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1 Running head: Short Communications

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

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23 **Abstract**

24 The objectives of the present study were to determine prevalence and intensity of
25 gastrointestinal parasites of dead or euthanized emaciated moose in central and southern
26 Sweden (n=50), and to assess parasite intensity as a major contributing factor in the poor
27 condition of these moose. All animals were infected and most had gastrointestinal nematodes.
28 Seven parasite species were found in the abomasa, and 10 species were found in the small
29 intestine. Co-infections were commonly found in the abomasum (*Ostertagia antipini* and
30 *Mazamastrongylus dagestanica*) and in the small intestine (*Nematodirella alcidis* and
31 *Trichostrongylus capricola*). Moose had many nematodes; highest numbers were 224,400 and
32 11,760 in the abomasum and in the small intestine, respectively. Fourteen moose had more
33 than 40,000 gastrointestinal nematodes (excluding large intestine nematodes, represented by
34 *Trichuris spp.* and *Oesophagostomum venulosum*). Additionally, moderate prevalence (36%)
35 of protostrongylid larvae (dorsal spine larvae) and low prevalence (2-4%) of protozoal
36 infections were identified at copromicroscopical examination. Adult moose had significantly
37 more parasites than subadults. The presented data can be useful to compare with the results
38 from other studies performed in this animal species. The results could not show parasite load
39 as a single or major cause of the moose mortality, but they provide an update on the species
40 composition of helminthofauna in moose in Sweden and illustrate the extreme infection
41 intensities that free-ranging moose can have.

42

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

45

46 Few recent data are available regarding gastrointestinal parasites of moose
47 (*Alces alces*), especially in Europe (Davidson et al., 2015; Milner et al., 2013; Nikander,
48 1989; Nilsson, 1971; Kutz et al. 2012). An example is provided by Davidson et al. 2015 on
49 hunted moose from the general population in Norway. However, these authors did not report
50 on small intestinal parasites. The aim of this study was to provide an update on species
51 composition and infection intensity of gastrointestinal (GI) parasites throughout the entire GI
52 tract of dead and euthanized emaciated moose in Sweden. Also, we aimed to assess the
53 contribution of parasite infection intensity as a risk factor contributing to the assumed
54 increased moose mortality event.

55 Whole moose carcasses (n=50) were collected in central and southern Sweden
56 between September 2012 and October 2014 and were submitted for necropsy to the National
57 Veterinary Institute (SVA, Uppsala, Sweden). Scavenging was negligible and only two
58 carcasses showed marked decomposition. Sex, age and nutritional condition were recorded.
59 Age determination was made by counting cementum annuli of the 1st molar. Nutritional
60 condition was based on a subjective assessment of fat around internal organs, in the coronary
61 groove and degree of serous atrophy of bone marrow. GI lesions were defined as present if
62 moose showed macroscopic and/or microscopic evidence of GI disturbance, including
63 mucosal thickening, hyperemia, ulceration and/or inflammation, diarrhea and fibrous
64 adhesions limiting gastrointestinal motility.

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

70 1991, Skrjabin et al. 1954). *Spiculopteragia alcis* was considered a synonym of
71 *Mazamastrongylus dagestanica* (Drózd 1965, Hoberg and Khrustalev 1996). Species
72 identification took into account male polymorphism occurring in nematodes of subfamily
73 Ostertaginae (major/minor morph: *Ostertagia leptospicularis*/*Ostertagia kolchida*,
74 *Spiculopteragia boehmi*/*Spiculopteragia mathevossiani*, *Teladorsagia*
75 *circumcincta*/*Teladorsagia davtiani*) (Drózd 1995, Liénard et al. 2006).

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

78 Ten grams of carefully washed abomasal mucosa were scraped and digested (1.8ml pepsin
79 660E/ml, 2.5ml hydrochloric acid 17.4% and 150ml water) at 37°C for 14 hours. After
80 sieving (38µm aperture), the material was microscopically examined to identify encysted
81 parasites (Soulsby 1982, MAFF 1986).

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

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

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

94 condition, abnormal behavior and/or inability to rise. Sex identification was possible in all but
95 one case, representing 36 females and 13 males. Moose were classified as adults (>2 years
96 old, n=29) and subadults (<2 years old, n=21). Based on nutritional condition, 29 moose
97 (60%) were considered emaciated, while 19 (39.5%) were in poor to average condition. Body
98 condition was not reported for two animals. Thirty-three moose (66%) had GI lesions present
99 whereas 17 did not. Animals belonging to the same body condition category and GI lesion
100 status were homogenously distributed regarding sex and age, excluding the possibility that
101 these independent variables played a role in nutritional status and GI health.

102 Fifty, 48 and 6 moose had parasites in the abomasum, small intestine and large intestine,
103 respectively. The number of worms ranged between 120 and 224,400 (\bar{x} =30 207 \pm 39 040) in
104 the abomasa and 0 and 11,760 in the small intestine (\bar{x} =2 123 \pm 2 283). Species composition
105 in the abomasa and in small intestine, their prevalence, and abundance are presented in Table
106 1.

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

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

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

118 (16%) and *Moniezia* sp. (2%). Larvae of protostrongylids were found in 36% of the examined
119 samples; cysts/oocysts of *Giardia* sp., *Entamoeba* sp. and *Eimeria* sp. were found in 4%, 2%
120 and 2% of the samples, respectively.

121 Sex had no effect on the intensity of infection. Adult animals had significantly higher
122 intensity of infection when considering the total parasite count (TPC) (48,174 vs 10,380;
123 $p=0.001$) as well as *O. antipini*, *O. leptospicularis/O. kolchida*, $p<0.001$; *T. capricola* and *M.*
124 *dagestanica*, $p<0.05$, and higher prevalence of *O. leptospicularis/O. kolchida* infection
125 ($p<0.01$).

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

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

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

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

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

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

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

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

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

166 Parasite counts in this study should be considered as extremely high regarding when referring
167 to moose GI parasitism. Recorded burdens contribute presumably to the poor condition of
168 sampled animals, considering the extensive abomasitis changes observed in some cases at
169 necropsy (data not shown). The higher worm burden recorded in adults suggests that
170 acquisition of infection accumulates over time. The high intensity of infection in moose also
171 indicates a high parasite infection pressure from the environment, in these parts of Sweden,
172 characterized by a density of approximately between 0.3 – 1.0 moose/km². It should be
173 highlighted that *Echinococcus* sp. and lungworm infection were not accounted for in the
174 present study, but can play a role in mortality (Kutz et al. 2012).

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

181

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187

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228

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

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