EXPERIMENTAL INFECTION OF MOOSE (*ALCES ALCES*) WITH *ELAPHOSTRONGYLUS* SPP. (NEMATODA, PROTOSTRONGYLIDAE) ORIGINATING FROM REINDEER (*RANGIFER TARANDUS*) AND MOOSE, WITH SPECIAL EMPHASIS ON CLINICAL SIGNS, GROSS- AND MICROSCOPIC LESIONS, AND PREDILECTION SITES

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ABSTRACT: Captive moose calves (*Alces alces*) were used to study symptoms of *Elaphostrongylus* infections that affect moose and reindeer (*Rangifer tarandus*) in Scandinavia. Seven calves were infected experimentally with *Elaphostrongylus alces* larvae and three with *E. rangiferi* larvae. Both parasites produced neurological and behavioral symptoms in calves. The *E. alces* animals showed mild to severe neurological signs and moderate pathological changes, mainly in the meninges and the peripheral nervous system. Other symptoms were posterior weakness, clockwise circling, and inwards bending of the hind legs. The *E. rangiferi* animals showed severe neurological signs and paralysis with mild to prominent pathological changes in the central nervous system; behavioral symptoms included legs kept wide apart, weakness, gait incoordination, and reluctance to rise. In general, the severity of symptoms was related to the parasitic dosage and whether the host was normal or aberrant. The symptoms and lesions identified here should aid in identifying the cause and extent of these parasites in mortality of wild moose typically observed at or near death.

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Key words: *Alces alces*; clinical signs; disease; *Elaphostrongylus alces*; *Elaphostrongylus rangiferi*; lesions; moose; parasites; symptoms.

Both moose (*Alces alces*) and reindeer (*Rangifer tarandus*) populations increased in Scandinavia during the 20th century, with moose numbers peaking in the 1980s. These increases were associated with multiple factors including changes in Swedish forest management, a decrease in the moose harvest, and the absence of large carnivores (Cederlund and Markgren 1987, Cederlund and Bergström 1996, Hörnberg 2001). During the last half of the 20th century, attention was directed towards nematodosis in the nervous system of moose and reindeer in Scandanavia (Roneus and Nordkvist 1962, Kummeneje 1974, Stéen and Rehbinder 1986, Stuve 1986, Stuve and Skorping 1987, Stéen and Roepstorpff 1990, Handeland and Norberg 1992, Olsson et al. 1995). Two species of *Elaphostrongylus* were associated with neurologic disease in these cervids, namely *E. rangiferi* (Mitskevich 1960) and *E. alces* (Stéen et al. 1989, 1997, Stéen and Johansson 1990, Gibbons et al. 1991, Gajadhar et al. 2000).

High density populations of moose and reindeer presumably facilitated the spread of *E. alces* and *E. rangiferi*, such that high rates of neurological disease in both during the 1980s and 1990s were likely due to elaphostrongylosis (Stéen and Rehbinder 1986, Stéen and Roepstorpff 1990, Handeland 2002, Davidson

et al. 2020). Elaphostrongylosis in reindeer causes general physical weakness, poor coordination in movement of the hind legs, and/or posterior paralysis (Roneus and Nordkvist 1962, Mitskevich 1964, Bakken and Sparboe 1973, Handeland and Nordberg 1992, Handeland 1994); other clinical signs include restlessness, dyspnea, and coughing (Kummeneje 1974). Elaphostrongylosis in wild moose is characterized by incoordination, swaying of hindquarters, broad and stamping gait, and hypermetria (Stéen and Roepstorpff 1990). Elaphostrongylid larvae (L_1) are passed via feces, thereafter penetrating a terrestrial gastropod and developing into the infective stage (L_3); cervids are exposed by ingesting infected gastropods (Lankester 2008). However, the routes taken by L_3s to become L_5s and adults are not fully understood and may differ among *Elaphostrongylus* spp. and their hosts.

Given the common occurrence of elaphostrongylosis and related morbidity in Swedish moose, it was important to describe the behavioral and physical symptoms associated with the disease in a controlled setting. Therefore, the objective of this study was to experimentally infect captive moose with high dosages of *E. alces* and *E. rangiferi* to induce symptoms of elaphostrongylosis. Specifically, I aimed to identify and describe the clinical signs, gross- and microscopic lesions, predilection sites, and growth of moose associated with the disease.

METHODS

Eighteen moose calves up to 7 months-old (1 May presumed universal birth date) were obtained from the wild (n = 11) and zoos (n = 7) (Table 1). They were maintained in separate stalls in an indoor stable rebuilt for this purpose (Stéen et al. 1997). Calves from zoos had been dewormed with Ivermectin (Ivomec® 0.2 mg/kg) 2–4.5 months before procurement. Fecal samples were collected and examined weekly from each calf prior to the experiment; all except C11 were initially negative for protostrongylid larvae. At 10–30

Table 1. Characteristics of moose calves experimentally infected with *Elaphostrongylus alces* or *E. rangiferi*.

