Effects of *Elaphostrongylus* spp. infection on haematology and blood chemistry

– in moose (*Alces alces*), reindeer (*Rangifer tarandus*), goat (*Capra aegagrus hircus*) and sheep (*Ovis aries*)

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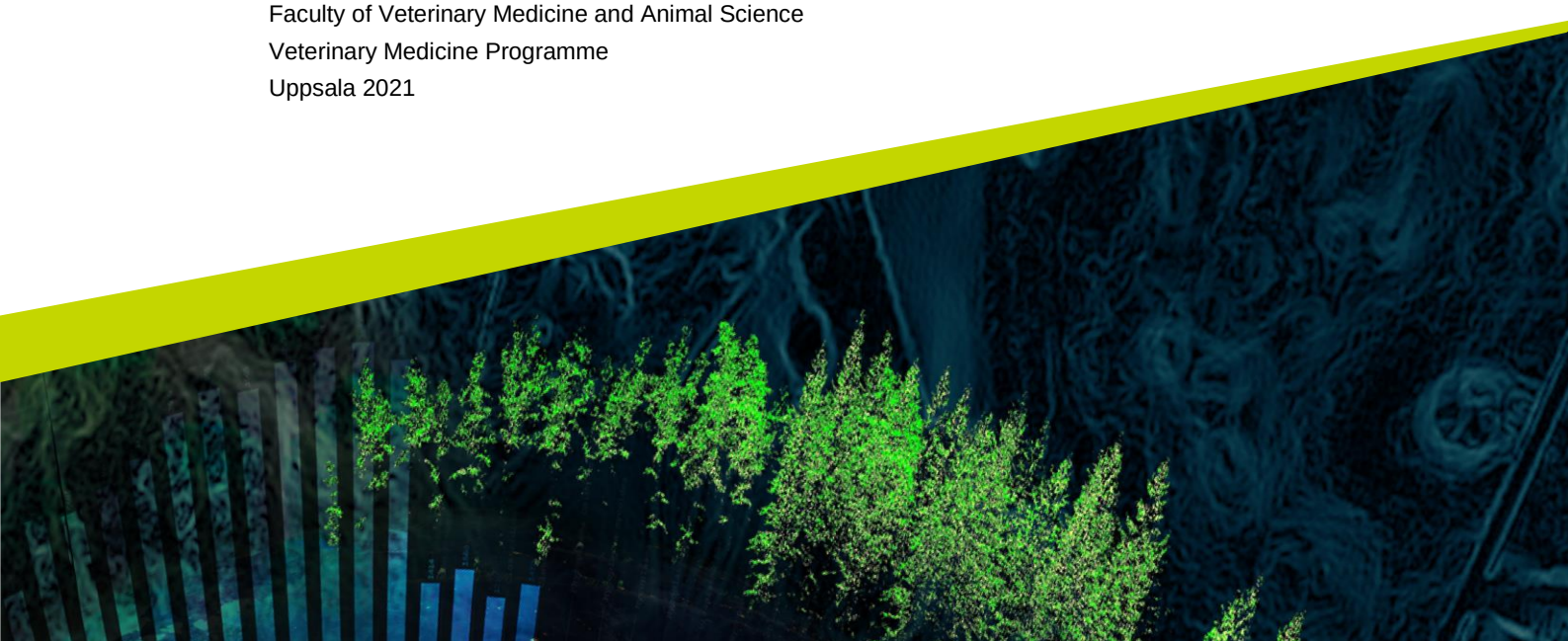
Degree project/Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
Veterinary Medicine Programme
Uppsala 2021



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Credits:	30 credits
Level:	A2E
Course title:	Independent project in Veterinary Medicine
Course code:	EX0869
Programme/education:	Veterinary Medicine Programme
Course coordinating dept:	Department of Clinical Sciences
Place of publication:	Uppsala
Year of publication:	2021
Keywords:	*E. alces, E. rangiferi,* elaphostrongylosis, muscleworm, brainworm, small ruminant, eosinophilia, biochemistry, serum chemistry

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Abstract

The aim with this study was to investigate the effects *Elaphostrongylus alces* and *Elaphostrongylus rangiferi* have on haematology and blood chemistry in experimentally infected animals. The nematodes of genus *Elaphostrongylus* spp. can cause neurologic disease, myositis, verminous pneumonia and increased mortality. Nine moose (*Alces alces*) calves, six reindeer (*Rangifer tarandus*) calves, six goat (*Capra aegagrus hircus*) kids and five lambs (*Ovis aries*) were experimentally infected with *Elaphostrongylus alces*. Three moose calves and one lamb were infected with *Elaphostrongylus rangiferi*. Each animal was orally inoculated with approx. 1000 infective third-stage larvae, two moose calves received a lower dose (approx. 400 infective larvae, *E. alces*). Blood samples were taken at around 14-day intervals from each animal and continued until the animal died or was euthanized, 12 to 158 days post inoculation.

The blood samples were analysed for haematology and biochemistry parameters including haemoglobin (Hb), haematocrit (HCT), total leukocyte count (LPK), band and segmented neutrophils, eosinophils, basophils, lymphocytes, monocytes, aspartate aminotransferase (ASAT), creatine kinase (CK), glutamate dehydrogenase (GLDH), total protein (TP), urea, cholesterol, calcium, albumin, α_1-globulin, α_2-globulin, β_1-globulin, β_2-globulin, γ-globulin and albumin-globulin (A:G) ratio.

The analysed parameters from the *E. alces* and *E. rangiferi* infected moose calves were compared with results from a control group of six uninfected calves. The six reindeer calves were their own control, comparing samples taken before and after *E. alces* infection. For the infected goat kids and lambs, changes over time after infection were studied; no samples were taken before infection.

The most marked finding in the blood analyses was eosinophilia, significantly elevated in the *E. alces* infected moose calves, increased also in reindeer calves, goat kids, one lamb, and in *E. rangiferi* infected moose calves, occurring one to three weeks after infection. In the reindeer calves increased numbers of basophils were often seen with the elevated eosinophils. Higher haematocrit levels compared to controls were seen in the moose calves, often together with clinical signs. The moose calves had a significant decrease of urea levels in the blood 1 to 14 days after infection compared to controls.

Less pronounced alterations occurring in moose calves, reindeer calves and goat kids were increased neutrophils within two weeks of infection. A slight increase of α_2-globulin (α_{1+2}-globulin in reindeer) in *E. alces* infected animals was seen mainly in goat kids and lambs week 5 to 6 of infection, and less noticeable in moose calves and reindeer calves week 3 to 4 after infection. In *E. rangiferi* infected animals the α_2-globulin levels increase close to the animal's death. No marked alterations of analysed liver or muscle enzymes were observed.

Keywords: E. alces, *E. rangiferi*, elaphostrongylosis, muscleworm, brainworm, small ruminant, eosinophilia, biochemistry, serum chemistry

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Abbreviations

ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase (μkat/L)
CI	Confidence interval
CK	Creatine phosphokinase (μkat/L)
CNS	Central nervous system
E. alces	*Elaphostrongylus alces*
E. cervi	*Elaphostrongylus cervi*
E. rangiferi	*Elaphostrongylus rangiferi*
GLDH	Glutamate dehydrogenase (nkat/L)
Hb	Haemoglobin (g/L)
HCT	Haematocrit (%)
L_1	First-stage larvae
L_2	Second-stage larvae
L_3	Third-stage larvae (infective)
L_5	Adult worm
LPK	Leukocytes (x 10^9/L)
PI	Post-inoculation
PNS	Peripheral nervous system
TP	Total protein (g/L)

1. Introduction

Sweden is inhabited by semi-domesticated reindeer (*Rangifer tarandus*) and wild cervid populations of moose (*Alces alces*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*) (Stéen *et al.* 1998a), some of these cervids are also farmed (National Veterinary Institute [SVA] 2019). Both wild and semi-domesticated cervids freely cross the border to Norway and Finland (National Veterinary Institute [SVA] 2019). Different cervid populations can share pastures and woodland, they can also graze in the same locations as domestic ruminants like goat and sheep (Handeland & Slettbakk 1995; Tryland 2013). From an economic perspective cervids, in particular moose, roe deer and reindeer, are important resources for hunting, tourism and animal husbandry (Stéen *et al.* 1998a).

The nematodes of genus *Elaphostrongylus* spp. (family *Protostrongylidae*), also called muscleworms, are found in cervids and can cause neurologic disease, myositis (Lankester 2001; Rollinson & Hay 2012) and increased mortality in populations (Stéen *et al.* 1998a). *Elaphostrongylus rangiferi* caused large outbreaks in the 1960s and early 1970s in Swedish and Norwegian reindeer herds (Davidson *et al.* 2020) with numerous dead animals (Stéen *et al.* 1998a). In the middle of 1980s, the moose population that had been increasing in Sweden since 1970, showed increased mortality and unhealthy animals were observed in both Norway and Sweden (Stéen *et al.* 1998a). By necropsy of sick and dead wild moose, 49% (Stéen *et al.* 1998a) to 98% (Stéen *et al.* 2016) were diagnosed with *Elaphostrongylus alces*. Studies of naturally infected animals and experimental studies have confirmed that cross-infection of *Elaphostrongylus* spp. is possible (Handeland 1991; Handeland & Sparboe 1991; Stéen *et al.* 1997, 1998b). Infection of other cervids than the normal host and to domestic ruminants like goat and sheep have been confirmed (Handeland 1991; Handeland & Sparboe 1991; Stéen *et al.* 1997, 1998b).

Diseases can alter normal physiological mechanisms in the affected animals resulting in alterations in the tested laboratory parameters (Thrall *et al.* 2012). By identifying the effects of a disease on blood parameters, laboratory testing and interpretation can give an increased understanding of the disease's effect on the host (Rostal *et al.* 2012; Thrall *et al.* 2012). Thereby are haematology and serum chemistry important tools to investigate and understand the health of an individual but also a tool for health surveillance on a population level (Rostal *et al.* 2012).

This study aims to investigate the effect of *E. alces* and *E. rangiferi* on haematology and blood chemistry in experimentally infected animals. The objectives are to identify how infection alters the analysed parameters and if the changes are similar in different host species (reindeer, moose, sheep and goat). To the author's knowledge haematology and blood chemistry have only been analysed for experimental infection with *E. rangiferi* in one goat kid (Handeland & Skorping 1992) and one lamb (Handeland *et al.* 1993), and white blood cells in goat kids (Handeland & Skorping 1993).

2. Literature Review

The nematode family *Protostrongylidae* (order *Strongylida*, superfamily *Metastrongyloidea*) consists of many parasites that affect the lungs in domestic and wild ruminants and lagomorphs (Carreno & Hoberg 1999). Most *Protostrongylidae* genera are lung-dwelling and the adults live in the lungs where the eggs are deposited and embryonate. Adults in two genera (*Elaphostrongylus* and *Parelaphostrongylus*) of the 13 in the family are tissue-dwelling and located more distant from the lungs (Anderson 2000). Adults are located around and in the spinal cord and in muscle fasciae (Olsson *et al.* 1995). Their eggs are deposited in the veins and are transported with the blood to the lungs where they embryonate (Anderson 2000). The first-stage larvae (L_1) are coughed up, swallowed and leave the host with the faeces for development in an intermediate host, a gastropod (slug or snail) (Anderson 2000).

The genera *Elaphostrongylus* and *Parelaphostrongylus* both belong to the subfamily *Elaphostrongylinae* (Rollinson & Hay 2012) and can cause cerebrospinal nematodiasis with tissue damage when the parasites migrate into and through the CNS (Olsson *et al.* 1993; Zachary 2017). *Parelaphostrongylus* spp. are Nearctic (temperate and Arctic areas of North America) consisting of three known species: *P. tenuis*, *P. andersoni* and *P. odocoilei* (Lankester 2001). *Elaphostrongylus* spp. are mainly found Palearctic (Eurasia north of the Himalayas) but through export of animals, *E. rangiferi*, spread to Newfoundland (Canada) and *E. cervi* to New Zealand (Lankester 2001). In the genus *Elaphostrongylus* four species have been described: *E. cervi* Cameron, 1931, *E. panticola* Lubimov, 1945, *E. rangiferi* Mitskevich, 1960, and *E. alces* Stéen *et al.*, 1989 (Stéen *et al.* 1997). *Elaphostrongylus cervi* and *E. panticola* are considered the same species, *E. cervi* (Anderson 2000), but it might consist of two subspecies, *E. cervi cervi* in red deer (*Cervus elaphus elaphus*) and *E. cervi panticola* in maral deer (*C. elaphus sibiricus*) and sika deer (*C. nippon*) depending on locality (European or Asian origin).

The normal definitive host of *Elaphostrongylus* spp. differs between the different parasite species, also differences in pathogenicity in normal (usual) and abnormal (aberrant) hosts are seen between the parasite species (Lankester 2001). *E. rangiferi* has reindeer and caribou as normal hosts but can also infect moose, goat, sheep, calves and probably also muskox (*Ovibos moschatus*) (Lankester 2001). *E. alces* has moose as its normal host (Stéen *et al.* 1989) but can also infect

reindeer, goat and sheep (Stéen *et al.* 1998b). *E. cervi* is found in different subspecies of red deer (*Cervus* spp.). In Europe and New Zealand where red deer are mostly feral, neurologic disease is not often seen, but in Asia it is a severe helminth disease in more densely held farmed cervids resulting in economic consequences (Lankester 2001).

The ability of *Elaphostrongylus* spp. to infect different hosts and their pathogenicity have been investigated in several studies. Through cross-infection of moose and reindeer it was shown that *E. rangiferi* and *E. alces* can infect other hosts than their normal host and develop patent infections (Stéen *et al.* 1997, 1998b). However, the parasites' ability to reproduce is significantly reduced in the abnormal host (Stéen *et al.* 1997, 1998b). The risk of hosts cross-infection exists in areas where small ruminants, reindeer and moose share habitat and pastures (Stéen *et al.* 1998b).

2.1. Life cycle of *E. rangiferi* and *E. alces*

The definitive host is infected accidentally when consuming the third-stage larvae (L_3) of *Elaphostrongylus* spp. within infected gastropods (intermediate host) located on the vegetation (Anderson 2000).

2.1.1. Migration route

The migration route of the L_3 from the gut of the definitive host to the central nervous system and musculature have some differences among the *Elaphostrongylus* spp. The venous transportation of the eggs from the tissue to the lungs, hatching, and L_1s leaving the host via faeces, are similar for all *Elaphostrongylus* spp. (Anderson 2000).

Elaphostrongylus rangiferi

In a study by Handeland & Skorping (1992) where goats were experimentally infected with *E. rangiferi*, the findings suggested that the parasites enter the tissue from abomasum and penetrate the abomasal venules. Histopathologic inflammatory reactions and larval findings in different tissues lead Handeland & Skorping (1992) to suggest that the L_3s were carried in the portal blood to the liver, migrated through hepatic tissue and continued in the caudal vena cava to the heart and lungs where they migrated to pulmonary venules and were transported out in the circulation to all tissue. After reaching the CNS (day 10), the larvae increased in diameter indicating that they had started to grow when in the predilection tissue. Findings also indicated that worms arrested in arterial vessels close to spinal nerves ultimately migrated along these to the CNS (Handeland & Skorping 1992). Handeland (1994) conducted an experimental study of *E. rangiferi* in reindeer with

similar pathologic findings and concluded the same migration route as Handeland & Skorping (1992) via the general circulation and a secondary lymphatic-vascular route. In reindeer the migration occurs earlier after infection than in sheep and goat, and a higher percentage of the given dose were recovered from the CNS (especially in subarachnoid spaces) at similar time PI (Handeland 1994). This indicate a more successful migration in reindeer compared to in small ruminants (Handeland 1994).

Olsson *et al.* (1998) emphasized that other parasites known to be disseminated via blood vessels (*Angiostrongylus mackerrasae* and *Angiostrongylus Cantonensis* in rats) reach the brain within one to two days. However, Handeland & Skorping (1992) indicated that *E. rangiferi* takes much longer to reach the CNS, an observation inconsistent with a blood-borne route (Olsson *et al.* 1998). Olsson *et al.* (1998) did an experimental study on guinea pigs (*Cavia porcellus*) to investigate the migration route of *E. cervi* and *E. alces*. Guinea pigs turned out to be an unsuitable host for the latter but for *E. cervi* the findings indicated a direct tissue migration by penetrating the stomach wall, entering muscles in the lateral body wall and following nerves and entering the spinal canal via spinal nerves, first being found in the CNS day 11 PI (Olsson *et al.* 1998).

