abomasal nematodes using histograms. Based on their resemblance - shape - I have decided to test infection intensity using generalized linear models with poisson errors and a logit link function (poisson distribution). The response was the intensity of infection (total number (count)) of "Strongylidae type" eggs (EPG) and adult abomasal nematodes. The explanatory variables were age and gender of animals, study area, short term body condition estimate from

hunters, long term body condition index, slaughter weight and interaction between age and gender.

I have also investigated the difference in infection intensity with adult abomasal nematodes among adult female moose with regard to their lactation status (non-lactating (N=9), lactating (N=3)).

2.5.4 Correlation between EPG and adult female abomasal nematodes counts

I have investigated the correlation between faecal count of Strongylidae type eggs and the total count of adult female nematodes found in the abomasum. I have used the female nematode count only, because this is the sex producing eggs. The correlation test used was Spearman's rank correlation based on few observation, because of uncertainty about the distribution and monotonic measurements.

3 RESULTS

3.1 Prevalence

3.1.1 Prevalence (%) of gastrointestinal parasite eggs in moose faeces

Parasite egg prevalence across all three study areas varied from 0 to 100%, depending on parasite species, geographic area and age class and gender of moose examined (Table 6).

Across all study areas, the prevalence of *Moniezia* sp. was strongly associated with calves - Figure 2 (logit slope ± SE= 23.830 ± 9505.8, $\chi^2_{2,11}$=29.36; p < 0.001), though the difference in prevalence among female and male calves was not statistically significant (p = 0.530).
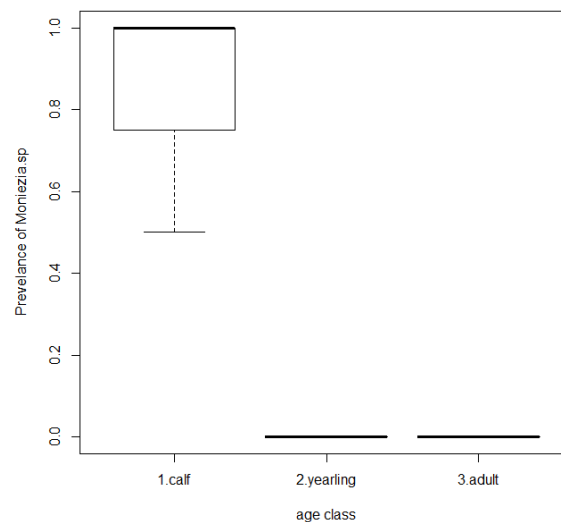

Figure 2: Prevalence (%) of *Moniezia*.sp in different moose age classes (N=45) in Hedmark county. Box plots show the spread of data from 25th-75th percentiles, black lines represent medians, and whiskers represent min and max values

Strongylidae-type eggs were present in all moose age classes and in both genders and the prevalence varied from 33.3 to 80%. The prevalence of *Strongyloides papillosus* varied from 0 to 50%, the eggs were present in all moose age classes and in both genders except in male calves. *Eimeria* sp. eggs were found in all moose age classes and in both genders except in male yearlings, the prevalence varied between 0 to 40%.

In none of the cases above were the relationships between prevalence and age class, prevalence and gender or prevalence and study area statistically significant (p > 0.05; Table 6).

Table 6: Prevalence (%) of gastrointestinal parasites (egg stage) in moose faeces (N=45).

Age class	Gender	N	*Moniezia sp.*	*Trichuris sp*	*Strongyloides papillosus*	*Strongylidae type*	*Nematodirus ssp.*	*Eimeria sp.*
calf	female	4	100	0	50	50	0	25
	male	5	60	0	0	40	0	40
yearling	female	3	0	0	33.3	33.3	0	33.3
	male	7	0	0	28.6	71.4	0	0
adult	female	11	0	0	18.2	63.6	0	18.2
	male	15	0	6.7	13.3	80	6.7	20

3.1.2 Prevalence of gastrointestinal parasite larvae in moose faeces

Parasite prevalence of hatched larvae across all three study areas varied from 0 to 75%, depending on the category of hatched larvae, age class and gender of moose. Prevalence of hatched GI larvae varied from 0 to 30% with highest being in adult female moose. The prevalence of S-shaped hatched larvae varied from 0 to 75%, with the prevalence being highest in calves (Table 7).

Table 7: Prevalence (%) of gastrointestinal parasites (larval stages) in moose faeces (N=41).

Age class	Gender	N	hatched GI larvae	*Dictyocaulus* larvae	S-shaped larvae
calf	female	4	25	25	75
	male	5	0	0	60
yearling	female	2	0	50	0
	male	6	16.7	16.7	33.3
adult	female	10	30	0	0
	male	14	14.3	0	7.1

3.1.3 Prevalence of abomasal parasites in moose by nematode species

Prevalence of abomasum nematode species varied from 0 to 100% depending on age class and gender of moose examined (N=30) (Table 8).

The most prevalent nematode species found in the abomasa were *Ostertagia antipini* and *Spiculopteragia alcis*. Both species were present in all age classes and in both genders of animals examined.

Table 8: Prevalence (%) of different abomasum nematode species found in moose (grouped by age class) (N=30)

Age class	Gender	N (30)	*Ostertagia leptospicularis*	*Ostertagia antipini*	*Ostertagia kolchida*	*Ostertagia* sp.	*Spiculopteragia alcis*	*Spiculopteragia* sp.	*Teladorsagia circumcincta*
calf	female	4	25	75	0	25	25	0	0
	male	5	40	100	0	0	60	0	0
yearling	female	3	0	100	0	0	66.7	0	0
	male	7	0	85.7	0	14.3	100	0	0
adult	female	8	37.5	100	12.5	12.5	100	0	12.5
	male	3	100	33.3	0	33.3	100	33.3	0

The prevalence of *Ostertagia antipini* varied from 33.3 till 100 %, being lowest among adult males and highest among male calves, female yearlings and adult female (all 100%). Age class and gender were both significant factors for the prevalence of *Ostertagia antipini* ($\chi^2_{2,9}$=8.33 and $\chi^2_{1,8}$=6.25; p = 0.015 and 0.012, respectively), together with their interaction ($\chi^2_{2,6}$=6.28, p = 0.043). Prevalence was positively associated with yearling age class (logit slope ± SE = 18.3788 ± 6858.5) and negatively with male gender (logit slope ± SE = -3.4657 ± 1.3229).

The prevalence of *Spiculopteragia alcis* varied from 25 till 100%, being lowest among female calves and highest among yearling males and adults of both sexes (all 100%). The prevalence was significantly affected by the interaction between age class and gender ($\chi^2_{2,6}$= 7.93, p = 0.018). In general odds of infection were lower for male adults (logit slope ± SE = -3.584 ±1.814).

The prevalence of *Ostertagia leptospicularis* varied from 0 to 100%, being lowest among both genders of yearling age class (0%) and highest among adult males (100%). None of the correlations between prevalence of *Ostertagia leptospicularis* and age class, gender or study area was statistically significant (p > 0.05).

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To summarize the important parasite prevalence results: among all age classes, calves were the one having the highest prevalence of *Moniezia* sp. and S-shaped larvae. *Moniezia* sp. were not found in yearlings and adults, but instead these animals had high prevalence of *Strongyloides papillosus* and Strongylidae-type eggs. All animals examined had at least one species of abomasal parasites present, with the most prevalent being *Ostertagia antipini* and *Spiculopteragia alcis*.

3.2   Probability of individual infection

3.2.1   Probability of infection with selected GI parasite eggs

The only significant factor associated with an increased probability of infection of an individual host with *Moniezia* sp. was its age (logit slope ± SE= -21.07 ± 3850.39, $F_{1,42}$=135.44, $p < 0.001$; Figure 3a). Mean age of infected animals was 0.5 years, median of un-infected animals was 2.5 years (min: 0.5 years; the spread of data from 25th-75th percentiles: 2.5 - 4.5 years; max: 13.5 years). Other explanatory variables, including indicators of body condition (SHBC, LTBC, slaughter weight) as well as gender, study area and interaction age*gender, were not significant (all $p > 0.05$).

The only significant factor associated with the increased probability of infection with Strongylidae type eggs was study area ($\chi^2_{2,40}$=6.73, $p = 0.034$; Figure 3b) while other explanatory variables were not significant (all $p > 0.05$).

None of the fitted explanatory variables explained significant variation in the probability of an individual being infected with *Eimeria* sp. (all $p > 0.05$).
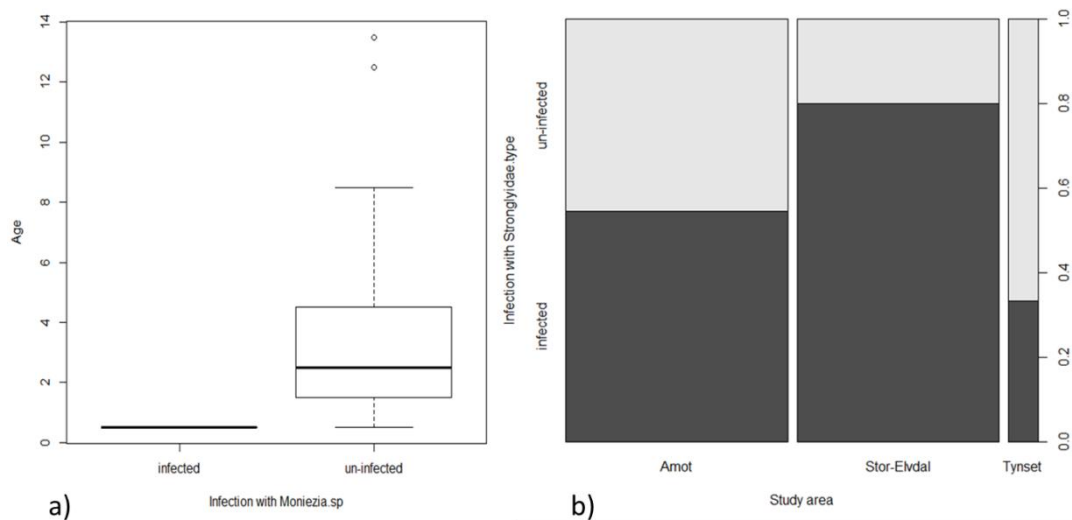

Figure 3: Different factors significantly affecting the probability of infection of *Moniezia sp* (Figure 3a) and Strongylidae type (Figure 3b) found in the faeces (all $p < 0.01$). Box plots Figure 3a show the spread of data from 25th-75th percentiles, black lines represent medians, and whiskers represent min and max values.