ID	Age (months) – (sex)	[1]Inoculum	Infection age (mo)	Initial symptom [2](DPI)	Mortality [3](DPI)
E. alces					
C2	< 1 (M)	1000	7	15	17 (D)
C3	2 (F)	1000	7	15	37 (E)
C4	5 (M)	1000	6	36	39 (D)
C5	5 (F)	1000	7	54	84 (E)
C6	5 (F)	1000	7	59	62 (D)
C7	2 (M)	1000	7	41	100 (E)
C8	7 (F)	1000	11	31	70 (E)
C9	1 (F)	400	11	80	75 (E)
C11	5 (F)	400	9	34	125 (E)
E. rangiferi					
C1	2 (F)	1000	5	9	12 (E)
C10	1 (F)	1000	5	14	94 (E)
C12	0 (M)	1000	5	64	152 (D)

[1]Inoculum = *E. alces* and *E. rangiferi* of larvae/dose. [2]DPI = days post-infection. [3]D = died, E = euthanized.

days post-stabling, all were treated with mebendazol for 10 days (6 mg/kg Mebenvet®) (Nordkvist et al. 1983, Stéen et al. 1997).

Twelve calves were infected at 5–11 months old and 6 were control animals. Two calves (C9, C11) were infected with ~ 400 *E. alces* L_3, 7 (C2–C8) with ~ 1000 *E. alces* L_3, and 3 (C1, C10, C12) with ~ 1000 *E. rangiferi* L_3 (Table 1). Calves were infected by administering crushed snails harboring infected larvae either in their bottle-fed milk formula or through a stomach tube (Stéen et al. 1997); a single exception was C8 fed grain containing crushed snails. Each calf was observed daily to document and photograph any abnormalities in behavior, gait, and posture.

Biweekly, the calves were immobilized with Rompun® (xylazine, 1.0 mg/kg), weighed, and clinically examined; immobilization was reversed with Antisedan® (atipamezol, 1.0 mL/40 mg xylazine). Animals with fever were treated with Streptocillin® (Dihydristreptomycin-bensylpenicillinprocain 0.25 g, 200,000 IE/mL, 1.0 mL/kg) and Finadyn® (analgeticum, antipyreticum 50 mg/mL, 2.0 mL/kg). If unable to rise from a prone position, calves were euthanized intravenously (Phenobarbitalum natricum 109.7 g, spiritus fortis 209 g aq. puris/100 mL ex. tempore); survivors were euthanized similarly at experiment end.

Elaphostrongylus spp.

Morphological criteria (Lankester et al. 1998) were used to determine the purity of the elaphostrongyline L_1s; the L_1 infection of the intermediate host is described in Stéen et al. (1997). The dosages of infective larvae (L_3) were similar to those employed in other infection studies with *Elaphostrongylus* spp. (Halvorsen et al. 1989, Handeland and Skorping 1992, Handeland et al. 1993, 2000, Hemmingsen et al. 1993). Because the objective was to induce observable symptoms, these purposefully severe infections did not equate or intend to reflect the rate of gastropod (parasite) acquisition through free-ranging consumption. Procedures to examine feces and lungs for infective larvae (L_1) are provided in Stéen et al. (1997), and clinical signs and lesions indicated sites to recover adult and juvenile *Elaphostrongylus* spp. (see RESULTS). Larvae were identified to family as described in Lankester et al. (1998).

Post Mortem Examination

All experimental calves were necropsied except 4 control animals. They were completely skinned after which the skeletal muscles, muscle fascia, and sub-cutis were inspected macroscopically for *Elaphostrongylus* spp. and lesions. The complete central nervous system (CNS) was removed by sawing the skull and vertebral column to expose the entire CNS. The dura mater was cut longitudinally, flattened, and examined for *Elaphostrongylus* spp. and lesions; spinal nerves were cut and removed intact. The brain and attached pia arachnoid was opened and examined for *Elaphostrongylus* spp. and lesions (Stéen et al. 1997, 1998). The brain was cut into sections (in accord with the National Veterinary Institute, Sweden) from the frontal lobe to the obex, including frontal and occipital poles of the cerebral hemispheres, the middle of the cerebral hemisphere, trigonum olfactorium, the optic chiasma, the pyriform lobes, cerebral peduncles, and all lobes of the cerebellum and the medulla oblongata. Spinal cord sections were taken from the 4 regions of the spinal cord (i.e., cervical, thoracic, lumbar, and sacral regions). The whole brain and spinal cord, and tissue samples from skeletal muscles and internal organs were fixed in 10% formalin for histology. Histological specimens were processed, cut at a thickness of 4µm, and stained with hematoxylin and eosin.

Nematodes were fixed in heated 70% ethanol and identified to genus using light microscopy.

RESULTS

All moose (fed *ad libitum*) were in moderate to good body condition with normal increase in body weight during the experiment regardless of experimental or control animal, parasitic treatment (*Elaphostrongylus* species), or infective dose.