In a study by Hemmingsen *et al.* (1993) on reindeer infected with *E. rangiferi*, the adult parasites were located in the subdural space of the CNS and on or just beneath fasciae of skeletal muscles. The location of the worms changed over time. Day 48 PI the majority of worms were found in the subdural space of the cranium and the rest in the subdural space of the spinal canal; some had reached the adult stage at this time. At day 90 PI the first worms were found in skeletal muscles and from day 182 PI all worms were found in skeletal muscles. Most findings in the skeletal muscles were associated with *Musculus latissimus dorsi, M. obliquus externus* and *M. longissimus dorsi*. These findings indicate that the most plausible main migration route for *E. rangiferi* is first to the CNS where the worms mature and then into muscle tissue (Hemmingsen *et al.* 1993). Handeland (1994) declare that the main sites of development took place in the subarachnoid spaces. Hemmingsen *et al.* (1993) discuss that the worms most likely locate a partner of opposite sex within the spinal canal. After developing to the adult (L_5) and gravid stage (Hemmingsen *et al.* 1993) the female deposits unembryonated eggs in the veins of the tissue where she is located (Anderson 2000). The eggs are transported with the blood to capillaries in the lungs where the eggs embryonate and are encapsuled by the host inflammatory response (Anderson 2000). From day 103 PI developing eggs were found in the lungs of infected reindeer (Handeland 1994). The first-stage larvae (L_1) hatch and enter the airways, advance up in the respiratory system before they are swallowed and follow the gastro-intestinal system out of the host with faeces (Anderson 2000).

Elaphostrongylus alces

An investigation by Handeland *et al.* (2001) of *E. alces* migration in goats concluded that the parasite has a direct migration route from the abomasum and small intestine, producing abdominal visceral lesions (abomasum, small intestine and liver with inflammatory cells and hepatocellular degeneration), and entering the epidural space in the caudal vertebral canal along spinal nerves. Stéen *et al.* (1998b) found only minor lesions, inflammatory response and parasites epidurally in the vertebral space and skeletal muscles in reindeer, sheep and goat infected with *E. alces.* They interpreted the findings to be evidence of a direct migration route to epidural space of CNS and that inflammation in and around body lymph nodes may indicate an alternative route via the lymph system (Stéen *et al.* 1998b). Both Handeland *et al.* (2001) and Stéen *et al.* (1998b) found no signs of haematogenous spread of *E. alces* as described by Handeland & Skorping (1992) in *E. rangiferi* infection.

Handeland & Gibbons (2001) studied randomly selected, naturally *E. alces* infected moose calves and yearlings by necropsy of head and eviscerated carcass, kidneys, and lungs. Depending on the time of the year the animal was killed the location of the parasite varied, in September the worms were found in the epidural space of the vertebral canal while in animals killed later in the year an increasing proportion of worms were found in skeletal muscles (Handeland & Gibbons 2001). The nematode developed to adults epidurally and migrated during the first 6 months after infection from the epidural space of the vertebral canal to skeletal muscles (predilection site thigh muscles) where they live in reproductive pairs. The lack of epidural space in the cranial cavity Handeland & Gibbons (2001) discussed as explanation to why no parasites or lesions were found in this region. Table 1 presents a summery of the parasites' migration route, location and prepatent period in normal and abnormal host.

2.1.2. Prepatent period

The prepatent period varies among the *Elaphostrongylus* spp. and infected host species. When Stéen *et al.* (1997) did the experimental study with cross-infection of reindeer and moose the prepatent period for *E. alces* was between 39 to 73 days in moose; in reindeer the shedding of L_1 was low and intermittent with a prepatent period of 39 to 133 days. When infecting sheep and goat with *E. alces* no shedding of L_1 in the faeces occurred (Stéen *et al.* 1998b).

For reindeer calves experimentally infected with *E. rangiferi,* the prepatent period was 4 to 4,5 months (Handeland *et al.* 1994) and for moose calves infected with *E. rangiferi* only one of three survived long enough to shed L_1 larvae, occurring after 133 days (4,4 months) PI (Stéen *et al.* 1997). When cross-infecting reindeer and moose the parasites' capability to reproduce decreased considerably (Stéen *et al.* 1997).

Table 1. E. rangiferi and E. alces migration route, location and prepatent period in normal and abnormal host

	E. rangiferi		*E. alces*	
	Normal host	**Abnormal host**	**Normal host**	**Abnormal host**
Host species	Reindeer and caribou	Moose, goat, sheep and calves. Probably muskox	Moose	Reindeer, goat and sheep
Migration route	Haematogenous and an alternative lymphatic-vascular route		Direct route and an alternative via the lymph system	
Location CNS	Subdural space and subarachnoid space of the cranium and of the spinal canal		Epidural space of the spinal canal	
Location muscle fasciae	Most findings: *M. latissimus dorsi, M. obliquus externus* and *M. longissimus dorsi*		Predilection site: thigh muscles	
Prepatent period	4 to 4,5 months	Moose: 4,4 months Lamb and goat kids: no L_1 shedding	39 to 73 days	Reindeer: 39 to 133 days (4,4 months) Lamb and goat kids: no L_1 shedding

2.1.3. Intermediate host

The L_1 invade the foot of terrestrial gastropods, their obligatory intermediate host, where they moult twice and develop to the third-stage (L_3) (Anderson 2000). Many protostrongylids have been shown experimentally to be capable of developing in a wide range of intermediate hosts (terrestrial and aquatic snails and slugs) (Anderson 2000).

In a specific area, the natural transmission to the definitive host probably occurs mainly by a few important species of gastropods (Anderson 2000). Olsson *et al.* (1995) studied the snail and slug population at Utö (Swedish island, latitude: 58° 56' 14.99" N, longitude: 18° 15' 22.20" E) 1990 to 1994 and found 18 snail species and 12 slug species. Of these different gastropod species larvae (L_1, L_2 and L_3) of *E. alces* were found only in four slug (*Arion subfuscus, Deroceras agreste, D. reticulatum* and *Limax cinereoniger*) and three snail species (*Succinea spp., Vitrina pellucida* and *Zonitoides nitidus*). As the gastropods differ in their preference of location (higher or lower in the vegetation) Olsson *et al.* (1995) suggest that this may affect which species are more prone to infect different age-groups of moose depending on their grazing pattern.

Skorping & Halvorsen (1980) tested the susceptibility of different gastropods collected in northern part of Norway (Tromsøya, latitude: 69° 39' 59.99" N, longitude: 18° 56' 59.99" E) to *E. rangiferi*. All species tested were susceptible to L_1 infection, but the degree of infection varied considerably. The shortest time for 50% of the L_1 to develop to infective L_3 was approx. 20 days at 20°C, but time

varied among gastropod species. Halvorsen & Skorping (1982) studied how temperature affects the development of *E. rangiferi* to the infective stage in the intermediate host (*A. arbustorum* and *E. fulvus*) and if the gastropod species affects development rate. In the study developmental zero (lowest temperature for development) appeared at 8 to 10°C and the highest rate of development occurred at 24°C in *E. fulvus* and at 28°C in *A. arbustorum* (Halvorsen & Skorping 1982).

Climate change with longer seasons to develop (Tryland 2013) and higher temperatures can speed up the development rate of *Elaphostrongylus* spp. larvae in the intermediate host and increase the winter survival of gastropods (Halvorsen & Skorping 1982; Halvorsen 2012; Handeland *et al.* 2019; Davidson *et al.* 2020). This can increase the abundance of the parasite resulting in increased frequency of disease in the final host population (Halvorsen & Skorping 1982; Tryland 2013; Handeland *et al.* 2019; Davidson *et al.* 2020). Halvorsen (2012) examined the abundance of *E. rangiferi* infection (L_1 in faeces) in reindeer and found a positive relationship with increased summer temperature. Lorentzen & Halvorsen (1986) investigated the effect humidity has on survival of free-living *E. rangiferi* L_1. At positive temperature low humidity (20% relative humidity, 22°C) had a negative effect on survival of the parasite compared with when parasites were submerged in water (Lorentzen & Halvorsen 1986).

2.2. Clinical signs

2.2.1. *Elaphostrongylus alces*

Normal host: moose

E. alces can cause marked neurologic disease and death primarily in moose calves and yearlings (Stéen & Rehbinder 1986; Stéen & Roepstorff 1990). *E. alces* infection in moose calves in an experimental study by Stéen *et al.* (1997) resulted in elaphostrongylosis in all calves with clinical signs of neurological abnormality, lost appetite, fever, and signs of pain. Many calves developed respiratory signs with persistent, deep coughing (Stéen 1990). Neurological abnormalities were often affecting the hindquarters with posterior paresis, positioning deviations or weakness. Ataxia, muscle stiffness and behavioural changes were also registered (Stéen 1990).

In wild moose calves with neurological abnormalities and emaciation, *E. alces* infection has been diagnosed by necropsy (Stéen & Roepstorff 1990). Stéen & Rehbinder (1986) necropsied 35 wild moose that were found dead (primarily calves), 49% had elaphostrongylosis and of these 94% had affected nutritional state, the majority being emaciated. Stéen *et al.* (2016) investigated the prevalence of *E. alces* in the counties of Sweden and found most infected moose in the middle south,

Uppsala county, and Södermanland county (mean prevalence 100%). The only county with 0% mean prevalence was Skåne, in the very south of Sweden (Stéen *et al.* 2016).

Abnormal hosts

Experimental infection of sheep, goat and reindeer with *E. alces* resulted in no or only very mild neurological signs (Stéen *et al.* 1998b).

When infecting reindeer with *E. alces,* even with a heavy infective dose, no or only mild clinical signs were seen (Stéen *et al.* 1998b). In the study by Stéen *et al.* (1998b), all reindeer calves had normal weight increase and good body condition during the experiment. Most calves appeared healthy the whole time, but slight lameness was noted in one calf (one hind limb, 116 days PI) and one calf started coughing persistently (day 50 and until euthanised) (Stéen *et al.* 1997). Halvorsen *et al.* (1989) experimentally infected three reindeer calves with *E. alces,* but none developed patent infection.

The six goat kids in the Stéen *et al.* (1998b) study infected with high doses (1000 L_3) of *E. alces* showed no progression of infection and no clinical signs. They had good body condition and normal weight until they were euthanized 125- and 129-days PI. In an experimental study by Handeland *et al.* (2001) where five goats were infected with *E. alces,* no clinical signs were seen.

Five lambs experimentally infected with high doses *E. alces* in the Stéen *et al.* (1998b) study were euthanized 126 PI days without showing any clinical signs during the experiment. They were all in good body condition and normal weight (Stéen *et al.* 1998b).

2.2.2. *Elaphostrongylus rangiferi*

Compared with *E. alces* infection in abnormal hosts, *E. rangiferi* causes more severe clinical signs.

Normal host: reindeer

Handeland (1994); Handeland *et al.* (1994) experimentally infected 12 reindeer calves with *E. rangiferi*, with doses of 200, 300 or 1000 L_3. To examine the life cycle of the parasite and its effect on the host the reindeer calves were euthanized at different times PI (2,5 to 196 days PI) and necropsy was performed (Handeland 1994). The seven calves euthanized 4,5 weeks or later PI all developed neurologic clinical signs with locomotive abnormalities, starting 4 to 5 weeks PI. At necropsy of these seven calves' worms were recovered from the subarachnoid space of the brain and in all but one (euthanized day 196 PI) worms were also found in the subarachnoid space of the spinal cord. The most common neurologic signs were asymmetric paraparesis, posterior ataxia and tail paresis. Higher infective dose resulted in more severe clinical signs. The calf kept for 196 days (6,4 months) PI

recovered from clinical signs around six months PI. The five calves without clinical signs were euthanized 2,5 to 20 days PI, probably affecting the result (Handeland *et al.* 1994).

Lankester (2001) declares that the most common form of disease is a subacute verminous pneumonia with dyspnoea, sometimes coughing, general weakness, and poor condition. Handeland *et al.* (1994) registered verminous pneumonia from day 103 PI in the experimentally infected calves.

Abnormal hosts

In the study by Stéen *et al.* (1997) on moose calves experimentally infected with *E. rangiferi,* the calves developed marked neurological disorders, anorexia, fever and signs of pain.

The one lamb infected in the experimental study by Stéen *et al.* (1998b) first developed severe neurologic disease with limping and uncoordinated locomotion at day 21 PI, becoming lethargic and unwilling to rise. On day 23 PI the limbs were completely paralysed with absence of rightening and placing reflex but with bright and alert eye expression (Stéen *et al.* 1998b). The lamb was euthanised day 24 PI with normal body condition and weight. Handeland *et al.* (1993) infected seven lambs with 150 to 3000 L_3; the one receiving highest infective dose developed coughing and increased respiratory rate while three lambs developed pruritus. In experimentally infected goat kids pruritic signs were seen 4 to 10 weeks PI and neurological signs 35 to 94 days PI (Handeland & Skorping 1993).

In the northern parts of Norway Handeland (1991) diagnosed *E. rangiferi* in lambs with neurological abnormalities in herds of sheep that were located in areas where reindeer were observed. Handeland & Sparboe (1991) have also identified *E. rangiferi* in dairy goats with severe neurological signs in northern Norway with summer pastures in areas also used by reindeer herds. When comparing *E. rangiferi* pathogenicity in goat and sheep, sheep were more resistant to developing cerebro-spinal elaphostrongylosis with locomotor and brain disturbances (Hande-land & Slettbakk 1995).

The muskox is also suspected of being susceptible to *Elaphostrongylus* spp. In a small herd of free-living muskox (*Ovibos moschatus*) on the border between Norway and Sweden five animals were observed with posterior ataxia (Holt *et al.* 1990). The herd was located in an area also inhabited by reindeer and moose. The muskoxen were killed to prevent further suffering (one was found dead, probably killed by predators) and necropsied to investigate the cause of ataxia. Lepto-meningeal lesions with incomplete nematode specimens were seen in the caudal lumbar and sacral part of the spinal cord (Holt *et al.* 1990). The lesions and nematodes resembled those of *Elaphostrongylus* spp. found in reindeer and moose, but further identification was not possible (Holt *et al.* 1990).

2.3. Pathology

2.3.1. *Elaphostrongylus alces*

Normal host: moose

Gross examination revealed that *E. alces* were located epidurally in the vertebral canal (Stéen & Roepstorff 1990; Stéen *et al.* 1997; Handeland & Gibbons 2001) from as early as 39 days PI (Stéen *et al.* 1997). Handeland & Gibbons (2001) found the majority of parasites caudally to the 10th thoracic vertebra; Stéen & Roepstorff (1990) located them in the lumbar and thoracic regions of the spinal canal and cauda equina, surrounded by haemorrhage and oedema. Mature parasites were also found in skeletal muscles, especially in the thighs, beneath the muscle fascia in the epimysium (Handeland & Gibbons 2001).

Histologically, the inflammatory reaction, consisted of oedema, haemorrhages and aggregates of mononuclear inflammatory cells (lymphocytes, plasma cells and macrophages) and eosinophils epidurally in the connective tissue surrounding the spinal cord (Stéen & Roepstorff 1990). Increased numbers of inflammatory cells also occurred in surrounding tissue (overlapping from epidural lesion) in the spinal cord, spinal nerves and ganglia (Handeland & Gibbons 2001). Epidural lesions can cause pressure on the nerves resulting in neurologic clinical signs (Handeland & Gibbons 2001). Around eggs and larvae granulomas with a thin connective tissue capsule occurred (Stéen & Roepstorff 1990). No surrounding tissue reaction was seen around adult *E. alces* in thoracic and lumbar muscle fasciae (Stéen & Roepstorff 1990); inflammatory reactions was most prominent around eggs (Stéen & Rehbinder 1986).

Abnormal hosts

Even with heavy infective doses of *E. alces,* pathological lesions were minor in reindeer and small ruminants and no adult *E. alces* were found in the infected goat or sheep.

Five of six experimentally infected reindeer also tested negative for adult parasites but as L_1s were retrieved from faeces from four of these calves, there must have been adult *E. alces* present (Stéen *et al.* 1997). One hundred- and fifty-eight days PI Stéen *et al.* (1997) found adult *E. alces* in the epidural space (lumbar region) in one reindeer calf.

Histological examination (reindeer) showed an inflammatory response around the lateral nerves of the spinal cord and in moderate amounts in the inner surface of the dura mater (Stéen *et al.* 1998b). In the lambs the brain parenchyma and cerebral meninges had a mild to moderate hyperaemia and oedema (Stéen *et al.* 1998b).

