Nematode Parasites of Reindeer in Fennoscandia

Population Dynamics, Anthelmintic Control and its Environmental Impact

Jackie T. Hrabok

Faculty of Veterinary Medicine and Animal Science
Department of Biomedical Sciences and Veterinary Public Health
Division of Parasitology and Virology
Uppsala, Sweden

Acta Universitatis Agriculturae Sueciae

Year: 2006:89

Abstract

Nematode infections of semi-domesticated reindeer of northern Finland were monitored by faecal egg counts. The tracer calf technique was also used to estimate the acquisition of nematode infection from pasture. The most abundant parasite in the worm counts of tracer animals and in faecal egg counts of adult female reindeer was *Ostertagia gruehneri*. *Capillaria* sp. eggs were detected in calves and adults, but Nematodirinae eggs were only recovered from calves. Egg output of *O. gruehneri* was most abundant from late summer to autumn whereas *Capillaria* sp. and the Nematodirinae dominated the winter months. The proportion of inhibited larvae of *O. gruehneri* and Nematodirinae steadily increased from summer to early winter, followed by a decline and a commensurate increase in the number of adult parasites in the second summer.

High prevalence and low intensity also characterized the nematode infections of winter-slaughtered reindeer from other northern herds examined. Our investigations showed that parasite transmission occurs throughout the year in this part of the sub-Arctic.

We demonstrated that reindeer are suitable hosts for important nematode parasites of sheep and goats (*Haemonchus contortus* and *Teladorsagia circumcincta*) and cattle (*Ostertagia ostertagi*), as well as for *Trichostrongylus axei*. However, it is not known if all of these parasite species reach maturity in reindeer. With the trend towards increasing numbers of livestock in the southern grazing regions of reindeer, these findings highlight the increased risks with parasites not normally associated with reindeer.

Approximately 80% of reindeer in Finland are de-wormed with ivermectin once annually in the winter. We analysed soil samples containing faeces from reindeer treated with ivermectin. Ivermectin degraded rapidly during the first spring, but residual levels were detected for more than two summer seasons following treatment. Residues were similar from ungrazed and grazed reindeer pastures, but the levels in faeces from reindeer treated with oral ivermectin were higher than for the subcutaneous formulation. Our results show that ivermectin persists on pasture longer than previously shown. However, the levels found had no detectable negative effects on the soil nematode communities beneath the faeces.

Keywords: Ostertagia gruehneri, Nematodirinae, epidemiology, cross-transmission, livestock, ivermectin, soil nematodes, sub-Arctic, Finland

Author's address: Jackie T. Hrabok, Department of Parasitology (SWEPAR), Swedish University of Agricultural Sciences (SLU) and National Veterinary Institute (SVA), SE-751 89 UPPSALA, Sweden

For my family, friends, and colleagues around the world who
encourage me to follow my Arctic dreams

Author's note

With the release of this doctoral thesis, I feel it necessary to mention a few points:
I am Canadian, not Sámi, Finnish, Swedish, nor U.S. American. In order to portray the noble parasites of the far north, I have lived a mere three years above the Arctic Circle among reindeer and their owners in the Sámi reindeer husbandry region of Finland. I have allowed my imagination to run free, with an open door to learning just about everything around me. In compiling this thesis, I have relied on academic research, traditional knowledge from the herders, and first-hand experiences within the villages.

It is my hope that as you read this original contribution to science you will be not only caught up in the mystery of nematode survivorship in the sub-Arctic, but intrigued by the beneficial use of ivermectin against reindeer parasites, as well as motivated to learn more about the reindeer husbandry, its native peoples, and the unique land they inhabit.

J. T. Hrabok

Jackie T. Hrabok
September 2006

Contents

Appendix

Papers I-V

This thesis is based on the following papers, which will be referred to by their Roman numerals:

  I. Hrabok, J. T., A. Oksanen, M. Nieminen, P. J. Waller. 2006. Population dynamics of nematode parasites of reindeer in the sub-Arctic. Veterinary Parasitology *(in press)*.

  II. Hrabok, J. T., A. Oksanen, M. Nieminen, P. J. Waller. 2006. Prevalence of gastrointestinal nematodes in winter-slaughtered reindeer of northern Finland (*submitted*).

  III. Hrabok, J. T., A. Oksanen, M. Nieminen, A. Rydzik, A. Uggla, P. J. Waller. 2006. Reindeer as hosts for nematode parasites of sheep and cattle. Veterinary Parasitology 136, 297-306.

  IV. Yeates, G. W., J. T. Hrabok, A. Oksanen, M. Nieminen, P. J. Waller. 2006. Soil nematode populations beneath faeces from reindeer treated with ivermectin. Acta Agriculturae Scandinavia Section B – Soil and Plant Science *(in press)*.

  V. Åsbakk, K., J. T. Hrabok, A. Oksanen, M. Nieminen, P. J. Waller. 2006. Prolonged persistence of faecally excreted ivermectin from reindeer in a sub-Arctic environment *(submitted)*.

Papers are reproduced by permission of the journals concerned.

Terms and abbreviations

Anthelmintic	drug with efficacy against parasitic worms
Arrested development	temporary cessation of nematode larval development in the host
EL4	early fourth-stage larva
Endectocide	macrocyclic lactone antiparasitic drug against internal nematodes and external arthropod parasites
epg	number of nematode eggs per gram faeces
FEC	nematode faecal egg count
GABA	glutamate gated butyric acid
GI nematode	nematode of the gastrointestinal tract
HPLC	high performance liquid chromatography
Ivermectin sc.	subcutaneous formulation of ivermectin
L3	infective third-stage nematode larva
O. gruehneri	*Ostertagia gruehneri*
Periparturient	soon to deliver offspring
Prepatent period	time from infection until eggs are detectable in faeces

Background

Circumpolar distribution of *Rangifer tarandus* (reindeer/caribou)

Reindeer and caribou play an important role in human inhabitation of the Arctic and sub-Arctic regions. Reindeer herding and wild reindeer/caribou hunting are traditional activities handed down through the generations. Caribou hunting dates to several thousands of years ago, and reindeer herding, at the very least to the 1500-1600's (Ulvevadet, 2004). The taxonomic classification of the genus *Rangifer* and its sole species *R. tarandus* has been widely debated. The conventional classification suggests 8 subspecies including: *R. t. granti* (Barren-ground), *R. t. caribou* (Woodland), *R. t. platyrhynchus* (Svalbard), *R. t. pearyi* (Peary), *R. t. tarandus* (Eurasian), *R. t. fennicus* (Eurasian forest reindeer), *R. t. groenlandicus* (Greenland) and *R. t. eogroenlandicus* (extinct from the east coast of Greenland) (Roed, 2005) (Figure 1). Although reindeer and caribou belong to the same species, there are several morphological differences (body size, distance between antlers, leg length, pelage, and length of rostrum) among sub-species. Basically, caribou includes all wild individuals in North America and reindeer refers to wild and domestic *Rangifer* in Eurasia. In 1892, reindeer from Siberia were introduced into Alaska, U.S.A. Today, the Western Arctic Caribou Herd and reindeer have overlapping ranges on the Seward Peninsula where crossbreeding occasionally occurs. In this habitat reindeer have smaller body size, are less wary of predators, have a breeding season beginning 2-4 weeks earlier in the autumn than the caribou, and have a lower propensity to migrate than caribou.

The classification and colonization of *R. tarandus* were assessed from the analyses of nuclear and mitochondrial DNA by Roed (2005), who studied the origin of the species. The gene pool of all existing sub-species arose from a large and continuous glacial population ranging across extensive areas of tundra in Eurasia and Beringia. The morphological differences among sub-species probably evolved as adaptive responses to post-glacial environmental changes, but an exception to this is the North American Woodland caribou, which has a sub-species specific refugium. The largest refugium is from Beringia, a second small and isolated refugium arose in western Eurasia in close connection to the extensive ice sheet that covered Fennoscandia, and the third refugial area was probably located south of the extensive North American continental ice sheet (from where the present day North American Woodland caribou likely originated).

There are over 3 million caribou in North America (Kofinas & Russel, 2004). In Alaska, there are approximately 20,000 semi-domesticated reindeer of which 10,000 - 13,000 are found on the Seward Peninsula where they are managed by 11 herders of native ancestry. While the development of the reindeer velvet antler market during the 1970's and 1980's enhanced profitability, most Alaskan reindeer herders believe that meat sales provide the economic backbone for the industry and manage their herds accordingly (Wiklund & Finstad, 2006). Russia has approximately two thirds of the global population of domesticated reindeer, which

constitutes 1.2 million individuals, and an additional 1.5 million wild reindeer (Klokov, 2004).
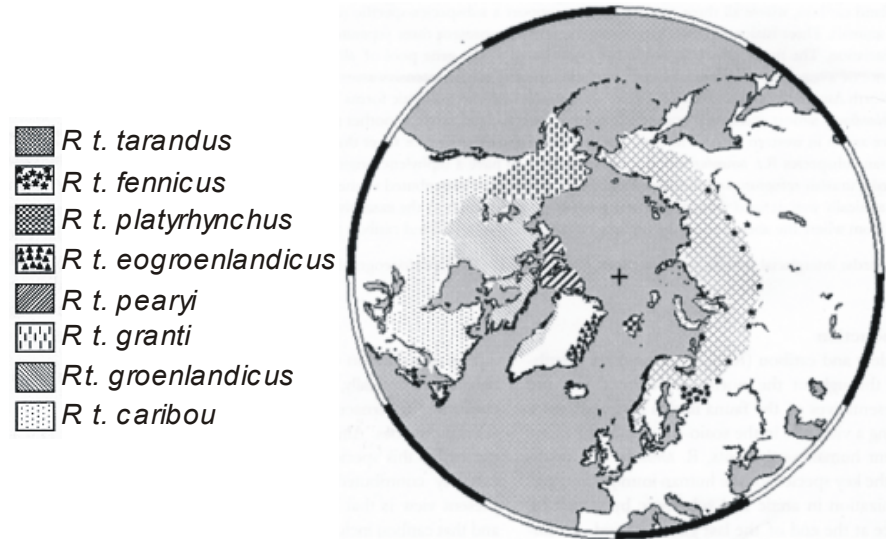

R t. tarandus
R t. fennicus
R t. platyrhynchus
R t. eogroenlandicus
R t. pearyi
R t. granti
Rt. groenlandicus
R t. caribou

Figure 1. Map of the circumpolar distribution of the sub-species of *Rangifer tarandus*. After Roed (2005).

Reindeer husbandry in Fennoscandia

In Sweden and Norway the management of reindeer is governed by the respective ministries of agriculture in each country, and in Finland by the Ministry of Agriculture and Forestry. Reindeer husbandry is a social, cultural, and economic family enterprise, occupying 30-40% of the landscape in predominantly the northern areas of each country. The husbandry is primarily geared towards meat production, with secondary income derived from the sale of reindeer handicrafts. Finland has a well-developed Reindeer Herders' Association using the reindeer and Sámi culture to promote tourism to attract foreign earnings. In addition to the domesticated reindeer of approximately 200,000 animals in each of these three Nordic countries, wild reindeer are present in Norway (*R. t. tarandus*) (~29,000) and in Finland (*R. t. fennicus*) (~2,500 animals) (Nieminen, 2005).

Every component of the reindeer industry is thoroughly regulated by laws, which vary between countries. The framework of this thesis was conducted in the northern Sámi reindeer husbandry area in Finland, where the husbandry retains many of the traditional methods of herding. Herds are free ranging year-round on natural pastures and are not confined during winter to corrals, or housed, to the same extent as domestic livestock, e.g. sheep, goats, and cattle. There are approximately 5,500 Finnish and Sámi owners of 200,000 reindeer in Finland. Eighty percent of calves, and a few of the older and sick animals are slaughtered each year during the annual winter harvest resulting in approximately 2.5 million kg of meat, 110,000 skins and 100 tons of dried antler. Combined with reindeer

travel tourism, the economic turnover of the annual reindeer husbandry amounts to approximately 60 million EUR (Filppa, 2005).

Reindeer husbandry is a unique link between wild and domestic livestock systems. The herding year begins 1 June and ends 31 May of the following year. In the north, the density of semi-domesticated reindeer is very low with 2.3-2.5 animals per km^2 with the size of grazing areas ranging from 6,000-7,000 km^2 (Paliskuntain Yhdistys, 2005). There are two major events each year involving the roundup of all herds belonging to each cooperative. These are the summer, most often early July, earmarking of calves and the annual cull during the winter from October to January when approximately 80% of the calf population is sent to slaughter. The permissible number of reindeer owned by individual herders is 500 in the north and the number permitted in each of the 56 cooperatives is reviewed each decade in collaboration between the Finnish Ministry of Agriculture and Forestry and the Finnish Reindeer Herders' Association, based upon carrying capacity of the landscape (Filppa, 2005). Each reindeer herding cooperative has defined geographical borders, and the families within each cooperative may construct fences, if the cooperative agrees, to ensure better control over their herds and to prevent mixing with reindeer from other herds and cooperatives. During years with harsh environmental conditions such as ice-covered ground, deep snow, or poor growing season, herders supplement natural browse with commercially produced reindeer fodder, hay, and water horse-tail (*Equisetum fluviatile*). The frequency of supplementary feeding is dependent upon the herder's preference and economic situation. Most herders distribute supplementary feed for only a few weeks at a time, especially so during late winter when fat reserves and body condition of the overwintered animals have decreased, which is the time when pregnancy requires a high plane of nutrition.