Clinical Signs – 400 and 1000 *E. alces* L_3

Most calves began showing clinical signs at 15–80 days post-infection (DPI) including weak hindquarters, swaying hind legs with wide-base stance, and uncoordinated gait (Tables 1, 2). Other clinical signs included reduced appetite, intermittent coughing, rattling and labored breathing, bruxism (grinding of the teeth), and evidence of aching muscles. Other symptoms shared of all calves included weakness in the back, shuffling of hind feet while walking, and lack of balance, except C2 which died at 17 DPI. Stiffness and recumbency were common in C2, C3, and C8; C3 was euthanized prematurely at 37 DPI due to injury. Clinical signs of C9 were mild as only weakness in the hind legs and knuckling were observed occasionally; it later recovered with no clinical signs evident prior to euthanasia. Only respiratory signs were observed of C11. Elevated body temperature (39.9–40.7°C) was measured in C2, C4, C5, and C6. Posterior weakness, clockwise circling, and inward bending of the hind legs with toeing out (Fig. 1) were observed in C4. Knuckling of the hind feet was observed in C5 and C7.

Gross Lesions – 400 and 1000 *E. alces* L_3

All moose that died or were euthanized were evaluated at necropsy for presence of gross lesions associated with *E. alces* infection.

Fig. 1. Moose calf infected experimentally with *Elaphostrongylus alces* displaying inward bending and toeing out of the hind legs.

Adult *E. alces* in varying amounts were located epidural in the spinal canal or the muscle fascia in 7 calves at 39 – 125 DPI (Table 2); no adult *E. alces* were found earlier at 17 (C2) and 37 DPI (C3). Most calves (C3 – C9) had macroscopic changes in the CNS and peripheral nervous system (PNS) including edema, hyperemia, and hemorrhage, especially in the epidural space of the spinal canal; C2 and C11 were the exceptions. Interestingly, C11 was the lone calf testing positive for protostrongylid larvae at arrival (Table 2).

There was moderate congestion and slight edema in the lungs of all calves. Additionally, C8 and C9 had multiple parasitic nodules spread widely in the lung parenchyma, with occasional hematomas. No gross pathological change was evident in the lungs of the other calves (C3 – C7, C11). L_1s were found in C4, C5, C7, and C9. Larvae without dorsal spines were found in the lungs of C3 (37 DPI); however, these larvae originated from the inoculum as larvae produced in the host have spines (Stéen et al. 1997).

The liver was somewhat enlarged and hyperemic in all calves. The liver of C5 was slightly fatty with a friable consistency and

the cut surface was greasy. Fibrin tags were noted on the liver capsule, indicating peri-hepatitis in C3. Larvae without spines (L_3–L_4) were found in the liver of C3 (Stéen et al. 1997). The parenchyma of the kidneys had multiple white-spots in C3, whereas slight hyperemia was found in the kidneys of C2, C4 – C9, and C11. The spleen was moderately blood-filled and the body lymph nodes were slightly to moderately enlarged with dark-red cut surfaces in all calves.

Microscopic Lesions – 400 and 1000 *E. alces* L_3

Brain and spinal cord

Moderate to severe non-purulent encephalitis with inflammatory cells (gliosis) in the cerebrum (Fig. 2), with lymphocytes, plasma cells, macrophages, and presence of lymphocytic perivascular cuffing were found in 4 calves (C2 – C5); the meninges of the cerebrum was similarly affected in C3 and C5. Endothelial hyperplasia of the vessel walls occurred in C4 and C6, and moderate non-purulent meningitis was found in the cerebral meninges of C6 and C7 (Fig. 3). The cerebrum was hyperemic and edematous in C8, and the cerebrum had moderate to severe non-purulent meningitis in C9 and C11 (Fig. 3), with inflammatory cells similar to those described above, as well as slight hyperemia and edema in the meninges and brain parenchyma (Table 2).

Hyperemia and edema were common in the spinal cords of all calves. A mild, non-purulent myelitis with perivascular cuffing and hyperemia occurred in the grey matter of the spinal cord of C2. Slight hyperemia, edema, and hemorrhages were observed in C2, C3, C4, and C7; slight meningitis with mononuclear perivascular cuffing and hemorrhages was also observed in C7. Accumulations of lymphocytes, macrophages, plasma cells, eosinophils with hyperemia, and hemorrhage were present in the peri-neurium and along the epidural side of the dura mater in C5, C6, and C8. Severe lesions associated with egg-granulomas (eggs surrounded by giant cells and fibrous tissue) were found in C9 and C11 along the epidural side of the dura mater in all spinal sections and around the nerves (Fig. 4). A heavy cellular reaction (plasma cells, lymphocytes, neutrophils, and eosinophils) was associated with both larvae and egg-granulomas in C9 (Fig. 4). The reaction included mainly eosinophils,

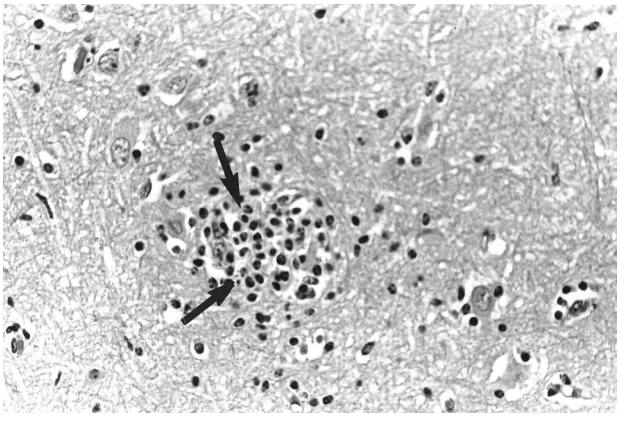

Fig. 2. Focal gliosis in the cerebrum (40X) of a moose calf infected experimentally with *Elaphostrongylus alces*.