3.2.2 Probability of infection with S-shaped larvae

Probability of infection with S-shape larvae was lower in older animals and the probability increased with decreasing age of animals (logit slope $\pm$ SE= -2.5024 $\pm$ 0.7992, $F_{1,36}$=36.09, p < 0.001; Figure 4a). The probability of infection was related with animal body condition, animals with poorer LTBC had a higher probability of infection (logit slope $\pm$ SE= -2.4389 $\pm$ 1.0239, $F_{1,35}$=7.57, p < 0.001; Figure 4b).
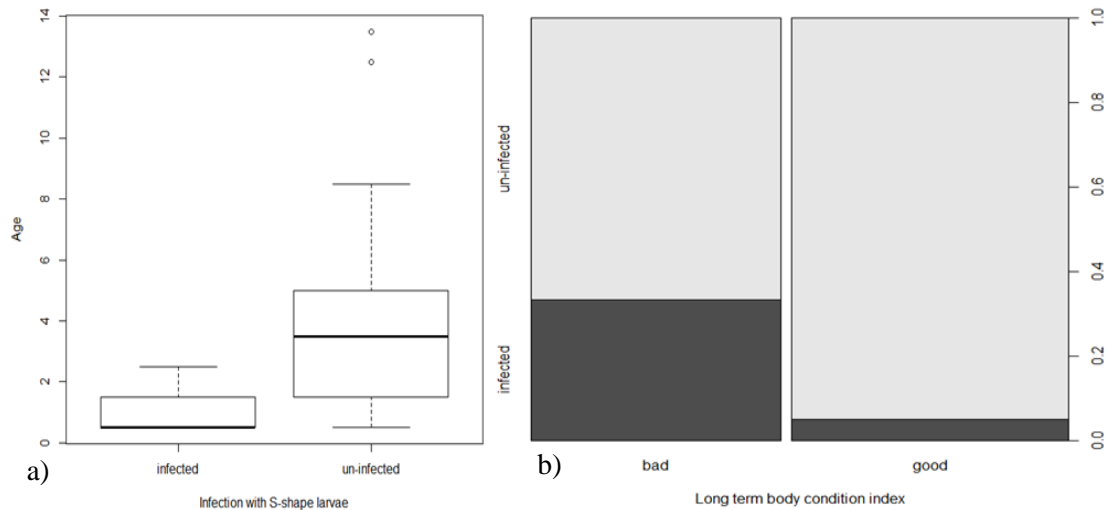

Figure 4: Different factors significantly affecting the probability of infection with S-Shape larvae (all p < 0.01) Box plots in Figure 4a show the spread of data from 25th-75th percentiles, black lines represent medians, and whiskers represent min and max values.

3.3   Intensity of infection

3.3.1   "Strongylidae type" eggs

Intensity of " Strongylidae-type" egg infection ranged from 0 till 1716 EPG, depending on age class and gender of animals (Table 9).

Table 9: Intensity of infection (EPG) with "Strongylidae-type" eggs found in moose faeces (N=45) (Q1- first quartile or the 25% spread of data; Q3- third quartile or the 75% spread of data)

Age class	calf		yearling		adult	
Gender N	female (4)	male (5)	female (3)	male (7)	female (11)	male (15)
min	0	0	0	0	0	0
Q1	0	0	0	39	0	78
median	39	0	0	78	78	156
Q3	78	234	78	78	78	234
max	78	312	156	156	312	1716

After dropping out the single outlier of 1716 EPG (7.5 year old male, 277 kg, STBC: Normal, LTBC: Good) - the nearest value of outlier was 312 EPG - the only significant factor for the intensity of " Strongylidae-type" infection left was gender ($\chi^2_{1,40}$=490.82, $p < 0.023$, Figure 5). The intensity of infection was higher in males (logit slope $\pm$ SE= 0.82844 $\pm$ 0.38732). Other explanatory variables, including indicators of body condition (SHBC, LTBC, slaughter weight) as well as age, study area and interaction age*gender, were not significant (all $p > 0.05$).
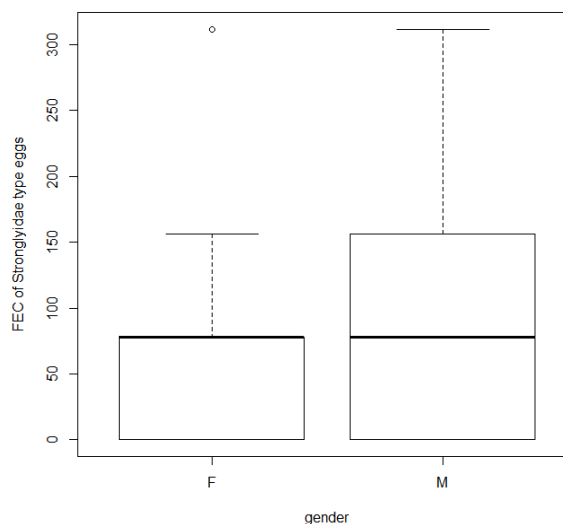

Figure 5: Intensity of infection (EPG) with "Strongylidae type" eggs found in moose faeces (N=45) depending on gender. Box plots show the spread of data from 25th-75th percentiles, black lines represent medians, and whiskers represent min and max values - outlier of 1716 EPG is excluded.

3.3.2 Adult abomasal nematodes

Intensity of infection with the adult stage of abomasum nematodes ranged from 60 till 56000 nematodes, depending on gender and age class of host animals (Figure 6).
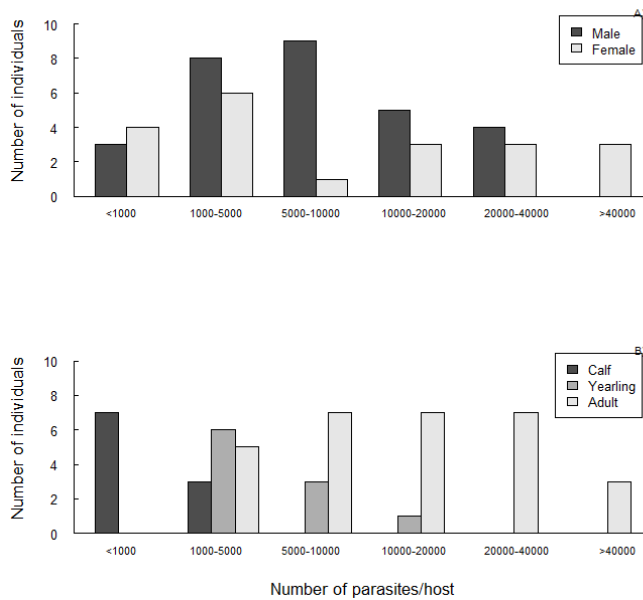

Figure 6: Frequency distribution of host individuals in relation to the number of adult worms per host. Figure 6A shows distribution in male (N=29) and female (N=20) moose and Figure 6B shows distribution in calf (N=10), yearling (N=10) and adult (N=29) moose.

Highest number of adult worms were found in adult females, and lowest number of adult worms were found in female calves (Figure 7).
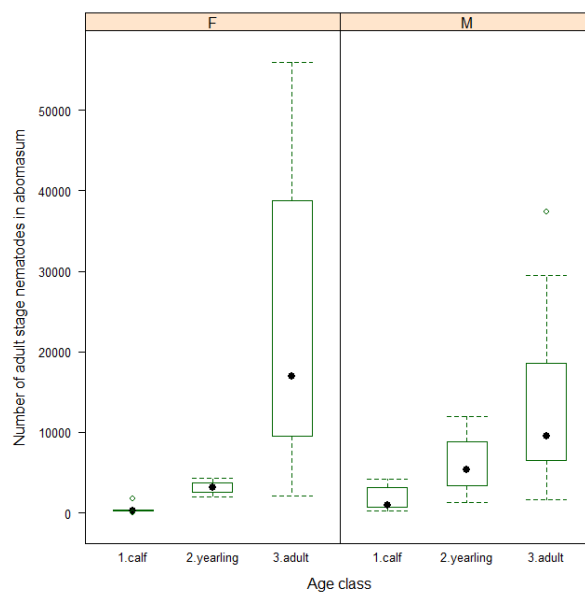

Figure 7: Intensity of infection with adult stage abomasal nematodes in female (F) and male (M) host moose (box plots show the spread of data from 25th-75th percentiles black circles represent medians, and whiskers represent min and max values).

The intensity of adult stage abomasal nematodes infection was significantly affected by age ($\chi^2_{1,41}$=272212, p < 0.001) and slaughter weight ($\chi^2_{1,39}$=44642, p < 0.001) of animals. The intensity of infection was lower in males (parameter estimate ± SE= -0.777 ± 0.297), and increased with slaughter weight (logit slope ± SE= 0.011 ± 0.003) of animals. The intensity of infection was lower in individuals with good index of long term body condition (parameter estimate ± SE= -0.457±0.234, $\chi^2_{1,38}$=23641, p < 0.051) (Figure 8).