Parasite fauna of reindeer

A parasite is an organism that lives in or on another organism of another species, known as the host (e.g. reindeer), from which the parasite derives its nutrition. Normally reindeer are infected by several species of parasites at the same time, with the most important consequences being parasite-induced reduction in growth rate, rather than parasite-induced mortality (Halvorsen, 1986). The range of parasites specific to, or primarily associated with reindeer include protozoa (unicellular eukaryotic organisms; e.g. *Besnoitia tarandi*, *Sarcocystis* spp., *Eimeria* spp.), cestodes (tapeworms; e.g. *Moniezia* spp.), nematodes (roundworms; gastrointestinal worms e.g. *Ostertagia gruehneri*, *Nematodirus tarandi*, *Nematodirella longissimespiculata*, lungworm *Dictyocaulus eckerti*, brainworm *Elaphostrongylus rangiferi*), pentastomids (sinusworm *Linguatula arctica*), and insects (e.g. the throat bot fly *Cephenemyia trompe* and warble fly *Hypoderma tarandi*). Some reindeer parasites are vector-borne, e.g. the extra-enteric nematodes *Onchocerca tarsicola* and *Setaria tundrae* by black fly and mosquito vectors, respectively. Other parasites reported in reindeer, for which reindeer are not normally the final host, include *Toxoplasma gondii*, *Brucella* spp., *Taenia* spp., and *Echinococcus granulosus*.

The three parasites of reindeer that have received most attention in reindeer husbandry areas are the arthropod parasites (warble fly and throat bot fly) and the brainworm. The decision of reindeer herders to treat their animals in winter with ivermectin is primarily based on the clinical signs they observe caused by the third-stage larvae of these insect parasites. Epidemic outbreaks attributed to *E. rangiferi* are occasionally reported in reindeer herds. However, this parasite is a particular problem in Northern Norway, in areas where sheep, goats, and reindeer have overlapping summer pastures. For the above reasons, I have chosen to describe the lifecycles of these specific parasites in more detail below.

External parasites (ectoparasites)

The warble fly (*H. tarandi*) and throat bot fly (*C. trompe*) were the first parasites for which control programmes were developed in the late 1970's for the Finnish reindeer husbandry. The drugs used in these early programmes were the organophosphates (e.g. fenthion and famphur).

Warble fly (*Hypoderma tarandi*)

The warble fly (*H. tarandi*) oviposit eggs on the hair, usually on the distal extremities of reindeer in the summer. The larvae hatch, crawl down the shaft of the hair and penetrate the skin. During the winter, after maturation in the subcutaneous tissues, the larvae perforate the skin of the back, creating a breathing hole. In the spring, the mature larvae emerge through the hole, fall to the ground where they pupate, and generally within one month emerge as adult flies (Nilssen & Haugerud, 1994). The intensity of infection in reindeer can be high with several hundred to thousands of larvae, which apart from the pathological effects on the animal significantly reduces the value of the hides.

Throat bot fly (*Cephenemyia trompe*)

The throat bot fly (*C. trompe*) is also referred to as the nasal bot. Its life cycle is principally similar to that of the warble fly. Female flies oviposit eggs around the muzzle and nostrils of the reindeer and the larvae develop in the throat and tonsillar region during the winter. In the spring, larvae are expelled when reindeer cough them up onto the ground. The larvae then pupate and continue development to adult flies (Nilssen & Haugerud, 1995). Oedema in the pharynx caused by bots may spread to the meninges and brain, and mature bots may accidentally enter the trachea causing fatal bronchopneumonia (Oksanen, Soveri & Nieminen, 1992a).

Internal parasites (endoparasites)

Reindeer brainworm (*Elaphostrongylus rangiferi*)

A widely recognized nematode pathogen of reindeer (and sheep and goats) in Fennoscandia is the brainworm *E. rangiferi*, which affects the central nervous system and skeletal muscles and may cause outbreaks of cerebrospinal elaphostrongylosis (CSE) (Bakken & Sparboe, 1973; Kummeneje, 1974). First-stage larvae (L1) with a characteristic protostrongylid dorsal-spine on its distal extremity are shed in reindeer faeces. They invade a terrestrial gastropod intermediate host (snail or slug) and develop to third-stage larvae (L3), which are

14

naturally infective for a variety of ruminant hosts such as reindeer and moose (*Alces alces*) (Stéen, Blackmore & Skorping, 1997), and goats and sheep (Handeland & Sparboe, 1991). The free-living L1 are exceptionally tolerant to freezing, and in laboratory-based experiments, survival exceeded one year at $-80^{\circ}C$ (Lorentzen & Halvorsen, 1986).

Gastrointestinal nematodes (GI nematodes)

Nematodes of the gastrointestinal tract, particularly those of the abomasum, are considered the most important production limiting parasites of sheep and cattle (Sykes, 1987). These parasites cause pathological changes in the animal that disrupt digestion and reduce appetite, which in turn affect the host immune status during infection and the ability to prevent parasite establishment and development. Reduced appetite causing anorexia has been demonstrated in semi-domesticated reindeer calves, where the feed-intake in infected animals was up to 20% less than for parasite-free animals (Arneberg, Folstad & Karter, 1996). Although much of the published information regarding abomasal nematodes is for domestic livestock, studies of the wild Svalbard reindeer suggest that sub-clinical parasitism influences the dynamics (especially the population density) of these latter animals (Albon et al., 2002; Stien et al., 2002a).

Gastrointestinal nematodes are transmitted via the intake of vegetation and have a direct life cycle. Eggs of trichostrongylid nematodes such as *Ostertagia* spp. are shed on pasture within the faeces of infected animals. A first-stage larva (L1) hatches from each egg, moults twice to become an infective third-stage larva (L3), and migrate on the surrounding vegetation. In the case of the subfamily Nematodirinae, the development of the L3 occurs within the egg and thereafter the egg hatches, releasing the infective larva on pasture (Figure 2).

The pre-parasitic L3 stages can withstand harsh climatic conditions of the Arctic, such as desiccation and freezing temperatures, before being ingested by grazing reindeer (Halvorsen et al., 1999). Once acquired by the host, the L3 moult to the fourth-stage (L4) in the abomasal mucosa before developing into adult worms. The time (prepatent period) between ingestion of the L3 and development to the egg producing adult stage can be as short as 21 days for *O. gruehneri*. However, depending upon environmental stimuli, the L4 may undergo arrested development in the abomasal mucosa, especially during the winter months (Halvorsen et al., 1999). For the subfamily Nematodirinae, infective L3 moult and develop into adult worms within the proximal part of the small intestine.

The life histories of the abomasal nematodes *O. gruehneri* and *Marshallagia marshalli* have been examined in wild Svalbard reindeer (Bye, Halvorsen & Nilssen, 1987). These species constitute more than 99% of the population of GI nematodes in the Svalbard reindeer (Irvine et al., 2000). A 6-year experiment on Svalbard using anthelmintics to render certain animals worm-free elucidated the confounding effects of climate, and showed that the probability of a treated reindeer having a calf, increased compared with untreated animals (Albon *et al*., 2002). It was also concluded that natural *O. gruehneri* worm burdens were sufficient to regulate the annual reindeer population by 1-5% by impairing the body condition and fecundity of female reindeer (Irvine et al., 2001).
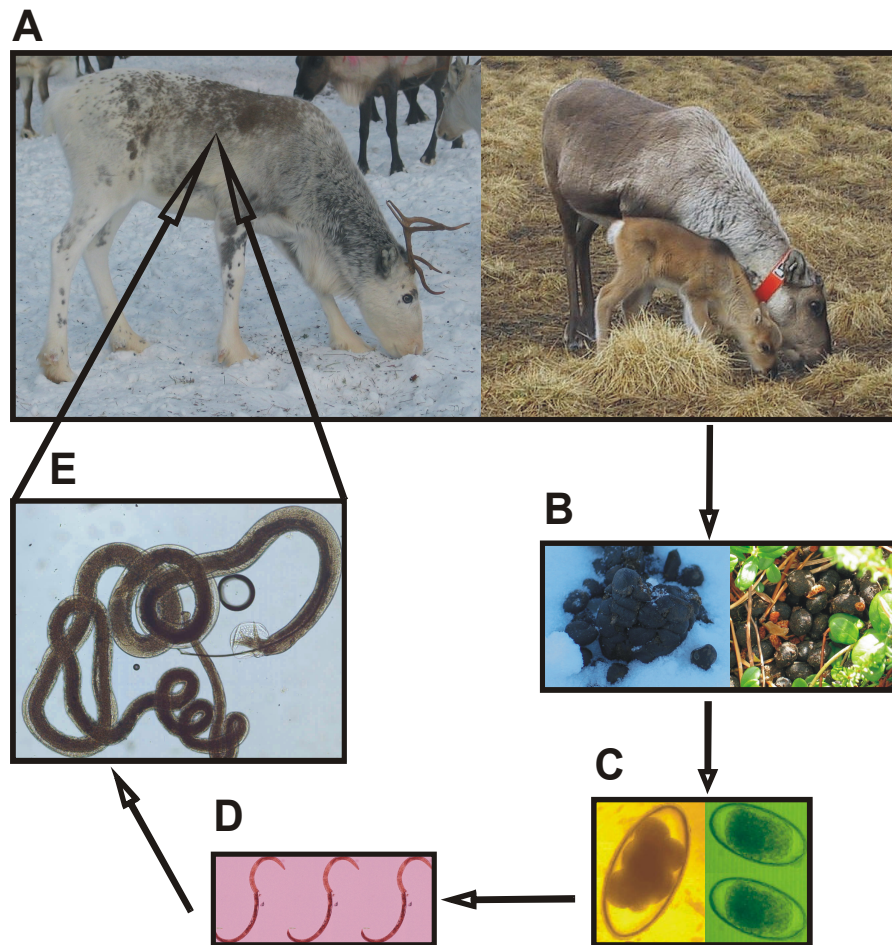
Figure 2. Lifecycle of directly transmitted gastrointestinal nematodes of reindeer. Reindeer grazing on either winter or summer ranges (A) deposit fresh faeces on pasture (B). Faeces of infected animals may contain nematode eggs (C), and dependent upon environmental variables, the nematode eggs will hatch, and first-stage larvae will moult to the second- and third- stage within the vegetation (D). As reindeer graze, these infective third-stage larvae are accidentally ingested, and development to the adult stage occurs at the parasite species-specific predilection site, e.g. the abomasum or a particular section of the intestine (E).

The winter transmission of reindeer abomasal nematodes has been studied. During this time reindeer spend approximately 50% of their time foraging, yet their body weight may be reduced by more than 50% due to the catabolism of the animal's stored fat reserves (Halvorsen & Bye, 1999). The extent of starvation during winter and spring is largely determined by the severity of weather conditions. Svalbard calves become infected with *O. gruehneri* larvae in their first winter, although the abundance of larvae decreases from October to April (Halvorsen *et al*., 1999). Arrested larval development was not considered important in the dynamics of *O. gruehneri* populations in Svalbard reindeer because arrested larvae were present in only a small fraction of the total

16

overwintering nematode populations in the animals during this time. Halvorsen & Bye (1999) also showed that the parasite burden increased from autumn to the following spring in both sexes of calves and adult reindeer, largely attributed to increasing numbers of adult *O. gruehneri*. The abundance of nematodes consistently increased until reindeer were 3-5 years old, and thereafter the level of infection declined. The high intensity of infection found in adult reindeer of Svalbard suggests that reindeer do not develop an efficient immune response towards infections with this parasite, as that demonstrated for abomasal parasite infections of sheep and cattle, which occurs during the end of their first grazing season on pasture (Skyes, 1994; Larsson *et al.*, 2006). The estimate of abomasal parasite life-expectancy in reindeer in the high Arctic is 2 years (Halvorsen *et al.*, 1999), compared with that of the traditional livestock nematodes of approximately 3-4 months (*O. ostertagi* in cattle, Smith & Grenfell, 1985; *Haemonchus contortus* in sheep, Smith, 1988).

Control of parasites in reindeer

Anthelmintic treatment

Parasites of ruminants are commonly controlled by the use of anthelmintic drugs (de-wormers). Ivermectin is a broad-spectrum anthelmintic that has a wide range of activity and a very high level of efficacy against both endo and ectoparasites, and is thus commonly referred to as an endectocide drug. Ivermectin belongs to the avermectin B1 group of anthelmintics and is a chemical derivative of the macrocyclic lactones that were initially isolated from fermentation broths of the actinomycete, *Streptomyces avermitilis* (Campbell, 1989). This drug was first launched in 1981 for commercial use to control parasites in cattle, sheep, horses, and swine (Waller, 1990). The main mode of action is by the interaction of avermectin with the nematodes' glutamate gated butyric acid (GABA) sensitive chloride channels. GABA binds to the GABA receptor-chloride channel causing an influx of chloride ions resulting in paralysis and death of the nematode (Turner & Schafer, 1989).

Use of ivermectin in reindeer

To gain knowledge about the safety, efficacy, and tissue residues of ivermectin in reindeer, a series of trials was performed during the 1980's in Alaska, Sweden, and Finland. The two methods of application that have received the most attention in reindeer are the subcutaneous injection (ivermectin sc.) and the oral paste formulations, with the manufacturers recommended dose of 200 micrograms per kg of animal body weight.

In Alaska, a January treatment with ivermectin sc. was virtually 100% effective in removing the warble fly (*H. tarandi*) infections from 40 reindeer freely grazing on the western Alaska Arctic tundra (Dieterich & Craigmill, 1990). The animals were culled approximately 5 months after treatment, and all larval stages of warbles were found to be dead and undergoing absorption. The body condition of the treated animals was also better than that of the non-treated control group. Tissue levels of ivermectin rapidly declined and approached undetectable levels 24 days after injection, although residues in back fat were initially twice as high as residues in liver, muscle, and kidney. These results lead to the official registration for the use of ivermectin in reindeer. In Sweden and Finland, there is stipulated a 28-day withholding period from treatment until animals are destined for human consumption.