In one reindeer calf and all lambs the liver parenchyma had white spots and lesions were also seen in the goat livers (Stéen *et al.* 1998b)

In all reindeer calves and lambs the lungs were moderately hyperaemic and oedemic (Stéen *et al.* 1998b). Nonpurulent bronchointerstitial pneumonia with mononuclear cell infiltration was seen in all lambs. The lungs in the goats were moderately oedematous and emphysematous with some haemorrhages, 50% having interstitial pneumonia with moderate lymphocytes. In one goat degenerated parasite eggs appeared in the alveolar walls (Stéen *et al.* 1998b).

2.3.2. *Elaphostrongylus rangiferi*

Normal host: reindeer

In reindeer the parasite was found subdurally (mainly in the subarachnoid spaces) over the brain and spinal cord, and in loose connective tissue in skeletal muscles (thoracic, neck, back and abdomen) (Handeland 1994). In the CNS encephalomyelitis, focal traumatic encephalomyelomalacia, meningitis and other inflammatory lesions related to the parasite infection were described by Handeland (1994). Also in the PNS degeneration, necroses and inflammation was observed. Pathological lesions were located in the abomasal wall, liver, kidneys, lungs, and myocardium with necroses and inflammatory cells. The calves developed verminous pneumonia and inflammatory oedema in connective tissue of skeletal muscles (Handeland 1994).

Abnormal hosts

In two of three moose calves infected with *E. rangiferi*, the parasite was found subdurally in the CNS penetrating the pia mater of the brain as well as in the spinal subdural or epidural spaces (Stéen *et al.* 1997).

In sheep haemorrhage was prominent macroscopically in epidural and subdural spaces in the thoracic and lumbar regions. Haemorrhages also occurred close to *N. brachialis* and *N. ischiaticus* (Stéen *et al.* 1998b). In the lungs a small number of *E. rangiferi* larvae were found and the lungs had numerous fresh and old nodules, yellow/reddish in colour. Histologically, an inflammatory response with mononuclear cells, hyperaemia and haemorrhages was present in the meninges, grey matter of spinal cord, subdural side of dura mater and around nerves. In the meninges and brain parenchyma moderate oedema and hyperaemia were seen. The lungs were slightly hyperaemic and oedematous with a few parasitic granulomas with eosinophils and mononuclear cellular infiltration. Liver, spleen and kidney were hyperaemic (Stéen *et al.* 1998b).

2.4. Haematology and blood chemistry

Depending on organ damage caused by the migrating parasite and the influence different larvae stages have on surrounding tissue where they are located, the host immune and inflammatory response might be reflected in haematology and blood chemistry. When the parasite starts to migrate through the host, cell injury and activation of inflammatory and immune responses can occur (Zachary 2017). The host's response to a parasitic insult depends on the damage that occurs and the parasite's capability to evade or overcome the immune response (Tizard 2012). *Elaphostrongylus* spp. uses the same migration pattern in their normal host as in the abnormal host (Stéen *et al.* 1997).

2.4.1. Haematology

In young animals the total count of red (erythrocytes) and white blood cells (leukocytes) are decreased compared to the levels seen in the adult animal. After birth haemoglobin (Hb) and haematocrit (HCT) decreases, within 1 to 1,5 months, starting to increase again thereafter (Harvey 2012). The ratio of the different leukocyte (LPK) cell lines in the young animal can also differ from the ratio seen later (Harvey 2012). In reference studies of moose Rostal *et al.* (2012) divided the animals into three age groups, the calves (<12 months) had lower levels of HCT, Hb and LPK compared with yearlings and adults. For reindeer Nieminen & Timisjärvi (1981) writes that at the age of 5 months these parameters were at the same level as for the adult animal.

Haemoglobin (Hb) and haematocrit (HCT)

Located in the erythrocytes, haemoglobin (Hb) plays a vital role in the transportation of oxygen in the blood (Kaneko *et al.* 2008). Haemoglobin also facilitates transportation of other gases and has a buffering effect by binding hydrogen ions (H^+) (Harvey 2012). Haemoglobin quantity is measured per unit volume (Thrall *et al.* 2012). The haematocrit (HCT) is the percentage of erythrocytes in the whole blood (Thrall *et al.* 2012). Changes in Hb and HCT parallel each other (Harvey 2012). High values can occur due to dehydration, erythrocytosis or splenic contraction. Blood loss, increased erythrocyte destruction, decreased erythrocyte production, inflammatory disease and over hydration causes low values. To interpret HCT together with total plasma protein (TP) can give valuable information. High HCT in combination with high TP is seen in dehydrated animals, splenic contraction occurs with low or normal TP. Low HCT with high TP can be seen in animals with anaemia with inflammatory disease cause's (Harvey 2012).

In a study by Franzmann & Bailey (1977) haemoglobin and haematocrit were the best parameters to reflect condition of a moose population. In wild moose populations the condition of the animals varies over the year, peaking in September-

October while the lowest values of these parameters were registered in March-May (Franzmann & Bailey 1977). The lowest values of Hb and HCT were registered in the animals in the worst condition. The condition of a moose was graded 1 to 10, where grade 1 was defined as "A point of no return. A generalized appearance of weakness. The moose walks with difficulty and can no longer trot, pace or canter" (Franzmann *et al.* 1976:13). Grade 10 definition was" A prime, fat moose with thick, firm rump fat by sight. Well fleshed over back and loin. Shoulders are round and full" (Franzmann *et al.* 1976:13).

Leukocytes, LPK

The total number of leukocytes (LPK) varies significantly between species (Harvey 2012). Lymphocytes and neutrophils are the most numerous types of leukocytes, but differs between species if lymphocytes or neutrophils are most abundant (Harvey 2012). Sheep and goats have more lymphocytes (Harvey 2012) while in reindeer Miller *et al.* (2013) reported that neutrophils were more numerous than lymphocytes. Kockum Adolfsson (1993) and Rostal *et al.* (2012) have studied reference values for moose blood with slightly differing result. Kockum Adolfsson (1993) registered higher levels of neutrophils than lymphocytes while in Rostal *et al.* (2012) study the number of neutrophils and lymphocytes was more equal, calves having more lymphocytes than neutrophils.

Neutrophils

In inflammatory responses to foreign material and organisms (primarily bacteria) the neutrophils are important actors through their capability of phagocytosis and killing of organisms (Thrall *et al.* 2012). Neutrophils are only present in the blood for a shorter period of time (half-life approx. 8,9-10,5 hours, in calves and horses (Carlson & Kaneko 1975; Carakostas *et al.* 1981)) after leaving the bone marrow and before entering into tissue where they can survive for a few days (Harvey 2012). In humans 50% or less of the total neutrophils in the blood are circulating (Summers *et al.* 2010) and assessed when taking blood samples, the rest are transiently retained in veins and capillaries as a marginating pool (Harvey 2012).

Cattle have a smaller reserve of neutrophils in the bone marrow compared with dogs, for example (Zachary 2017). Neutropenia in cattle can therefore be seen also in smaller increase of demand (Zachary 2017).

Lymphocytes

Only a small part of the lymphocytes circulate in the blood, the majority are located in lymphoid organs and some also form a marginating pool in pulmonary capillaries (Harvey 2012). They are only present in the blood for a short period of time before migrating through lymphatic tissue (Harvey 2012).

Eosinophils

Eosinophils are important for the host defence against helminthic infections (Harvey 2012) and as a response to parasites, eosinophilia can occur (Zachary 2017). Proteins from the eosinophil granules can damage the parasite membrane and thereby protect the host from a larval stage of parasitic infestation (Thrall *et al.* 2012). The eosinophils also have a phagocytic ability but not as effective as neutrophils (Harvey 2012). Eosinophils can remain in tissue for weeks or months, but in the circulating blood their half-life is much shorter (8 to 18 hours in humans) (Harvey 2012).

Basophils

Like eosinophils basophils can occur as a response to parasites (Zachary 2017) and are important in the immunity against helminths with a similar function as mast cells (Harvey 2012). The basophils only migrate into peripheral tissue as a response to parasites or an allergic reaction (Eberle & Voehringer 2016). The basophil granules contain histamine, proteases and other pro-inflammatory mediators that are released when the basophils are activated (Eberle & Voehringer 2016).

Monocytes

An increased number of monocytes in the blood, monocytosis, can occur as an inflammatory response (Zachary 2017). Monocytes are located in the blood before migrating to the tissue where they differentiate into macrophages (Harvey 2012). The macrophages play an important role as they phagocytize foreign substances like debris, bacteria and complexes of organisms but they also clean up the tissue from injured cells and cellular debris (Thrall *et al.* 2012). Macrophages are important for the innate and adaptive immune system. As sentinel cells they detect antigens and activate the host's defence by present the antigen and by release of cytokines (Tizard 2012). Macrophages also activate and modulate the inflammatory response by releasing pro-inflammatory mediators when they recognize tissue damage (Tizard 2012).

2.4.2. Blood chemistry

Creatine kinase (CK)

The enzyme creatine kinase is mainly found in the cytoplasm of muscle cells and is considered a specific muscle-leakage enzyme (Thrall *et al.* 2012). Creatine kinase increases rapidly after muscle injury; the half-life in the blood is only about two hours (in horses (Hinchcliff 2014)) and it decreases fast when the damage stops (Thrall *et al.* 2012). Parasitic disease causing myopathies can result in increased CK; the magnitude of the increase depending on the extent of the injury of the muscles (Thrall *et al.* 2012). Two to three times the normal value is considered a

mild increase of CK, four to ten a moderate and ten times the normal or more, a severe increase (Zachary 2017).

Aspartate aminotransferase (ASAT)

Aspartate aminotransferase is an enzyme present in hepatocytes and muscle cells and leaks out when cells are injured (Thrall *et al.* 2012). Muscle injury results in a slower increase of ASAT compared to CK. The half-life of ASAT is approx. 7 to 8 days in horses (Thrall *et al.* 2012). The elevation is graded the same way as CK indicated as mild, moderate or severe (Zachary 2017).

Glutamate dehydrogenase (GLDH)

Glutamate dehydrogenase is a leakage enzyme coming from the hepatocytes (Thrall *et al.* 2012). Some amounts are also found in the kidneys and small intestine, but increased serum levels are considered liver specific (Kaneko *et al.* 2008). Kaneko *et al.* (2008) declared that because of the location of GLDH in the mitochondria the enzyme is only released when irreversible hepatocyte injury, or necrosis, has occurred.

Urea

In the Krebs-Henseleit cycle ammonia and bicarbonate are synthesised into urea by the hepatocytes (Kaneko *et al.* 2008). The major nitrogen elimination is by making urea which then is filtered in the kidneys and the excess amount is passed out in the urine (Kaneko *et al.* 2008). Decreased liver function and malnutrition can lead to decreased urea concentration in the blood and decreased glomerular filtration can result in increased urea concentration (Thrall *et al.* 2012).

Cholesterol

Plants do not synthesize cholesterol and therefor herbivores must synthesize their own which is done primarily in the liver (Thrall *et al.* 2012). Consequently, hepatic failure may result in decreased blood cholesterol. Cholesterol is mainly excreted through the bile and disturbance in the bile flow (cholestasis) can therefore lead to increased serum cholesterol (Thrall *et al.* 2012).

Total protein (TP)

Serum proteins are separated into albumin and globulins, the latter consists of hundreds of different globular proteins diverging in size and properties (Thrall *et al.* 2012). With electrophoresis the proteins can be divided into different fractions (albumin, α-globulins, β-globulins and γ-globulins) depending on size and charge of the protein. Total proteins can remain within reference intervals during acute inflammation when the decreased amount of albumin evens out the increase of globulins (Thrall *et al.* 2012). The immunoglobulins are produced by B-lympho-

cytes while the majority of the other plasma proteins are produced by hepatocytes (Kaneko *et al.* 2008).

Albumin

A fall in osmotic pressure stimulates production of albumin and decreased synthesis occurs as a response to acute inflammation, infection or trauma (Kaneko *et al.* 2008). Albumin is synthesized in the liver, but it is not until 60-80% of the liver function is lost that hypoalbuminemia is noted (Thrall *et al.* 2012). Decreased amounts can also be caused by kidney disease, gastrointestinal disease, malnutrition and blood loss. Hyperalbuminemia is primarily caused by dehydration (Thrall *et al.* 2012).

Globulins

The α- and β-globulins consist of many different positive acute phase proteins that increase in response to acute inflammation (Thrall *et al.* 2012). An increase, primarily of α_2-globulin, but also α_1-globulins, is seen in acute inflammatory diseases due to the acute phase response (Kaneko *et al.* 2008). Transferrin (iron transport) is a negative acute phase protein included in the β_1-globulin fraction (O'Connell 2005) which can decrease due to inflammation and infection (Kaneko *et al.* 2008). The β_2-globulin fraction mainly consists of different compliment proteins which can participate in opsonization of parasites (Kaneko *et al.* 2008). By opsonization the parasite is tagged with molecules (opsonins) that facilitates phagocytosis (Tizard 2012). Immunoglobulins (Ig) of all types make up the major part of the gamma globulins, but IgM and IgA can sometimes migrate into the β-globulin fraction (Thrall *et al.* 2012). The immunoglobulins are important in the host adaptive immunity as defences against pathogens (Tizard 2012).

2.4.3. Cerebrospinal nematodiasis: haematology and blood chemistry

No previous studies have looked at the haematologic and biochemical alterations in *E. alces* infected animals or in *E. rangiferi* infected moose calves. In studies of blood parameters in other lung-, brain- and/or muscle-worm infections with migration to CNS, in different host species, the main finding has been eosinophilia while blood chemistry has remained unchanged in most cases.

Elaphostrongylus spp

In a study of *E. rangiferi* in goats, blood samples were taken daily from one infected goat kid and one control kid (Handeland & Skorping 1992). Differential leukocyte counts and blood serum analyses for enzyme activity (alanine aminotransferase (ALAT), ASAT, CK and GLDH) were reported. No clinical signs were noted in the

infected animals and the serum enzymes of the tested kids did not differ between infected and not infected. From day 8 PI with L_3s the blood analysis showed marked eosinophilia in the infected goat kid. Handeland & Skorping (1992) discussed that the migration of larvae from the lungs through pulmonary venules and out in the general circulation started between day 6 to 10 PI and that this resulted in marked eosinophilia. Although, as proposed, the *E. rangiferi* penetrate abomasal venules and follow the portal blood to the liver where hepatic lesions were seen starting from day 2 to 4, the enzyme activity (ALAT, ASAT, CK and GLDH) remained unchanged (Handeland & Skorping 1992).

Handeland & Skorping (1993) did an experimental study of Norwegian dairy goat kids. Twelve goat kids were infected with different doses (100 to 700 L_3) of *E. rangiferi*. Four goat kids were kept as controls and the infected kids were divided into three groups depending on infection dose. Blood samples were taken on days 14 and 32 PI. Total and differential leukocyte counts were analysed and the result showed a significant increase of eosinophils on day 14 in the group that had received the highest L_3 dose (700 larvae) compared with the control animals. On day 32 this group had more eosinophils than the other groups, but the eosinophils had decreased and were no longer significantly elevated (Handeland & Skorping 1993).

In another experimental study of *E. rangiferi* seven lambs were infected with 150 to 3000 L_3 (Handeland *et al.* 1993). From one lamb that received 3000 L_3 blood samples were taken daily from two days prior to inoculation to day 30 PI when it was euthanized; blood was also taken from a control lamb during the same time period. A differential leukocyte count was performed and blood serum was analysed for enzyme activity of ALAT, ASAT, CK and GLDH. The infected lamb had shown moderate coughing and increased respiratory frequency in the second week and a bit higher body temperature than the other animals. Around one week after infection the eosinophils increased, peaking on day 17 PI and then decreasing starting to normalize at the end of the test period. The other tested parameters were within the normal range and did not differ from the control lamb (Handeland *et al.* 1993).

Valcárcel *et al.* (2004) investigated the effect on blood cells in *E. cervi* infection by sampling 26 red deer when killed; of these, 5 were diagnosed with *E. cervi*. Red blood cell parameters were analysed, total leukocyte counted and differentiated. Valcárcel *et al.* (2004) study showed no differences between infected and non-infected animals regarding the blood parameters.

Parelaphostrongylus tenuis

Parelaphostrongylus tenuis is present in eastern North America where the normal host is the white-tailed deer (*Odocoileus virginianus*) but it can infect alternative cervid hosts and also species in the families Camelidae, Antilocapridae and Bovidae

(Lankester 2001). The parasite uses gastropods as an intermediate host and in the final host the L_3 migrate from the digestive tract to the spinal cord and subdural space with cerebrospinal nematodiasis (Ismail *et al*. 2011). In an experimental study of Ismail *et al.* (2011) five llamas (*Lama glama*) were each inoculated with five infective larvae (L_3) of *P. tenuis*, three llamas were given 10 L_3 and three llamas received 25 L_3. Ten of the eleven animals that were infected developed varying degree of neurologic deficit during the experiment. Four weeks pre-infection and every two weeks until euthanized (day 77 to 140) blood samples were taken. Haematology analysis included total red blood cell count and red blood cells parameters, total leukocyte count and differential cell count of leukocytes. Blood chemistry was analysed for: TP, albumin, globulin, fibrinogen, glucose, blood urea nitrogen, creatinine, sorbitol dehydrogenase, ASAT, phosphorus, potassium, and magnesium. The only significant difference was seen in eosinophils which was elevated in all infected animals' post-infection (Ismail *et al.* 2011).