a)

b)

c)

d)
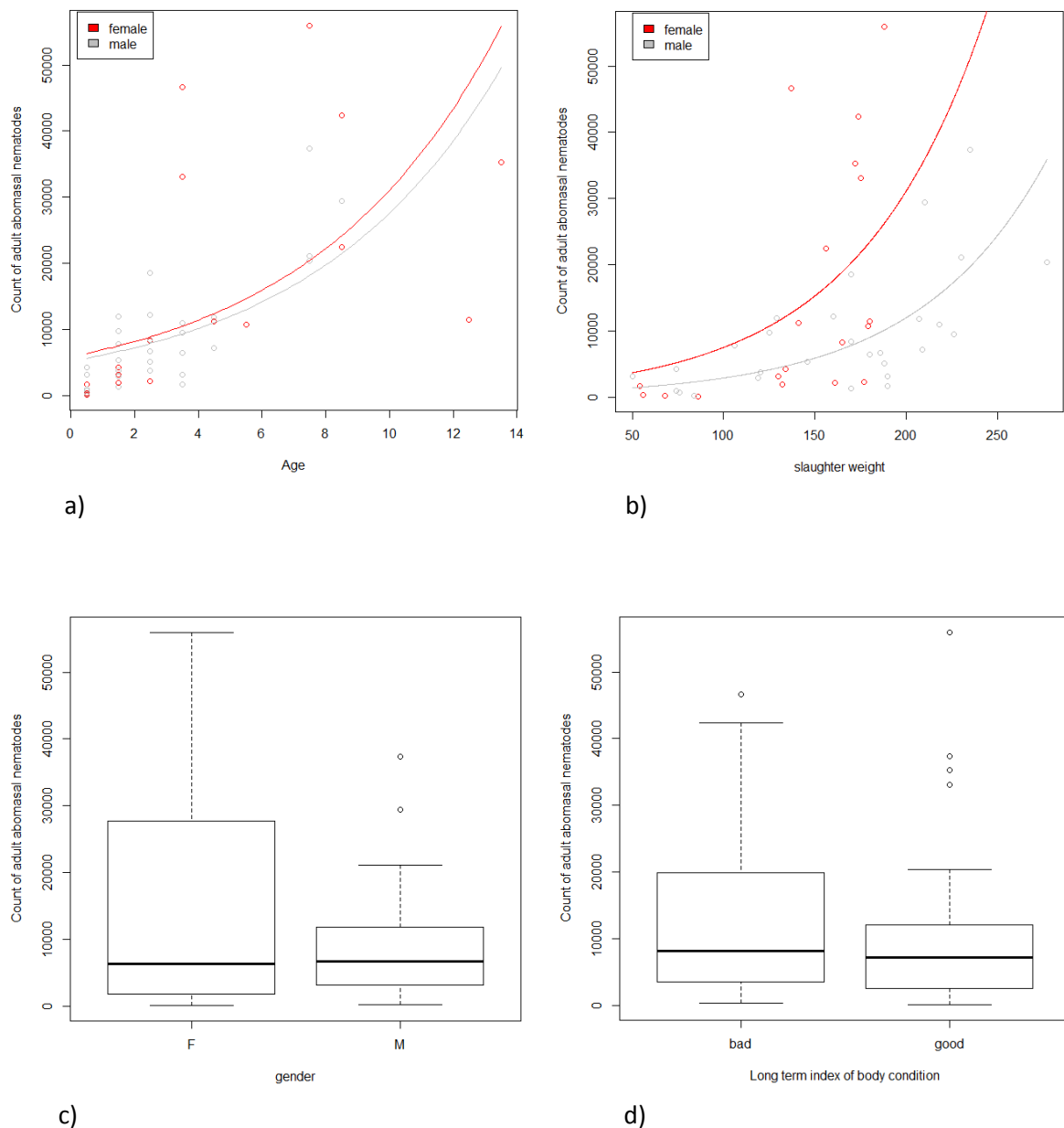
Figure 8: Intensity of infection with adult stage of abomasal nematodes (N=49) depending on age (Figure 8a), slaughter weight [kg] (Figure 8b), gender (Figure 8c) and long term index of body condition (Figure 8d). Lines in Figures 8a and 8b are fitted lines using model predictions, red and gray, for females and males respectively. Box plots in figures 8c and 8d show the spread of data from 25th-75th percentiles, black lines represent medians, and whiskers represent min and max values.

Figure 9, represents the relative importance of age class and the gender of animals in explaining variation in intensity of infection with adult stage abomasal nematodes. From the figure we can conclude that the age class of the animals is more important than their gender.
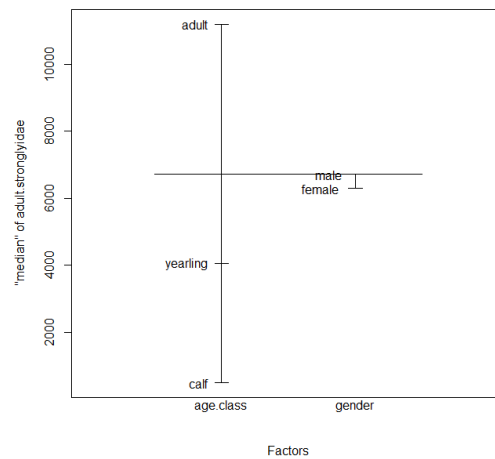
Figure 9: Factors influencing the intensity of infection with adult stage of abomasal nematodes (N=49)

The intensity of adult stage abomasal nematodes infection in adult females was not significantly affected by lactation status ($\chi^2_{1,10}$=1789.2, p < 0.739) .

3.4   Correlation between Strongylidae egg and adult counts

The correlation between the number of " Strongylidae type" eggs in the faeces and adult stage nematode worms in the abomasum (only female adult nematodes included) was positive (Spearman, r= 0.424, p= 0.003; Figure 10) for all host animals (N=45).

Figure 10: Relationship between logarithm of Strongylidae type eggs and logaritmn of adult worms count - female worms.

4   DISCUSSION

" Because moose live normally at relatively low density
levels and in colder northern latitudes, the heavy
gastrointestinal infestations frequently encountered in animals
such as deer are probably uncommon".

Anderson (1975)

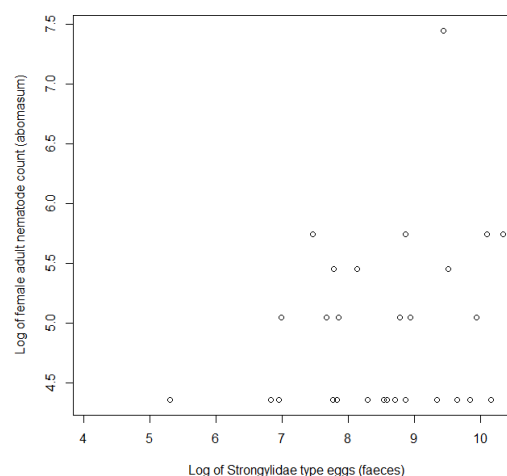This quote was taken from an article written about disease and management of moose living in North America. Even though the article is almost 40 years old and it discusses parasites in moose from North America, I have decided to use this quote as an opening statement, because the findings in my study of moose gastrointestinal helminths here in Norway, reveal a completely different story.

A brief summary of my findings reveals that, in general, the prevalence and infection intensity of eggs, larvae and adult stage of helminth parasites in moose of different age classes and genders examined, were high. The highest prevalence was found for adult nematodes in moose abomasa - all animals examined were infected with them. Reasons for high prevalence and intensity of infection, which differed from Anderson's (1975) belief, are discussed below. One important finding, that should be clearly stated at the beginning is, that when it comes to estimation of parasite prevalence of host animals, the reliability of data gained from postmortem examinations is much higher in comparison with the data from their faeces. This should be kept in mind when new research on gastrointestinal parasites is planned in the future. Furthermore monitoring should be standardized and repeated at regular intervals and across seasons.

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4.1   Faecal analysis

Even though most of the "egg stage" prevalence results were not statistically significant, moose of all age classes examined in Hedmark county, were infected with either *Strongyloides papillosus*, *Eimeria* sp. and Strongylidae type eggs. The later were also found by Milner et al (2013a) in their study in 2009 and 2010 of moose from Hedmark county. Milner et al. found, not surprisingly, that the faecal egg count varied depending on year and month of sampling. The prevalence of Strongylidae type eggs in adult moose in January 2009 was similar to that in October 2013 (74% and 72%, respectively), but the prevalence in calves

in January 2009 was higher (65%, 45%, respectively). Milner at al. found a higher prevalence of *Nematodirus* sp. in 2009 and 2010 than was found by this study but the prevalence of *Trichuris* sp. was similarly very low. Milner et al. did not detect *Moniezia* sp., *Eimeria* sp, or *Strongyloides papillosis* eggs in the faeces of 106 animals examined.

Annual and seasonal fluctuations in fecal egg output (production of eggs by adult female nematodes), which may affect faecal egg counts (FEC, Houtert & Sykes, 1996), are well known phenomena, with multiple causes (Taylor et al., 2007). Firstly, egg output of gastrointestinal parasites depends on parasite fecundity - some nematode parasites can produce many thousands of eggs per day, others like *Trichostrongylus* produce less, only a few hundred eggs daily. For example *Ostertagia ostertagi* in cattle during summer produces between 20 and 320 epg/day (Stien et al., 2002b). Secondly FEC will depend on host immunity which can decrease egg production of existing adult worms or modify the development of new infections either by their destruction or arrest at the larval stages (Taylor et al., 2007). Host immunity affects egg production of *Ostertagia circumcincta* indirectly, by having an impact on worm number and worm size (Stear et al., 1995). Because the number of eggs is positively correlated with the size of female worms any inhibition of worm development decreases egg production (Stear et al., 1995; Houtert & Sykes, 1996; Stien et al., 2002b). If host immunity is depressed, either because of malnutrition, stress, concomitant disease, pregnancy, lactation or other factors, the egg output of parasite species increases (Taylor et al., 2007). Season is a very important factor which causes fluctuations in egg output and consequently FEC. Some of the species of *Ostertagia* genus found in ruminants of northern hemisphere, start their hypobiosis (arrested or inhibited development) in the autumn (cited after Almería et al., 1996) because of adverse environmental conditions - low temperatures during cold winter. Transmission rates are commonly low in late autumn - winter period due to decrease in egg production (Almería et al., 1996; Eysker, 1997; Halvorsen et al., 1999; Stien et al., 2002b; Taylor et al., 2007; Boey et al., 2011) and because of reduced survival and development rate of the free-living stages (Halvorsen et al., 1999). It is estimated that 91% of all egg output takes place in three months, from the beginning of June to the end of August (Stien et al., 2002b). Climatic conditions during this period are believed to be the most optimal (Taylor et al., 2007; Kutz et al., 2012). The most favourable conditions for development of free-living stages are found during mild and wet weather (Halvorsen et al., 1999). The most important environmental factors for a high success rate and speed of development are: precipitation, moisture in the faeces, soil moisture and temperature

(O´Connor et al., 2006; Khadijah et al., 2013). Alterations of egg output could also be a consequence of changes in host diet quality or quantity, intake of tannin-rich forages, daily volume of faeces produced by the host or changes in feed intake or host metabolism (diarrhea) resulting from parasitism itself (Houtert & Sykes, 1996; Taylor et al., 2007; Zajac & Conboy, 2012). Wilson et al. (2002) mentioned repeatability of parasite counts as being one of the factors influencing FEC variation - repeatability of FEC of samples collected 2-3 days apart was around 75%. They concluded that individuals varied in their faecal egg production from one day to the next and in order to accurately determine heterogeneities in parasite loads multiple samples over several days may be required.

