Gastrointestinal parasitic infections in dead or debilitated moose (alces alces) in SWEDEN

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Gastrointestinal parasitic infections in fallen and debilitated moose (*Alces alces*) in Sweden.

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Abstract

The objectives of the present study were to determine prevalence and intensity of gastrointestinal parasites of dead or euthanized emaciated moose in central and southern Sweden (n=50), and to assess parasite intensity as a major contributing factor in the poor condition of these moose. All animals were infected and most had gastrointestinal nematodes. Seven parasite species were found in the abomasa, and 10 species were found in the small intestine. Co-infections were commonly found in the abomasum (*Ostertagia antipini* and *Mazamastrongylus dagestanica*) and in the small intestine (*Nematodirella alcidis* and *Trichostrongylus capricola*). Moose had many nematodes; highest numbers were 224,400 and 11,760 in the abomasum and in the small intestine, respectively. Fourteen moose had more than 40,000 gastrointestinal nematodes (excluding large intestine nematodes, represented by *Trichuris spp*. and *Oesophagostomum venulosum*). Additionally, moderate prevalence (36%) of protostrongylid larvae (dorsal spine larvae) and low prevalence (2-4%) of protozoal infections were identified at copromicroscopical examination. Adult moose had significantly more parasites than subadults. The presented data can be useful to compare with the results from other studies performed in this animal species. The results could not show parasite load as a single or major cause of the moose mortality, but they provide an update on the species composition of helminthofauna in moose in Sweden and illustrate the extreme infection intensities that free-ranging moose can have.

Keywords: abomasa, emaciated, gastro-intestinal, moose, parasites, parasitic count, small intestine

Few recent data are available regarding gastrointestinal parasites of moose (*Alces alces*), especially in Europe (Davidson et al., 2015; Milner et al., 2013; Nikander, 1989; Nilsson, 1971; Kutz et al. 2012). An example is provided by Davidson et al. 2015 on hunted moose from the general population in Norway. However, these authors did not report on small intestinal parasites. The aim of this study was to provide an update on species composition and infection intensity of gastrointestinal (GI) parasites throughout the entire GI tract of dead and euthanized emaciated moose in Sweden. Also, we aimed to assess the contribution of parasite infection intensity as a risk factor contributing to the assumed increased moose mortality event.

Whole moose carcasses (n=50) were collected in central and southern Sweden between September 2012 and October 2014 and were submitted for necropsy to the National Veterinary Institute (SVA, Uppsala, Sweden). Scavenging was negligible and only two carcasses showed marked decomposition. Sex, age and nutritional condition were recorded.

Age determination was made by counting cementum annuli of the 1st molar. Nutritional condition was based on a subjective assessment of fat around internal organs, in the coronary groove and degree of serous atrophy of bone marrow. GI lesions were defined as present if moose showed macroscopic and/or microscopic evidence of GI disturbance, including mucosal thickening, hyperemia, ulceration and/or inflammation, diarrhea and fibrous adhesions limiting gastrointestinal motility.

Abomasa and first 7 meters of small intestines – as separate samples - were rinsed in tap water; their content was sieved (150µm aperture) and suspended in 4 liters of tap water. Four deciliter and 1 deciliter aliquots were stored at 4°C until examination. The larger aliquot was examined if the smaller one was negative. Nematodes in each examined aliquot were counted and identified to species (based on 50 males per sample) using morphological keys (Barth

1991, Skrjabin et al. 1954). *Spiculopteragia alcis* was considered a synonym of *Mazamastrongylus dagestanica* (Drózdz 1965, Hoberg and Khrustalev 1996). Species identification took into account male polymorphism occurring in nematodes of subfamily Ostertaginae (major/minor morph: *Ostertagia leptospicularis*/*Ostertagia kolchida*, *Spiculopteragia boehmi*/*Spiculopteragia mathevossiani*, *Teladorsagia circumcincta*/*Teladorsagia davtiani*) (Drózdz 1995, Liénard et al. 2006).

Caecum and spiral colon from all animals were opened and macroscopically examined for parasites.

Ten grams of carefully washed abomasal mucosa were scraped and digested (1.8ml pepsin 660E/ml, 2.5ml hydrochloric acid 17.4% and 150ml water) at 37°C for 14 hours. After sieving (38µm aperture), the material was microscopically examined to identify encysted parasites (Soulsby 1982, MAFF 1986).

Three to five grams of faeces (n=50) were examined with a modified formalin/ether sedimentation technique (Thienpont et al., 1986).