Angiostrongylus cantonensis

Angiostrongylus cantonensis (family Metastrongylidae), rat lungworm, has rodents *Rattus rattus* and *Rattus norvegicus* as final hosts and gastropods as intermediate hosts (Garcia *et al.* 2014). It is a zoonotic parasite that causes eosinophilic meningitis in humans if infective larvae (L_3) are ingested (Federspiel *et al.* 2020). Symptoms seen in humans include headache, cough, nausea and diarrhoea, apathy, fever, neck stiffness, paresthesia and weakness (Xie *et al.* 2019). The final host ingests the infective larvae which migrate from the intestines through tissue, enter the bloodstream and continues the migration to CNS causing neurologic symptoms (Federspiel *et al.* 2020). After moulding twice and developing to an adult worm migration continue to the pulmonary arteries where eggs are deposit, these hatch in the alveoli and the L_1 are coughed up, swallowed and passed out with faeces, ready to infect the intermediate host (Federspiel *et al.* 2020).

In an experimental study of Garcia *et al.* (2014) 60 adult *Rattus norvegicus* were infected with 100 L_3, ten rats at a time were killed 1, 2, 3, 4, 6 and 8 weeks after infection; ten rats were kept uninfected as controls. When the animals were euthanized blood samples were collected and analysed for: total red blood cells count (RBC) and red blood cells parameters, platelets, total leukocyte count, differential cell counts of leukocytes and blood chemistry (ASAT, CK, CK-MB fraction (cardiac marker) and lactate dehydrogenase (LDH)) (Garcia *et al.* 2014). All exposed animals developed infection and compared with control animals the haematocrit and RBC were significantly lower in animals killed on week 3 and later, platelet counts were lower from week 6. For white blood cells, a significant increase was seen on neutrophils (week 3 to 6), eosinophils (all weeks), basophils (week 4 to 6), lymphocytes and leukocytes (week 3 to 8). In blood chemistry a

significant increase was seen of CK (week 6 to 8), CK-MB (week 4 to 8), LDH (week 2 to 8) and ASAT increased only week 2 (Garcia *et al.* 2014).

In a literature review of 22 reported human cases of *A. cantonensis* in Europe, 1988 to 2019, Federspiel *et al.* (2020) found marked eosinophilia in 89% of the cases. In two case reports of infected infants' blood, analyses were performed by counting red and white blood cells in peripheral blood (Xie *et al.* 2019). Both patients had a marked increase of eosinophils, hypereosinophilia (Xie *et al.* 2019).

3. Material and Methods

The blood samples for this study were part of an experimental study with clinical trials conducted between May 1989 and June 1990. Data concerning parasitic findings, pathology and prepatent period originate from earlier published material by Stéen *et al.* (1997, 1998b). Twelve moose calves, six reindeer calves, six goat kids and six lambs were infected with *Elaphostrongylus* spp. Six moose calves were also kept as controls. The study design was as described by Stéen *et al.* (1997) and Stéen *et al.* (1998b).

For each animal a daily journal was kept with information about type and quantity of food and supplements, water intake, general condition and behaviour, and whether treatments were given (doses and duration). The animals were observed daily for gait, posture and behavioural abnormalities. With approx. 14-day interval the animals were weighed and clinical examined. Animals were euthanized if showing severe neurological signs (Stéen *et al.* 1997).

The experiments conformed to Swedish regulations regarding animal care and usage (Stéen *et al.* 1997, 1998b). They were approved by the regional ethical committee for animal experiments (The National Board of Agriculture, Sweden).

3.1. Experimental design and animals

Moose calves

Of the total 18 moose calves, 11 were obtained from the wild and 7 from Swedish zoos (three different zoos) between May 1989 to January 1990 (Stéen *et al.* 1997). Some of the wild calves were only around one week old when caught after being observed alone without signs of moose cow. The calves from zoos were dewormed with Ivermectin (Ivomec® 0.2 mg/kg intramuscular) 2 to 4,5 months prior to arrival at the test facility. The calves were approximately 4,5 to 9,5 months old when infected; nine with *E. alces* and three with *E. rangiferi*, Table 2.

Table 2. Moose calves: controls and experimentally infected with E. alces or E. rangiferi

Calf no.	Sex	Origin	Approx. age when infected	Dose of L_3	*E.* spp	No. blood samples pre infection	No. blood samples PI
1	F	Wild	5,0 mon.	1000	*E. r.*	5	2
2	M	Wild	6,5 mon.	1000	*E. a.*	9	1
3	F	Wild	6,5 mon.	1000	*E. a.*	9	1
4	M	Zoo	5,5 mon.	1000	*E. a.*	3	2
5	F	Zoo	6,5 mon.	1000	*E. a.*	6	5
6	F	Zoo	6,5 mon.	1000	*E. a.*	6	3
7	M	Wild	6,5 mon.	1000	*E. a.*	9	3
8	F	Wild	9,5 mon.	1000	*E. a.*	2	5
9	F	Wild	9,5 mon.	400	*E. a.*	13	5
10	F	Wild	6,0 mon.	1000	*E. r.*	5	6
11	F	Wild	8,0 mon.	400	*E. a.*	5	9
12	M	Wild	4,5 mon.	1000	*E. r.*	5	10
13	F	Zoo	-	-	Control	14	-
14	M	Wild	-	-	Control	12	-
15	F	Zoo	-	-	Control	6	-
16	F	Zoo	-	-	Control	7	-
17	M	Wild	-	-	Control	19	-
18	F	Zoo	-	-	Control	10	-

Reindeer calves

The reindeer calves, semi-domesticated animals from the Sangis area in Lapland were captured 27th August 1989 (Stéen *et al.* 1997). They were approx. 3 months old when captured and 6,5 to 10 months old when infected with *E. alces*, Table 3. All age-groups in the herd were treated annually with Ivermectin (Ivomec® 0.2 mg/kg), intramuscularly (Stéen *et al.* 1997). As a control, health and parasite checks were done on the original herd during the entire experiment (Stéen *et al.* 1998b).

Table 3. Reindeer calves infected with E. alces

Calf no.	Sex	Approx. age when infected	Dose of L_3 *E. alces*	No. blood samples pre infection	No. blood samples PI
1	F	10 months	1000	13	5
2	F	8,0 months	1000	9	9
3	F	8,0 months	1000	9	8
4	M	8,0 months	1000	9	9
5	M	6,5 months	1000	8	10
6	M	6,5 months	1000	7	10

Small ruminants

Six lambs of Rya sheep (Table 4) and six kids (Table 5) of Swedish dairy goats were collected from their original herds in June 1989, before the flocks were released on pasture (Stéen *et al.* 1998b). At the age of two months, before they were obtained for the experiment, all animals were dewormed with Fenbendazol (Axilur® vet, 10 mg/kg) orally (Stéen *et al.* 1998b). Control groups for the lambs and kids were the original herds, which were health and parasite checked continuously while the experiment was ongoing (Stéen *et al.* 1998b).

Table 4. Lambs infected with E. alces or E. rangiferi

Lamb no.	Sex	Approx. age when infected	Dose of L_3	*E.* spp.	No. blood samples pre infection	No. blood samples PI
1	M	9,0 months	1000	*E. a.*	-	7
2	M	9,0 months	1000	*E. a.*	-	7
3	M	9,0 months	1000	*E. a.*	-	7
4	M	8,0 months	1000	*E. r.*	-	1
5	M	9,0 months	1000	*E. a.*	-	7
6	M	9,0 months	1000	*E. a.*	-	7

Table 5. Goat kids infected with E. alces

Goat kid no.	Sex	Approx. age when infected	Dose of L_3 *E. alces*	No. blood samples pre infection	No. blood samples PI
1	M	9,0 months	1000	-	8
2	F	9,0 months	1000	-	8
3	F	9,0 months	1000	-	8
4	M	9,0 months	1000	-	8
5	M	9,0 months	1000	-	8
6	M	9,0 months	1000	-	8

The stable and feeding

The animals were kept in a stable (indoors) rebuilt for this project and reared in separate stalls (Stéen *et al.* 1997, 1998b). The stalls were cleaned daily, and the animals observed and status noted in the diary two or more times daily. The animals were kept for totally 6 to 12 months.

The feeding routine and feed for the moose calves was based on Kolmården Zoo's (veterinary Bengt Röken) feeding regime. The calves were weaned at approximately 4 to 6 months of age after being bottle-fed with deer-milk formula. All calves were fed alfalfa pellets, mineral supplement, mixed grain and hay, moose were also given limited quantities of browse (Stéen *et al.* 1997).

Faecal examination for parasites was done weekly on all animals prior to experimentation, with negative result for protostrongylids and other helminths for all but

one, a moose calf that initially tested positive for protostrongylids (Stéen *et al.* 1997, 1998b). After, 10 to 30 days following installation in the stable, deworming with Mebendazole (Mebenvet®, Telmin® 6 mg/kg oral) was done for 10 days (Stéen *et al.* 1997, 1998b). The choice of anthelmintic was according to the study by Nordkvist *et al.* (1983) on efficacy against *E. rangiferi* (Stéen *et al.* 1997, 1998b). After deworming all animal tested negative for protostrongylids before the experiment started (Stéen *et al.* 1997).

E. alces and E. rangiferi infection

For the infections first-stage larvae of *E. alces* were obtained from necropsy of heavily infected wild moose from Utö island (Stockholm archipelago) and of *E. rangiferi* L_1 from a kept reindeer at the University of Tromsø, Norway (Stéen *et al.* 1997, 1998b). The only cervids on Utö are roe deer and moose (Olsson *et al.* 1995) and no domesticated ruminants occur (Stéen *et al.* 1998b). The purity of *E. alces* L_1 was determined according to morphological criteria described by Lankester *et al.* (1998).

Third-stage larvae used in infections were obtained by exposing the snail intermediate hosts, *Lymnea stagnalis* to L_1 larvae. Counting of L_{3s} in several snails in every batch was done to estimate infection dose given to each animal in the experiment (Stéen *et al.* 1997). The L_3 dose each animal received is presented in Table 2, Table 3, Table 4 and Table 5. One moose calf (no 8, Table 2) was given crushed L_3 infected snails in the grain feed while the rest of the calves were fed the snails in their milk or through stomach tube (Stéen *et al.* 1997).

3.2. Blood samples

Blood samples were collected from the jugular vein using the Vacutainer system and an 18 or 21G needle.

Table 2 presents the number of blood samples taken from each moose calf. From the control moose calves, median time interval between blood samples included in the statistical analysis were 14 days (varying between 14 to 35 days). After *E. alces* infection of moose calves the first set of blood samples were taken 7- or 8-days PI (one calf 14 days PI). Median time interval between blood samples were 14 days (11 to 35 days). From the *E. rangiferi* infected moose calves the first set of blood samples were taken 7- or 8-days PI. Median time interval between blood samples were 14 days (4 to 35 days).

Table 3 presents the number of blood samples taken from each reindeer calf. Included blood samples in the statistical analysis before infection were taken with a median interval of 14 days (14 to 35 days). After *E. alces* infection all calves were

sampled day 7 or 8 PI. Median time interval between blood samples were 14 days (13 to 35 days).

From the lambs and goat kids no blood samples were taken before infection, Table 4 and Table 5 presents the number of samples taken PI. The *E. alces* infected lamb were first sampled day 22 PI, median time interval between samples were 13,5 days (12 to 16 days). From the lamb experimentally infected with *E. rangiferi* one blood sample was taken 19 days after infection. From the goat kids experimentally infected with *E. alces* first samples PI were taken day 8, median time interval between samples were 14 days (12 to 16 days).

3.3. Haematology and blood chemistry

The blood was in general collected between 09.00-12.00 am and analysed the same afternoon. For haematology and fibrinogen EDTA tubes with anticoagulant were used. For biochemistry serum tubes were used.

The blood samples were analysed by Clinical Pathology Laboratory, Swedish University of Agricultural Sciences (SLU), Uppsala. Haemoglobin (Hb), haematocrit (HCT) and total leukocyte count (LPK) were analysed with a haematology instrument, Sysmex F-800 (Sysmex, Kobe, Japan). The leukocytes were manually differentiated for band and segmented neutrophils, eosinophils, basophils, lymphocytes and monocytes.

Blood chemistry aspartate aminotransferase (ASAT), creatine kinase (CK), glutamate dehydrogenase (GLDH), total protein (TP), urea, cholesterol and calcium were analysed using an automated chemistry analyser, Cobas Mira (Roche Diagnostics, Risch, Switzerland). Serum protein electrophoresis was performed and differentiated into six fractions: albumin, α_1-globulin, α_2-globulin, β_1-globulin, β_2-globulin and γ-globulin using a Paragon Electrophoresis System (Beckman Instruments Ltd, US) and albumin-globulin (A:G) ratio was calculated.

3.4. Statistical analyses

Minitab 18 (Minitab Inc., Pennsylvania) was used to plot all of the analysed blood parameters to identify any trends relative to time post-inoculation. Day 0 equals the day when the animals were given the infective dose of *Elaphostrongylus* spp. Scatterplots with connection and groups were used. The clinical signs described in each animal's journal and in published material (Stéen *et al.* 1997, 1998b) were graded depending on severity (none = 0, mild =1, moderate = 2 and severe = 3) and plotted against time of occurrence. This enable comparison of clinical signs and changes in the analysed parameters. Clinical signs graded as 1 (mild) were for example: mild diarrhoea, some coughing, increased respiratory sounds, less active,

lifting limbs higher or dragging the hooves, posterior stiffness, slightly wobbly and/or partly reduced appetite. Example of clinical signs graded as 2 (moderate): severe diarrhoea, coughing (deep, persistent), fever, lethargic, increased time lying down, difficulty to move, stiffness, wobbly and/or inappetent. Signs graded as 3 (severe) were: severely affected general condition, severe coughing (deep and persistent), recumbent position almost the entire time, no food or water intake, fever, severe diarrhoea, complete paralysis, and/or falling when standing.

The data were analysed separately by host species and parasite, uninfected (control group for the moose calves) and infected. The latter set was split into smaller groups based on elapsed post-inoculation when the blood sample was taken. Blood samples from the control moose calves at the age 5-11 months were included in the analysis, not samples taken prior to that. For the reindeer calves the uninfected samples only included samples taken at age ≥4 months. Most samples were taken approx. 14 days apart, therefore each group contained samples from a period of 14 days. Distribution was investigated using histograms. Due to small numbers of samples in each group and not normally distributed data in the groups, a non-parametric method for analyse was chosen. The groups were compared with the uninfected samples using median confidence interval (CI), for 95% confidence six or more observation is requires, less data decreases the confidence. The median CI was calculated with Mood's Median Test (Minitab18). Mood's Median Test tested if significant median difference ($P < 0.05$) between control and deviating group existed. The test is non-parametric and requires independent data between the groups tested. Therefore, tests of the significant difference could only be performed on moose calves using data from the control animals in one group and infected moose calves (minimum of 6 samples) in the other group.

In the control group some calves showed clinical signs during the sampling period. Less severe clinical signs seen in the control group were milder diarrhoea and decreased general condition with increased recumbent time. Blood samples (totally three) from moose calves (two calves) with moderate to severe clinical signs in the control group were not included in the statistical analyses. One of the two calves stopped eating, became lethargic and recumbent, developed a fever and started losing fur. The calf was treated, later euthanized due to the severity of the clinical signs. The second calf had diarrhoea that became severe, the calf became inappetent, had increased recumbent time and was found dead. The control calves were dewormed and regularly checked for protostrongylids in faeces (all tests were negative) to assure they had not become *Elaphostrongylus* infected.

4. Result

In Table 6, Table 8, Table 12 and Table 13 an overview of the major clinical signs are listed with a grading of how severely affected each animal was, 0 = no clinical signs, 1 = mild, 2 = moderate and 3 = severe.

4.1. Elaphostrongylus alces

Appendix 1 contains median values for all tested blood and blood chemistry parameters in the different host species infected with *E. alces* (Table 14 moose calves, Table 15 reindeer, Table 16 lambs and Table 17 goat kids).