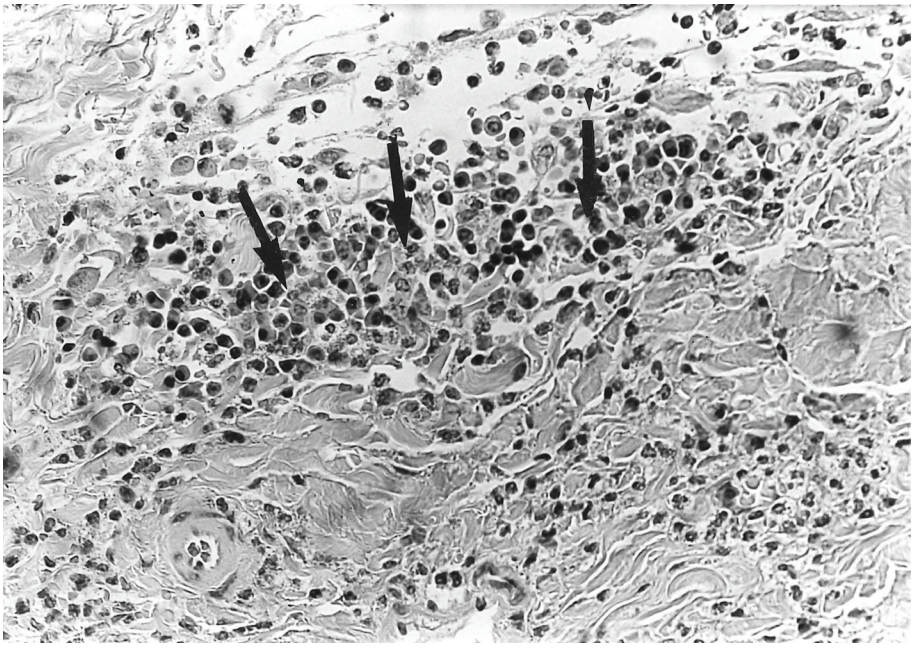

Fig. 3. Evidence of non-purulent meningitis with mononuclear cells (40X) in a moose calf infected experimentally with *Elaphostrongylus alces*.

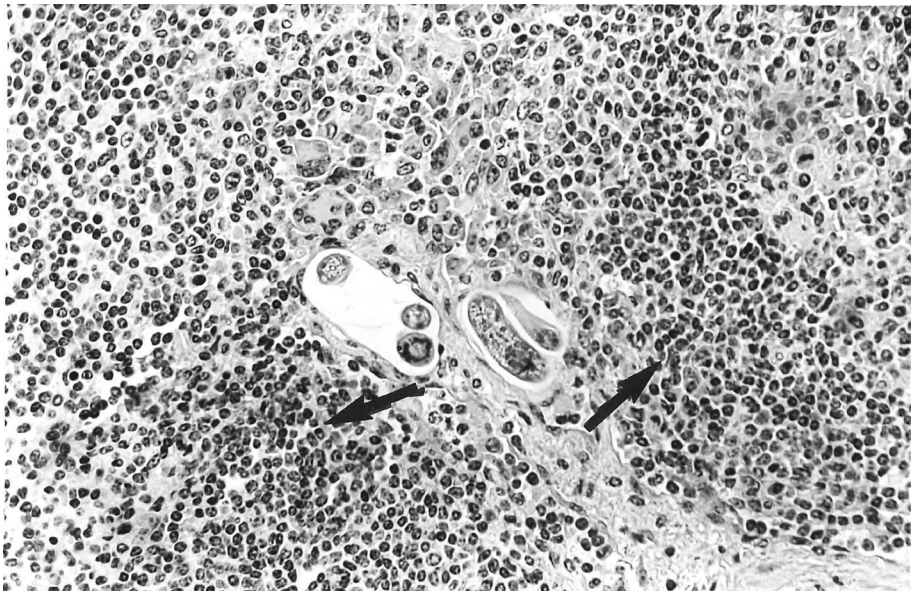

Fig. 4. Evidence of microscopic reaction towards egg-granulomas and larvae with peri-neuritis in a nerve-root (40X) of a moose calf infected experimentally with *Elaphostrongylus alces*.

although a significant part of the cell population also consisted of macrophages and lymphocytes, with a few foreign-body giant cells. Parasitic inflammation and egg-granulomas (Fig. 4) were accompanied by purulent exudate in C11 (Table 2).

Table 2. Characteristics and symptoms of moose calves experimentally infected with *Elaphostrongylus alces* or *E. rangiferi*.