A reason why different parasite species were found in the faeces examined in the two moose studies conducted till now in our study area could be, next to reasons pointed before, associated with the fact that material used in 2009 and 2010 was frozen before parasitological examination (Milner et al., 2013a). It is known that if frozen fecal material is used, parasite egg abundance is biased low because of damage on the outer egg shell which causes decrease in egg flotation ability (Zajac & Conboy, 2012). Even though we cannot make final conclusions about parasite prevalence only from egg counts, we must in order that the data gained from this method are trustworthy, use fresh fecal material or material stored at low T (4°C). At this temperature faeces can be stored for at least two months with minimal development (Foreyt, 2001).

4.1.1 *Moniezia* sp. infections

The only statistically significant difference in the prevalence of egg stage of parasites found in moose faeces was for *Moniezia* sp., which was only found in calves. *Moniezia* sp., is a tapeworm, that requires an intermediate host - oribatid mite (Acarina) - and a definitive host to complete its cycle. Oribatid mites are important components of the soil fauna and have a cosmopolitan distribution. Forage mites ingest the eggs and after 1-4 months embryos develop to so called cysticercoids. Infection of the final host is by ingestion of infected mites during grazing. Seasonal fluctuations in the incidence of *Moniezia* sp. infections can be related to activity periods of the forage mite vectors during the summer in temperate regions (Taylor et al., 2007). Genus *Moniezia* sp. can be found all over the world and can cause economical losses due to infections in domestic ruminants (Denegri et al., 1998). The genus includes six main species (Chroust, 1998) with two of them being the most important: *M. benedeni,* mainly infecting cattle and *M. expansa* principally infecting sheep (Denegri et al., 1998).

Moniezia sp. is reported commonly from moose as well (Hoeve et al., 1988). Infection with *M.benedeni* is common in cattle calves during their first year of life and less common in older animals (Taylor et al., 2007). Hansen et al. (1950) found out that lambs infected with *M. expansa* were retarded in growth compared to a similar group of uninfected ones. Even though it is believed that *Moniezia sp*, in case of heavy infection, reduce growth in young animals (Table 3), our results showed that infection intensity was not correlated with either LTBC, STBC or slaughter weight of animals. The only significant variable was age. Reasons why there was such a distinct decline in *Moniezia sp*. prevalence with age could indicate that older animals acquired immunity towards this tapeworm. Acquired immunity (alt. adaptive immunity) is long lasting and specific protection against re-infection that follows previous exposure or infection by a pathogen or by immunization against it (Lawrence, 2008). In case of nematode infections, immune response depends on antigenic stimulation by secretory or excretory products released during the development of the L_3 larvae to the adult (Taylor et al., 2007). Acquired immunity acts to decrease parasite establishment, survival, reproduction and maturation (Wilson et al., 2002). In the case of acquired immunity, highest infection levels should occur in juveniles experiencing their first invasion (Thomas et al., 2005).

In general immune response of vertebrates to macroparasites and protozoan microparasites tend to be weaker compared to their immune response to bacteria and virus infection because the infections themselves tend to be persistent and hosts may be subject to repeated reinfection (Begon, 2007). Immune adult sheep may ingest around 50 000 *Ostertagia* sp. daily without showing any clinical signs of parasitic gastritis (Taylor et al., 2007). Because host defensive responses are costly - energy and material invested in response are diverted away from other important bodily functions, there must be a trade-off between the response and growth or reproduction of animals (Thomas et al., 2005; Wobeser, 2005; Begon, 2007). Under conditions of continuous infection, optimum resource allocation by the host allows tolerance of some parasitic infection - permitting a proportion of invading parasites to survive. The parasite burden tolerated depends on the costs of immunity - if nutrition of the host is poor, the investment in immune system declines and parasite burden increases (Thomas et al., 2005).

4.1.2 Protostrongylidae larvae

Prevalence of "Dorsal-spine" or "S-shaped" larvae was high for calves and yearlings and probability of infection was statistically correlated with decreasing age of animals. "Dorsal-spine" larvae are first stage larvae (L_1) with a dorsal, posteriorly directed spine (Taylor et al.,2007), and parasite species with this morphology of L_1 belong to Protostrongylidae family. These are pathogenic nematodes of free ranging and domestic ungulates across the world (Kutz et al,. 2012). Based on previous knowledge, species of Protostrongylidae larvae found in the moose faeces could belong either to *Elaphostrongylus* spp. or *Varestrongylus* spp. (Rebecca K. Davidson, "personal communication"). In both, species are transmitted indirectly, with definite hosts getting infected during summer through accidental ingestion of intermediate host - snails containing L_3 larvae (cited after Handeland & Gibbons, 2001). Definitive identification of the genus or species of Protostrongylidae from L_1 larval stages found in the faeces is not possible. Definitive identification of species is possible only from recovery of adult parasites or by applying molecular techniques and DNA sequencing (Kutz et al., 2007).

Adults of *Varestrongylus* spp. can be found in the respiratory system of their host. Eggs produced by the females hatch to release first stage larvae that move up the respiratory tract, are swallowed and passed in the faeces (Kutz et al,. 2012). *Varestrongylus sagittatus* parasitise mainly red deer and *Varestrongylus capreoli* mainly roe deer (Taylor et al., 2007). *Varestrongylus* sp. is a miniscule lungworm (1-2 cm) found deep in the airways or parenchyma of the lungs, with a broad range of hosts, naturally infecting muskoxen, caribou and rarely moose across most of arctic and subarctic North America (cited after Kutz et al., 2012). Abundance of this larvae L_1 in the faeces is usually low (Kutz et al., 2007). The general pathology and impacts of this parasite in its hosts are not yet known, till now no gross lesions in lungs have been observed (Kutz et al,. 2012).

Three species from *Elaphostrongylus* genus, *E. alces* (occurring in moose)*, E. cervi* (occurring in red deer), and *E. rangiferi* (occurring in reindeer), have similar first stage larvae and are hard to distinguish (Lankester et al., 1998). Different species are not strictly host specific (Franzzman & Schwartz, 2007). *Elaphostrongylus alces* in moose of Sweden and Norway was first described by Steen in 1989 (cited by Handeland & Gibbons, 2001), and is believed to be less pathogenic compared to the other two because its development takes place in the epidural space of the vertebral canal and not within the CNS. After ingestion, the L_3

larvae of *E. alces* migrates directly from the gut to the epidural space of the caudal vertebral canal where it develops to adult stage. During development, the nematode produces inflammation of the epidural tissue and spinal nerves. Development in the caudal vertebral canal is followed by some anterior dispersion of nematodes along the canal, and final migration into skeletal muscles (Lankester et al., 1998; Handeland & Gibbons, 2001). Infections with *Elaphostrongylus* spp. normally occur in summer and fall, and clinical disease can appear in the following spring (Stéen et al., 2005). There are different reports concerning pathogenicity of *Elaphostrongylus alces,* from being regarded as a moderately pathogenic parasite, which may reduce chance of moose surviving harsh winters (cited after Handeland & Gibbons, 2001) to being the cause of neurological disturbances, locomotive abnormalities, muscle atrophie and ataxia - the latter was observed in two 2 month old calves (Stéen & Roepstorff, 1990). Stéen et al. (2005) found out that elaphostrongylosis was the main cause of mortality of 18% (N=724) of moose examined in Sweden and that the disease was more common for younger animals.

I speculate, based on the high prevalence of "S-shaped" larvae among younger animals, that possibility of Protostrongylidae spp. observed belonging to the genus *Elaphostrongylus* is much higher that the possibility of it belonging to the genus *Varestrongylus*.

Prevalence of "dorsal spine" larvae among calves (N=9) from our study was approximately 67.5%. Prevalence with *E. alces* among calves (N=7) found by Handeland & Gibbons (2001) was higher, approximately 85%. Similarly high prevalence was found also in calves (N=54) from southern Norway, approximately 86% (cited after Handeland & Gibbons, 2001). Handeland & Gibbons (2001) examined 14 moose, 7 calves and 7 yearlings, and they found out that except 1 calf and 1 yearling all animals were infected, with adult nematodes found in either vertebral canal (mainly calves shot before the hunting season) or skeletal muscles (mainly yearlings, calves shot in October). They concluded that yearlings must have been infected during their first summer and have created immunological protection against new infection during the second one. The findings in the current study found a greater than 50% drop in prevalence between calves and yearling, providing further evidence in support of Handeland and Gibbons previous conclusions. The probability of infection with S-shaped larvae in our study was correlated also with poorer LTBC. Vicente et al. (2007) found that L_1 counts of *E. cervi* were negatively associated with red deer body condition.

4.2 Abomasal nematodes

Prevalence of adult worms in abomasa was 100%. All animals examined were infected. High prevalence of infection by abomasal nematodes, approaching 100% has been a common observation in all parasite surveys carried out among cervids (cited after Santin-Durán et al., 2004). There was no evidence of strong parasite aggregation in this current study population, with 37 % (N=49) of the animals examined carrying around 80% of the total parasite burden. Macroparasite aggregation hypothesis states that most host individuals are harbouring low number of parasites, but a few individuals are host to many. As a rule, generally less than 20% of individuals harbour 80% of the helminth parasite population (Wilson et al., 2002). Degree of aggregation influences regulatory role of parasites - parasites can regulate host population when only few individuals exhibit heavy infections (Tompkins et al., 2002). Factors contributing to the observed heterogeneities in parasite burdens among host animals are generated by variation between individuals in their exposure to parasite infective stages - seasonality and spatial heterogeneity - and by differences in their susceptibility - age, gender, genetic differences in susceptibility to infection, condition of the host, host behaviour, stress level etc. (Shaw et al., 1998; Wilson et al., 2002; Thomas et al., 2005; Begon, 2007).