Differences in prevalence and intensity of infection of nematode species among different risk groups were determined for each identified GI helminthic species using the Chi-square and the Kruskal-Wallis tests, respectively, to identify potential risk factors. Explanatory variables were sex, age, body condition (emaciated or average/good condition) and presence of GI lesions. Chi squared tests were performed to evaluate variations in the frequency of classes of body condition and GI lesions related to sex and age classes. Statistical analyses were performed using the software IBM SPSS Statistics 20 (IBM Corp. Armonk, NY, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

Fifteen, 21 and 14 carcasses were collected during 2012, 2013 and 2014. Twelve of the collected moose were found dead, while 38 were euthanized by gunshot due to poor

condition, abnormal behavior and/or inability to rise. Sex identification was possible in all but one case, representing 36 females and 13 males. Moose were classified as adults (>2 years old, n=29) and subadults (<2 years old, n=21). Based on nutritional condition, 29 moose (60%) were considered emaciated, while 19 (39.5%) were in poor to average condition. Body condition was not reported for two animals. Thirty-three moose (66%) had GI lesions present whereas 17 did not. Animals belonging to the same body condition category and GI lesion status were homogenously distributed regarding sex and age, excluding the possibility that these independent variables played a role in nutritional status and GI health.

Fifty, 48 and 6 moose had parasites in the abomasum, small intestine and large intestine, respectively. The number of worms ranged between 120 and 224,400 ($\bar{x}$=30 207 ± 39 040) in the abomasa and 0 and 11,760 in the small intestine ($\bar{x}$=2 123 ± 2 283). Species composition in the abomasa and in small intestine, their prevalence, and abundance are presented in Table 1.

No single species infections were recorded, while co-infections with *Ostertagia antipini* + *M. dagestanica* and *O. antipini* + *M. dagestanica* + *O. leptospicularis*/*O. kolchida* were common in the abomasa (82% and 64% of examined moose, respectively) and co-infection with *Nematodirella alcidis* + *Trichostrongylus capricola* was dominant in the small intestine (58% of moose).

Trichuris sp. and *Oesophagostomum venulosum* were found in the cecum of 5 (10%) moose and in the ascending colon of one (2%) moose, respectively. Larvae and adults belonging to the genera *Ostertagia* and *Spiculopteragia* were found in 4 (8%) samples of digested gastric mucosa.

The following parasitic eggs were found in fecal samples: trychostrongylid (80%), *Nematodirella*/*Nematodirus*-like (48%), *Capillaria* sp. (6%), *Dicrocoelium dendriticum*

(16%) and *Moniezia* sp. (2%). Larvae of protostrongylids were found in 36% of the examined samples; cysts/oocysts of *Giardia* sp., *Entamoeba* sp. and *Eimeria* sp. were found in 4%, 2% and 2% of the samples, respectively.

Sex had no effect on the intensity of infection. Adult animals had significantly higher intensity of infection when considering the total parasite count (TPC) (48,174 vs 10,380; p=0.001) as well as *O. antipini*, *O. leptospicularis/O. kolchida*, p<0.001; *T. capricola* and *M. dagestanica*, p<0.05, and higher prevalence of *O. leptospicularis/O. kolchida* infection (p<0.01).

Despite no statistical difference could be demonstrated in intensity of infection in moose with and without GI lesions, the former had a higher prevalence and intensity of *T. capricola* infection in the small intestine (p<0.05 and p<0.01 respectively). Presence of GI lesions was similar both in the animals found dead (69%) or euthanized (65%). Abomasitis, followed by trauma were the most frequently identified main finding or cause of death in moose showing GI lesions (66% had multiple main findings), while trauma and parasitic meningitis due to *Elaphostrongylus* were the most commonly identified main causes of death in those animals free from GI lesions (23% had multiple main findings).

Geographic origin did not seem to influence infection status (data not shown).

Despite the compromised health status, parasite species composition observed here is consistent with previous reports on European moose (Davidson et al. 2015; Milner et al. 2013; Nikander 1989; Nilsson 1971).

Differently from the present report, Nilsson et al. 1971, did not find *T. circumcincta/T. davtiani* and *Nematodirus filicollis* and described the GI worm-burden as almost always low.

Abomasal parasite species composition – small intestines were not examined – in Norway (Davidson et al. 2015) was consistent with our study, even if recorded intensity of infection was lower (only 6% of the animals – all females – exceeded 40 000 TPC, the highest count being 56,000), likely because the study refers to hunted population. Davidson et al. (2015) considered TPC above 40 000 as extreme and 14 animals (28%, 78% being females) in our study exceeded this burden.