In Sweden, a trial conducted with 37 reindeer calves showed that the efficacy of ivermectin sc. was 100% against the warble fly (*H. tarandi*) and the nasal bot fly (*C. trompe*), following slaughter 50 days after treatment (Nordkvist *et al.,* 1983). The efficacy against gastrointestinal trichostrongylids and *Dictyocaulus* lungworm was also 100%, but it was lower for *E. rangiferi,* probably because these worms were located in the central nervous system behind the blood-brain barrier where ivermectin has limited penetration (Nordkvist *et al.*, 1983).

Ivermectin was introduced to the Finnish reindeer husbandry in 1982. Approximately 80% of reindeer are treated with this anthelmintic once annually during the winter. Host plasma concentrations are one of the best indicators of the expected level of endectocidic efficacy of anthelmintics. The injectable formulation is most commonly used, and was shown to be virtually 100% effective against larvae of the warble fly (*H. tarandi*) and throat bot fly (*C. trompe*) (Oksanen & Nieminen, 1998; Oksanen *et al.*, 1992b). The efficacy of oral ivermectin was found to be 100% against the warble fly, but less effective than the injectible formulation against nematodes. Both formulations increased the weight gain of pregnant female reindeer, compared to non-treated animals, all of which received adequate nutrition by pen-feeding (Oksanen *et al.*, 1992b). Oksanen, Soveri & Nieminen (1993) interpreted from their results that the efficacy of the subcutaneous formulation of ivermectin was superior to oral and topically applied ivermectin, against inhibited GI nematodes. The subcutaneous formulation of ivermectin in reindeer makes the best possible use of the active ingredient by being most readily absorbed than other routes, and thus treatment by injection is recommended to be used wherever possible (Oksanen *et al.*, 1995). In addition, it is much easier to administer ivermectin sc. to reindeer compared with the oral paste.

Potential for ivermectin resistance in reindeer husbandry

There are a number of recommendations that are aimed at reducing the selection pressure for anthelmintic resistance amongst nematode populations of livestock. These include targeted or selective use of anthelmintics to treat individual animals within a herd or flock, treatment at a time when there is a significant proportion of the parasite population on pasture which thus escapes anthelmintic selection pressure (i.e. a population in refugia), and the use of non-chemical alternatives, or adjuncts, for control (Waller, 1990, 1994, 2005). It has also been suggested to treat only animals that display clinical signs. However, the importance of sub-clinical infections in which pathological signs are not apparent, is unknown for the GI nematodes of reindeer. Therefore, it may be best to continue current management practises employed in Finland of whole herd treatment once annually.

A few studies have evaluated the efficacy of narrow- and broad-spectrum anthelmintics in reindeer in Fennoscandia. In Sweden, the efficacy of ivermectin sc. was deemed superior to fenbendazole, mebendazole, and fenthion, against warble and bot fly larvae, lungworms, and GI nematodes (Nordkvist *et al.*, 1983). Oksanen & Nieminen (1998) found that the endectocide moxidectin was effective against nematodes in the Kutuharju experimental reindeer herd, but less effective than ivermectin against insect pests. Moxidectin eliminated 100% of abomasal adult nematodes, more than 99% of abomasal larvae, and the majority of nematode eggs in faeces, as compared to non-treated Svalbard reindeer (Irvine, 2000). Complete efficacy against warble and bot flies was observed after reindeer were treated subcutaneously with another endectocide, doramectin, but the impact on nematodes was not examined (Oksanen & Nieminen, 1996). All of these studies showed that there is more than one choice of effective chemical treatments for the control of reindeer parasites. However, it must be recognised that ivermectin,

moxidectin, and doramectin are all members of the macrocyclic lactone group of anthelmintics. Studies on anthelmintic resistance have repeatedly shown that when resistance to one member of an anthelmintic group occurs, then side-resistance to all other drugs in the same group, or 'action family', automatically occurs (Waller, 1997).

The general recommendation to owners of reindeer is to treat the animals just once annually, during the early months of winter, before the warble fly larvae damage the hides. At such a low frequency of treatment the likelihood for the emergence of resistant individuals within the target pest populations (arthropods and nematodes) is very low. However, it must be recognised that the vast majority, if not all, of these parasite populations are likely to reside within the reindeer host at this time, thus exposing almost the entire population for individuals who possess the genes for resistance. Although the numbers of such genetically resistant parasites might initially be low, over time they will increase as they have a survival advantage when the same drug (group) is used (Waller, 1990, 2005).

Grazing management and nutritional supplementation

Caribou herds of the Canadian Arctic migrate thousands of kilometres annually, the extent of this being primarily dictated by food supply. With the development of reindeer husbandry, the sizes of foraging areas have been reduced by fenced boundaries, albeit separate areas for winter and summer grazing. The combined areas of winter and summer reindeer pastures for individual reindeer herding cooperatives in northern Finland are rather extensive compared to traditional livestock pastures, with ranges from 6,000 - 7,000 km^2 and with population densities of 2.3 - 2.5 reindeer per km^2. This strategy of seasonal pasture use may have a beneficial effect on nematode parasite infections by reducing the magnitude of the free-living stages on a particular pasture, when animals are put to areas that they have not grazed, and thus non-contaminated for extended periods of time.

Population density of many herds however has also increased through human intervention, which has lead to over-grazed winter pastures, and in most districts of Norway and Finland, carrying capacity of the land has been exceeded (Kumpula, Copaert & Nieminen, 2002; Moen & Danell, 2003). Restricting the movement of animals to a more sedentary system of management increases the chances of animals being exposed to parasite infection (Waller, 2005). During late winter and early spring, the population density of reindeer herds may reach high numbers when supplementary feed is distributed in piles on the surface of the snow, or in troughs in the forest. Some herders also confine their reindeer in corrals during the winter for up to three months with the goal of increasing animal growth and productivity by additional supplementary feeding regimes. Large quantities of potentially parasite contaminated faecal material accumulate in and around these feeding areas, thereby increasing the chance that more individuals in the herd may become infected as they continue to graze.

Some reindeer herders also construct specially designated fenced calving areas in which hinds and newborn calves are confined for 1 to 2 months. The objective is for herders to monitor the daily health of calves and to apply ear tags. Strategic

20

feed supplementation, particularly to these young animals and to the periparturient lactating females is offered during this time in which these classes of animals are most susceptible to nematode infection (Coop & Kyriazakis, 1999). However, the density of animals within these calving enclosures may be very high and similar to the high densities of reindeer held in winter corrals. From inference with studies on the ecology of the free-living stages of nematodes of sheep and cattle, it can be assumed that under favourable conditions of temperature and moisture, the time for development of the newly shed eggs to infective larvae on pasture is approximately three weeks (Stromberg, 1997). However such translation of eggs to infective larvae is likely to be much longer during reindeer calving when temperatures are cool.

Communal pastures and cross-transmission threats

Wild reindeer populations in, e.g., mountainous tundra regions in southern Norway share summer pastures with sheep (Bye, 1987). In the above mentioned study, the density of reindeer was 1.1 animals per km^2 and 12.5 sheep per km^2 on 1600 km^2 of forested pasture. The sheep nematodes (*Teladorsagia davtiani*, *T. circumcincta*, and *Trichostrongylus axei*) were found to be transmitted to reindeer, but the extent to which these parasites might adversely affect reindeer is unknown.

In northern Norway, all goats and more than 90% of the sheep are located in Nordland and the Troms region. Herds of reindeer have been observed in summer on the shared pastures with these domestic livestock. Handeland & Slettbakk (1995) reported that in late autumn, following an exceptionally warm and moist summer, approximately 30% of dairy goat farms in the Balsfjord district of northern Norway had cases of cerebrospinal elaphostrongylosis (CSE) on areas shared with reindeer. They reported this as an epidemic outbreak attributed to the reindeer brainworm (*E. rangiferi*) in the goat flocks and sporadic occurrence in sheep flocks. Locomotor and brain disturbances with posterior paresis and blindness were the main clinical manifestations, however recovery was possible. Handeland *et al*. (1994) reported that although the natural cross-transmission of the brainworm to goats occurred, the parasite could not complete its life cycle in domestic livestock.

Environmental impact of parasite control

The environmental impact of ivermectin and other macrocyclic lactones on non-target organisms, such as soil or dung dwelling fauna (nematodes, dipterans, and coleopterans) is inconclusive. There is concern about the effect of ivermectin residues on the natural processes of dung degradation and nutrient cycling (Herd, 1995). Ivermectin is excreted unaltered in faeces, irrespective of the route of administration (Sommer *et al*., 1992). Ivermectin is not phytotoxic, antibacterial, or antifungal, it is practically immobile in soil, and there is little uptake by plants (Halley, VandenHeuvel & Wislocki, 1993). When present in water, or as thin films on surfaces, ivermectin is rapidly degraded to less bioactive compounds (Halley, VandenHeuvel & Wislocki, 1993).

Many studies on the impact of ivermectin on pasture have been conducted in temperate climatic zones of the northern hemisphere, where earthworms play a

21

major role in the degradation process (Holter, 1979). In the Arctic, earthworms are scarce or absent, so soil micro-organisms are particularly important in decomposition of organic matter and nutrient cycling in this environment (Sjursen, Michelsen & Holmstrup, 2005).

Nilssen *et al.* (1999) determined that the concentration of ivermectin in reindeer faeces reached maximum levels approximately 4 days after subcutaneous treatment, followed by a gradual decrease, but residual levels were detected for more than 30 days after treatment. The persistence of faecally excreted ivermectin from reindeer in the sub-Arctic has not previously been examined.

Aims of the study

The aim of this study was to obtain epidemiological data on the prevalence, intensity, and seasonal distribution of nematode parasites of reindeer raised in an area that typifies the northern reindeer herding region of Finland. In addition, studies were conducted to examine the potential role of reindeer to act as carriers of nematode parasites of conventional livestock (sheep and cattle). Further, environmental impact studies were conducted on the fate and impact of ivermectin residues excreted from animals treated according to the annual treatment recommendation. Thus, the objectives of this project were:

1. To investigate the seasonal prevalence and abundance of GI nematode parasites, and their distribution among various age and sex classes of semi-domesticated reindeer (papers I and II).

2. To explore the potential of reindeer to act as hosts for GI nematode parasites of sheep and cattle (paper III).

3. To determine the environmental impact of faecally excreted ivermectin in the sub-Arctic (papers IV and V).

Methodological considerations

Background of Kutuharju experimental reindeer herd

The present studies were mainly conducted at the Kutuharju experimental reindeer research field station (69° 48' N, 26° 49' E), using the experimental reindeer herd (approx. 200 animals of mixed age and gender) maintained by the Finnish Reindeer Herders' Association in northern Finland (Figure 3). The station is approximately 350 km north of the Arctic Circle within the Muotkatunturi reindeer cooperative region, encompassing 42 km^2 of fenced perimeter, designated for reindeer research. Laboratory procedures were conducted at the Finnish Game and Fisheries Reindeer Research Station in Kaamanen, approximately 15 km east of Kutuharju. The main objective of maintaining this herd, which was founded in 1964, is to permit long-term studies on reindeer physiology, ecology, parasitology, heredity, and improvements in breeding, supplementary feeding, and meat production. The herd is rounded up monthly for sampling as required for research project demands, but otherwise is free-ranging on natural pastures with supplementary feed (fodder and hay) being distributed during the winter and spring, whenever the ground is snow-covered. The herd is also treated with ivermectin once each winter to reduce warble and throat bot fly larvae.

Grazing management is an important practice of the annual cycle of the reindeer husbandry so that designated winter and summer pastures are not overgrazed. The various pastures of Kutuharju are also used to separate the herd into smaller experimental units, especially during calving and rut in late April to mid-June and September-October, respectively. Kutuharju is situated in the Northern Boreal vegetation zone straddled between the Forest Lapland and Fjall Lapland phyto-geographic regions. The most common trees are pine (*Pinus sylvestris*) and mountain birch (*Betula pubescens*) with an underlayer of fruticose, foliose, and crustose lichens (*Cladonia* spp.). Both ground and arboreal lichens are important in the winter diet of reindeer. Common bushes are juniper, dwarf birch, and willow, with a dwarf shrub layer dominated by berries (blue-berry, lingon-berry, cloud-berry, marsh tea), and the bottom layer consisting of moss and hairgrass, which are important in the summer diet of reindeer. Herbs and sedges surrounding the numerous fens and bogs are also an important feed source for reindeer in the autumn. An elongated ridge of glacial drift, bedrock crops, and steep walls form the southern boundary of Kutuharju, of which much is above the tree line. The composition of Kutuharju soils is striated between being dry and dominated with mineral sediments of granite, sand, and silt, to inundated areas comprising hummocks and peat bogs, small lakes, rivers, and streams.
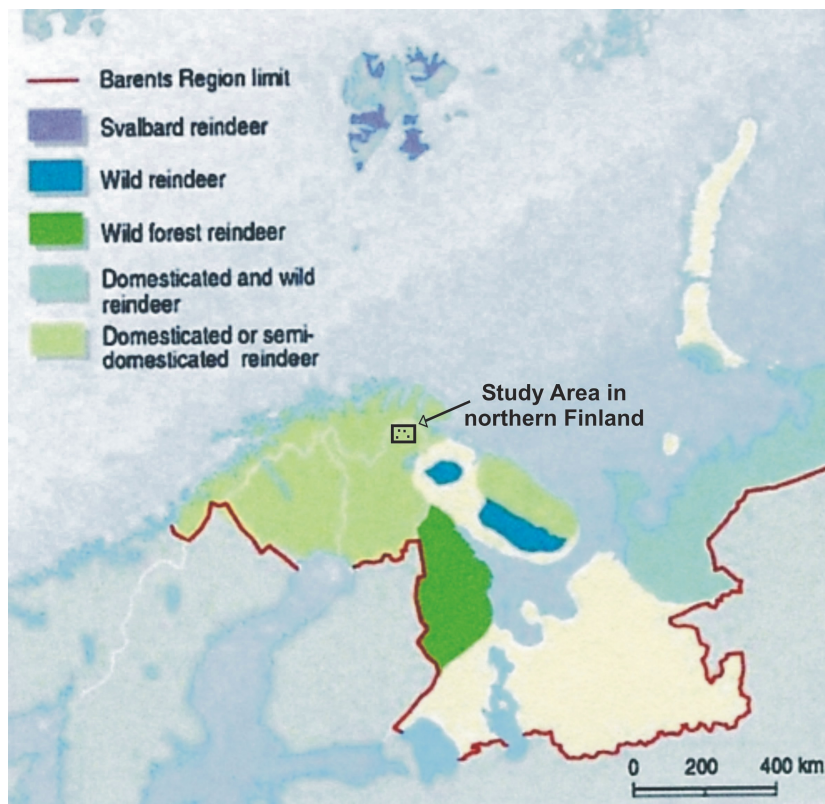
Figure 3. Map of the study area in which semi-domesticated reindeer were examined for nematode parasites of northern Finland. (After Rekacewicz, 1998).