One of the possible reason for the non-strongly aggregated outcome could be connected to the lack of large predators in our study area. It is believed that predators may predate prey with the highest parasite burden and by so doing decrease the parasite prevalence in the prey population and reduce long term parasite impact on host numbers (Hudson et al., 1992b). Animals with highest parasite burdens are responsible for most parasite transmission and play an important role in persistence of parasites (Wilson et al., 2002). With hunters being the main predators, the elimination of highly parasitized animals may not occur to the same extent as with large carnivores.

4.2.1 High parasite burdens detected

Infection intensity varied from 60 adult worms found in female calves to 56 000 adult worms found in adult females. Because this study is one of the first studies conducted on moose GI parasites, references regarding how many abomasal parasites moose in good body condition usually harbor were not available. For reindeer, Stien et al. (2002b) found between year fluctuations in number of abomasal parasites *Ostertagia gruehneri*. One year they ranged from 5 000-10 000, and the other year from 9 000-17 000 parasites per host. Range of abomasal parasites in caribou from Canada was 495-12 847 (Hughes et al., 2009) and mean

abundance of adult worms in female reindeer in late summer was 9 852 ± 4 562 (Irvine et al., 2000). Hoberg et al. (2001) wrote that between 12 000 - 15 000 adult *Ostertagia ostertagi* is sufficient to induce mortality in cattle calves less than one year of age. In adult cattle excess of 40 000 nematodes can be present in their abomasums and a minimum of 10 000 is considered necessary for development of severe gastric disease (cited in Hoberg et al., 2001). In the past it was suggested that abomasal parasite counts (APC) can be used as one of the parameters for estimating deer abundance, as high APC was correlated with high population density (Eve & Kellogg, 1977). Demarain et al. (1983) suggested that the use of APC as a health index is not practical because of multifactoral influence on it - host age and gender to name two. Hoberg et al. (2001) suggested that the use of values for intensity of infection and their linkage to pathogenicity must not be overinterpretated, based on different factors that may influence the interaction between hosts and parasites, including nematode species, host species, weight, age, immune status, nutrition, environmental setting (availability of forage and water, T°) etc. Nevertheless, larger host species are expected to sustain a greater absolute numbers of parasites (Morand & Poulin, 1998) and have, because their voluntary food intake requirements are higher, greater likelihood of infection (Gunn & Irvine, 2003).

Even though quantitative relationship between worm burden and pathogenic effect is unclear (Holmes, 1990), many different studies performed on variety of hosts showed that high infection intensity can have variety of negative impacts (Gulland, 1992; Hudson et al., 1992a; Stien et al., 2002a; Hughes et al., 2009). Gulland (1992) found high parasitic burdens in dead malnourished Soay sheep. She conducted an experiment which showed that survival rate increased when animals were treated with antihelminthics. Macroparasites tend to generate morbidity rather than mortality in the host, reducing the condition and the ability of the host to search for resources, so heavily infected individuals tend to have lower reproductive success and increased vulnerability to secondary causes of mortality such as predation or secondary infection (Thomas et al., 2005). Hudson et al. (1992a) found that reduction in parasite burden increased winter survival and breeding production of red grouse and Stien et al. (2002a) found that reduction of parasite burden lead to increased reindeer body mass, fat depth and fecundity. High infection intensity in red grouse has been shown to have an indirect impact on mortality, by increasing vulnerability to mammalian predators (Hudson et al. (1992b).

One of the reasons why we have stored washed abomasa is that they could later be used to asses the abundance of larvae within the mucosal lining of the abomasa. Hughes et al. (2009) found out that overall, caribou generally carried more larval stage than adult parasites and that

spatial abundance of larvae varied - in April a majority were found in lumen and in October, the majority were found in mucosa. Mucosal larvae directly affect body weight by damaging the abomasal wall which reduces appetite and digestive efficiency (Fox, 1997).

In my moose study population sample, infection intensity varied between genders and age classes and animals with lower LTBC had higher worm counts.

4.2.1.1   Age and gender influences on parasite burden

This study found that median abomasal infection intensity was similar between males and females, although the three animals with the highest parasite burdens (more than 40 000 worms) were all females. In general it is believed that in mammal taxa males are more susceptible for parasite infection than females of all age classes (Zuk & Mckean, 1996; Klein, 2000; Hudson et al., 2001; Wilson et al., 2002; Body et al., 2011), especially in sexually dimorphic species (Zuk & Mckean, 1996). The difference between genders is believed to be due to difference in immune function (endocrine-immune relationship: the protective function of estrogen; immunosuppressive function of testosterone (Zuk & Mckean, 1996; Klein, 2000), different mortality and senescence rates, ecological differences between sexes (behaviour, diet, habitat choice; Wilson et al., 2002) and differences in body size (Hudson et al., 2001; Wobeser et al., 2005). Irvine et al. (2006) found that female red deer from Scotland had higher parasite burdens than males, but the result is not comparable because the timing of data collection varied among the gender - males were culled in August and September, and females during the winter. Because pregnancy and lactation represent high energetic costs for females, plus some hormones produced during parturition and lactation can have an immunosuppressive effect, the susceptibility of females to parasites at some times of year may increase (Wilson et al., 2002).

The intensity of adult stage abomasal nematode infection was significantly affected by age of animals, with younger animals harboring lower number of parasites than older ones. Results about age related parasite burden in different studies vary, with age-intensity curves either showing a continual increase in parasite load or a gradual leveling-off of parasite burden with age (Body at al., 2011). This levelling off is thought to be generated by acquired immunity (Hudson et al., 2001; Wilson et al., 2002). Because parasite burden increased with age, the increase of burden by slaughter weight was expected. There did not appear to be evidence for the development of immunity to the abomasal nematodes detected given that calves had lower parasite burdens than adults. No evidence of age acquired immunity was found in three other

studies of red deer (Irvine et al, 2006; Santin-Durán et al., 2008) and reindeer (Halvorsen et al., 1999).

In the current study the age of animals carried greater importance for the intensity of infection than their gender.

4.2.1.2   Parasite burden and estimates of body condition

Animals in lower body condition (LTBC) had higher intensity of infection of adult abomasal worms and higher probability of infection with S-shape larvae.

Even though the density of animals in Hedmark county is not currently increasing it is still considered to be high. Between 1986 and 2008 a decline in carcass weight of calves, yearlings and adults was observed (Milner et al., 2012; Milner et al., 2013b; Solberg et al., 2012), which could be associated with a decrease in forage availability (Milner et al., 2012; Milner et al., 2013c). Availability of successional forest (the highest availability of natural browse) per moose has declined since the 1960s because of changes in forestry - decline of clear-felling and herbicide treatment of regenerating stands (Milner et al., 2013c; Mathisen et al. 2014). At the same time hunting interests are keeping moose population high - a long period of high moose density relative to the natural forage availability in the area is indicated by a high accumulated browsing impact on Scots pine at the landscape scale. Low forage availability is indicated as well with browsing on a, for moose non-preferred, tree species such as alder and spruce (Mathisen et al., 2014). Concurrently, increased summer temperatures in central and southern Norway are considered to be responsible for a general reduction in food quality (Solberg et al., 2012). Danielsen (2001) found that the increase in moose population size in south and southeast Norway has led to a reduction of growth (weight) and fecundity of moose. Moose carcass weights are generally higher in areas with low browsing pressure (Solberg et al., 2012).

I hypothesise that low long term body condition could be a consequence of the sustained high moose population density in Hedmark county (Mathisen et al. 2014).

Many different studies have pointed out the negative impact of high host densities on parasite prevalence and intensity of infection (Hudson et al., 1992a; Arneberg et al., 1998; Arneberg, 2001; Albon et al, 2002; Stian et al., 2002; Santin-Durán et al., 2004; Stewart et al., 2005; Gortázar et al., 2006; Bonenfant et al., 2009; Body et at., 2011). It is generally accepted that overabundance increases the risk of infection by altering host resistance to parasites because

of poor nutrition and/or increased stress levels (Gasbarre, 1997; Hoberg et al., 2001; Wobeser et al., 2005; Body et al., 2011). Competition or shortage of food may make a host more vulnerable to infection or to the effects of infection (Begon et al., 2007). Host overabundance increases competition for scarce resources (Solberg & Saether, 1994) and consequently leads to poorer nutrition and a decrease in body mass and body condition (Lavsund et al., 2003; Irvine et al, 2006; Bonenfant et al., 2009; Body et at., 2011). Hughes et al. (2009) found a significant negative relationship between body mass of caribou and their parasite burden. The increase in host densities leads to increased environmental contamination of free living stages of parasites and to increased transmission potential (Arneberg, 2001; Hoberg et al., 2001). Albon et al. (2002) found that the abundance of the nematodes themselves was significantly related to the host density 2 years earlier. Thus, increases in host density, will, subject to a delay, lead to an increase in nematode abundance.

The level of nutrition is extremely important in coping with the consequences of parasitism (resilience) and to constrain and eventually overcome the parasitism (resistance) by limiting the establishment, growth rate, fecundity and/or persistence of a parasite population. It can also affect the parasite population through the intake of antiparasitic compounds (Coop & Kyriazakis, 1999; Halvorsen et al., 1999; Coop & Kyriazakis, 2001; Wobeser et al., 2005). Most wild species deal with periodic, seasonally occurring, food shortage in a variety of ways that are partially determined by the ability to store reserves (Kistner et al., 1980; Wobeser et al., 2005). Malnutrition involves an overall shortage of food, a deficiency of one or more specific elements, or an inappropriate balance among nutrients (Wobeser et al., 2005). Parasites can contribute to mortality in protein-energy malnourished hosts, exacerbating the effects of food shortage by inducing anorexia, decreasing digestion and absorption of food, and increasing the loss of endogenous protein into the gut (Parkins & Holmes, 1989; Gulland 1992; Houtert & Sykes, 1996). Malnutrition suppresses the immune system, which allows infection to become pathogenic (Gulland, 1992). GI parasites can reduce nutrient availability in ruminants through reduction in voluntary food consumption. The latter can range in average from 10 till 30% depending on the parasite species and the number of L_3 larvae (Houtert & Sykes, 1996).