Moose

Less severe clinical signs of limited duration were observed in all nine of the *E. alces* infected moose calves. Six of the moose calves developed severe clinical signs close to the death or euthanasia. The remaining three calves (no. 8, 9 and 11) had no to moderate clinical signs when euthanized.

Table 6. Moose calves experimentally infected with E. alces

Calf no.	Prepatent period, days	Infection length, days	Cause of death	Clinical signs: 0 (none) to 3 (severe)	No. and localization of *E. alces*
2	-	17	D	3 (N, R, F)	-
3	-	37	E	3 (N, GC, A, R)	-
4	39	39	D	3 (N, GC, A, F)	4: ce, mf
5	55	84	E	3 (N, GC, A, F)	57: ce
6	-	62	D	3 (N, GC, A)	23: ce
7	68	68	E	3 (N, GC, A, R)	33: ce
8	42	70	E	1 (N, R)	7: ce
9	73	75	E	0	6: ce
11	20 (P)	125	E	2 (R)	42: ce, mf (P)

Codes - Cause of death: D = found dead, E = euthanized. Clinical signs: 0 = no clinical signs, 1 = mild, 2 = moderate and 3 = severe, N = neurologic, R = respiratory, F = fever, GC = general condition affected, A = inappetence, M = muscular. Localization of E. alces: ce = cavum epidurale, cs = cavum subdurale, mf = muscle fasciae. (P): Protostrongylid positive when captured.

Table 7 presents the median values and median confidence intervals (CI) of the analysed blood and serum parameters that displayed a noticeable alteration in the calves with 95% confidence, grouped after time for sampling PI. Haematocrit (HCT), eosinophils and urea showed significant changes from the control group.

Table 7. Median values of the most deviating tested blood parameters in E. alces infected moose

Day PI	Control	Day 1-14	Day 15-28	Day 29-42	Day 43-56	Day 57-70	Day 71-84	Day 85-126
HCT,	32 (51)	32 (9)	*35,6 (4)*	30,9 (6)	36,6 (6)	32,3 (4)	42,6 (2)	33 (3*)
%	[31,0; 33,7]	[(27,3; 36,6]	[34; 38]	[27,4; 33,6]	[33,7; 38,1]	[29; 36]	[33; 52,2]	
Eosinoph.,	0,05 (51)	0,23 (9)	*0,66 (4)*	0,17 (6)	0,35 (6)	0,40 (4)	0,33 (2)	0,40 (3*)
x 10^9/L	[0,04; 0,07]	[0,14; 0,74]	[0,49; 1,9]	[0; 0,5]	[0,32; 0,73]	[0; 2,21]	[0; 0,7]	
Urea,	7,2 (52)	6,3 (9)	*6,4 (4)*	5,9 (6)	5,8 (6)	*6,5 (4)*	6,0 (2)	6,0 (3*)
mmol/L	[6,8; 7,5]	[5,5; 6,9]	[4,9; 7,1]	[4,2; 7,1]	[5,0; 7,3]	[5,5; 7,0]	[5,3; 6,6]	

*Codes - (n) number of samples included in the group. [x; y] median confidence interval (when <6 samples [x;y] represents lowest; highest result). * all samples are from the same individual. Underlined =significant change compared with controls (≥ 6 samples). Cursive = changes with lower confidence (4 samples)*

HCT fluctuated with a significant increase (95% confidence) at days 43-56 compared with controls. Eosinophils increased and fluctuated, significant increase was seen days 1-14 and 43-56, Figure 1. Urea decreased significantly at days 1-14 after infection, Figure 2.

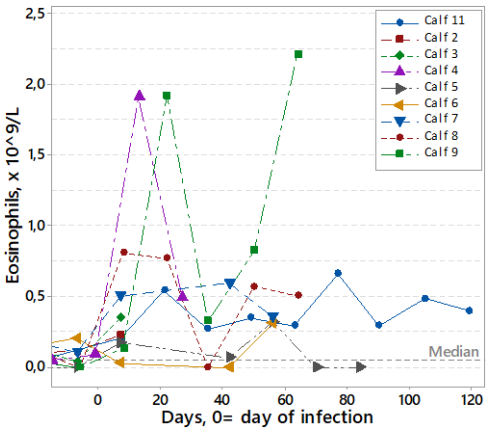

Figure 1. Eosinophils in moose infected with E. alces. Median line of controls.

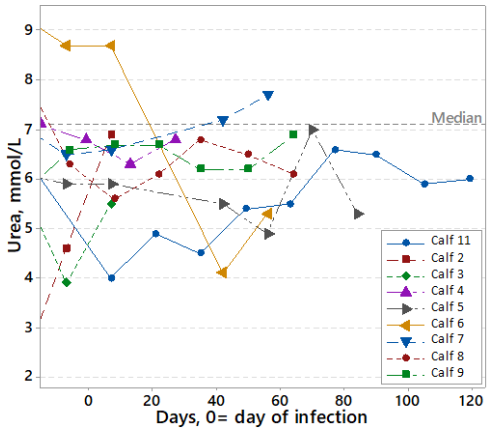

Figure 2. Urea in E. alces infected moose. Median line of controls.

With lower confidence (4 samples in the group) alterations of these parameters were also seen at time intervals other than mentioned above (marked with *cursive* in Table 7). In time intervals with only four sample alterations were also seen in other parameters: Hb increased (day 15-28), neutrophils increased (day 57-70) and protein increased (day 57-70), appendix 1 Table 14. Neutrophils initially increased

and after day 14 decreased to controls' level, Figure 3. Fluctuations with later increase were observed.

At the individual level some changes were observed. Calves no. 2, 4, 5, 6 and 7 all had elevated levels of lymphocytes in their last blood sample before death (severe clinical signs) compared with earlier samples.

For glutamate dehydrogenase (GLDH) one calf, no. 8, showed the highest values (>500 nkat/L), the other calves had levels ≤47 nkat/L. The last blood sample from calf no. 5 had noticeably deviating test result for a number of parameters (Hb, HCT, leukocytes, neutrophils, monocytes, calcium, protein and albumin), this occurred the day it was euthanized with severe clinical signs. No similar results were seen in the other calves with severe clinical signs.

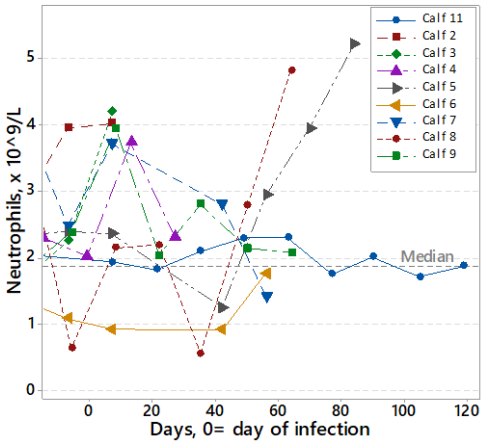

Figure 3. Neutrophils in E. alces infected moose calves. Median line of controls.

Reindeer

Only two reindeer calves showed clinical signs during the experiment, Table 8. Calf no. 2 was temporary limping on a hind limb and quickly recovered. Calf no. 3 coughed from day 50 and until euthanized.

Table 8. Reindeer calves infected with E. alces

Calf no	Prepatent period, days	Infection length, days	Cause of death	Clinical signs: 0 (none) to 3 (severe)	No. and localization of *E.a.*
1	39	75	E	0	0
2	96	124	E	1 (N/M)	0
3	55	124	E	2 (R)	0
4	133	133	E	0	0
5	91	158	E	0	4: ce
6	95	158	E	0	0

Codes - E = euthanized. Clinical signs: 0 = no clinical signs, 1 = mild, 2 = moderate, 3 = severe, N = neurologic, R = respiratory, M = muscular. Localization of E. alces.: ce = cavum epidurale.

Table 9 presents the median values of the sampled blood parameters with the highest deviation from pre- infection levels. The majority of parameters did not show any obvious trends PI. The leukocytes were slightly elevated after infection, most noticeably on days 1-28 PI, initially caused by an increase of neutrophils (day 1-14), Figure 6. Eosinophils and basophils exhibited the most noticeable changes

(Figure 4 andFigure 5). The median CI for eosinophils and basophils deviated most from pre-infected levels on days 15-28.

Table 9. Median values for leukocytes, eosinophils, basophils and α_{1+2}-globulin in E. alces infected reindeer calves

Day PI	Pre-infection	Day 1-14	Day 15-28	Day 29-42	Day 43-56	Day 57-70	Day 71-84	Day 85-98	Day ≥99
LPK	4,5 (23)	7,3 (5)	6,4 (3)	6,7 (6)	5,3 (6)	5,5 (5)	5,4 (5)	4,9 (5)	5,55 (14)
x 10^9/L	[4,2; 5,4]	[4,3; 8,8]	[5,6; 8,5]	[4,5; 7,5]	[3,5; 7,1]	[3,3; 6,1]	[3,7; 6,7]	[3,2; 8,2]	[3,4; 6,5]
Eosinoph.,	0,6 (23)	1,1 (5)	1,8 (3)	1,2 (6)	1,0 (6)	0,7 (5)	0,7 (5)	0,4 (5)	0,6 (14)
x 10^9/L	[0,3; 0,7]	[0,4; 3,8]	[1,3; 3,3]	[0,6; 1,8]	[0,4; 1,8]	[0,4; 1,1]	[0,5; 1,0]	[0,2; 0,9]	[0,4; 0,9]
Basophils,	0,04 (23)	0,00 (5)	0,58 (3)	0,22 (6)	0,18 (6)	0,09 (5)	0,13 (5)	0,16 (5)	0,13 (14)
x 10^9/L	[0,02;0,07]	[0; 0,5]	[0,5; 1,1]	[0,1; 0,5]	[0,02; 0,3]	[0,3;0,37]	[0,07;0,3]	[0,03;0,4]	[0,06;0,1]
α_{1+2}-glob.	4,0 (10)	4,0 (4)	7,0 (3)	5,0 (6)	4,5 (6)	4,5 (6)	5,0 (5)	6,0 (5)	6,5 (14)
g/L	[3,7; 5]	[3; 5]	[6; 11]	[4,4; 6]	[3,4; 8,6]	[4; 7,3]	[4; 7]	[4; 7]	[6; 7]

Codes – Pre-infection represents samples taken before inoculation of parasites, age approx.4,5 to 9,5 months. (n): group size, [x;y]: median CI (when <6 samples [x;y] represents lowest;highest result)

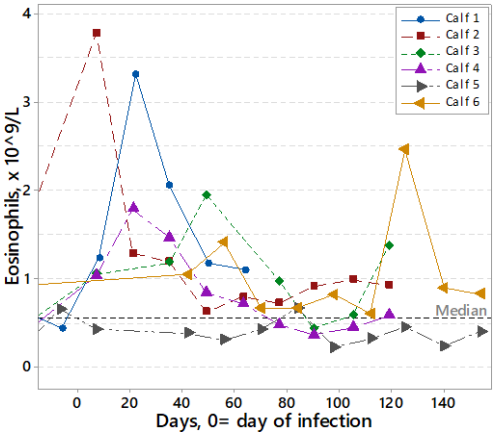

Figure 4. The change of eosinophils over time in reindeer. Median line pre-infection.

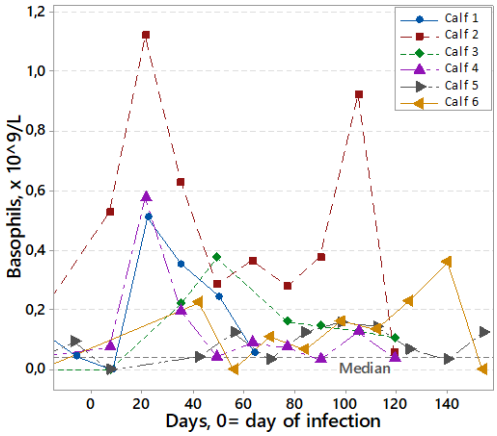

Figure 5. Basophils in E. alces infected reindeer calves. Median line pre-infection.

In reindeer some of the samples were not differentiated in α_1- and α_2-globulin, therefore all samples were converted to α_{1+2}-globulin to make comparison possible. α_{1+2}-globulin deviated most on days 15-28 (three samples) compared with before infection, Figure 7. One calf (no. 6) had elevated CK levels (16,9 µkat/L) 42 days PI.

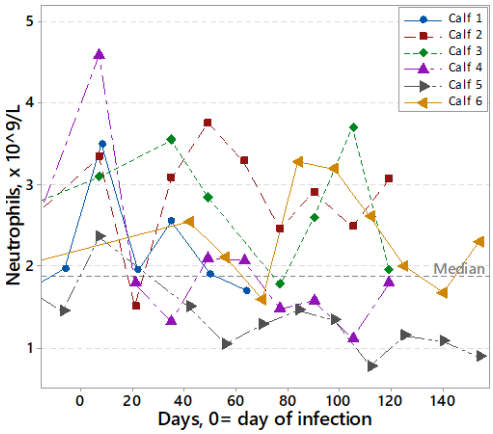

Figure 6. Neutrophils in E. alces infected reindeer. Median line pre-infection.

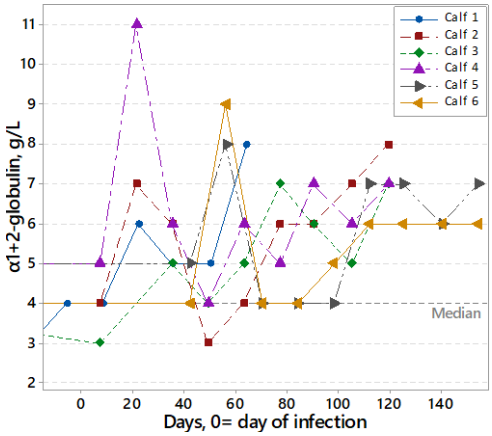

Figure 7. α_{1+2}-globulins in E. alces infected reindeer. Median line pre-infection.

Small ruminants

None of the small ruminants infected with *E. alces* showed clinical sings or shed any L_1. The lambs were euthanized day 126 PI. Goat kids no. 1 and 3 were euthanized on day 129 PI and the other goat kids on day 125 PI. No worms were recovered at necropsy.

Most of the tested blood parameters did not change noticeably over time PI, Table 16 and Table 17. Lamb no. 1 had higher eosinophils (1,25 x 10^9/L) in the first blood sample (day 22) compared with the other lambs, Figure 8. At group level (lambs) the number of eosinophils did not exhibit any remarkable variations (Table 10).

Table 10. Median values for eosinophils, urea and α_2-globulin in five lambs infected with E. alces

Day PI	Day 15-28	Day 29-42	Day 43-56	Day 57-70	Day 71-84	Day 85-98	Day 99-112
Eosinoph., x 10^9/L	0,40 (5) [0,23; 1,27]	0,44 (5) [0,23; 0,57]	0,49 (4) [0,28; 0,74]	0,18 (5) [0; 0,39]	0,50 (3) [0,5; 0,82]	0,65 (5) [0,4; 0,8]	0,33 (2) [0,2; 0,5]
Urea, mmol/L	2,5 (5) [2,3; 3,4]	2,1 (5) [1,6; 3,4]	4,7 (5) [4; 6,6]	6,5 (5) [5,5; 9,4]	8,4 (5) [7,9; 9,5]	7,9 (5) [7,7; 9,6]	4,7 (5) [3,6; 9,4]
α_2-globulin g/L	2 (5) [2; 3]	5 (5) [5; 8]	2 (5) [2; 2]	2 (5) [2; 2]	3 (5) [2; 3]	3 (5) [2; 3]	3 (5) [2; 3]

Codes – (n): group size, [x;y]: median CI (when <6 samples [x;y] represents lowest;highest result)

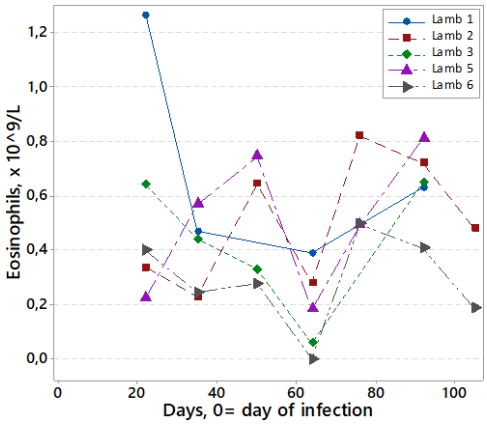

Figure 8. Eosinophils in lambs PI of E. alces.