Selection of animals

For the long-term epidemiology study (paper I), 30 reindeer calves (15 male and 15 female) and 30 adult female reindeer (>1 year of age) from the Kutuharju herd were rectally faecal sampled approximately each month for 2.5 years. These samples were used for nematode faecal egg counts. It was not possible to locate specific reindeer in the forest on each sampling occasion, therefore the first 60 animals to be rounded up were sampled each month. Animals designated as tracer calves (paper I) were selected from the Kutuharju herd such that there were 2 male and 2 female calves of the same age treated with ivermectin in the beginning of each month. The tracer animals ranged in age from 3.5 to 14 months at time of anti-parasitic treatment.

Abomasums and small intestines were acquired from the reindeer slaughterhouse (Toivoniemi Kaamanen) and winter reindeer roundups (paper II) during the winters of 2002-2004. This material was obtained from the neighbouring reindeer herds, including Kutuharju, and the co-operatives of Paistunturi, Kaldoaivi, and Muddusjärvi. All of these latter animals had not been de-wormed with ivermectin during the preceding 12 months. Efforts were made to obtain samples from equal

numbers of calves and adult reindeer, but this was not always possible as the reindeer owners selected which animals were to be slaughtered.

The animals examined in the cross-transmission of cattle and sheep nematodes to reindeer (paper III) were derived from different sources. Twelve, 4 to 5 month old male reindeer calves were purchased from the Kutuharju experimental herd and re-located to the zoological garden at Oulu University, Finland. Six Gotland breed male sheep and 6 Swedish black and white bovine cattle calves, all aged approximately 4 months, were obtained from commercial sources and re-located to the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. All reindeer, sheep, and cattle were de-wormed with ivermectin and maintained on concrete floored group pens to preclude the possibility of re-infection. They received fodder, hay, and water, *ad libitum* for the duration of the trial. All animals were artificially inoculated with nematode larvae, approximately 6 weeks after the ivermectin treatment.

The reindeer selected for use in the field trial to investigate the impact of ivermectin on soil nematodes (paper IV) and on the prolonged persistence of ivermectin in the sub-Arctic environment (paper V), were acquired from the Kutuharju herd and re-located to the Finnish Game and Fisheries Reindeer Research Station, Kaamanen. These eight, 25-week old female animals were maintained on concrete flooring and fed lichen *ad libitum* during the study period, and thereafter returned to Kutuharju to rejoin the herd grazing on natural pastures.

Diagnostic methods used

Faecal samples

The presence of nematode eggs in faeces was analysed using the methods described by Gordon & Whitlock (1939) based on 3 g of faeces. A modified McMaster technique with a sensitivity of 50 eggs per gram faeces (epg) was performed with reindeer faeces (paper I, II, III) and cattle calves and lamb faeces (paper III).

Faecal cultures (paper III)

Infective larvae (L3) of cattle and sheep GI nematodes were obtained from respective bulk cultures of faeces. Larvae were harvested from the faeces and stored in flasks at 8°C before species differentiation according to Borgsteede & Hendriks (1974).

Tracer animal technique (paper I)

Estimating the abundance of infective larvae on pasture was conducted using the tracer animal technique. In extensive grazing situations, such as in reindeer husbandry in Fennoscandia, the direct pasture sampling technique of analysing vegetation for larvae is inappropriate. The tracer calf method utilized young 4 to 5 month old, parasite naive reindeer calves, which were treated with ivermectin sc., and then released to natural pastures to continue freely grazing with the Kutuharju herd for 7-8 weeks. The aim of this procedure was to initially render the animals worm-free and after a period of 3-4 weeks allow for ivermectin levels to fall to

negligible levels, when the tracer calf would acquire new infection from pasture. We allocated 2 male and 2 female reindeer calves as tracers in each month for 20 consecutive months. The abomasum and small intestine from each calf was examined for all stages of GI nematodes to determine the seasonal magnitude of parasite infections.

Nematode worm counts

Prevalence and intensity of nematode infections were determined not only by regular faecal egg counts, but also by necropsy of tracer calves (paper I), winter-slaughtered reindeer acquired from the Kaamanen slaughterhouse and neighboring co-operatives (paper II), and from reindeer calves, cattle calves, and lambs for the cross-transmission study (paper III). Either two (paper II), or four (paper I, III) 20 ml replicate sub-samples from each abomasal (3 L) and small intestinal contents (3 L), and abomasal mucosal digest (1 L), were examined. Prior to examination, these samples were preserved by freezing at -18°C (Dobson, Waller & Donald, 1990). When required for worm counting and species differentiation, these sub-samples were thawed on a 30 μm sieve using a gentle stream of warm tap water. All material, including nematodes retained on the sieve, were back-washed into a beaker, stained with Lugol's iodine and examined with a stereomicroscope.

The lungs of all tracer calves were perfused 3 times with up to 8 L of water, and the major bronchial airways were opened and thoroughly flushed with warm tap water. The total volume of fluid collected was allowed to settle at 4°C for approximately 36 h before decanting to a final volume of 50 ml. Samples were preserved by freezing at -18°C. Total sediment was examined macroscopically for the presence of large lungworms, and in addition, 1 ml sub-samples were examined to determine the presence of larval stages of parasites.

Identification of nematodes (papers I and II)

Nematode eggs

The McMaster slide chambers were examined at 100 X with an Olympus BX40 compound microscope. Trichostrongylid eggs could not be positively identified to genus. However, *O. gruehneri* adults were the only species of nematode recovered from abomasal contents, and the eggs in uteri of the adult female worms were measured, and digital photo microscopy was used to confirm identifications. Eggs of *O. gruehneri* were oval-shaped and had a thin and transparent outer membrane, and had a length ranging between 87-96 μm and width of 42-49 μm. Eggs of *Capillaria* sp. were dark brown, barrel-shaped with a slightly protruding polar plug, and thicker shelled than the trichostrongylids, and they had a length of 49-52 μm and width of 23-27 μm. There were two types of Nematodirinae eggs each with a thick outer envelope, and they were approximately twice as large as all of the other nematode eggs. Of the Nematodirinae eggs measured, the length ranged from 194-235 μm and the width was 91-109 μm. Based on the identification of the adult nematodes in the small intestine, these eggs were *Nematodirus tarandi* and *Nematodirella longissimespiculata*. It was not possible to confirm with certainty species level identification for the Nematodirinae. Therefore these two types of eggs were classified at the subfamily level, Nematodirinae.

Nematode adults and larvae

A representative collection of gastrointestinal adult and larval nematodes (paper I, II, III) were cleared and mounted in polyvinyl lacto-phenol (PVL), photographed and measured using an Olympus digitizer, Olympus SZX9 Stereomicroscope, Olympus BX40 compound microscope, and Soft Imaging System AnalySIS software. Identification of *O. gruehneri and the* Nematodirinae was possible using scientific keys (Fruetel & Lankester, 1989; and Barth, 1991) and by comparison with the nematode reference collection of the Svalbard reindeer from the University of Oslo Zoological museum (O. Halvorsen personal communication).

The identification to species level of worms from the small intestine from the subfamily Nematodirinae was not conclusive. Generally the spicules of *N. longissimespiculata* were fully extruded (5 mm) to approximately one third the total body length of the worm and the spicules of *N. tarandi* were significantly shorter at approximately 2 mm. However, sometimes the spicules of *N. longissimespiculata* were withdrawn into the body cavity of the worm, making the length of the protruding spicules similar to *N. tarandi*. For this reason *N. longissimespiculata* and *N. tarandi* worms were grouped at the subfamily level, Nematodirinae. Bovine and ovine adult and early fourth-stage larvae (EL4) (paper III) were identified using the scientific keys of Borgsteede & Hendriks (1974), Durette-Desset (1983), and Barth (1991).

Environmental impact following ivermectin treatment (papers IV and V)

In November 2001, eight 25-week-old female reindeer calves obtained from the Kutuharju reindeer herd were transferred to the Kaamanen Reindeer Research Station and retained indoors and fed lichen *ad libitum*. The daily faecal production (control faeces) was collected during a 2-week period, pooled, and stored at 4°C. One week later, the calves received ivermectin by sc. injection (Ivomec® 10 mg per ml vet. inj.; Merial Inc., Haarlem, The Netherlands), or by the oral route (Ivomec® 0.8 mg per ml vet. mixt., Merial). The dose rate was 200 µg ivermectin per kg body mass, as recommended by the manufacturer. The daily faecal production was collected during the first 9 days following treatment and pooled.

In December 2001, all faeces from the ivermectin treated and non-treated groups were distributed onto experimental plots. These plots were established in two separate enclosures of forested reindeer pasture at the Reindeer Research Station, 1 fenced to prevent reindeer access (ungrazed) since 1995, the other (grazed) on an area which had been heavily stocked by reindeer for 5 years up to the commencement of the experiment (see Figure 4). During the experiment both enclosures were fenced to prevent access by reindeer.
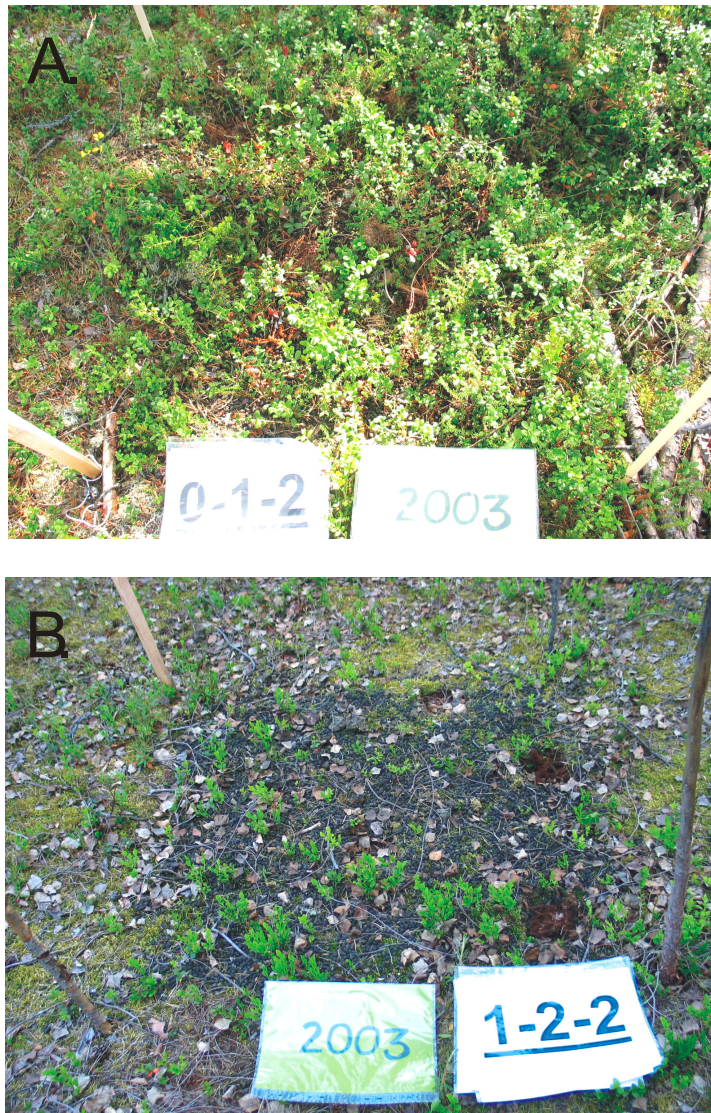
Figure 4. Experimental plots in which reindeer faeces treated with ivermectin were distributed on pastures representative of grazing conditions in the reindeer husbandry area of northern Finland. A. ungrazed pasture. B. grazed pasture.