Calf ID	[1]Inoculum	[2] Parasite Recovery	[3]Inflammation	[4]Clinical symptom
E. alces				
C2	1000	-	2	3 (N, R, F)
C3	1000	-	3	3 (N, R, GC, A, M)
C4	1000	4: ce, mf	2	3 (N, F, GC, A, M)
C5	1000	57: ce	3	3 (N, F, GC, A, M)
C6	1000	23: ce	2	3 (N, GC, A, M)
C7	1000	33: ce	2	3 (N, GC, A, R)
C8	1000	7: ce	1	1 (N, R)
C9	400	6: ce	1	1 (N)
C11*	400	42: ce, mf	3	2 (R)
E. Rangiferi				
C1**	1000	-	1	3 (N)
C10	1000	11: ce, cs	2	3 (N, R, F, GC, A, M)
C12	1000	5: ce, mf	2	3 (N, R, F, GC, A)

[1]Inoculum = infection of *E. alces* and *E. rangiferi*. [2]Parasite recovery = number and locale of recovered *E. alces* and *E. rangiferi* larvae/adult, where ce = cavum epidurale, cs = cavum subdurale, mf = muscle fasciae. [3]Degree of brain inflammation where 1 = mild, 2 = moderate, and 3 = severe. [4]Clinical level of symptoms where 1 = mild, 2 = moderate, 3 = severe, and N = neurologic, R = respiratory, F = fever, GC = general condition affected, A = inappetence, and M = muscular. *C11 was protostrongylid positive at capture. **Injury induced euthanasia at 12 DPI.

Lungs

Verminous pneumonia with moderate numbers of nematode eggs and larvae, hyperplasia of the bronchial epithelium, and endothelial hyperplasia of the vessel walls were found in C5 with maximal LPG of 214 L_1, C7 with maximal LPG 281 L_1, C9 with maximum LPG 18 L_1, and C11 with a maximal LPG of 808 L_1. Emphysema occurred in C5 and C7, along with a moderate number of mononuclear cells in the interlobular septae; some desquamated epithelial cells were in the bronchi with severe hemorrhage. A mild to moderate bronchoepithelial and peribronchial lymphoid hyperplasia with slight interstitial emphysema occurred in C8 with maximal LPG of 131 L_1. A pronounced purulent pneumonia with main infiltrations of eosinophils, neutrophils, and mononuclear perivascular cuffing occurred in C11. All these calves were euthanized at a later stage of the experiment (Tables 1, 2).

Other organs

The liver showed hyperemia in all infected calves. Mononuclear cellular infiltration in the liver capsule and in the portal triads was present in C5 and C7; focal liver necrosis with cell debris and surrounding lymphocytes was also observed in C5 (Table 2). Interstitial nephritis was pronounced in C5, characterized by severe mononuclear cell infiltrations in the medullary part of the kidney. C7 had acute perisplenitis in the form of hyperemia and granulocytes on the splenic capsule. Lymphadenitis in mesenteric lymph nodes with granulocytic cellular infiltrations in the sinuses, capsule, and trabeculae was observed in C5 and C11, and lymph node hyperplasia was observed in C7 and C8. A few nematode eggs and L_1 were found in the

cortical part of the right popliteal lymph node in C11.

Clinical Signs – 1000 *E. rangiferi* L_3

The 3 calves began showing weakness and uncoordinated gait in the hind legs at various times from 9 to 64 DPI (Tables 1, 2). As early as 9 DPI, C1 was listless and reluctant to rise while placing its weight forward on the front legs; its condition progressively worsened as it increasingly lost balance. It continuously lifted its hind legs keeping them spread and pointing forward near the front legs; C12 later displayed similar posture. At 12 DPI, C1 had posterior paralysis, broke its right front leg, and was euthanized (Table 1).

C10 and C12 walked on the tips of their hooves with C12 markedly weak in the hindquarters. The metacarpal-phalanges articulations were weak and over-extended during locomotion. This caused the phalanges to bend inwards with hyperextension of the fetlock, resulting in the dewclaws touching the ground (Fig. 5). Weakness in the hind legs progressed, the joints becoming severely swollen. Both calves were unable to rise, ultimately suffering paralysis; C10 was euthanized at 94 DPI and C12 succumbed at 152 DPI after it stopped eating, became lethargic, and collapsed (Table 1).

C10 had respiratory distress with labored breathing and intermittent cramps through 30 DPI, and C12 had coughing and labored breathing (Table 2). Both had intermittent high fever up to 41°C, shivering with continuously deep coughs; the fever declined after treatment with antibiotics and analgeticum. At 90 DPI (4 days prior to euthanasia), C10 exhibited nystagmus (uncontrolled repetitive movements of the eyes), loss of appetite, bruxism (teeth grinding), stiffness of the hind leg muscles, and obvious pain in the body muscles and joints. It was generally weak, inactive and recumbent, and tired quickly when exercised. It moved in a stumbling fashion with hypermetria, leaning against walls to gain balance and by pressing its forehead against a wall.