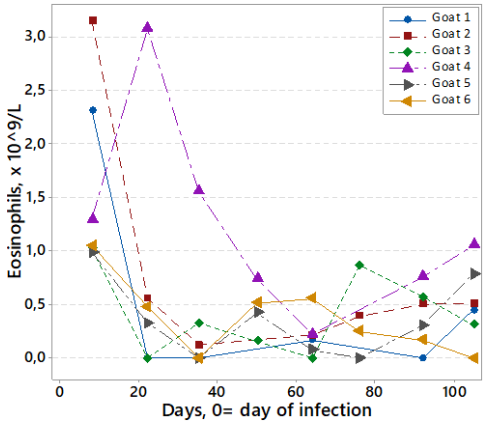

Figure 9. Eosinophils in goat kids PI of E. alces.

In the goat kids the eosinophil levels on day 8 (Figure 9), when the first blood samples were taken, were all higher than samples taken later in the study (Table 11).

Table 11. Median values for leukocytes, eosinophils, urea and α_2-globulin in six goat kids infected with E. alces

Day PI	Day 1-14	Day 15-28	Day 29-42	Day 43-56	Day 57-70	Day 71-84	Day 85-98	Day 99-112
LPK.,	19,1 (6)	19,4 (6)	14,3 (6)	14,2 (4)	13,75 (6)	12,65 (4)	16,0 (6)	14,5 (6)
x 10^9/L	[17,6; 20,3]	[11,6; 25]	[9,4; 24]	[10,5:25]	[8; 21]	[8; 17,3]	[9,4; 19]	[10,3; 19]
Eosinoph.,	1,17 (6)	0,40 (6)	0,06 (6)	0,47 (4)	0,19 (6)	0,32 (4)	0,40 (6)	0,48 (6)
x 10^9/L	[1,0; 2,6]	[0; 2,1]	[0; 1,1]	[0,2; 0,7]	[0; 0,4]	[0; 0,9]	[0,1; 0,7]	[0,1; 1,0]
Urea,	6,65 (6)	5,6 (6)	4,55 (6)	3,65 (6)	4,45 (6)	5,05 (6)	2,15 (6)	7,3 (6)
mmol/L	[5,8; 7,9]	[4,9; 7,6]	[3,5; 6,0]	[3,0; 4,6]	[3,3; 5,5]	[3,9; 5,5]	[1,4; 3,0]	[5,2; 8,9]
α_2-globulin	4 (6)	3 (6)	7 (6)	2 (6)	2 (6)	4 (6)	3 (6)	4 (6)
g/L	[3; 4,65]	[2; 5,92]	[6;8]	[2; 2,64]	[2; 2]	[2; 4,64]	[2; 4]	[3; 4,64]

Codes – (n): group size, [x;y]: median CI (when <6 samples [x;y] represents lowest;highest result)

In all lambs urea started to increase after day 35, for all but one the levels decreased after day 92 PI, Figure 10. All goat kids had a marked decrease of urea in the blood samples taken day 92 PI, increasing in the following samples day 105, Figure 11. In the goat kids the leukocytes were elevated compared with reference intervals (Constable *et al.* 2017), most noticeable in the first four weeks (Table 11). This increase was mainly caused by elevated levels of neutrophils and lymphocytes (appendix 1, Table 17).

Both in lambs (Figure 12) and goat kids (Figure 13) the α_2-globulin were increased at sampling day 35 compared to the levels registered before and after.

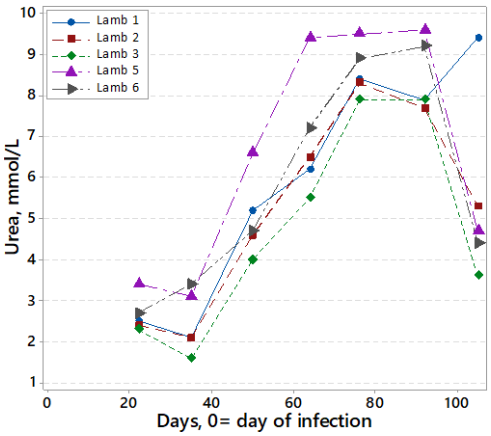

Figure 10. Urea levels in lambs PI of E. alces.

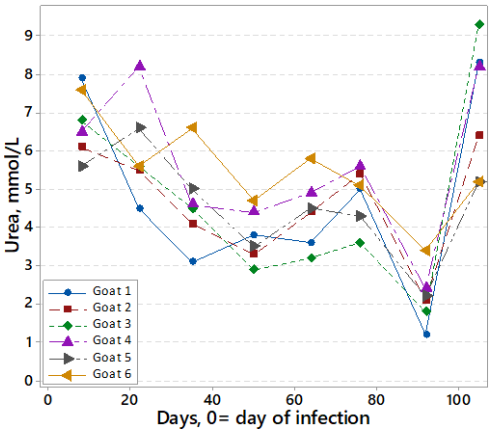

Figure 11. Urea levels in goat kids PI of E. alces.

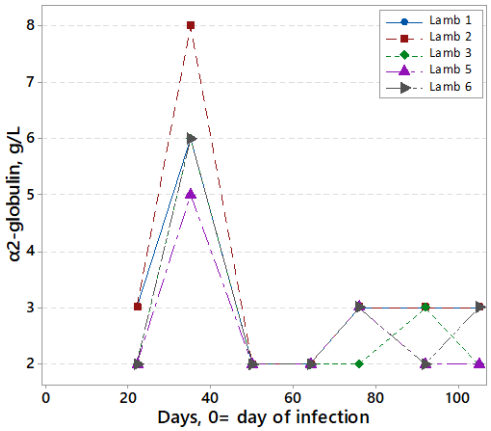

Figure 12. α_2 -globulin in E. alces infected lambs.

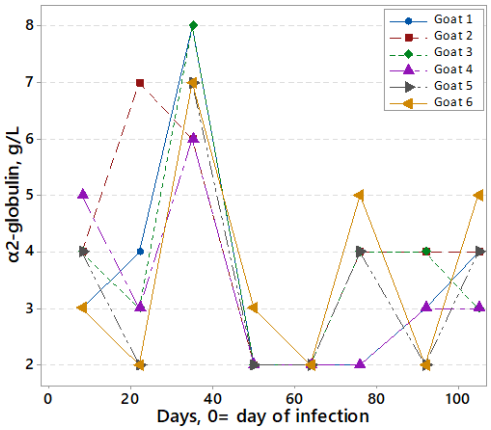

Figure 13. α_2-globulin in E. alces infected goat kids.

4.2. *Elaphostrongylus rangiferi*

Moose

All three *E. rangiferi* infected moose calves showed neurologic abnormalities when they were euthanized or died, (Table 12). Calf no. 10 and 12 showed clinical signs starting 1,5-2 months prior to their death. The signs had varied over time, periodic fever, increased time laying down, and sever coughing were some of the clinical signs besides the neurologic.

The median values of blood samples from the moose calves are presented in appendix 2, Table 18.

Table 12. Moose calves experimentally infected with E. rangiferi

Calf no.	Prepatent period, days	Infection length, days	Cause of death	Clinical signs: 0 (none) to 3 (severe)	No. and localization of *E.r.*
1	-	12	E	2 (N, M)	-
10	-	94	E	3 (N, GC, A, R)	11: ce, cs
12	133	152	D	3 (N, GC, A, R)	5: cs, mf

Codes - cause of death: D = found dead, E = euthanized. Clinical signs: 0 = no clinical signs, 1 = mild, 2 = moderate and 3 = severe, N = neurologic, R = respiratory, GC = general condition affected, A = inappetence, M = muscular. Localization of E. rangiferi: ce = cavum epidurale, cs = cavum subdurale, mf = muscle fasciae

The day 12 blood sample from calf no. 1 showed a marked increase in eosinophils, Figure 14. All calves showed an initial increase of eosinophils compared with previous (before infection) and following blood samples (Figure 15). For all three calves Hb and HCT increased in the last blood sample taken from each animal (before death) compared with previous samples. LPK increased after infection, control animals having a median of 4,4 x 10^9/L (CI 4,2; 4,6) and 1-14 days PI the median was 6,65 x 10^9/L (lowest 5,6; highest 8), eosinophils and neutrophils contributing to this.

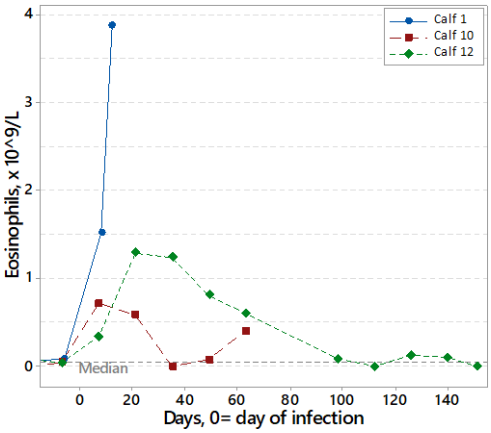

Figure 14. Eosinophils in E. rangiferi infected moose calves. Median line of controls.

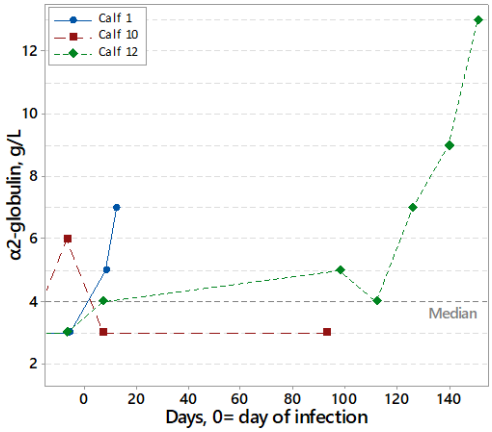

Figure 15. α_2-globulin in E. rangiferi infected moose calves. Median line of controls.

The protein levels increased PI for all three calves. Around day 50 PI protein levels peaked and started to decrease in the two calves that were alive. Alterations in other parameters differed between the calves. In calf no. 10 ASAT (43,1 µkat/L), GLDH (246 nkat/L), CK (583 µkat/L) and urea (29,9 mmol/L) showed a marked increase in the last blood sample taken of these parameters (day 93 PI). This was not seen in the other calves. In calf no. 1 an increase of α_2-globulin was observed day 12 PI (last sample before death), the other two calves were not sampled at this time PI. For those calves no test results were received for α_2-globulin from day 7 until day 93 (calf no. 10) and 98 PI (calf no. 12). In calf no. 12 increasing levels of α_2-globulin were seen from day 126 PI (Figure 15), the calf having moderate-severe clinical

signs with posterior paresis, inappetent, coughing, increased recumbent time and fever. Calf no. 12 also had a marked decrease in albumin from day 126 and increased γ-globulin after day 100, most pronounced day 140 (22 g/L).

Sheep

The haematology and serum chemistry for the *E. rangiferi* infected lamb is presented in appendix 2, Table 19. The lamb (no. 4) developed severe neurologic clinical signs three weeks PI with a rapid deterioration, Table 13.

Table 13. Lamb infected with E. rangiferi

Lamb no.	Prepatent period, days	Infection length, days	Cause of death	Clinical signs: 0 (none) to 3 (severe)	No. and localization of *E.r.*
4	-	24	E	3 (N)	0

Codes - cause of death: E = euthanized. Clinical signs: N = neurologic

Only one blood sample was taken, day 19 PI. Compared with blood samples taken from the other lambs at the same time PI some parameters were deviating, median given is for *E. alces* infected lambs 22 days PI. Haemoglobin 126 g/L (median 114 g/L) and HCT 41% (median 34%), increased neutrophils 4,48 x 10^9/L (median 1,43 x 10^9/L), GLDH increased to 169 nkat/L (median 51 nkat/L), urea 6,9 mmol/L (median 2,5 mmol/L), protein 68 g/L (median 60 g/L) and an increase of α_2-globulins 7 g/L (median 2g/L) was also seen.

5. Discussion

Result from the experimental study indicate that infection with *E. alces* (in normal and abnormal host) and *E. rangiferi* (abnormal host) causes alterations in some haematological and blood chemistry parameters.

A common finding was the significant increase of eosinophils noted in *E. alces* infected moose calves, also seen in reindeer calves, goat kids and one lamb, and in *E. rangiferi* infected moose calves. Depending on the time for sampling these changes appeared with varying clarity. The increased number of eosinophils were first noticed day 7-8 PI when moose calves, reindeer calves and goat kids sampled first time PI. This agreed with the findings from *E. rangiferi* infection in one goat kid of Handeland & Skorping (1992), twelve goat kids of Handeland & Skorping (1993) and a lamb of Handeland *et al.* (1993). In this study eosinophilia was not seen in the *E. rangiferi* infected lamb and only in one of five *E. alces* infected lamb, which may be explained by the time chosen for first sampling (19- and 22-days PI respectively). Handeland *et al.* (1993) recorded the peak of eosinophils on day 17 PI in an *E. rangiferi* infected lamb (dose 3000 L_3). In this study the first samples of the *E. rangiferi* infected calves (day 7 or 8 PI) exhibited mildly elevated levels of eosinophils compared with control group. Day 12 PI the highest eosinophil registration was seen in the one calf sampled that day. The other two calves were not sampled until 21 days PI, thereby probably missing this peak. Only one *E. alces* infected moose calf (no. 4) was sampled around two weeks PI (day 13 PI). This sample also showed among the highest levels of eosinophils (in the *E. alces* moose group) and for all but one moose calf the samples taken day 21-22 PI were near or within normal levels. When comparing the levels of eosinophils in *E. rangiferi* (day 12) and *E. alces* (day 13) infected calves, the level in an *E. rangiferi* infected calf was twice as high as the *E. alces* infected calf. There are several possible explanations. It could be related to differences in the parasite's migratory route (haematogenous or direct), differences in how well the parasite has adapted to immune systems of the normal (*E. alces*) and abnormal host (*E. rangiferi*), individual differences, or exact timing of blood sampling. Tizard (2012) concluded that well-adapted parasites avoid causing damage in the host leading to noticeable host's immune reaction against it, so the reaction caused by *E. alces* in moose might be the least aggressive.

The elevated levels of eosinophils in *E. alces* infected normal and abnormal hosts and in *E. rangiferi* infected abnormal host is also in line with other parasites causing cerebrospinal nematodiasis where eosinophilia is seen, *P. tenuis* in llamas (Ismail *et al.* 2011) and *A. cantonensis* in humans (Xie *et al.* 2019; Federspiel *et al.* 2020) and in rats (Garcia *et al.* 2014). Garcia *et al.* (2014) registered the highest eosinophil increase during the last few weeks of infection; in this study the increase was seen in the first weeks. Garcia *et al.* (2014) discuss that the increase might be related with the oviposition of the female worm and hatching of L_1. From the results in our study it is difficult to evaluate how much oviposition and hatching results in increased eosinophils. When comparing the first day recovering L_1 in faeces and the levels of eosinophils prior to that some animals show an increase of varying degree, but the majority of animals in our study show a much greater increase early in the infection. No obvious trends associated with the first shedding of *E. alces* L_1 could be seen in the other haematologic or serum chemistry parameters in this study either.

An increase of basophils was seen in reindeer calves, most noticeably in samples taken at day 21 PI. Also in comparison with reference values by Miller *et al.* (2013) and Swenson *et al.* (1999) the basophil levels demonstrate an increase. The interpretation from this study is that in reindeer *E. alces* also can trigger a basophilic response in the host. The same individuals had the highest levels of both basophils and eosinophils. The time when blood samples were taken PI can have affected how well the deviation was registered, no blood samples were taken from the reindeer calves in the time period when the highest eosinophil registrations were done in the other species (12-13 days PI). In the *A. cantonensis* study in rats by Garcia *et al.* (2014) increased numbers of basophils were also seen, as described above with the eosinophils the increase was seen later in the rats, registered week 4 to 6 PI.

In reindeer calves LPK levels were increased in the first weeks PI compared to pre-infection and over reference values by Miller *et al.* (2013). Also in goat kids LPK were increased 1 to 4 weeks PI and were over reference values by Constable *et al.* (2017). In the goat kids this could be due to young age (9 months) compared to reference and a physiologic response due to stress as these blood samples were the first taken from the goat kids. An increase was also seen in the moose calves, deviating the most from control animals in the *E. rangiferi* infected calves but not significantly in the *E. alces* infected calves. Parts of the increase comes from the eosinophils but the main contributor in moose and reindeer calves came from neutrophils while goat kids had an initial increase of both neutrophils and lymphocytes. The increase of neutrophils fits well considering the migration of parasites through different organs causing damage and an inflammatory response, supported by the pathological findings of Stéen *et al.* (1997, 1998b). The inflammatory response with increased neutrophil demand were not to the extent that band

neutrophils occurred. In *A. cantonensis* infected rats Garcia *et al.* (2014) registered increased numbers of neutrophils week 3 to 6 and LPK week 3 to 8.