Replicate sub-samples which consisted of faeces, vegetation, and soil were collected from the plots in June, July, and September 2002, and in June, July, August, and October 2003, spanning 25 to 95 weeks after faecal deposition. This experiment was repeated with another set of reindeer calves in November 2002, with new plots established approximately 1 m away from the original plots. Sub-samples were collected from these plots in June, July, August, and October 2003, and in June, July, August, and October 2004, spanning 25 to 95 weeks after faecal deposition. Each sub-sample collected was examined and the density and diversity

of soil nematodes was determined (paper IV). The residue of ivermectin within the sub-samples was determined by high performance liquid chromatography (HPLC) (paper V).

Meteorology

The Finnish Meteorological Institute provided precipitation data from the Ivalo airport, and sunlight hours were recorded at the township of Kevo for the duration of all fieldwork studies. Both localities are within 90 km from the experimental sites. Snow depth and ambient temperature were measured at the Reindeer Research Station in Kaamanen, 10 km south-east of the experimental sites. Data is presented in various forms in the research papers.

Results and discussion

Seasonal dynamics of reindeer nematode infections

Egg counts in calves

The magnitude of nematode infections, as reflected by faecal egg counts (FEC) of reindeer calves, varied between the 2.5 years of study. The abundance of eggs between seasons also varied, but eggs were shed in all months throughout the study. Of the 30 calves that were sampled each month, there were always a few animals that shed fewer than 50 eggs per gram faeces (epg), which was the detection limit of our test. However, there were also calves that shed a few hundred nematode eggs each month, but overall, the mean monthly faecal egg counts was generally very low, with no individuals shedding more than 600 epg on any occasion. At all times during the study, calves appeared healthy and displayed no obvious clinical signs of being infected with GI nematodes.

The trends in monthly abundance of eggs being shed were nematode species specific. *Capillaria* sp. and *O. gruehneri* were most abundant in 2004, whereas the Nematodirinae (*N. tarandi* and *N. longissimespiculata*) were most common in 2002. *Capillaria* sp. was the dominant winter (November–December) parasite with approximately 60% of calves shedding eggs. The Nematodirinae FEC also tended to increase over the winter, but less than 20% of reindeer calves sampled each month, shed eggs. Generally, egg output did not differ between male or female calves, but in 2004, male calves shed statistically more eggs of *O. gruehneri* than female calves.

The trend in monthly faecal egg count patterns was also associated with calf age. In June 2002, the calves were only 1-2 months old when faecal samples were collected, and all recorded negative egg counts. In the following months, egg counts appeared and increased in accordance with the acquisition of infection from pasture. The abundance of *Capillaria* sp. and the Nematodirinae peaked in the reindeer's first winter (November–December) when calves were between 6 and 7 months old. With the exception of June 2002, the group mean egg counts for *Capillaria* sp. was always greater than zero. The rise in abundance for *O. gruehneri* eggs occurred in the calves' second spring/summer (April–June) when they were 11–12 months of age. Ninety percent of reindeer calves shed *O. gruehneri* eggs during their second spring/summer (April–June), but the prevalence of infection decreased to 17% during the winter months (November–January). Egg output of *O. gruehneri* from wild Svalbard reindeer was also shown to peak in the summer months and was low during the winter (Irvine *et al*., 2000, 2001).

The whole Kutuharju herd was treated with ivermectin in December each year. Faecal samples were also collected at the time of treatment. Based on the post-treatment faecal samples in January (30 days following ivermectin treatment), the efficacy of this drug against *Capillaria* sp. was poor. Not only were *Capillaria* sp. egg counts higher in January than prior to treatment in December, but also egg counts of this species continued to increase in the subsequent winter months to reach maximum levels (approx. 600 epg) in March. Both in December 2002 and

2003 the faecal egg counts of *O. gruehneri* and the Nematodirinae had naturally declined to near zero values prior to anthelmintic treatment, thus it was not possible to detect if there was any benefit of ivermectin treatment on these species of nematodes. However, the efficacy of ivermectin against *O. gruehneri* has been shown to be virtually 100% in reindeer (Oksanen & Nieminen, 1998). These authors also reported that ivermectin appeared to reduce the *Capillaria* sp. egg production, but the difference between treated and non-treated control groups was not significantly different.

Egg counts in adult female reindeer

During the experimental period *O. gruehneri* was the most abundant GI nematode species according to the egg output from adult female reindeer. *Capillaria* sp. eggs were also detected, but in lower numbers than for reindeer calves. *Ostertagia gruehneri* was more abundant in 2004 than in 2003 and 2002, with peaks occurring during summer and autumn. In 2002 and 2004, the peak in abundance of *O. gruehneri* occurred in early September, and in 2003, in July, which also coincided with 100% prevalence of adult females shedding eggs. The monthly trends and the magnitude of *O. gruehneri* egg counts of adult female reindeer were similar to those seen in the reindeer calves. Oksanen & Nieminen (1998) reported that the peaks in trichostrongylid egg output (presumably *O. gruehneri)* in adult female reindeer occurred in March and May, but their trial was only performed from December until May, so these workers are likely to have missed recording the annual peak of egg output, which from our study appears to be in late summer/autumn.

Nematodirinae eggs were not detected in adult reindeer. Adult female reindeer shed statistically fewer *Capillaria* sp. eggs than calves, in all years sampled. However, the overall mean monthly abundance of *O. gruehneri* and *Capillaria* sp. eggs from adult female reindeer sampled in this study was regularly below the detection limit of 50 epg faeces, irrespective of year sampled.

Worm burdens of tracer calves

Worm burdens of tracer calves tended to increase with increasing calf age. *Ostertagia gruehneri* was the only species of nematode recovered from the abomasum of these animals. Over the entire study period, the number of adult *O. gruehneri* was not significantly different between male or female reindeer calves. Adult *O. gruehneri* were more abundant in 2004 than in 2003, with highest numbers recorded in spring-summer. This was similar to the pattern of highest egg counts of this parasite species in reindeer calves sampled. The seasonal dynamics of *O. gruehneri* nematodes was similar in both years. When the adult parasite worm burdens declined, the abundance of inhibited early fourth stage (EL4) larvae increased. The highest numbers of adult *O. gruehneri* were recorded in June of each year, when the tracers were entering their second summer (approximately 12 months of age). This trend differs somewhat from the seasonal patterns of trichostrongylid infections in ruminant livestock in temperate areas, where calves or lambs generally have their highest infections recorded during their first summer

to autumn on pasture, but then infection levels tend to decline over winter (Waller, Donald & Dobson, 1981).

Ostertagia gruehneri EL4 were prevalent in virtually every tracer calf during the months from September to March, which coincides with the cold temperatures of winter, and the presence of snow and ice. The mean number of EL4 was highest in October with mean worm burdens of approximately 3000 in tracer calves. Although EL4 were recorded in other months of the year, the number of larvae was low. The abundance of EL4 did not differ between years and calf gender. The numbers of EL4 declined in late winter (April) with the onset of warmer weather, and at the same time there was a commensurate increase in the number of adult *O. gruehneri*. In the Svalbard studies (Halvorsen *et al.*, 1999), the overall natural infection of *O. gruehneri* in reindeer calves also tended to increase from October to March, but there was little evidence of arrested larval development. However, only limited data was collected during the course of those investigations, so extrapolations to predict seasonal trends in parasite population dynamics needs to be made with caution.

Intestinal nematodes were all identified as belonging to the subfamily Nematodirinae (*N. tarandi* and *N. longissimespiculata*). The overall abundance of Nematodirinae was higher in 2004 than in 2003, and they were recorded in every month, except May 2003. The low abundance of Nematodirinae larvae was variable within and between months. It is however, important to note that we did not detect high efficacy of ivermectin against Nematodirinae eggs. The number of eggs shed approximately thirty days after ivermectin treatment were surprisingly higher than the egg output on the day of treatment. Therefore, the adult Nematodirinae worms that we detected in the tracer calves may have developed from larvae that had been acquired from pasture prior to the commencement of the tracer calf grazing period.

Moniezia sp. tapeworms were detected in the small intestine of approximately 25% of the tracer calves, but details regarding levels of infection and seasonality were not were recorded. Adult *Dictyocaulus* sp. was found in only one of the 80 lungs that were inspected. From the perfusions of these lungs, approximately 70% contained first-stage larvae, some with the characteristic dorsal-spine of the brainworm *E. rangiferi.* All of these genera have previously been described in wild (Halvorsen & Bye, 1999) as well as in semi-domesticated reindeer (Nordkvist *et al.*, 1983). Observations on the low efficacy of ivermectin against *E. rangiferi* larvae were reported by Nordkvist *et al.* (1983) and Oksanen *et al.* (1992b).

Worm burdens of winter-slaughtered calves

Ostertagia gruehneri was recorded in all calves collected during the winter slaughter from herds of northern Finland. One of the herds was the Kutuharju herd in which the tracer calves co-grazed and the other animals were derived from surrounding reindeer co-operatives. There was no difference in the number of *O. gruehneri* found in male and female calves, and the majority of the worm population (~80%) was EL4. At the time of slaughter in mid-winter, calves would have been grazing on natural pasture since their birth in the preceding May, and they would not have received anthelmintic treatment prior to slaughter. Thus, their

worm burdens represent 8 months of accumulation of infective nematode larvae acquired from pasture. In comparison with the worm burdens of the tracer calves (paper I), with the worm burdens of calves from the 3 semi-domesticated herds (paper II), the results are surprisingly similar. This is despite the fact that the tracer calves had only a short period (approx. 4 weeks) of being exposed to pickup from pasture. The prevalence of *O. gruehneri* was 100% in tracer calves and the slaughterhouse samples of reindeer calves in the winter of 2002-03 and 2003-04. The mean intensity of infection was also very similar and the percentage of EL4 was similar between the two classes of calves.

Very high efficacy of ivermectin has been confirmed against inhibited overwintering *Ostertagia* spp. in domestic ruminants (Benz, Roncalli & Gross, 1989). It has also been reported that late winter treatment of wild reindeer with another macrocyclic lactone anthelmintic, moxidectin, reduced the arrested larval stages of *O. gruehneri* by more than 95% (Irvine, 2000). Oksanen & Nieminen (1998) reported that moxidectin and ivermectin were equally effective against GI nematodes of reindeer. This implies that the use of ivermectin in tracer reindeer would have effectively eliminated any infections with *O. gruehneri* that these animals would have acquired before treatment. Given that this drug persists within the animal for several weeks following treatment (Nordkvist *et al*., 1983; Dieterich & Craigmill, 1990), the grazing interval of 7-8 weeks was chosen in our study to allow at least 4 weeks for any infection acquired by the tracers to successfully establish in these animals prior to slaughter.

The prevalence of intestinal nematode infections of the subfamily Nematodirinae (*N. tarandi* and *N. longissimespiculata)* during winter in each year of study was higher in the Kutuharju calves than in calves from the neighbouring co-operatives. There was no significant difference in the abundance of Nematodirinae between male and female reindeer calves, and approximately 30% of this worm burden occurred as EL4.

Worm burdens of winter-slaughtered adult reindeer

Arneberg, Folstad & Karter (1996) claim that GI nematode abundance is generally higher in adult reindeer than in calves, and males are probably more susceptible than female reindeer. This implies that female calves are likely to have lower intensities of GI nematodes than reindeer from other age and sex groups. We found *O. gruehneri* in all but one of the 43 adult reindeer processed. Overall, *O. gruehneri* was statistically more abundant in adult reindeer than in calves as Arneberg, Folstad & Karter (1996) suggested, but the percentage of EL4 was significantly less in adult reindeer than in calves. Similar findings were reported from wild reindeer from Svalbard where the higher *O. gruehneri* burdens in adult reindeer were attributed to the increase in the adult worm population (Halvorsen & Bye, 1999).

We found no parasites in the small intestine of adult reindeer, which was also the case in Irvine (2000), for adult reindeer slaughtered from Svalbard. During the winter of 2003-2004, the 8 adult female reindeer slaughtered from the Kutuharju herd had been sharing the same range as the tracer calves for the preceding 7-8 months. We found that these adult females harboured higher numbers of *O.*

gruehneri than the tracer calves. Sheep and cattle have been shown to tolerate abomasal nematode burdens far exceeding those found in our studies without showing clinical signs. Sub-clinical burdens of trichostrongylids have, on the other hand, been shown to alter the gastrointestinal function, sometimes with severe consequences (Sykes, 1978).

Reindeer as hosts of domestic livestock GI nematodes

The reindeer husbandry range of Scandinavia overlaps with sheep, goat, and cattle pastures. Through experimental dosing, we determined that young reindeer are suitable hosts to important gastrointestinal parasites of sheep (*T. circumcincta*, *H. contortus*) and cattle (*O. ostertagi*), as well as being a suitable host for the less host-specific nematode parasite, *T. axei*. Very poor establishment of bovine derived *Cooperia oncophora* was recorded in reindeer calves (2%) compared with bovine calves (59%). A study from Svalbard showed that reindeer were infected with *T. circumcincta*, *Ostertagia occidentalis*, and *Ostertagia trifurcata* when grazing on areas where cattle, pigs, and horses had previously been kept (Bye & Halvorsen, 1983). The overall mean intensity of *H. contortus*, *T. axei*, and *T. circumcincta*, in our study did not differ between reindeer and sheep; however, early fourth-stage larvae of *H. contortus* were more abundant in reindeer. These species represent the economically most important nematode parasites of sheep and goats throughout the world. Reindeer calves were most susceptible to L3 derived from sheep, however, the experiment was terminated by slaughter 40 days post-infection, and thus we did not establish whether *H. contortus* larvae would resume development to result in egg producing adult female nematodes.