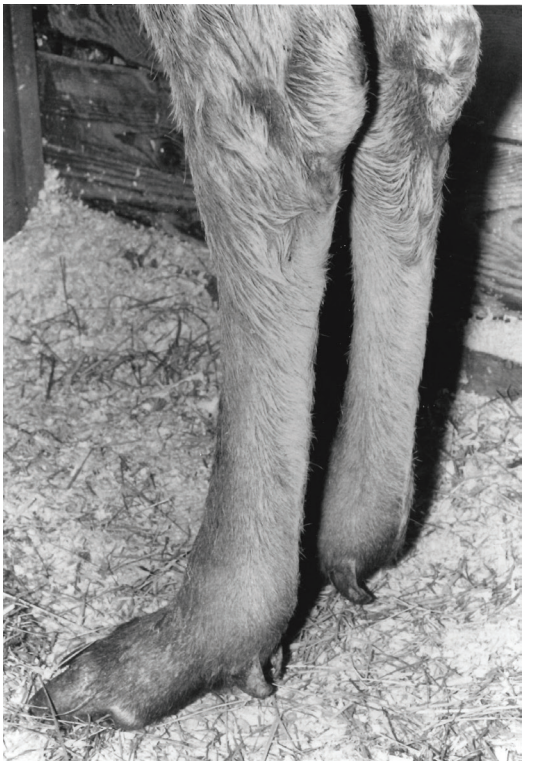

Fig. 5. Moose calf (*Alces alces*) infected experimentally with *Elaphostrongylus rangiferi* displaying severely swollen joints, and weak and flexed metacarpus-phalanges articulation causing the phalanges to bend inwards with hyperextension of the fetlock, resulting in the dewclaws touching the ground.

Gross Lesions – 1000 *E. rangiferi* L_3

No adult *E. rangiferi* were found in the CNS or muscle fascia of C1 euthanized at 12 DPI (Table 2); L_3–L_4 were found in the lungs, but not eggs or L_1s. Eleven adult *E. rangiferi* were located subdural and epidural of the spinal cord in C10 euthanized at 94 DPI (Table 2), and 5 adult *E. rangiferi* were located subdural, penetrating pia mater of

the cerebrum (Fig. 6) and in muscle fascia of C12 at 152 DPI (Table 2) (see Stéen et al. 1997). Multiple hemorrhages and some edema were found in the meninges of the CNS in C1 and C12; despite the presence of 11 adult *E. alces*, no lesions were found in the CNS of C10.

Hyperemia and congestion were common in the lungs of all 3 calves. The lungs of C1 exhibited fresh and old hematomas, plus several parasitic nodules. A moderate amount of fibrin was attached to the pulmonary pleural surface indicating pleuritic in C12, that also shed larvae in the feces at 133 DPI (see Stéen at al. 1997). The liver of C1 was moderately fatty and covered with multiple white-spots and fresh focal hemorrhages and the cut surface was slightly greasy;

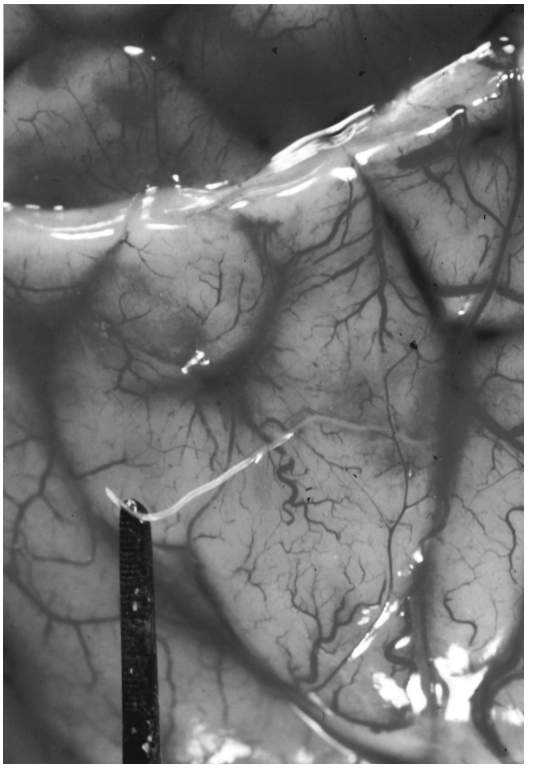

Fig. 6. An adult *Elaphostrongylus rangiferi* worm penetrating the pia mater in the cerebrum of a moose calf infected experimentally with *E. rangiferi.*

L_3 and L_4 were not present. The kidneys of C12 had white-spots on the surface beneath the capsule. The mesenteric lymph nodes and spleen were swollen, and the cut surfaces were intensely hyperemic in C1 and C12. All calves displayed petechial hemorrhages in the body muscles and edematous joints.

Microscopic Lesions – 1000 *E. rangiferi* L_3

Brain and spinal cord

A moderate extravascular hemorrhage and hyperemia were found in the cerebrum of C1 (Table 2). The CNS of C10 and C12 had moderate meningo-encephalitis with severe hyperemia, edema, accumulation of mononuclear inflammatory cells, and perivascular cuffing (Table 2). Endothelial hyperplasia was present in some vessel walls in the grey matter of the spinal cord, especially in the area of filum terminale of C12. Diffuse hemorrhages with hyperemia, edema, and a moderate mononuclear cellular reaction were in the connective tissue between the spinal nerves in the subdural space and along the subdural side of the dura mater of the spinal cord down to the cauda equine in all 3 calves, but most pronounced in C1 (Table 2).