In the *E. alces* infected moose calves a significant increase of HCT was seen compared with control. The significant increase coincided with clinical signs like inappetence, coughing, neurologic abnormalities, muscular stiffness and/or lying down more than normal, present in four of the six calves. Three of the calves with elevated HCT also had increased TP, dehydration being a plausible explanation. Also, the *E. rangiferi* infected calves had elevated HCT levels prior to their death, being severely sick.

At necropsy, white-spots were observed in liver and kidneys in many of the moose calves and to a minor extent also in the other species (Stéen *et al.* 1998b). The enzyme activity of ASAT, GLDH and CK did not indicate that the parasitic migration through the host caused sufficient cell injury to alter the tested parameters. Only one animal, moose calf no. 10 infected with *E. rangiferi,* had substantially increased ASAT, GLDH and CK when euthanized. The calf had severe neurologic abnormalities (ataxia, weak hindquarters and bruxism), increased time laying down and from five days prior to death almost total recumbency, not eating and very limited water intake. At necropsy, multiple haemorrhages were seen in the leg muscles most likely caused by trauma, and the liver was severely enlarged (hepatic steatosis). The deviating parameters in this calf were interpreted as being secondary to the neurologic effects caused by the parasite, 11 worms recovered in the CNS at necropsy, rather than a response to direct cellular damage caused by the parasite in the organs (liver and muscles).

In goat kids and lambs a slight increase of α_2-globulin was seen 5 to 6 weeks after infection with *E. alces.* A lesser increase was also seen in *E. alces* infected moose calves and reindeer calves (α_{1+2}-globulin in reindeer), occurring week 3 to 4 after infection. In the *E. rangiferi* infected animals α_2-globulin levels increased in the last samples taken before death. The increase of α_2-globulin (α_{1+2}-globulin in reindeer) seems a bit late to be a response to migrating parasites from gastro-intestinal tract to CNS but may be related to some other parts of the parasites life-cycle.

In the *E. alces* infected moose calves the urea levels were significantly decreased (days 1-14) after infection compared to controls. Pre-infection these moose calves urea levels were slightly lower than control calves. All calves were eating with good appetite; compared with the control group the diet and amount food given were the same. Two of the infected moose calves had milder diarrhoea at the time. The moose calves were infected at four different dates between November to March, no obvious food related explanations were seen. The explanation to the decreased urea is therefore unknown. Deviating urea levels were also registered in goat kids (decreased days 85-99) and lambs (increased days 71-84). At necropsy (Stéen *et al.* 1998b), there were no findings that explained the alterations recorded in all the

animals. The goat kids and lambs (all but the *E. rangiferi* infected lamb) were infected the same date and fed the same feed. Therefore, the changes observed are believed to be nutritionally related.

In this study the time of the first blood sampling PI varied among the different host species and parasite administered. The majority of moose and all reindeer calves were tested on days 7 or 8 PI, followed by next sample between days 21-42 PI. All goats were tested on day 8 PI and second sample on day 22 while lambs were first sampled on day 22 (19 for *E. rangiferi* infected lamb) and the next sample on day 35 PI. Increased numbers of days between sampling increases the risk of missing important haematologic and biochemistry changes. Handeland & Skorping (1992) sampled the goat kid in their study daily from two days prior to inoculation to 14 days PI, which made it possible to better follow the deviations in tested parameters. This study, however followed the animals for a longer period of time after infection giving more information on blood changes over time.

For wildlife and semi-domesticated species the available reference ranges of haematology and blood chemistry are limited and the results from different methods varies. Rostal *et al.* (2012) investigated reference ranges for chemically immobilized, apparently healthy wild Norwegian moose and presented the findings according to age groups: calves, yearlings and adults. The animals were sampled January to March, dated from helicopter and anaesthetized (Rostal *et al.* 2012). In Sweden Kockum Adolfsson (1993) has investigated the normal haematology and blood chemistry for wild moose, immobilized from helicopter and sampled in February to March. The two studies have reported similar mean values for the tested parameters but differ in the way data is presented. The conditions for calves in the present study differ in many aspects from those of a "normal" moose life, for example with good feed supply, very restricted space to move around and not being exposed to shifting weather conditions. The conditions for blood sampling also differ considerably, wild moose are immobilized by darting from helicopter while the calves in this experiment became familiar with the routine and remained calm. Therefore, it can be misleading to compare tested parameters with those "wild" reference values. The same difficulty was noted with reference values for reindeer.

In this study a separate control and experimental groups sampled under the same conditions existed only for the moose calves while the reindeer calves were their own control with blood samples taken before infection with *E. alces*. For both lamb and goat kids the first samples were taken PI. To evaluate the data, reference samples taken before infection are very usable. Breed and age of the animal are two important factors that influence the haematology and biochemistry parameters (Mbassa & Poulsen 1992, 1993). This makes it challenging to find relevant reference intervals for comparison.

The sample size varied between the groups in this study, one lamb (*E. rangiferi*), three moose calves (*E. rangiferi*), five lamb (*E. alces*), six goat kids (*E. alces*), nine

moose calves (*E. alces*) and six moose calves as control. With only one or three animals in a group it was difficult to draw any conclusions, especially if a diversity is seen between the test results in a small group or if only one blood sample is taken (*E. rangiferi* infected lamb).

Statistical analysis was only performed on the blood samples from *E. alces* infected moose calves due absence of independent control animals in the other groups and/or very small groups. In the control group all blood samples taken from the age 5 to 11 months were included (multiple samples from the same individual), this could have caused bias as they were not all independent of each other.

5.1. Conclusion

The results from this study indicate that *E. alces* infection in normal (moose calves) and abnormal (reindeer calves, lambs and goat kids) hosts and *E. rangiferi* infection in abnormal (moose calves) hosts mainly causes haematological changes with increased levels of eosinophils one to three weeks after infection. In reindeer calves an increase of basophils and eosinophils were often seen in the same animal.

Other changes showed more deviation over time and between species. An increase of LPK, mainly neutrophils occurred in the first weeks PI in some moose calves, reindeer calves and goat kids. In all species a slight increase of α_2-globulin (α_{1+2}-globulin in reindeer) occurred, the increase was more noticeable in goat kids and lambs occurring 5 to 6 weeks after infection. The alterations of these parameters were not significant and need further investigation to better understand how the parasitic infections influences them.

In moose calves extensive terminal neurological impairment by the *Elaphostrongylus* spp. parasite can lead to severe clinical signs which alter many haematologic and blood chemistry parameters, most commonly an increase of HCT.

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Acknowledgements

I would like to thank my supervisors Margareta Stéen, Department of Anatomy, Physiology and Biochemistry, and Inger Lilliehöök, Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU) for generous support. I would also like to thank Murray W. Lankester (Professor Emeritus, Lakehead University, Canada) for his constructive feedback on the manuscript.

Popular Science Summary

The roundworms of genus *Elaphostrongylus* spp., muscleworms, are found in different deer species and can cause neurologic disease, muscle inflammation and increased death. In the 1960s and early 1970s *Elaphostrongylus rangiferi* caused large outbreaks in reindeer herds in Sweden and Norway. In the middle of 1980s increased mortality and sick moose (mainly calves and yearlings) were observed in Sweden and Norway, by examining dead animals this was linked to infection with the newly discovered *E. alces* parasite. To learn more about elaphostrongylosis, both naturally and experimentally infected animals of different species have been studied. The aim with this study was to investigate if the disease causes alterations that can be seen in blood samples.

Part of the muscleworm's development to the reproductive adult form must take place in a warm-blooded animal (a definitive host) and it is possible only in some animal species. The parasites often have some animal species in which this development normally takes place, the normal definitive host. For *E. alces* the normal host is moose while it is reindeer for *E. rangiferi*. These two muscleworm species can also infect other species (abnormal definitive hosts), for example other deer species and domestic animals like goat and sheep. When the definitive host browse or graze, they accidentally swallow snails and slugs located in the vegetation. Both snails and slugs are intermediate hosts where some of the development stages of the parasite must take place for it to become an infective larva. When the definitive host has ingested the parasite, it starts to move (migrate) through the host to reach specific, preferred locations. The larvae penetrate the stomach wall and either continuing into blood vessels (*E. rangiferi*) to be transported with the blood to nerve tissue and central nervous system, CNS (the brain and spinal cord), or using a direct route (*E. alces*), penetrating the abdominal wall and entering into muscles and nerve tissue to reach CNS. There might also be other routes that they use. *E. rangiferi* penetrate the outermost of the membranes surrounding the spinal cord and brain, in this location the worm starts to develop, it grows and mature. *E. alces* worms do not penetrate the membrane around the spinal cord, it stays in the spinal canal outside the outermost membrane. The inflammatory reaction the worm causes, with bleeding, oedema and inflammatory cells can cause neurologic effects due to increased pressure on nerves. After development in the CNS the worms continue their migration to the connective

tissue surrounding muscle tissue. The female worms deposit eggs in blood vessels where she is located, the eggs are carried with the blood to the smallest lung vessels. After hatching the larvae penetrate and enter the lungs, they work their way up the respiratory system, are swallowed and following the gastro-intestinal system, leaving with faeces. The larvae penetrate the foot of snails or slugs, then starts to develop to infective larvae.

A parasite that is well adapted to its host avoid triggering the immune system in different ways, it is also important for them not to cause the host too much harm as they are depending on them for development and reproduction. Sometimes the parasites cause alterations in the host that can be seen in blood samples. This can give important information for a better understand of how the parasite affects the host and also some help in diagnostics of disease, treatment and disease surveillance.

In an earlier published study conducted in 1989 to 1990 (Stéen *et al.* 1997, 1998b) different animal species (moose calves, reindeer calves, goat kids and lambs) were experimentally infected with *E. alces* or *E. rangiferi*. In the published material from the study the parasites possibility to infect abnormal host species and clinical signs in infected animals were described. The publications also describe the findings from examination of the animal bodies after death, migratory route of the parasite, morphology (shape and form) of the parasite and the parasites capability to reproduce leading to parasite larvae in the animal's faeces. During the experiment bloods samples were taken from the infected animals.

The aim with this study was to investigate the effects *E. alces* and *E. rangiferi* have on haematology and blood chemistry in experimentally infected animals. If and how the diseases altered different parameters in the blood samples over time after infection were examined. No previous studies have looked at the changes in blood parameters in *E. alces* infected animals or in *E. rangiferi* infected moose calves.

When the experimental study was conducted the animals were kept in a stable (indoors) in separate stalls. Nine moose calves, six reindeer calves, six goat kids and five lamb were experimentally infected with *E. alces*. Three moose calves and one lamb were infected with *E. rangiferi*. The animals were between 4,5 to 9 months old when fed with a dose infective parasite larva. A high dose (approx. 1000) infective larva were given all animals but two, two moose calves were given a lower dose (approx. 400) of *E. alces.* Blood was collected and analysed for white and red blood cells and biochemical parameters. In moose the analysed parameters from the infected calves were compared with results from a control group of six calves. The control calves were not infected, they were kept and taken blood samples from under the same conditions as the infected moose calves. The six reindeer calves were their own control, comparing blood samples taken before and after infection. For the six goat kids and six lambs the alteration over time after infection were studied, no control group was included. Blood samples were taken

with around 14 days interval from each animal and continued until the animal died or was euthanized, occurring between 12 to 158 days after infection. The majority of infected (*E. alces* and *E. rangiferi*) moose calves develop severe clinical signs (often neurologic abnormalities, coughing, lost apatite and decreased general condition) and the *E. rangiferi* infected lamb developed severe neurologic disease. The *E. alces* infected reindeer calves, goat kids and lambs showed no or very mild clinical signs.

The most marked finding in the blood samples were the eosinophils, increased numbers were seen in *E. alces* infected moose calves, reindeer calves, goat kids and one lamb, and in *E. rangiferi* infected moose calves. This occurred one to three weeks after infection. In the reindeer calves an increase of eosinophils was often seen together with increased numbers of basophils. Eosinophils are a type of white blood cells that can play an important role in the hosts defence against parasitic worms, this blood cell can release proteins with the ability to damage the parasite and engulf it. Basophils are also white blood cells and can be important in the immunity against parasites.

Elevated red blood cells in the blood, haematocrit levels, were seen in the moose calves, often together with clinical signs. The increase can be due to dehydration as the calves with clinical sings were laying down more, less interested in eating and drinking and with neurological signs affecting balance and mobility.

Less pronounced alterations but that occurred in moose calves, reindeer calves and goat kids were increased neutrophils the first weeks after infection. Neutrophils are white blood cells and are important in the immune system, fighting foreign material/pathogens and cleaning up dead cells in the tissue. The increase of these cells can be a response to damage that the migrating parasites causes the host. In goat kids and lambs a slight increase of α_2-globulin was seen 5 to 6 weeks after infection with *E. alces.* A lesser increase was also seen in *E. alces* infected moose calves and reindeer calves (α_{1+2}-globulin in reindeer) week 3 to 4 after infection. In *E. rangiferi* infected animals the increase was seen in the last blood samples before death. α_2-globulin increases as a reaction to acute inflammation.

Even though the parasite migrates through different organs in the host, for example the liver, this was not reflected with increased enzyme levels in the blood samples. Enzymes that normally are found within certain cell types can leak out into the blood if the cells are injured. The blood samples were taken with approx. 14 days interval, changes that occurred could also have be missed because of this.

Appendix 1

Table 14. Median values of haematology and blood chemistry for E. alces infected moose calves. Control group age 5-11 months

Sampling day PI	Control	Day 1-14	Day 15-28	Day 29-42	Day 43-56	Day 57-70	Day 71-84	Day 85-126
Hb, g/L	110 (51)	117 (9)	121 (4)	113 (7)	118,5 (6)	118,5 (4)	142,5 (2)	118 (3*)
HCT, %	32 (51)	32 (9)	35,6 (4)	30,9 (6)	36,6 (6)	32,3 (4)	42,6 (2)	33 (3*)
LPK, x 10^9/L	4,4 (51)	6,3 (9)	5,85 (4)	3,95 (6)	5,15 (6)	6,85 (4)	6,4 (2)	4,2 (3*)
Neutroph. x 10^9/L	1,85 (51)	3,72 (9)	2,12 (4)	1,68 (6)	2,22 (6)	3,13 (4)	3,48 (2)	1,88 (3*)
Eosinoph., x 10^9/L	0,05 (51)	0,23 (9)	0,66 (4)	0,17 (6)	0,35 (6)	0,40 (4)	0,33 (2)	0,40 (3*)
Basophils, x 10^9/L	0	0	0	0	0	0	0	0
Lymphoc., x 10^9/L	2,26 (51)	2,38 (9)	2,24 (4)	1,98 (6)	2,38 (6)	2,04 (4)	2,40 (2)	1,72 (3*)
Monoc., x 10^9/L	0,05 (51)	0,07 (9)	0,06 (4)	0,06 (6)	0,02 (6)	0,042 (4)	0,19 (2)	0,09 (3*)
Calcium, mmol/L	2,6 (52)	2,6 (9)	2,6 (4)	2,6 (6)	2,65 (6)	2,65 (4)	2,45 (2)	2,6 (3*)
Urea, mmol/L	7,15 (52)	6,3 (9)	6,4 (4)	5,85 (&)	5,8 (6)	6,5 (4)	5,95 (2)	6,0 (3*)
Cholest., mmol/L	1,39 (52)	1,43 (9)	1,28 (4)	1,26 (6)	1,33 (6)	1,43 (4)	1,48 (2)	1,27 (3*)
ASAT, µkat/L	1,1 (52)	1,0	1,1	1,0	1,1	1,1	2,9	1,0 (3*)
CK, µkat/L	1,8 (52)	1,5 (9)	2,05 (4)	1,95 (6)	2,35 (6)	2,15 (4)	6,7 (2)	3 (3*)
GLDH, nkat/L	< 20 (50)	<20 (9)	<20 (4)	<20 (6)	<20 (6)	<20 (4)	<20 (2)	<20 (3*)
Protein, g/L	63 (52)	63 (9)	67 (4)	64 (6)	65 (6)	68 (4)	56 (2)	72 (3*)
Albumin, g/L	41,5 (36)	44,0 (3)	40,0 (3)	40,5 (6)	41,5 (6)	42,5 (4)	33,0 (2)	43,0 (3*)
α_1-globulin, g/L	4,0 (36)	3,0 (3)	4,0 (3)	4,0 (6)	3,5 (6)	4,5 (4)	3,5 (2)	4,0 (3*)
α_2- globulin, g/L	4 (36)	3 (3)	5 (3)	4 (6)	3 (6)	5 (4)	5 (2)	5 (3*)
β_{1+2}-globul, g/L	6,0 (36)	6,0 (3)	7,0 (3)	6,0 (6)	7,0 (6)	7,0 (4)	6,5 (2)	8,0 (3*)
γ-globulin, g/L	8,0 (36)	7,0 (3)	10,0 (3)	10,0 (6)	9,5 (6)	10,0 (4)	7,5 (2)	13,0 (3*)
Alb-glob. ratio	2,0 (36)	2,4 ((3)	1,7 (3)	1,8 (6)	1,8 (6)	1,7 (4)	1,5 (2)	1,5 (3*)