Most reindeer herders in the southern cooperatives in Finland maintain their herds in corrals from December to February for ease of supplementary feeding in winter. Sheep may occasionally be penned in the same fenced-in areas during spring lambing season, or during the summer. Troell, Waller & Höglund (2005) showed *H. contortus* survives overwinter in Sweden, as faecal egg counts were positive in all tracer lambs, and upon slaughter, low numbers of adult parasites were recovered. Our study showed that 43% of experimentally infected *H. contortus* established in reindeer as EL4. It is a common practice in Finland that adult female reindeer are gathered in enclosures close to the herder's home during spring calving. During this time of parturition and lactation, it may be possible that arrested populations of *H. contortus* acquired by reindeer grazing on sheep contaminated pasture, may resume development to result in contamination of these confined reindeer calving pastures.

With regard to bovine nematodes, our study showed that *O. ostertagi* was capable of infecting reindeer to the same extent as in their definitive hosts, i.e. young, previously worm-free cattle. There appeared to be some host-induced effect on the incoming infection with this species, as significantly greater numbers of *O. ostertagi* remained arrested in development in reindeer compared to cattle, however, this parasite also reached patency (egg laying adults) in reindeer. The mean total body lengths of male and female adult *O. ostertagi* were marginally longer in reindeer than in their natural definitive host (cattle), and thus, it appeared that *O. ostertagi* was not unduly affected using the reindeer as host.

35

The implications of this work are important. We demonstrated experimentally that reindeer are suitable hosts for the nematode parasites of sheep and goats (*H. contortus* and *T. circumcincta*) and cattle (*O. ostertagi*), as well as for *T. axei*. Reindeer grazing on pastures shared with other ruminants may acquire natural nematode parasite infections of sheep or cattle origin. This is particularly so in areas in northern Norway, where for example around the Troms region, there is a significant goat dairy industry sharing pasture with reindeer (Handeland & Sparboe, 1991). However, it is not known if all of these aberrant livestock parasite species reach maturity in reindeer. With the trend towards increasing numbers of livestock in the southern grazing regions of reindeer, these findings highlight the increased risk of infections with parasites not normally associated with reindeer.

Impact of climate warming on parasites

The Finnish Meteorological Institute has published a report highlighting the remarkably mild winters in the 1990's. The mean temperature in Lapland for the winter months from December to March has increased by approximately 1°C between the periods 1961-1990 and 1971-2000 (http://www.fmi.fi/weather/climate_6.html#5; 5 September 2006). Although the amount of precipitation of 1961-90 was similar to that of 1971-2000, there were more freezing and thawing events occurring during the winters during the latter period.

Meteorological variables, principally precipitaion and temperature, are the main abiotic factors that determine the success in the development and survival of free-living stages of parasites. Thus any changes in conditions that favour these processes could be expected to result in an exacerbation of parasite infections in livestock. Experimental work with the muskoxen lungworm *Umingmakstrongylus pallikuukensis* revealed the importance of summer temperatures for its development and northward spread. It is notable that lungworm infections have become conspicuous during a time when summer temperatures have been increasing (Kutz, Hoberg & Polley, 2001). Early summer precipitation is essential for the survival of intermediate stages of many helminths. Changing climatic patterns are likely to affect the abundance and the epidemiology of these parasite infections.

The mean monthly temperatures at our study site for July, August, November, and December 2004 were 1-2 degrees warmer than for the same time period in 2002 and 2003. The abundance of *Capillaria* sp. and *O. gruehneri* nematode eggs, as well as the intensity of *O. gruehneri* worm burdens of tracer calves and adult female reindeer were higher in 2004 than in each of the 2 preceding years of our epidemiology study, implying that warmer temperatures are a stimulus for the development of larvae on pasture.

Environmental impact of ivermectin in the sub-Arctic

There has been concern about the possible impacts of excreted ivermectin on non-target organisms, such as soil or dung dwelling fauna, responsible for dung degradation and nutrient cycling (Strong 1993). Many studies on the impact of ivermectin on dung fauna and dung degradation were conducted in temperate climatic zones of the northern hemisphere, where earthworms play a major role in the degradation process (Curry, 1987). Very little is known of the possible impacts in relation to springtails, mites, enchytraeids, and soil nematodes, which are the most important microfauna of Arctic and sub-Arctic ecosystems. Through their feeding activity and digging ability, these organisms promote a fine-grained structure of surface soil layers, which improve aeration and water drainage. Reduced abundance or activity of some of these organisms may render the soil structure less stabile and more compact, and this could in turn bring about increased soil erosion.

Faeces production in reindeer varies seasonally in relation to feed availability, the digestibility of feed, and to water re-absorbency rates in the large intestine (Stien et al., 2002b). This suggests low faecal output over the long winter period, and a peak in faecal output in the summer associated with peak plant biomass. Changes in faecal consistency occur between seasons - from dry winter pellets that are associated with low faeces production, to soft faecal pats in June. Apart from the obvious importance of anthelmintic treatment at epidemiologically critical times with regard to parasite infections, the timing of ivermectin treatment of reindeer herds in the winter might be of less environmental impact in the sub-Arctic. Coprophilic organisms are attracted to fresh dung not to the dry pelleted winter faeces, which may contain the potentially toxic residual levels of ivermectin from previously de-wormed reindeer.

We analysed soil samples containing ivermectin treated reindeer faeces for drug residues using high performance liquid chromatography (HPLC). Ivermectin degradation rapidly took place during the first spring, but residual ivermectin could be measured for more than two summer seasons following treatment. The highest concentration determined, 650 ng/g dry soil, was in June 2004, 80 weeks after deposition. Concentrations appeared similar irrespective of whether samples were collected from ungrazed or grazed areas that typify the reindeer habitat of the region. Our results show that considerable amounts of ivermectin can persist on reindeer pasture for a time exceeding two grazing seasons following treatment, and thus for considerably longer than shown in any earlier reported studies (Lumaret *et al*., 1993; Nilssen *et al*., 1999).

A report on the toxicity of ivermectin for two soil dwelling species, the springtail *Folsomia fimetara* and the enchytraeid *Enchytraeus crypticus* (Jensen, Krogh & Sverdrup, 2003) demonstrated a toxicity threshold value for the springtail to be 0.26 mg/kg (260 ng per g) dry soil. During our entire study period of approximately 20 months, from December 2002 to August 2004, mean concentrations of ivermectin exceeded 74 ng per g soil. The ivermectin threshold toxicity value for the springtail was within the range of sample concentrations

determined in our study. Thus, there are grounds to suggest further environmental evaluation studies on the use of ivermectin to control parasites of reindeer in the sub-Arctic.

Soil nematodes are particularly important in the decomposition of organic matter and nutrient cycling in the Arctic (Sjursen, Michelsen & Holmstrup, 2005). No long-term studies have been conducted on the effect on soil nematode communities of ivermectin residues in faeces of reindeer treated in early winter. Soil nematode diversity has been shown to be much less in extreme environments, such as in the Arctic region, than in more temperate climates (Boag & Yeates, 1998). This makes extreme environments particularly sensitive to any man-induced changes. On this basis, it is important to conduct environmental impact studies on the effect of any chemical application that may ultimately end up in an Arctic environment. This particularly applies to any chemical, such as ivermectin, that has been shown to have detrimental effects on non-target organisms in more equitable environments.

We examined soil containing reindeer faeces that was treated with ivermectin for the abundance and diversity of soil nematodes. This study (paper IV) was performed in parallel with an investigation on the concentration of ivermectin in reindeer faeces (paper V). A total of 30 nominal genera of soil nematodes were identified. The 3 most abundant taxa were *Tylenchus*, *Aphelenchoides*, and Rhabditidae. Although significant differences in numbers of soil nematode fauna were observed between treatments on individual occasions, none of the differences occurred consistently with treatment, or with time. There were also no consistent significant differences in soil nematode communities recovered from soils beneath dung of reindeer treated with either ivermectin oral or sc. formulations in subsequent years.

Arthropods, particularly coprophiles, were rarely found during the two-year sampling period of our study. This finding is not surprising as hard, dry faeces of reindeer (Nilssen *et al*., 1999), cattle (Bryan, 1976), or horses (English, 1979) are shown to be unattractive to dung beetles. We were unable to ascertain what role soil-dwelling nematodes play in the breakdown of winter-deposited reindeer faeces, but it is presumed that their activity, together with that of other microorganisms (e.g. bacteria, fungi, and protozoa) minor. Therefore, we presume that physical, abiotic forces such as snow, rain, and temperature fluctuations, resulting in repeated freezing and thawing of the progressively ageing dung are likely to be dominant factors in the eventual disappearance of winter-deposited reindeer faeces in the sub-Arctic environment.

Future perspectives

Parasitism is the most successful form of life and occurs ubiquitously in nature. Every vertebrate is the natural host to a variety of parasites. A parasite can infect the host with little or no effect, or can cause dysfunction in the animal, resulting in disease or death. The effects of parasites on the long-term animal health and productivity as well as on the economy of the reindeer husbandry of Fennoscandia are unclear because, still, much knowledge is lacking.

Little is known about the factors that impact the rate of development and length of survival of the free-living stages of nematode parasites of reindeer in the Arctic and sub-Arctic. From our studies we conclude that winter pickup of *O. gruehneri* occurs with high prevalence, but at a rather low intensity. In more temperate regions, temperature and precipitation have been shown to be important stimuli for optimum hatching of GI nematodes for traditional domestic livestock (Stromberg, 1997). The peak abundance in faecal egg count shedding patterns was distinct and varied from summer/autumn for *O. gruehneri*, to winter for *Capillaria* sp. and the Nematodirinae. Ecological studies on the development and survival of the free-living stages of reindeer parasites in both field and laboratory conditions (that simulate environmental events of the field) are of importance to determine the fate of the seasonal peaks of nematode egg output as observed in our study. In mid-winter, reindeer were frequently observed to dig in 30-40 cm deep snow to reach the lichen layer of vegetation. The subnivean temperature, i.e. the temperature beneath the snow at the ground layer interface, remains just around freezing. It is probable that the infective larvae of nematodes inhabit this zone, where they are protected from extremes of wind and cold. However, this phenomenon deserves future attention. It is reasonable to assume that the more active calves whom spend extra energy foraging during the winter are most likely to ingest such infective larvae. However, the calves that prefer to frequent the feeding areas where daily supplemental feed is distributed probably maintain a high plain of nutrition during the winter. Thus they may be able to counter any increased risk of larval exposure with the development of an earlier and a better immune response to infection. There are many areas of research that need to be conducted on the ecology and on the translation processes (development and survival), which are important for the free-living stages of nematode parasites of reindeer in the sub-Arctic.

Inhibited development of nematode parasites of sheep and cattle has attracted a lot of research interest in recent years (Eysker, 1993, 1997). Principal factors responsible for this phenomenon are unfavourable weather conditions (cold or dry), or host induced effects (acquisition of immunity). Arrested larval development of *O. gruehneri* was present in all of the semi-domesticated reindeer herds we examined. It has been established that for some parasites found at the limit of their environmental tolerance, inhibition of development can be programmed to occur irrespective of environmental conditions or host age (Waller *et al.*, 2004). Does the same occur with parasites of reindeer?

Cross-transmission of sheep, goat, and cattle nematodes to reindeer, and *vice versa* is likely to increase due to climate warming and the obvious increasing trend

of co-grazing in the zones where the distribution of these animals overlap. Goat and sheep farmers in Norway are reporting adverse effects in their animals caused by a parasite (e.g. the brainworm *E. rangiferi)* derived from reindeer. It is not unlikely that the reciprocal may also occur, whereby reindeer become infected with nematode parasites conventionally associated with sheep, goats, and cattle. What would be the economic impact of such 'adventitious' parasites to the respective ruminant industries?

We have already witnessed monthly increases in mean ambient temperatures at Kaamanen, and this warming trend is apparent across the globe. Global warming knowledge in the Canadian Arctic has shown that caribou are migrating further north and west to seek cool relief provided by the Arctic Ocean. Hoberg *et al*. (1995) found a new lungworm that has been carried further north by muskoxen. Global warming may extend the migratory behaviour of wildlife and thereby potentially extend the range of parasites to the north. Regarding Fennoscandia, if sheep, cattle, and goats are continued to be herded further north, the size of communal grazing areas will certainly continue to increase. Can the free-living stages of *H. contortus* and *O. ostertagi* survive on pasture over a full Arctic winter? Is it the embryonated egg stage, the infective L3 stage, or both that survive on pasture over winter? An increase in the risk of cross-transmission potential affects all hosts involved because a few parasitic species of domestic livestock can establish within reindeer and *vice versa*, with the potential to cause sporadic epidemic outbreaks when weather conditions are favourable for the parasites.

Semi-domesticated reindeer with white pelage attract egg-laying warble flies. An investigation of the susceptibility of reindeer to external parasites such as warble flies and concomitant infections with internal parasites (GI nematodes) would be of interest. Since white reindeer are a visual target for warbles, are these individuals also more susceptible to nematode parasites due to a potential weakening of the immune system caused by the fly larvae? This could be monitored by worm and warble estimations on white and brown pelage adult reindeer.

Further studies on the environmental impact of ivermectin are warranted, specifically on its impact on springtails and enchytraeids, which are numerous in the soil/pasture interface of the Arctic environment. However, it is important to keep the issue of environmental impact in perspective. Detecting the presence of drug residues in samples using super sensitive analytical procedures is one thing, but whether these have any more than an ephemeral effect on the bio-system under question, is another matter. A non-emotional approach to environmental impact studies and their interpretation is essential, otherwise it could precipitate non-scientific reactions, halting the sale of important endectocidic drugs for use in the reindeer industry.