There is no published normal reference range for intensity of infection in moose. In domestic animals counts as high as 12,000-15,000 for *Ostertagia ostertagi* and >140,000 for *Trichostrongylus* spp. can be fatal in calves (Hoberg et al., 2001). In the present study, *Ostertagia antipini*, *M. dagestanica* and *O. kolchida* showed counts over 12,000, respectively, in 22, seven and three moose, the majority of them (respectively 68%, 71% and 67%) being emaciated, but it was not possible to estimate the contribution of abomasal parasitism to this condition.

Copromicroscopical results in our study were generally in agreement with those of parasitic count and identification, except for markedly higher prevalence of whipworm and *Dicrocoelium dendriticum*. Because other organs, such as liver and lungs did not undergo systematic parasitological examination, it was not possible to ascertain if protostrongylid larvae present could be ascribed to either *Elaphostrongylus* sp. or *Varestrongylus* sp. (or both) infections.

Differently from Davidson et al. 2015, the fecal sedimentation technique we used allows recovery of cysts of protozoan parasites (e.g. *Giardia* and *Entamoeba*) and heavier helminth eggs (e.g. *Trichuris* and *Dicrocoelium*), so the results cannot be fully compared. Anyhow, we found a lower proportion of animals shedding *Eimeria* sp., *Strongyloides* sp., *Moniezia* sp. and *Dictyocaulus* sp. and a higher proportion of animals shedding strongyle – type egg,

Nematodirella/*Nematodirus* sp., *Trichuris* sp., dorsal spine larvae, *Capillaria* sp., *Giardia* sp., *Entamoeba* sp. and *Dicrocoelium* sp.

Parasite counts in this study should be considered as extremely high regarding when referring to moose GI parasitism. Recorded burdens contribute presumably to the poor condition of sampled animals, considering the extensive abomasitis changes observed in some cases at necropsy (data not shown). The higher worm burden recorded in adults suggests that acquisition of infection accumulates over time. The high intensity of infection in moose also indicates a high parasite infection pressure from the environment, in these parts of Sweden, characterized by a density of approximately between 0.3 – 1.0 moose/km^2. It should be highlighted that *Echinococcus* sp. and lungworm infection were not accounted for in the present study, but can play a role in mortality (Kutz et al. 2012).

The importance of these parasitic infections in moose requires further studies. No significant difference in TPC could be found between animals with and without GI lesions, but e.g. *T. capricola* was more prevalent and abundant in the former. Targeted studies investigating GI parasites, GI lesions (both macroscopic and microscopic) and body condition of both debilitated and healthy moose from groups as homogenous as possible (particularly with respect to sex and age) are needed.

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229 Table 1: Prevalence and intensity of infection of GI parasites in the abomasum and in the

230 small intestine of 50 fallen or debilitated moose (*Alces alces*) from Sweden.

Identified species	Total		Abomasum		Small intestine	
	Number infected/number examined (%)	$\bar{x}$ ± SD	Number infected/number examined (%)	$\bar{x}$ ± SD	Number infected/number examined (%)	$\bar{x}$ ± SD
Ostertagia antipini	49/50 (98.0)	20070±30299	49/50 (98.0)	19961±30275	14/50 (28.0)	109±347
Mazamastrongylus dagestanica	42/50 (84.0)	6356±10871	42/50 (84.0)	6252±10767	12/50 (24.0)	105±407
Trichostrongylus axei	11/50 (22.0)	781±2058	11/50 (22.0)	753±1942	1/50 (2.0)	28±198
O. kolchida[a]	36/50 (72.0)	2474±4336	36/50 (72.0)	2472±4335	2/50 (4.0)	2±13
Spiculopteragia boehmi/S. mathevossiani)	18/50 (36.0)	750±1848	16/50 (32.0)	714±1820	2/50 (4.0)	6±29
Haemonchus sp.	3/50 (6.0)	24±116	3/50 (6.0)	24±116	-	-
Teladorsagia davtiani[b]	1/50 (2.0)	33±233	1/50 (2.0)	33±233	-	-
Nematodirella alcides	37/50 (74.0)	814±1713	-	-	37/50 (74.0)	814±1713
Trichostrongylus capricola	39/50 (78.0)	997±1681	-	-	39/50 (78.0)	997±1681
Trichostrongylus vitrinus	2/50 (4.0)	10±58	-	-	2/50 (4.0)	10±58
Nematodirus filicollis	3/50 (6.0)	51±268	-	-	3/50 (6.0)	51±268
Cooperia oncophora	1/50 (2.0)	1±6	-	-	1/50 (2.0)	1±6
Dicrocoelium dendriticum	1/50 (2.0)	NA	-	-	1/50 (2.0)	NA
Moniezia sp.	1/50 (2.0)	NA	-	-	1/50 (2.0)	NA

231 [a] minor morph of *O. leptospicularis*; [b] minor morph of *T. circumcincta*