*Codes - (n) number of samples included in the group, * all samples are from same individual*

Table 15. Median values of haematology and blood chemistry for E. alces infected reindeer calves. Pre-infected (pre-inf.) represents the samples taken before inoculation of parasites, age 4,5-9 months

Sampling day PI	Pre-inf.	Day 1-14	Day 15-28	Day 29-42	Day 43-56	Day 57-70	Day 71-84	Day 85-98	Day ≥99
Hb, g/L	154 (25)	153 (5)	155 (3)	146 (5)	149 (6)	150 (6)	142 (5)	144 (5)	139 (14*)
HCT, %	42 (25)	42 (5)	45 (3)	41,7 (6)	41 (6)	41 (6)	39,8 (5)	41 (5)	38,5 (14*)
LPK, x 10^9/L	4,5 (23)	7,3 (5)	6,4 (3)	6,7 (6)	5,3 (6)	5,5 (5)	5,4 (5)	4,9 (5)	5,6 (14*)
Neutroph. x 10^9/L	1,98 (23)	3,34 (5)	1,79 (3)	2,55 (6)	2,11 (6)	1,71 (5)	1,78 (5)	2,60 (5)	1,88 (14*)
Eosinoph., x 10^9/L	0,56 (23)	1,06 (5)	1,79 (3)	1,19 (6)	1,01 (6)	0,72 (5)	0,67 (5)	0,44 (5)	0,59 (14*)
Basophils, x 10^9/L	0,04 (23)	0,00 (5)	0,58 (3)	0,22 (6)	0,18 (6)	0,09 (5)	0,13 (5)	0,16 (5)	0,13 (14*)
Lymphoc., x 10^9/L	1,89 (23)	1,63 (5)	2,18 (3)	2,23 (6)	1,62 (6)	1,65 (5)	2,02 (5)	1,41 (5)	1,91 (14*)
Monoc., x 10^9/L	0,06 (23)	0,20 (5)	0,06 (3)	0,02 (6)	0,06 (6)	0,00 (5)	0,07 (5)	0,07 (5)	0,00 (14*)
Calcium, mmol/L	2,6 (25)	2,45 (6)	2,5 (3)	2,5 (6)	2,5 (6)	2,5 (6)	2,5 (5)	2,5 (5)	2,5 (14*)
Urea, mmol/L	9,3 (25)	8,3 (6)	8,3 (3)	10,1 (6)	9,4 (6)	9,1 (6)	8,3 (5)	8,8 (5)	7,9 (14*)
Cholest., mmol/L	1,6 (25)	1,6 (6)	1,5 (3)	1,4 (6)	1,5 (6)	1,6 (6)	1,5 (5)	1,5 (5)	1,5 (14*)
ASAT, µkat/L	0,9 (25)	0,8 (6)	0,8 (3)	1,0 (6)	0,9 (6)	1,0 (6)	1,1 (5)	1,0 (5)	0,9 (14*)
CK, µkat/L	2,1 (25)	1,8 (6)	2,2 (3)	2,5 (6)	2,1 (6)	2,3 (6)	2,2 (5)	2,1 (5)	2,2 (14*)
GLDH, nkat/L	<20 (26)	<20 (6)	<20 (3)	<20 (6)	<20 (6)	<20 (6)	<20 (5)	<20 (5)	<20 (14*)
Protein, g/L	64 (25)	63,5 (6)	63 (3)	64,5 (6)	67 (6)	65,5 (6)	61 (5)	61 (5)	61 (14*)
Albumin, g/L	47,0 (10)	45,5 (4)	48,0 (3)	45,0 (6)	47,5 (6)	47,0 (6)	43,0 (5)	46,0 (5)	43,0 (14*)
α_{1+2}-glob, g/L	4,0 (10)	4,0 (4)	7,0 (3)	5,0 (6)	4,5 (6)	4,5 (6)	5,0 (5)	6,0 (5)	6,5 (14*)
β_{1+2}-globul, g/L	7 (10)	8 (4)	6 (1)	7 (6)	7 (5)	6 (6)	6 (5)	5 (5)	6 (14*)
γ-globulin, g/L	5,0 (10)	5,5 (4)	6,0 (3)	5,5 (6)	5,5 (6)	7,0 (6)	6,0 (5)	4,0 (5)	5,0 (14*)
Alb-glob. ratio	2,65 (10)	2,55 (4)	2,70 (3)	2,50 (6)	3,10 (5)	2,25 (6)	1,90 (5)	2,70 (5)	2,50 (14*)

*Code: (n) = number of samples in the group, * = 2 to 4 samples from the same individual*

Table 16. Median values of haematology and blood chemistry for the five lambs infected with E. alces

Sampling day PI	Day 15-28	Day 29-42	Day 43-56	Day 57-70	Day 71-84	Day 85-98	Day 99-112
Hb, g/L	114 (5)	105 (5)	106 (5)	107 (5)	94 (3)	111 (5)	115,5 (4)
HCT, %	34 (5)	24 (5)	31 (5)	30 (5)	25 (3)	32 (5)	33 (4)
LPK, x 10^9/L	7,5 (5)	6,7 (5)	8,3 (5)	7,8 (5)	8,2 (3)	9 (5)	9 (4)
Neutroph. x 10^9/L	1,43 (5)	0,83 (5)	1,16 (4)	1,40 (5)	1,89 (3)	2,40 (5)	3,33 (2)
Eosinoph., x 10^9/L	0,40 (5)	0,44 (5)	0,49 (4)	0,18 (5)	0,50 (3)	0,65 (5)	0,33 (2)
Basophils, x 10^9/L	0	0	0	0	0	0	0
Lymphoc., x 10^9/L	4,42 (5)	4,42 (5)	6,21 (4)	6,01 (5)	5,25 (3)	4,80 (5)	5,74 (2)
Monoc., x 10^9/L	0,00 (5)	0,03 (5)	0,00 (4)	0,00 (5)	0,00 (3)	0,08 (5)	0,05 (2)
Calcium, mmol/L	2,4 (5)	2,6 (5)	2,4 (5)	2,5 (5)	2,5 (5)	2,6 (5)	2,6 (5)
Urea, mmol/L	2,5 (5)	2,1 (5)	4,7 (5)	6,5 (5)	8,4 (5)	7,9 (5)	4,7 (5)
Cholest., mmol/L	1,41 (5)	1,28 (5)	1,51 (5)	1,37 (5)	1,47 (5)	1,35 (5)	1,45 (5)
ASAT, µkat/L	1,6 (5)	1,6 (5)	1,7 (5)	2,1 (5)	2,1 (5)	2,0 (5)	2,1 (5)
CK, µkat/L	2,8 (5)	3,2 (5)	4,3 (5)	2,0 (5)	3,8 (5)	5,7 (5)	2,9 (5)
GLDH, nkat/L	51 (5)	111 (5)	93 (5)	86 (5)	129 (5)	110 (5)	127 (5)
Protein, g/L	60 (5)	61 (5)	61 (5)	66 (5)	69 (5)	68 (5)	70 (5)
Albumin, g/L	37 (5)	36 (5)	38 (5)	39 (5)	35 (5)	35 (5)	35 (5)
α_1-globulin, g/L	2 (5)	4 (5)	2 (5)	3 (5)	2 (5)	2 (5)	2 (5)
α_2- globulin, g/L	2 (5)	6 (5)	2 (5)	2 (5)	3 (5)	3 (5)	3 (5)
β_{1+2}-globul, g/L	9 (5)	6 (5)	9 (5)	9 (5)	11 (5)	11 (5)	13 (5)
γ-globulin, g/L	10 (5)	12 (5)	11 (5)	12 (5)	15 (5)	18 (5)	17 (5)
Alb-globul. ratio	1,6 (5)	1,5 (5)	1,7 (5)	1,4 (5)	1,2 (5)	1,1 (5)	1,0 (5)

Code: (n) = number of samples in the group

Table 17. Median values of haematology and blood chemistry for the six goat kids infected with E. alces

Sampling day PI	Day 1-14	Day 15-28	Day 29-42	Day 43-56	Day 57-70	Day 71-84	Day 85-98	Day 99-112
Hb, g/L	123,5 (6)	114,5 (6)	117 (6)	118,5 (6)	124 (6)	114,5 (4)	120 (6)	121,5 (6)
HCT, %	37,0 (6)	34,5 (6)	33,0 (5)	34,5 (6)	34,0 (6)	28,5 (4)	39,0 (6)	37,0 (6)
LPK, x 10^9/L	19,1 (6)	19,4 (6)	14,3 (6)	14,2 (4)	13,8 (6)	12,7 (4)	16,0 (6)	14,5 (6)
Neutroph. x 10^9/L	4,07 (6)	3,41 (6)	2,71 (6)	2,14 (4)	2,75 (6)	2,62 (4)	3,67 (6)	2,95 (6)
Eosinoph., x10^9/L	1,17 (6)	0,40 (6)	0,06 (6)	0,47 (4)	0,19 (6)	0,32 (4)	0,40 (6)	0,48 (6)
Basophils, x10^9/L	0	0	0	0	0	0	0	0
Lymphoc, x 10^9/L	12,25 (6)	12,66 (6)	10,90 (6)	12,31 (4)	9,56 (6)	9,26 (4)	12,01 (6)	10,58 (6)
Monoc., x 10^9/L	0,09 (6)	0,00 (6)	0,06 (6)	0,00 (4)	0,00 (6)	0,00 (4)	0,05 (6)	0,00 (6)
Calcium, mmol/L	2,4 (6)	2,5 (6)	2,5 (6)	2,6 (6)	2,6 (6)	2,6 (6)	2,6 (6)	2,5 (6)
Urea, mmol/L	6,65 (6)	5,60 (6)	4,55 (6)	3,65 (6)	4,45 (6)	5,05 (6)	2,15 (6)	7,30 (6)
Cholest., mmol/L	2,02 (6)	1,85 (6)	1,98 (6)	1,99 (6)	2,07 (6)	2,24 (6)	1,72 (6)	1,75 (6)
ASAT, µkat/L	1,30 (6)	1,25 (6)	1,20 (6)	1,20 (6)	1,30 (6)	1,25 (6)	1,30 (6)	1,45 (6)
CK, µkat/L	1,80 (6)	2,45 (6)	2,00 (6)	2,05 (6)	1,60 (6)	3,70 (6)	2,25 (6)	2,05 (6)
GLDH, nkat/L	90,5 (6)	56,0 (6)	71,5 (6)	77,0 (6)	76,5 (6)	86,0 (6)	57,0 (6)	107,0 (6)
Protein, g/L	62,5 (6)	64,0 (6)	62,5 (6)	64,0 (6)	64,0 (6)	64,0 (6)	64,5 (6)	63,5 (6)
Albumin, g/L	37,0 (6)	37,0 (6)	38,5 (6)	39,0 (6)	39,0 (6)	40,0 (6)	40,5 (6)	39,0 (6)
α_1-globulin, g/L	6,0 (6)	4,0 (6)	5,0 (6)	5,0 (6)	5,5 (6)	3,0 (6)	3,0 (6)	2,0 (6)
α_2- globulin, g/L	4 (6)	3 (6)	7 (6)	2 (6)	2 (6)	4 (6)	3 (6)	4 (6)
β_{1+2}-globul, g/L	7,0 (6)	9,5 (6)	7,0 (6)	9,0 (6)	9,0 (6)	9,0 (6)	9,5 (6)	10,5 (6)
γ-globulin, g/L	10,0 (6)	8,0 (6)	7,5 (6)	7,5 (6)	7,0 (6)	7,5 (6)	7,5 (6)	9,0 (6)
Alb-globul. ratio	1,4 (6)	1,4 (6)	1,7 (6)	1,6 (6)	1,6 (6)	1,7 (6)	1,5 (6)	1,6 (6)

Code: (n) = number of samples in the group

Appendix 2

Table 18. Median values of haematology and blood chemistry for E. rangiferi infected moose calves

Sampling day PI	Control	Day 1-14	Day 15-28	Day 29-42	Day 43-56	Day 57-70	Day 85-126
Hb, g/L	110,0 (51)	116,5 (4*)	110,5 (2)	131,0 (2)	141,5 (2)	131,5 (2)	121,5 (4**)
HCT, %	32,0 (51)	34,5 (4*)	32,5 (2)	37,5 (2)	42,0 (2)	37,8 (2)	37,0 (4**)
LPK, x 10^9/L	4,40 (51)	6,65 (4*)	6,05 (2)	4,90 (2)	4,60 (2)	5,25 (2)	8,85 (4**)
Neutroph. x 10^9/L	1,85 (51)	3,32 (4*)	2,87 (2)	1,82 (2)	2,04 (2)	1,75 (2)	4,31 (3**)
Eosinoph., x 10^9/L	0,05 (51)	1,12 (4*)	0,94 (2)	0,62 (2)	0,44 (2)	0,50 (2)	0,09 (3**)
Basophils, x 10^9/L	0	0 (4*)	0,04 (2)	0 (2)	0 (2)	0 (2)	0,18 (3**)
Lymphoc., x 10^9/L	2,26 (51)	2,14 (4*)	2,16 (2)	2,43 (2)	2,10 (2)	2,95 (2)	3,78 (3**)
Monoc., x 10^9/L	0,05 (51)	0,03 (4*)	0,05 (2)	0,03 (2)	0,02 (2)	0,05 (2)	0,53 (3**)
Calcium, mmol/L	2,6 (52)	2,55 (4*)	2,82 (2)	2,75 (2)	2,6 (2)	2,65 (2)	2,35 (4**)
Urea, mmol/L	7,15 (52)	6,85 (4*)	6,50 (2)	6,50 (2)	7,35 (2)	7,15 (2)	8,25 (4**)
Cholest., mmol/L	1,39 (52)	2,45 (4*)	2,08 (2)	1,80 (2)	1,58 (2)	1,44 (2)	1,24 (4**)
ASAT, µkat/L	1,1 (52)	1,0 (4*)	1,1 (2)	1,0 (2)	1,0 (2)	0,8 (2)	3,55 (4**)
CK, µkat/L	1,8 (52)	3,5 (4*)	3,3 (2)	2,3 (2)	2,0 (2)	1,8 (2)	7,45 (4**)
GLDH, nkat/L	< 20 (50)	<20 (4*)	20,5 (2)	56 (2)	29,5 (2)	<20 (2)	<20 (4**)
Protein, g/L	63,0 (52)	60,5 (4*)	69,0 (2)	69,5 (2)	78,0 (2)	66,5 (2)	58 (4**)
Albumin, g/L	41,5 (36)	35,5 (4*)					33,0 (4**)
α_1-globulin, g/L	4,0 (36)	3,5 (4*)					4,0 (4**)
α_2- globulin, g/L	4 (36)	4,5 (4*)					4,5 (4**)
β_{1+2}-globul, g/L	6,0 (36)	7,0 (4*)					6,5 (4**)
γ-globulin, g/L	8,0 (36)	8,5 (4*)					10,5 (4**)
Alb-glob. ratio	2,0 (36)	1,5 (4*)					1,5 (4**)

*Code: (n) = number of samples in the group. * = two samples from the same individual. ** = three samples from the same individual*

Table 19. Haematology and blood chemistry for one lamb infected with E. rangiferi

Sampling day PI	Day 19
Hb, g/L	126
HCT, %	41
LPK, x 10^9/L	7
Neutrophils. x 10^9/L	4,48
Eosinophils., x 10^9/L	0,42
Basophils, x 10^9/L	0,07
Lymphocytes., x 10^9/L	2,03
Monocytes., x 10^9/L	0
Calcium, mmol/L	2,8
Urea, mmol/L	6,9
Cholesterol., mmol/L	2,09
ASAT, μkat/L	1,8
CK, μkat/L	6,6
GLDH, nkat/L	169
Protein, g/L	68
Albumin, g/L	37
α_1-globulin, g/L	3
α_2-globulin, g/L	7
β_1-globulin, g/L	10
β_2-globulin, g/L	4
γ-globulin, g/L	16
Albumin-globulin ratio	20