References

Albon, S.D., Stien, A., Irvine, R.J., Langvatn, R., Ropstad, E., & Halvorsen, O. 2002. The role of parasites in the dynamics of a reindeer poulation. *Proceedings of the Royal Society London, Series B 269*, 1625-1632.

Arneberg, P., Folstad, I. & Karter, A.J. 1996. Gastrointestinal nematodes depress food intake in naturally infected reindeer. *Parasitology 112*, 213-219.

Bakken, G. & Sparboe, O. 1973. Elaphostrongylosis in reindeer. *Nordisk veterinærmedicin 25,* 203-210.

Barth, D. 1991. *Magen-Darm-Nematoden des Rindes*, Diagnostischer Atlas 284 Einzelabbildungen. Ferdinand Enke Verlag Stuttgart. 104 pp. [In German].

Benz, G.W., Roncalli, R.A. & Gross, S.J. 1989. Use of ivermectin in cattle, sheep, goats, and swine. In *Ivermectin and abamectin*. Edited by W.C. Campbell. Springer-Verlag. New York. pp 215-229.

Boag, B., & Yeates, G.W. 1998. Soil nematode biodiversity in terrestrial ecosystems. *Biodiversity and Conservation 7*, 617-630.

Borgsteede, F.H. & Hendriks, J. 1974. Identification of infective larvae of gastrointestinal nematodes in cattle. *Tijdschrift voor Diergeneeskunde 99*, 103-113.

Bryan, R.P. 1976. The effect of the dung beetle, *Onthophagus gazella*, on the ecology of the infective larvae of gastrointestinal nematodes of cattle. *Australian Journal of Agricultural Research, 24*, 567-574.

Bye, K. & Halvorsen, O. 1983. Abomasal nematodes of the Svalbard reindeer (*Rangifer tarandus platyrhynchus vrolik*). *Journal of Wildlife Disease 19*, 101-105.

Bye, K. 1987. Abomasal nematodes from three Norwegian wild reindeer populations. *Canadian Journal of Zoology 65*, 677-680.

Bye, K., Halvorsen, O. & Nilssen, K. 1987. Immigration and regional distribution of abomasal nematodes of Svalbard reindeer. *Journal of Biogeography 14*, 451-458.

Campbell, W.C. 1989. *Ivermectin and abamectin*. Springer-Verlag. New York. 360 pp.

Coop, R.L. & Kyriazakis, I. 1999. Nutrition-parasite interaction. *Veterinary Parasitology 84*, 187-204.

Curry, J.P. 1987. The invertebrate fauna of grassland and its influence on productivity: the composition of the fauna. *Grass Forage Science 42*, 103-120.

Dieterich, R. A. & Craigmill, A.L. 1990 Safety, efficacy, and tissue residues of ivermectin in reindeer. *Rangifer 10*, 53-56.

Dobson, R.J., Waller, P.J., & Donald, A.D. 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep: the effect of infection rate on the establishment of infective larvae and parasite fecundity. *International Journal for Parasitology 20*, 347-352.

Durette-Desset, M.C. 1983. Keys to genera of the superfamily Trichostrongyloidea. In *CIH keys to the nematode parasites of vertebrate No. 10.* Edited by R.C. Anderson & A.G. Chabaud. Farnham Royal Bucks Commonwealth Agricultural Bureaux. England. 86 pp.

English, A.W. 1979. The effects of dung beetles (Coleoptera and Scarabaeinae) on the free-living stages of strongylid nematodes of the horse. *Australian Veterinary Journal 55*, 315-321.

Eysker, M. 1993. The role of inhibited development in the epidemiology of *Ostertagia* infections. *Veterinary Parasitology 46*, 259-269.

Eysker, M. 1997. Some aspects of inhibited development of trichostrongylids in ruminants. *Veterinary Parasitology 72*, 265-283.

Filppa, J. 2005. Reindeer husbandry in Finland. *Rangifer 25*, 59-62.

Finnish Meteorological Institute. Climatological statistics for the normal period 1971-2000. http://www.fmi.fi/weather/climate_6.html#5/6. [Accessed 5 September 2006].

Fruetel, M. & Lankester, M.W. 1989. Gastrointestinal helminths of woodland and barren ground caribou (*Rangifer tarandus*) in Canada, with keys to species. *Canadian Journal of Zoology 67*, 2253-2269.

Gordon, H.M. & Whitlock, H.V. 1939. A new technique for counting nematode eggs in sheep faeces. *Journal of the Council of Scientific and Industrial Research 12*, 50-52.

Halley, B.A. VandenHeuvel, W.J.A. & Wislocki, P.G. 1993. Environmental effects of the usage of avermectins in livestock. *Veterinary Parasitology 48*, 109-125.

Halvorsen, O. 1986. Epidemiology of reindeer parasites. *Parasitology Today 2 (12)*, 334-339.

Halvorsen, O. & Bye, K. 1999. Parasites, biodiversity, and population dynamics in an ecosystem in the high Arctic. *Veterinary Parasitology 84*, 205-227.

Halvorsen, O., Stien, A., Irvine, J., Langvatn, R. & Albon, S. 1999. Evidence for continued transmission of parasitic nematodes in reindeer during Arctic winter. *International Journal of Parasitology 29*, 567-579.

Handeland, K., & Sparboe, O. 1991. Cerebrospinal Elaphostrongylosis in dairy goats in Northern Norway. *Journal of Veterinary Medicine B 38*, 755-763.

Handeland, K. Skorping, A., Stuen, S. & Slettbakk, T. 1994. Experimental studies of *Elaphostrongylus rangiferi* in reindeer (*Rangifer tarandus tarandus*): clinical observations. *Rangifer 14*, 83-87.

Handeland, K. & Slettbakk, T. 1995. Epidemiological aspects of cerebrospinal elaphostrongylosis in small ruminants in northern Norway. *Journal of Veterinary Medicine B 42*, 110-117.

Herd, R. 1995. Endectocidal drugs: ecological risks and counter-measures. *International Journal of Parasitology 25*, 875-885.

Hoberg, E.P., Polley, L., Gunn, A. & Nishi, J.S. 1995. *Umingmakstrongylus pallikuukensis* gen.nov. et sp.nov. (Nematoda: Protostrongylidae) from muskoxen, *Ovibos moschatus*, in the central Canadian Arctic, with comments on biology and biogeography. *Canadian Journal of Zoology 73*, 2266-2282.

Holter, P. 1979. Effect of dung-beetles (*Aphodius spp.*) and earthworms on the disappearence of cattle dung. *Oikos 32*, 393-402.

Irvine, R.J. 2000. Use of moxidectin treatment in the investigation of abomasal nematodiasis in wild reindeer (R*angifer tarandus platyrhynchus*). *Veterinary Record 147*, 570-573.

Irvine, R.J., Stien, A., Halvorsen, O., Langvatn, R. & Albon, S.D. 2000. Life-history strategies and population dynamics of abomasal nematodes in Svalbard reindeer (R*angifer tarandus platyrhynchus*). *Parasitology 120*, 297-311.

Irvine, R. J., Stien, A., Dallas, J.F., Halvorsen, O. & Langvatn, R. 2001. Contrasting regulation of fecundity in two abomasal nematodes of Svalbard reindeer (*Rangifer tarandus platyrhynchus). Parasitology 122*, 673-681.

Jensen, J., Krogh, P.H. & Sverdrup, L.E. 2003. Effects of the antibacterial agents tiamulin, olanquindox and metronidazole and the anthelmintic ivermectin on the soil invertebrate species *Folsomia fimetaria* (Collembola) and *Enchytraeus crypticus* (Enchytraediae). *Chemosphere 50*, 437-443.

Klokov, K. 2004. Russia. In *Family-based reindeer herding and hunting economies, and thestatus and management of wild reindeer/caribou populations*. Edited by B. Ulvevadet & K. Klokov. Centre for Sami studies. University of Tromso. pp 55-92.

Kofinas, G. & Russel, D. 2004. North America. In *Family-based reindeer herding and hunting economies, and thestatus and management of wild reindeer/caribou populations*. Edited by B. Ulvevadet & K. Klokov. Centre for Sami studies. University of Tromso. pp 21-52.

Kummeneje, K. 1974. encephalomyelitis and neuritis in acute cerebrospinal nematodiasis in reindeer calves. *Nordisk veterinærmedicin 26*, 456-458.

Kumpula, J., Copaert, A. & Nieminen, M. 2002. Productivity factors of the Finnish semi-domesticated reindeer (*Rangifer t. tarandus*) stock during the 1990's. *Rangifer 22*, 3-12.

Kutz, S.J., Hoberg, E.P. & Polley, L. 2001. A new lungworm in muskoxen: an exploration in Arctic parasitology. *Trends in Parasitology* 17, 276-280.

Larsson, A., Dimander, S-O., Rydzik, A., Uggla, A., Waller, P.J. & Höglund, J. 2006. A 3-year field evaluation of pasture rotation and supplementary feeding to control parasite infection in first-season grazing cattle: effects on animal performance. *Veterinary Parasitology* DOI: 10.1016/j.vetpar.2006.07.017.

Lorentzen, G. & Halvorsen, O. 1986. Survival of the first stage larva of the metastrongylid nematode *Elaphostrongylus rangiferi* under various conditions of temperature and humidity. *Holarctic Ecology 9*, 301-304.

Lumaret, J.P., Galante, E., Lumbreras, C., Mena, J., Bertrand, M., Bernal, J.L., Cooper, J.F., Kadiri, N. & Crowe, D. 1993. Field effects of ivermectin residues in reindeer faeces on dung beetles. *Journal of Applied Ecology 30*, 428-436.

Moen, J. & Danell, Ö. 2003. Reindeer in the Swedish mountains: an assessment of grazing impacts. *Ambio 32*, 397-402.

Nieminen, M. 2005. Reindeer: Semi-domesticated. In *The Saami: A Cultural Encyclopedia*. Edited by U.M. Kulonen, I. Seurujärvi- Kari & R. Pulkkinen. Suomen Kirjallisuuden Seura. Vammala. 498 pp.

Nilssen, A.C. & Haugerud, R.E. 1994. The timing and departure rate of larvae of the warble fly *Hypoderma* (=*Oedemagena*) *tarandi* (L.) and the nose bot fly *Cephenemyia trompe* (Modeer) (Diptera: Oestridae) from reindeer. *Rangifer 14*, 113-122.

Nilssen, A.C. & Haugerud, R.E. 1995. Epizootiology of the reindeer nose bot fly *Cephenemyia trompe* (Modeer) (Diptera: Oestridae) in reindeer, *Rangifer tarandus* (L.) in Norway. *Canadian Journal of Zoology 73*, 1024-1036.
.
Nilssen, A.C., Asbakk, K., Haugerud, R.F., Hemmingsen, W. & Oksanen, A. 1999. Treatment of reindeer with ivermectin: effect on insect dung fauna. *Rangifer 19*, 61-69.

Nordkvist, M., Rehbinder, C., Christensson, D. & Rönnbäck, C.A. 1983. A comparative study on the efficacy of four anthelmintics on some important reindeer parasites. *Rangifer 3*, 19-38.

Oksanen, A., Soveri, T. & Nieminen, M. 1992a. Fibre-optical pharyngoscopy for diagnosing throat bots in the reindeer. *Bulletin of the Scandinavian Society for Parasitology 2*, 30-32.

Oksanen, A., Nieminen, M., Soveri, T. & Kumpula, K. 1992b. Oral and parenteral administration of ivermectin to reindeer. *Veterinary Parasitology 41*, 241-247.

Oksanen, A., Nieminen, M. & Soveri, T. 1993. A comparison of topical, subcutaneous, and oral administration of ivermectin to reindeer. *Veterinary Record 133*, 312-314.

Oksanen, A., Norberg, H., Nieminen, M. & Bernstad, S. 1995. Influence of route of administration on the plasma concentrations of ivermectin in reindeer. *Research in Veterinary Science 58*, 286-287.

Oksanen, A. & Nieminen, M. 1996. Larvicidal effectiveness of doramectin against natural warble (*Hypoderma tanadi*) and throat bot (*Cephenemyia trompe*) infections in reindeer. *Medical and Veterinary Entomology 10*, 395-396.

Oksanen, A. & Nieminen, M. 1998. Moxidectin as an endectocide in reindeer. *Acta Veterinaria Scandinavica 39*, 483-489.

Paliskuntain Yhdistys 2005. Suomen porotalous. [The Finnish reindeer industry]. Rovaniemi, Finland.

Rekacewicz, P. 1998. Distribution of reindeer population in the Barents Region. *UNEP/ GRID- Arendal Maps and Graphics Library*. http:// maps.grida.no/go/graphic/ distribution of_reindeer_population_in_the_barents_region [Accessed 14 September 2006].

Roed, K.H. 2005. Refugial origin and postglacial colonization of holarctic reindeer and caribou. *Rangifer 25*, 19-30.

Sjursen, H., Michelsen, A. & Holmstrup, M. 2005. Effects of freeze-thaw cycles on microarthropods and nutrient availability in a sub-Arctic soil. *Applied Soil Ecology 28*, 79-93.

Smith, G. & Grenfell, B.T. 1985. The population biology of *Ostertagia ostertagi*. *Parasitology Today 1*, 76-81.

Smith, G. 1988. The population biology of the parasitic stages of *Haemonchus contortus*. *Parasitology 96*, 185-195.

44

Sommer, C., Steffansen, B., Nielsen, B.O., Jensen, V., Jespersen, K.M.V., Springborg, J.B. & Nansen, P. 1992. Ivermectin excreted in cattle dung after subcutaneous injection or pour-on treatment: concentrations and impact on dung fauna. *Buletin of Entomological Research 82*, 257-264.