Supplemental feeding can interfere in the objective measurements of overabundance by maintaining almost optimal body condition despite animals living at artificially high densities (Eve & Kellogg, 1977; Fox, 1997; Gortázar et al., 2006). It has been shown that resilience of hosts can improve with the increased protein intake (Coop & Kyriazakis, 2001; Ezenwa,

2004a). Milner et al. (2013a) compared parasite egg output in moose faeces between animals that used feeding station and no-users and found no difference in parasitism among them.

Assessing the short term nutritional condition of individual animals is usually based on the amount of fat in different indicator sites and body protein mass present on a carcass (Kistner et al., 1980; Stephensen et al., 1998; Wobeser et al., 2005). Animals can metabolize muscle mass during dietary protein inadequacy or in starvation, after exhaustion of fat reserves. Accumulation of fat differs between gender and age - fawns use available energy for growth and are unable to store fat reserves at levels comparable to older animals (Kistner et al., 1980). Even though, animals in lower LTBC had higher intensity of infection, LTBC and STBC were not correlated. This indicates, that even though animals had sufficient amount of fat in different indicators sites (kidneys and heart) for evaluating reliable body condition of animals in the future only LTBC index should be used.

4.2.2 Egg counts positively correlated with abomasal counts

The correlation between the number of " Strongylidae type" eggs in the faeces and adult stage nematode worms in the abomasum (only female adult nematodes included) was positive. It is not possible to calculate from EPG the actual worm population of the host, since many factors, mentioned above influence egg production of worms. Nevertheless egg counts in excess of 1 000 (Taylor et al., 2007 - host animal not determined) or in excess of 1 550 (Ezenwa, 2004a - data for Impala (*Aepyeros melampus*), modified from values used to classify sheep) are generally considered indicative of heavy infection and those over 500 of moderate infection (Ezenwa, 2004a; Taylor et al., 2007). Low EPG is not necessarily indicative of low infection, since patency may just be newly established or EPG may be affected by developing immunity (Taylor et al., 2007). Majority of moose examined had under 500 EPG, which is considered to be a low infection. Interestingly males had higher EPG counts than females. Reasons why in males faeces egg output was higher could be connected to rut period - male moose in rut feed less (Miquelle, 1990) and their amount of faeces is low. Consequently this low faeces amount could contribute to higher concentration of parasite eggs in them (Wilson et al., 2002).

4.2.3   The parasite species detected

Different studies have found different numbers of parasite nematode species present in moose abomasa. In Canada Stock & Barrett (1983) found six species, for North America Hoberg et al. (2001) mentioned eight, and Dróždž and Bylung (1970) found five species in Finland. In my study, six species were identified, the two most prevalent being - *Ostertagia antipini* and *Spiculopteragia alcis*. Both of them were present in all age classes and in both genders of animals examined and were found previously in Scandinavian moose (Dróždž & Bylung, 1970; (Nilsson, 1971; Nikander, 1989 (cited after Milner et al., 2013a)).

Both species belong to Ostertagiines, or medium stomach worms. Nematodes of this subfamily represent a dominant component of abomasal nematode fauna from both cervids and bovids, and are believed to be the most pathogenic of ruminant Strongyles (Fox, 1997; Hoberg et al., 2001; Santin-Durán et al. 2004; Kutz et al., 2012). Because not all of the species have the same pathogenic potential, it is important to identify the specific organism present. Large populations of *Ostertagia ostertagi* (main host is cattle) can induce extensive pathological and biochemical changes and these are maximal when the parasites are emerging from the gastric glands. In heavy infection of 40 000 or more adult worms acidity of the abomasal fluid declines which leads to failure in the activation of pepsinogen to pepsin, demise in bacteriostatic effect and enhancement in permeability of the abomasal epitelium to macroparasites (Taylor et al., 2007). Developing protective immune responses against them is very difficult and the immune response are very week and delayed in their onset (Gasbarre, 1997). *O. ostertagi* can be found in wild ruminants only when range is shared with cattle (Hoberg et al., 2001). For *Ostertagia circumcincta,* found in Soay sheep, Gulland (1992) suggested that it contributes to mortality in malnourished hosts, exacerbating the effects of food shortage. *O. leptospicularis* has been reported pathogenic for red deer (Hoberg et al., 2001). *Ostertagia gruehneri*, found in reindeer abomasa is also considered to be highly pathogenic, which at high intensity of infection (more than 5 000 adult nematodes/host; Kutz et al.., 2012), can have a negative impact on body condition (Stien et al., 2002; Hughes et al., 2009) and fecundity (Albon et al., 2002; red deer, Stewart et al., 2005). *Spiculopteragia boehmi* (hosts: mule deer, white-tailed deer and elk) is believed to be potentially pathogenic by decreasing body condition of their hosts (Kutz et al., 2012).

I have found no records about pathogenicity of either *Ostertagia antipini* or *Spiculopteragia alcis.*

4.2.4   Host - parasite relationships in the future

Presence of parasites could be used as a possible indicator of ecological changes (Body et al., 2011). Even though spread of parasites can be enhanced by different factors, it is believed that parasites infections will change in the future because of global climate change, habitat alterations, changes in agricultural and forestry practices and their concomitant impact on the distribution of hosts (Hoberg et al., 2001; Murray et al., 2006; Franzzman et al., 2007; Sinclair, 2007, Kutz et al. 2014). When it comes to climate change, it is believed that northern ecosystems will undergo the greatest changes.

The diversity and abundance of parasites are constrained by suitable climatic conditions (Thomas et al., 2005, Kutz et al., 2009). Most helminth and protozoan parasites have life cycle stages that are free in the environment or within intermediate hosts. The development and survival of these stages are sensitive to climatic conditions (Kutz et al., 2009). Because of climate change, increases in temperature and alternation in precipitation patterns are expected (Thomas et al., 2005). This will influence transmission rate, prolong transmission period and shift spatial and temporal patterns of pathogen diversity and associated disease (Hoberg et al., 2008; Kutz et al., 2009; Polley et al., 2010). The response of abomasal parasites with direct life cycle (for example *O. Gruehneri)* may be variable, extremes in maximum temperatures (soil T) may actually reduce survival rate of their free-living parasite stages (Kutz et al., 2009; Kutz et al., 2014a). Under laboratory conditions, mortality of *O. gruehneri* is 100% at 35 C° or higher and is suggested that under warmer climatic conditions their fitness will be reduced (Kutz et al., 2014a).

Climate change can influence host, among other, by having a potential to mediate stress and hence host susceptibility to infectious diseases. Changes in behaviour due to increased environmental temperatures may also result in suboptimal foraging, and thus in compromised body condition and immune function (Plowright et al., 2012). Thermal stress in moose is thought to contribute, in part, to reduced immune function and body weight (van Beest & Milner. 2013) and ability to handle parasite burden (Murray et al., 2006).

5   CONCLUSIONS AND MANAGEMENT APPLICATIONS

This is one of the first studies of moose gastrointestinal parasites in Norway. The moose population density in Hedmark county remains at a high level, and reduced slaughter weights have been recorded in all classes during recent years. This study confirms that abomasal parasite abundance is high in this population and high parasite burdens were associated with poorer body condition. Intensity of infection was correlated with host age and gender. The most common abomasal nematodes found were *Ostertagia antipini* and *Spiculopteragia alcis*. Calves have higher prevalences of *Monizia* sp. and "Dorsal spine larvae" compared to adults.

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All wildlife hosts parasites and determinating the threshold of their pathogenicity is difficult as it depends on many factors. Even though moose density hasn`t been increasing it is considered, next to decrease in natural forage availability, to be important factor contributing to lower body condition. It is well accepted that parasites have a greater negative effect on individuals that have already lowered body condition. Future wildlife management strategies of moose in Norway should take into account increased parasite transmission in dense populations and suitable countermeasures to keep populations at a more sustainable level should be introduced (Wobeser, 2005; Gortázar et al., 2006). Many of the GI nematode parasites which were found in moose can infect domestic ruminants (sheep, cattle and goats) as well. Because Hedmark county is strongly agriculturally oriented and farmers graze - free-range - their livestock in the areas used by moose, their is a potential for parasite transmission between them. Even though, the pathogenicity is much higher for wild ruminants compared to domestic ones (Hoberg et al., 2001), the increased transmission potential could induce economic loss for the livestock farmers.

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Knowledge gained about parasite status of Norwegian moose in this study provides vital baseline data for future research. Without future studies, which will help detect fluctuations in parasite prevalence and infection intensity and maybe identify causes responsible for it (experimental study), the final answer about my assumption for the high prevalence and infection intensity in this study (i.e. the relationship between natural forage availability and moose density) will never be confirmed.

6   ACKNOWLEDGMENTS

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8 Appendix 1:

PARASITTER HOS ELG – FELTPROTOKOLL

GENERELL INFORMASJON

Elg ID:
Dato, klokkeslett:
GPS posisjon av skuddstedet UTM 32☐   N   Ø   UTM 33☐
Navn på jaktlag:
Jaktleder:
Ansvarlig jeger for elgen:

Informasjon om jaktsituasjonen (var dyrets atferd normal, foregikk selve jaktsituasjon uten spesielle hendelser, dyret skutt under los eller ikke, andre anmerkninger):

INFORMASJON OM DYRET (Kryss av/ring inn)

Alder	Kalv		1,5 år	2,5år eller eldre
Kjønn	Hanndyr		Hunndyr	
For voksne kyr	Med kalv **Antall:** **Kjønn:**		Uten kalv	Ukjent
	Har melk i juret		Ikke melk i juret	Ukjent
	Utseende på melk			
	Klart	Melkeaktig	Tykt	
Slaktevekt (uten innvoller, hodet, nedre del av beina og skinnet): **kg**				

Beskriv eventuelle tegn til sykdom eller skader (byller, sår, benfraktur, øyeskader, sykdommer i huden og lignende). Please describe any signs of illness or injury (boils, wounds, benfraktur, damage, diseases of the skin and the like).

SNU ARKET!

Vurdering av kondisjon (Basert på vurdering av fettlaget på nyrene og hjertet (bildene) og underhudsfett. Ring inn).