Lungs

The lungs were hyperemic, edematous, and emphysematous in the 3 calves. Interstitial pneumonia characterized by severe consolidation, mononuclear cellular infiltration, and focal necrosis with cell debris were evident in C1 that had L_3–L_4 but no L_1. A mild interstitial mononuclear cellular reaction was visible in C10, also without L_1 (Table 2). Lymphadenitis characterized by severe granulocytic and mononuclear cellular infiltration in the capsule and sinuses of the

mediastinal lymph nodes were observed in all 3 calves.

Other organs

The liver of all 3 calves exhibited moderate bile duct proliferation with mononuclear cell reaction in the portal spaces. Their kidneys had severe interstitial nephritis, consisting of multi-focal necrosis with cell debris, calcified tubular walls, and mononuclear cellular reaction.

Control animals

The control calves showed no clinical signs of elaphostrongylosis. Necropsy of 2 calves revealed no macroscopic or microscopic changes which could be associated with elaphostrongylosis, and no larvae or adult *Elaphostrongylus* spp. were found. Fecal analyses indicated no shedding of elaphostrongyline larvae during the experiment.

DISCUSSION

Overall, the clinical signs in moose calves with *E. rangiferi* infections were more severe than those associated with *E. alces*, as indicated by the relative abundance of lesions in the CNS, PNS, and internal organs, as well as the parasite-host relationship. However, certain calves in each treatment had an inflammatory reaction (Lankester 2008), or a response of sudden onset of neurological signs and rapid progression to the point where an animal became incapacitated or died. Further, dosage appeared to be influential – calves infected with 1000 *E. alces* L_3 showed moderate to severe locomotor signs with intermittent paresis, and those infected with 1000 *E. rangiferi* L_3 showed severe incoordination, neurological signs such as paresis, total paralysis, and other clinical signs. Conversely, calves infected with 400 *E. alces* L_3 had only mild and non-aggravating uncoordinated gait, although some histopathological changes were found in the CNS and PNS.

The extent of damage varies with the activity and numbers of migrating *Elaphostrongylus* spp., and the degree of inflammatory reaction by the host. Paresis and ataxia in *E. rangiferi*-infected moose were attributed to meningitis and spinal-nerve-root lesions in the lumbosacral regions of the spinal cord (Handeland 1994). Ingested *E. rangiferi* L_3 develop to adults in the arachnoid along the spinal cord, spinal canal, ventricles, and deep within nerves (Handeland 1994), and Anderson (1968) believed that elaphostrongyline had to undergo development to adult stages in the nerve parenchyma. In reindeer infected with *E. rangiferi*, parasites are recovered in the CNS where pathological changes also occur (Roneus and Nordkvist 1962, Handeland and Norberg 1992, Stéen et al. 1997). The 2 *E. rangiferi*-infected calves euthanized at 90+ DPI had lesions in the meninges, whereas histopathological changes were confined to the subdural space of the dura mater and around the spinal nerves of the spinal cord in C1 euthanized at 12 DPI. Unlike in reindeer infected with *E. rangiferi* (Roneus and Nordkvist 1962, Handeland and Norberg 1992), eggs and larvae were not found in these locations. The lesions subdural in moose calves infected with *E. rangiferi* indicate that an aberrant parasite, such as *E. rangiferi,* keeps its route and predilection site regardless of host (Lankester 1977, 2008, Handeland 1991, 2002, Stéen et al. 1998).

In calves infected with *E. alces*, lesions were concentrated epidural along the spinal canal and larvae were not inside the CNS parenchyma (Stéen et al. 1997, 1998). In this study, lesions from *E. alces* were mostly concentrated to the PNS and meninges of the CNS. The frequency of histopathological changes in the adipose and connective

tissues around nerve roots, with the presence of eggs and larvae along the epidural side of the dura mater, indicate that *E. alces* move and reproduce at the epidural side of the spinal cord, not the parenchyma of the CNS.

Migrating and/or developing *E. alces* L_3s caused edema, hyperemia, hemorrhages, encephalitis, and meningitis in the CNS, meningitis of the spinal cord, as well as egg-granulomas with cellular reaction close to the spinal nerves. Lesions even occurred in C3 (1000 L_3) that was euthanized at 37 DPI and C4 (1000 L_3) that died at 39 DPI, but not in C2 (1000 L_3) that died at 17 DPI or C11 infected with 400 L_3s. Migrating *E. alces* L_3s in the abdomen, or via hematogenous route or the lymph system, produced focal inflammation, necrosis, and hemorrhages in the liver, kidneys, and spleen, and *E. alces* eggs and L_1s caused congestion, edema, and microscopically verminous pneumonia.

The CNS meninges of *E. rangiferi*-infected calves C1 and C12 displayed hemorrhages, edema, and meningo-encephalitis. Migrating L_3s caused white spots and hemorrhages in the liver (C1 only), white spots in the kidneys, and several hemorrhages in the body muscles. The 3 *E. rangiferi*-infected calves (C1, C10, C12) displayed lung hyperemia, congestion, and hematomas; only C1 had parasitic nodules in the lungs. Similarly, Spratt and Anderson (1968) found that L_3s moved through the liver, penetrated the diaphragm and pleural cavity, and were present in the lungs of *P. tenuis*-infected guinea pigs (*Cavia porcellus*).