SVAK (ubetydelige mengder fett, 1 og 2 på bildene)

NORMAL (moderate mengder fett, 3 på bildene)

GOD (rikelige mengder fett, 4 på bildene)

| 1 | 2 | 3 | 4 |
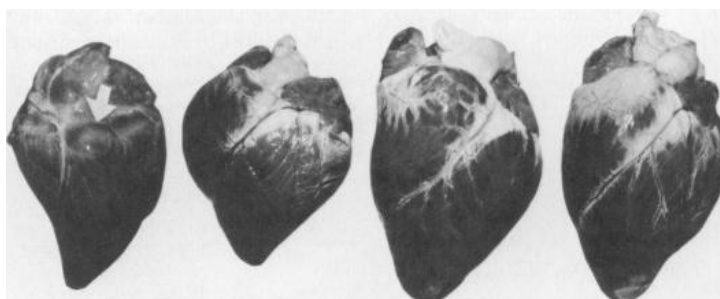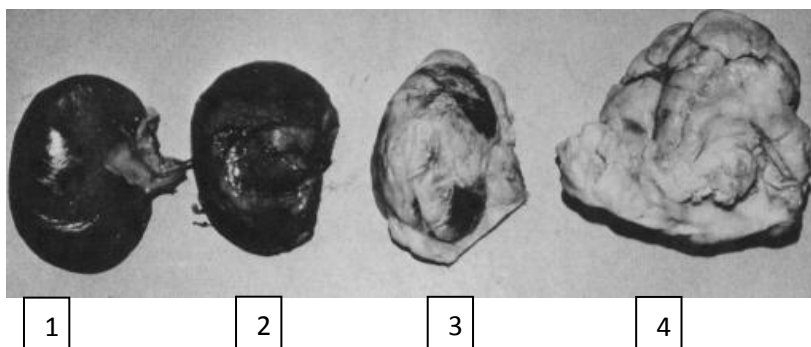
Skriv gjerne eventuelle kommentarer på vurdering av kondisjon her.

INFORMASJON OM PRØVETAKING (Kryss av)

PRØVEMATERIALE		JA	NEI	BEMERKNINGER
Blod	EDTA (lilla)			
	Uten konserveringsmiddel (rød)			
Melk				

Andre kommentarer.

Additional part of Appendix 1, not included in the hunters protocol:

DAY OF COLLECTING THE INTESTINE, TIME:_____

ANY SPECIAL EVENTS AT COLLECTING THE SAMPLES (SCAVANGERS, PROBLEMS, WEATHER...)

_____
_____
_____
_____
_____
_____

Faeces:

consistency:	SOFT	WATERY	VERY HARD

colour:

presence of blood :   YES   NO

presence of mucus: YES   NO

presence of parasites (tapeworm segments):   YES   NO

> The condition of the stool should be noted: colour (unusual colour should always be reported, light gray-excessive fat, poor intestinal absorption), presence of blood (severe parasitism other intestine diseases), mucus (intestinal parasitism, metabolic disease), consistency (soft, watery (diarrheic), very hard) and presence of parasites (tapeworm segments. Amount of faeces: rule of thumb: size of adults man`s thumb

ABOMASUM: wall damage: YES   NO

glove test – presence of parasites: YES   NO

CAECUM: wall damage: YES   NO

Trichuris sampling on the field   YES   NO

ORGANS COLLECTED ON THE FIELD/CABIN: **MANDIBULA**   YES   NO

ABOMASUM   YES   NO

OVARIES   YES   NO

OTHERS   _____

COMMENTS:

_____
_____

9 Appendix 2:

MOOSE POPULATION in three municipalities of HEDMARK COUNTY, Autumn 2013

	AREA	SEX	WEIGHT (kg)	AGE	age.class	STBC	LTBC	Abomasum count
2	Stor-Eldval	male	235	7.5	adult	2.normal	good	37420
2a	Stor-Eldval	male	84	0.5	calf	2.normal	good	260
3	Stor-Eldval	male	226	3.5	adult	2.normal	good	9520
4	Stor-Eldval	female	175	3.5	adult	3.good	good	33060
5	Stor-Eldval	female	174	8.5	adult	2.normal	bad	42320
6	Stor-Eldval	male	210	8.5	adult	2.normal	bad	29440
7	Stor-Eldval	female	132	1.5	yearling	2.normal	bad	1980
8	Stor-Eldval	female	137	3.5	adult	2.normal	bad	46700
9	Stor-Eldval	male	170	2.5	adult	2.normal	bad	8380
13	Stor-Eldval	female	56	0.5	calf	2.normal	bad	320
14	Stor-Eldval	male	180	3.5	adult	3.good	bad	6480
15	Stor-Eldval	male	160	2.5	adult	2.normal	good	12200
22	Stor-Eldval	male	209	4.5	adult	2.normal	good	7200
23	Stor-Eldval	male	120	1.5	yearling	2.normal	bad	3800
25	Stor-Eldval	female	156	8.5	adult	2.normal	bad	22400
26	Stor-Eldval	male	170	2.5	adult	2.normal	bad	18540
27	Stor-Eldval	male	106	1.5	yearling	2.normal	bad	7840
28	Stor-Eldval	male	190	3.5	adult	2.normal	good	1700
29	Stor-Eldval	female	134	1.5	yearling	2.normal	bad	4300
30	Stor-Eldval	male	190	3.5	adult	2.normal	good	3140
32	Stor-Eldval	female	172	13.5	adult	2.normal	good	35260
33	Stor-Eldval	male	119	1.5	yearling	2.normal	good	2940
34	Amot	female	161	2.5	adult	3.good	good	2140
35	Amot	female	179	5.5	adult	2.normal	good	10700
37	Amot	female	NA	0.5	calf	2.normal	NA	260
38	Amot	male	230	7.5	adult	2.normal	bad	21140
39	Amot	female	54	0.5	calf	2.normal	bad	1720
40	Amot	male	74	0.5	calf	2.normal	bad	4220
41	Amot	male	50	0.5	calf	2.normal	bad	3120
42	Amot	female	141	4.5	adult	2.normal	bad	11200
43	Amot	female	188	7.5	adult	2.normal	good	56000
44	Amot	male	146	1.5	yearling	2.normal	good	5400
45	Amot	male	76	0.5	calf	2.normal	bad	660
46	Amot	male	NA	2.5	adult	2.normal	NA	3800
47	Amot	male	207	4.5	adult	2.normal	good	11840
52	Amot	female	180	12.5	adult	3.good	NA	11440
56	Amot	male	186	2.5	adult	3.good	good	6720
57	Amot	male	129	1.5	yearling	2.normal	bad	11920
58	Amot	male	188	2.5	adult	3.good	good	5060

| ID	AREA	SEX	WEIGHT (kg)	AGE	age.class	STBC	LTBC	Abomasum count
59	Amot	female	68	0.5	calf	1.poor	good	220
63	Amot	female	165	2.5	adult	2.normal	good	8280
64	Amot	male	218	3.5	adult	2.normal	good	10960
65	Amot	male	74	0.5	calf	2.normal	good	980
66	Amot	male	125.2	1.5	yearling	NA	bad	9740
67	Amot	male	277,2	7.5	adult	2.normal	good	20400
BORIS 1	Tynset	female	86	0.5	calf	2.normal	good	60
BORIS 2	Tynset	female	177	NA	adult	3.good	NA	2240
BORIS 3	Tynset	male	170	1.5	yearling	2.normal	NA	1320
BORIS 4	Tynset	female	130	1.5	yearling	2.normal	NA	3140

NA- NOT AVAILABLE

10 Appendix 3:

IDENTIFICATION KEYS AND EPG, LPG FORMULA

1) IDENTIFICATION OF EGGS

Common ruminants eggs (http://www.rvc.ac.uk/review/parasitology/RuminantEggs/Common.htm):

Fig. 15.3 Worm eggs from ruminants.
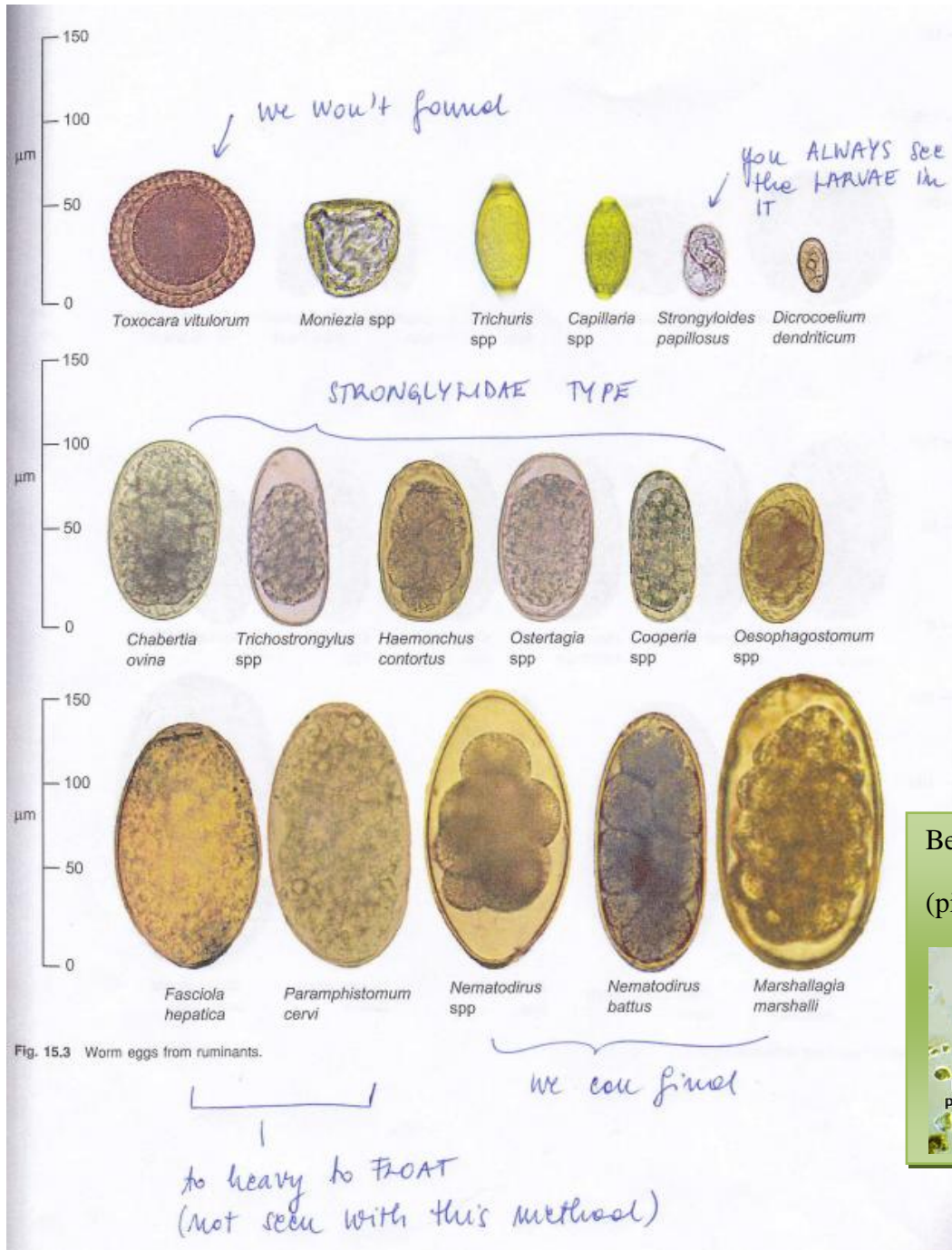
Taylor et al., 2007