The severity of lesions and clinical signs should reflect the infective dosage and the interaction between the parasites and its normal or aberrant host. The inflammatory lesions were presumably the cause of illness and neurological signs documented in both groups of infected calves, and histopathological changes were confirmed in the CNS and PNS. Pathological lesions caused by elaphostrongyline may be single or multiple, and occur anywhere in the CNS (Anderson 1968). Indeed, Handeland et al. (2000) found extensive changes and lesions in the CNS of a sheep (*Ovis aries*) experimentally infected with *E. cervi,* a closely related nematode species in red deer (*Cervus elaphus*; Lankester et al.1998). Nordkvist (1971) and Lankester (2008) previously speculated that the severity of clinical signs is probably parasite dose-dependent. I found that the severity of clinical signs was generally related to the infective dose of larvae as reflected by pathological changes. For example, C9 and C11 infected with 400 *E. alces* showed mild clinical signs throughout, surviving to euthanasia at 75 DPI and 125 DPI, respectively.

Olsson et al. (1998) found that the dosage of *E. cervi* L_3 given to guinea pigs was inversely related to the time when, or if the parasite reached the CNS. None reached the CNS at a dose of 150 L_3, whereas the parasite entered the CNS by 11 days at a dose of 1000 L_3. I believe that the lower dose of *E. alces* (400 L_3) was related to the lack of progression in clinical signs. A similar 10-day period to reach the CNS in moose was reported with *Parelaphostrongylus tenius* (a parasite of *Odocoileus virginianus*) larvae (Anderson and Strelive 1968, Anderson and Prestwood 1981), as well as *E. cervi* in a study with guinea pigs (Demiaszkiewicz 1989).

In elaphostrongyline infections, the immune response in different hosts varies from remarkable tolerance to severe inflammatory reaction. The reasons for these marked differences are largely unknown, particularly those related to the cellular immune response. The subarachnoid space has been recognized as an active immunological harbor (Hemmingsen et al. 1993). Spratt and Anderson (1968) suggested that nematodes which develop early life stages in immunologic tissues of their host, yet do not

trigger an immune response, have an evolutionary advantage. They found that *P. tenuis* L_3 were largely destroyed when introduced to an abnormal host (i.e., guinea pig), but if entering the CNS successfully, survived and developed.

In addition to neurologic signs, respiratory distress occurred in calves infected with both parasites and at two intervals, 7–15 DPI and 23–50 DPI. The first onset was probably due to the migration of L_3 and L_4 found in the lungs. The second phase was correlated with, and probably due to the irritation of mucous membranes in the upper respiratory tract when L_1 were coughed up and swallowed prior to their shedding in feces.

There are different conclusions and hypotheses about the migration route of *Elaphostrongylus* spp. Infective larvae without a spine found in the lungs and liver point to a hematogenus route (Stéen et al. 1997). And, Handeland and Skorping (1992) and Handeland (1994) believed that via general circulation, infective larvae penetrate the venules of the abomasal wall, travel through the hepatic-portal circulation to the heart and lungs, and spread to tissues including the CNS. But, Handeland (2002) found an L_3 in a sinus of a lymph node in an *E. cervi*-infected goat (*Capra hircus*), suggesting that migration may also occur via the lymph system. Likewise, eggs and L_1 of *E. alces* were found histologically in a popliteal lymph node of C11, which may indicate: 1) that L_3s can be trapped in the lymph system and develop to adults that produce eggs and hatch L_1, and 2) that the migration to the lungs can take place via the lymph system from hatched L_1s.

My study and Handeland et al. (2002) found that *Elaphostrongylus* larvae (L_3 and L_1) can migrate via the lymph system, and Handeland (1994) described a lymphatic-vascular migratory route from the gastrointestinal wall via the gastrointestinal lymph nodes to the lungs. In guinea pigs, however, *E. cervi* L_3s in the liver and omentum migrated by penetrating the stomach wall into the peritoneal cavity, moving through the diaphragm into the pleural cavity along the lateral body wall to enter the vertebral canal, likely along spinal nerves, entering the CNS after 11 days (Olsson et al. 1998). Migration via a hematogenous route or the lymph system was not evident, and Lankester (2008) also believed that L_3 spread is inconsistent via a hematogenous route. Spratt and Anderson (1968) described both L_3 migration directly through tissues entering the CNS via nerves or along blood vessels and that L_3s were carried via blood to the CNS. Anderson and Strelive (1968) suggested that *P. tenius* migrated directly along the spinal nerves. In summary, it is not entirely clear how the different elaphostrongylids migrate successfully and differences are possible.

At *ad libitum* consumption, body weight of calves increased similarly in the treatment and control groups. Likewise, Anderson (1964) reported that moose experimentally infected with *P. tenuis* were in good condition despite symptoms of neurologic disease. However, moose naturally infected with *E. alces* are often found emaciated (Stéen and Rehbinder 1986, Stéen and Roepstorpff 1990); albeit, animals are typically discovered near death with severe neurological symptoms. This deterioration is likely caused by the direct influence of parasite location that leads to neurological disorder that inhibits mobility and foraging, ultimately causing starvation (Stéen et al. 2005). The symptoms and characteristics of infection reported here will help identify the cause of debilitation and mortality in such moose.

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