EPG (eggs per gram) =

$$\text{No eggs counted} \times \frac{\text{(Volume of water (in mix master) + weight of faeces)}}{\text{Weight of faeces} \times \text{volume supernatant examined}}$$
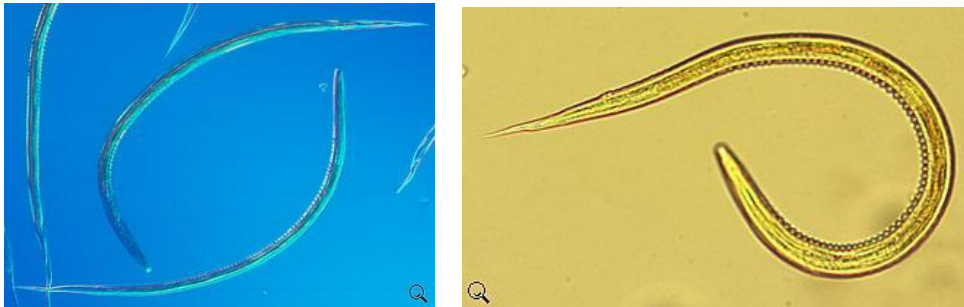
2) IDENTIFICATION OF LARVAE

based on: - shape of the tail
- body shape and size

SHAPE OF TAIL:

a) **STREIGH & POINTED END** (Hatched GI eggs)

http://www.studyblue.com/notes/note/n/parasitology-1/deck/5570959

b) **ROUND & CHUBBY END** (*Dictyocaulus* spp. - lungworm)

http://www.studyblue.com/notes/note/n/internal-parasites-lungworm

c) **S-SHAPE= DORSAL SPINE LARVAE** (*Varestrongylus* spp.& *Elaphostrongylus*

http://www.studyblue.com/notes/note/n/parasitology-1/deck/5570959

LPG (larvae per gram) =

Larval count   x   (total volume in tube (in microliters)/volume of subsample (in microliters))
weight of faeces in original sample (g)

Volume of fluid left in tube before subsample take: 1 ml: (ml*1000 to get microliters

61

3) IDENTIFICATION OF ADULTS

CAECUM

Trichuris sp.

http://www.savalli.us/BIO385/Diversity/13.Nematoda.html).

ABOMASUM

Subfamilie Ostertagiinae (description just for the most prevalent species in this study)

Males: Identification is based on the structure of the bursa, genital cone, spicules and on the dimensions of the esophageal valve (Hoberg et al., 2001).

Identification keys based on personal notes (Tina Ličina):

- SPICULES import to look in:
  - are both spikules the same or one of them is smaller
  - where do they split
  - slippers or nob at the end, the size of slipers;
  - do we see a fish hook structure in the end of the inner part of the spicules

- CAPITULORY BURSA (what structure we see (one V, double V...)

- RAY PATTERN ("or the fingers"): 212 (*Ostertagia sp*.) or 221 (*Spiculopteragia sp.*)
  (2: two of the fingers are more together)

- VALVA / ESOPHAGUS RATIO (more than 2 (*Ostertagia sp)*, less than 2 (*Spiculopteragia sp.*))

the end of esophagus   ratio : ↕ versus ↔ (more than 2, less than 2)
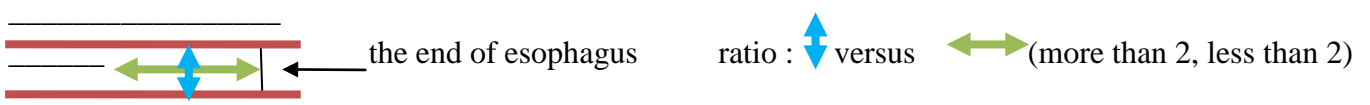
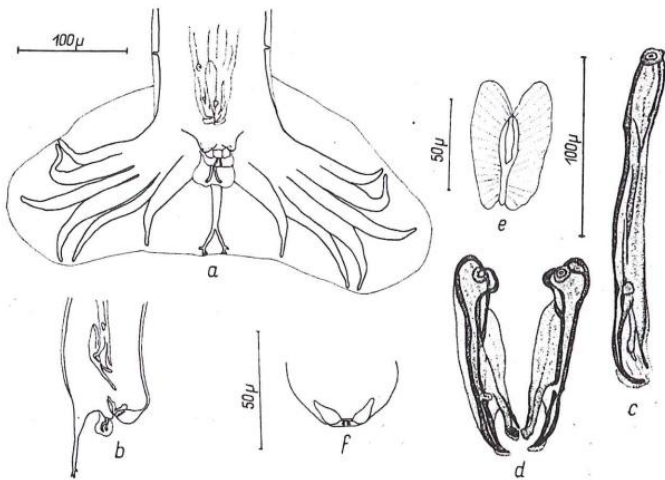muscle layer ▬

Ostertagia antipini

Fig. 3. *Ostertagia antipini*. a, b — posterior body part of male, ventro-dorsal and lateral view, resp.; c — spicule; d — teratologic spicules; e — gubernaculum, f — telamon, ventro-dorsal view

Tina Ličina personal notes:
- RAY PATTERN: 212
- VALVA/ESOPHAGUS: more than 2
- not well distinguish slippers (small) at the bottom of spicules
- inner part of the spicules has a fish hook ending - you always see a horizontal line on them
- capitulary bursa: we see the first V, the second V as a hearth shape and square shape

Skrjabin et al., 1954
- light brown color
- body length: 8.5-10.8 mm
- bursa consists of two large lateral lobes and one small medial one
- spicules are equal size, dark yellow till dark brown color - in the end of the central branch is a hook resembling a harpoon

Ostertagia leptospicularis

Tina Ličina personal notes:
- RAY PATTERN: 212
- VALVA/ESOPHAGUS: more than 2
- well distinguish slippers at the bottom of spicules
- spicules divides in the end (3/4, bottom quarter)
- we dont see the horizontal line in the end of the spicules
- capitulary bursa: we see the first and the second V (actually they have 3) + rounded blob shape (the small V comes out there)
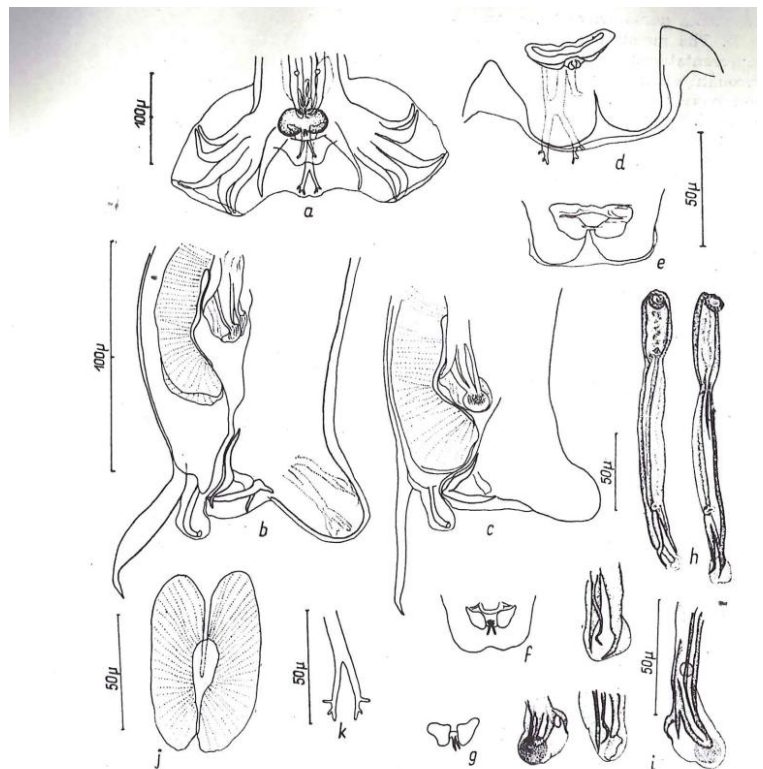

Fig. 4. *Ostertagia leptospicularis*. a, b, c — posterior body part of male, a — ventro-dorsal, b, c — lateral view; d—g — variability of shape of telamon depending on functional phases of genital cone, ventral view; h — spicules; i — ends of spicules, various views; j — gubernaculum; k — dorsal ray of bursa

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Spiculopteragia alcis

Tina Ličina personal notes:
- RAY PATTERN: 221 (hard to see)
- VALVA/ESOPHAGUS: less than 2
- spicules split in the end
- no slippers in the end of spicules, instead nods
 in the end of spicules

Skrjabin et al., 1954
- male: length of the body: 8.0-10.1 mm
- bursa consists of two large lateral lobes devided
by a deep incision
- equal spicules, golden-brown color and
characteristic form - tubular structures of almost
width over their entire length, distantly they branch
into two offshoots

FIGURE 119   *Spiculopteragia alcis* Schulz, Kadenatsiĭ, Evranova and
Schaldybin, 1952
(After Schulz, Kadenatsiĭ, 1954)

= MARAMASTRONGYLUS
DAGESTANICA