Stien, A., Irvine, R.J., Ropstad, E., Halvorsen, O., Langvatn, R. & Albon, S.D. 2002a. The impact of gastrointestinal nematodes on wild reindeer: experimental and cross-sectional studies. *Journal of Animal Ecology 71*, 937-945.

Stien, A., Irvine, R.J., Langvatn, R., Albon, S.D. & Halvorsen, O. 2002b. The population dynamics of *Ostertagia gruehneri* in reindeer: a model for the seasonal and intensity dependent variation in nematode fecundity. *International Journal of Parasitology 32*, 991-996.

Stéen, M., Blackmore, C.G. & Skorping, A. 1997. Cross-infection of moose (*Alces alces*) and reindeer (*Rangifer tarandus*) with *Elaphostrongylus alces* and *Elaphostrongylus rangiferi* (Nematoda, Protostrongylidae): effects on parasite morphology and prepatent period. *Veterinary Parasitology 71*, 27-38.

Stromberg, B.E. 1997. Environmental factors influencing transmission. *Veterinary Parastiology 72*, 247-256.

Strong, L. 1993. Overview: the impact of avermectins on pastureland ecology. *Veterinary Parasitology 48*, 3-17.

Sykes, A.R. 1987. Endoparasites and herbivore nutrition. In *The nutrition of herbivores*. Edited by J.B. Hacker & J.H. Ternouth. Proceedings of the second International Symposium on the Nutrition of Herbivores, 6-10 July. Brisbane Australia. pp 211-232.

Sykes, A.R. 1994. Parasitism in farm animals. *Animal Production 59*, 155-172.

Troell, K., Waller, P.J. & Höglund, J. 2005. The development and overwintering survival of free-living larvae of *Haemonchus contortus* in Sweden. *Journal of helminthology 79*, 373-379.

Turner, M.J. & Schafer, J.M. 1989. Mode of action of ivermectin. In *Ivermectin and abamectin*. Edited by W.C. Campbell. Springer-Verlag. New York. pp 73-88.

Ulvevadet, B. 2004. Introduction. In *Family-based reindeer herding and hunting economies, and thestatus and management of wild reindeer/caribou populations*. Edited by B. Ulvevadet & K. Klokov. Centre for Sami studies. University of Tromso. pp 9-14.

Waller, P.J., Donald, A.D. & Dobson R.J. 1981. Arrested development of intestinal Trichostrongylus spp. in grazing sheep and seasonal changes in the relative abundance of T. colubriformis and T. vitrinus. *Research in Veterinary Science 30*, 213-216.

Waller, P.J. 1990. Resistance to chemotherapy: what are the risks for the reindeer industry? *Rangifer 4*, 14-18.

Waller, P.J. 1994. The development of anhelmintic resistance in ruminant livestock. *Acta Tropica 56*, 233-243.

Waller, P.J. 1997. Anthelmintic resistance. *Veterinary Parasitology 72*, 391-412.

Waller, P.J., Rudby-Martin, L., Ljungström, B.L., & Rydzik, A. 2004. The epidemiology of abomasal nematodes of sheep in Sweden, with particular reference to overwinter survival strategies. *Veterinary Parasitology 122*, 207-220.

45

Waller, P.J. 2005. Domestication of ruminant livestock and the impact of nematode parasites: possible implications for the reindeer industry. *Rangifer 25*, 39-50.

Wiklund, E. & Finstad, G. 2006. Circumpolar reindeer management. In *Proceedings of the 4th World Deer Farming Congress*, 20-22 April 2006. Melbourne, Australia.

It is never easy to leave the people and places you love; even to pursue a dream. Change can be frightening, but with time, more satisfying than the previous challenges. Eventually we land on our feet again; this moment occurred for me the day this doctoral thesis went to the print shop. It was a risk giving up the security of my family home in Canada and the home I made in Fairbanks, Alaska for the unknown awaiting me in Uppsala, Sweden and in Kaamanen, Finland. I am glad I took those risks because I never would have met each of you, nor would I have found how much more I was capable of doing with my research interests.

Acknowledgements

This project was performed at the Department of Parasitology (SWEPAR) of the National Veterinary Institute and the Swedish University of Agricultural Sciences; Finnish Game and Fisheries Reindeer Research Station Kaamanen; and the Kutuharju reindeer field station in northern Finland. Research was funded by the Swedish Research Council of Environment, Agricultural Sciences and Spatial Planning (FORMAS); Nordic Arctic Research Program (NARP); the Finnish Ministry of Agriculture and Forestry (MAKERA); and the Nordic Council for Reindeer Husbandry Research (NOR). The Finnish Reindeer Herders' Association provided reindeer from the Kutuharju experimental herd for this project.

Peter Waller, my main scientific advisor, has pressed me to produce, and unlike many North American academic advisors, who are content to let students hang by the rope of their own expectations, Dr. Waller kept a noose on me in the final year whenever I entered into Swedish territory. He instilled in me the importance of standing up for what I believe in, to always work to the best of my abilities, and the benefits of controlling emotions during discussions. Equally important is the necessity of a daily well-balanced diet and post-lunch walk; these are the ingredients to productive writing! The English communications, surrogate fatherly hugs, and genuine concern for my well-being during my explorations in Finland are much appreciated. I am evermore grateful for the funding you secured which enabled my extensive travelling to collaborate with world leading parasitologists, reindeer biologists, and soil nematode specialist.

Arvid Uggla, my pure-blooded Swedish departmental advisor, was first to instruct me in Swedish grammar, first to introduce me to Surströmming (fermented herring), and first to believe in me. Your knack for managing students and employees by putting a smile on their face is world class. I appreciate your promptness and outstanding manner in which you reviewed this thesis, and for being available regardless of your physical location. When my confidence was wavering in the last few months before thesis completion, your encouragement was a guiding light. I would have written this Acknowledgement section about you på Svenska, but only a limited audience would then understand how you have helped me.

Antti Oksanen, my Finnish co-advisor, toiled over each one of our manuscripts as much as I have. You have a critical eye for detail Antti. Thank You for sticking with me until the very end. Just so you know, your name is known among the budding young Arctic ungulate parasitologists in North America. It took me four years to finally meet you face to face, an additional year to be able to communicate in Finnish with you, and only a few seconds to enjoy your humour.

Mauri Nieminen, my Finnish co-advisor, taught me about reindeer biology and history. Your passion for reindeer via artistic expression, photography, and even your wardrobe, supersedes mine. I have enjoyed your companionship during our fishing adventures, family dinners, and sauna evenings. I look forward to your eventual unfortunate retirement so that I might have a chance of purchasing your wonderful home, finely decorated with worldly acquired reindeer paraphernalia. I

in RenProjekt as the hundreds of samples you examined for nematodes was certainly demanding of 4 eyes. I wish you a life of happiness lived with youthful intensity!; **Göran** ensured that I was content with computers and programs. When my laptop crashed the first time, Göran made sure that it was back in business ASAP. I really appreciated your support when I needed help with software, and for the times you tracked down English versions of certain programs, as opposed to Swedish, which saved me a lot of frustration; **Johnny** from SVA-IT also came to my rescue numerous times, especially when my motherboard was electrocuted, and the operating system decided to repeatedly fail whenever I was 1600 km north in Kaamanen. I think the number 4645 will be emblazoned in my brain forever; **Owe** made the SWEPAR hallways an enjoyable environment. Thank You for letting me test your patience and for teaching me a few important Swedish phrases such as 'Lägg av' and 'Skärp dig'. Keep smiling… you brighten the day for many; **David Morrison** of SWEPAR came to my rescue many times when the help function of my statistics program would not do what I asked it to. This applies also to the spontaneous difficulties I encountered with MS Excel. David, you cheered me, and taught me more than a textbook; **Johan** was available when I needed to discuss matters through the eyes of a biologist. Johan, I value your energy for sticking up for what you think is the correct answer!; **Dan & Bodil** stuffed me with strawberries and always made me feel welcome; **Janne** teased and harassed me until I would return the favour with a smile. I think you are a great father; **Annie** shared her smile and musical talents with me; **Eva O-L** was a role model for showing me that managing a family of 3 kids and husband, completing a PhD, and daily exercise is possible without clinical stress; **Eva M** introduced me to boxing and also stressed the importance of eating healthy and daily exercise; **Malin** kept life realistic and shared her love of dogs with me, which made me homesick for my Goliath; **Anna Ro** helped with every managerial task I ever asked for help with; **Karin** drew me maps to shopping areas and made SWEPAR coffee room a pretty place to hang out; **Per** shared his passion about birds with me and taught me about the freshwater seal 'Saimaannorppa' *Phoca hispida botnica* in Finland. I admire your enthusiasm Per!; **Susanne**, **Birgitta**, **and Maria** always made me laugh, listened to my experiences from Kaamanen, and encouraged me to follow my dreams (and heart); **Anna Lu** kept my academic records up to date and was first to teach me about skating on lakes with extra long skate blades. Can you imagine - skates but no hockey stick?! **Sara** and **Anita** reminded me that I better get busy and make some babies while I am still a student; **Katrin** calmed my nerves with access to web-based email and to the SLU proxy server whenever I travelled; **Katarina** reminded me as the Finns often did that silence is more appropriate than being a chatter box; **Eva W** (and Anna Lu) have been role models for me, as women in science; **Kenneth** and **Eva-Britt** made me feel comfortable at coffee by not forcing me to speak Swedish, but when I tried, they were patient with my skills; **Set** taught me a bit about parasites of camels, and offered his home to my parents. Thank You.

Kaamanen Reindeer Research Station was my home for the most part of the first three years of my PhD studies. This is where I got my start and I have many people to thank for helping me to overcome the difficulties in communication, daily routines, and differences between Finnish and Canadian cultures. **Heikki** provided

50

I

Population dynamics of nematode parasites of reindeer in the sub-arctic

J.T. Hrabok [a,*], A. Oksanen [b], M. Nieminen [c], P.J. Waller [a]

[a] Department of Parasitology (SWEPAR), National Veterinary Institute and Swedish University of Agricultural Sciences, SE-751 89 Uppsala, Sweden
[b] Finnish Food Safety Authority Evira, Oulu Research Unit (FINPAR), P.O. Box 517, FIN-90101 Oulu, Finland
[c] Finnish Game and Fisheries Research Institute, Reindeer Research Station, FIN-99910 Kaamanen, Finland

Abstract

Nematode parasite infections of semi-domestic reindeer grazing in their natural habitat in northern Finland were monitored for approximately 2 years. This was achieved by monthly faecal egg counts of male and female calves and adult females from an experimental reindeer herd, in addition to estimating the acquisition of nematode infection from pasture using tracer reindeer calves. The most abundant parasite was *Ostertagia gruehneri* in the worm counts of tracer animals and in faecal egg counts of adult female reindeer. *Capillaria* sp. eggs were detected in calves and adults, but Nematodirinae eggs were only recovered from calves. Faecal egg counts showed variations between months for each nematode species, with male and female calves shedding similar numbers of eggs. During each year, calves shed more *Capillaria* sp. eggs than adult female reindeer, but similar numbers of *O. gruehneri* eggs. Egg counts of *O. gruehneri* were more abundant in late summer–autumn (July–September), whereas *Capillaria* sp. and the Nematodirinae dominated the winter months (November–February). The seasonal trends of adult worm burdens of *O. gruehneri* in the tracers paralleled the egg count patterns. *Capillaria* sp. was not detected in tracer worm counts. Tracer worm burdens showed that the proportion of inhibited larvae of *O. gruehneri* and Nematodirinae steadily increased from spring to early winter, followed by a decline and a commensurate increase in the number of adult parasites in the second summer. This investigation showed that parasite transmission occurs continuously throughout the year for nematode parasites of reindeer in northern Finland.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Semi-domesticated reindeer; Nematode parasites; *Ostertagia gruehneri*; *Capillaria* sp.; Nematodirinae

1. Introduction

Much of what is known about gastrointestinal parasites of ruminants is based on knowledge of the nematode family Trichostrongylidae, which parasitize the conventional ruminant livestock species, namely, cattle, sheep, and goats. Nematode parasites have been shown to impose substantial economic penalties on livestock production systems that are focused on maximizing product output, whether meat, fibre, or milk (Perry and Randolph, 1999).

Reindeer (*Rangifer tarandus*) are an integral part of the culture and economy of the Sámi people of Fennoscandia, and large herds of these semi-domesticated animals are maintained in co-operatives that encompass the northern sectors of Norway, Sweden, and Finland (Nieminen, 2005). Reindeer represent the most important ruminant production system in northern Finland, and thus any factor that imposes a constraint on their productivity is important for the local economy.

* Corresponding author. Tel.: +46 70454 7032; fax: +46 1867 4304.
E-mail address: Jackie.Hrabok@sva.se (J.T. Hrabok).

Reindeer are susceptible to nematode parasites of sheep and cattle (Hrabok et al., 2006), but their unique parasitic fauna is more adapted to the extreme environmental conditions of the sub-arctic, which is characterized by profound seasonal changes in temperature, day length, and precipitation. Similar to nematode parasites of the conventional ruminant livestock species, reindeer parasites have an obligatory free-living stage on pasture.

The life history of *Ostertagia gruehneri*, the abomasal parasite of reindeer, has been comprehensively examined in wild reindeer populations on the Norwegian archipelago of Svalbard in the high Eurasian arctic (Bye and Halvorsen, 1983; Halvorsen, 1986; Bye, 1987; Halvorsen and Bye, 1999; Halvorsen et al., 1999; Irvine et al., 2000; Stien et al., 2002a,b; Albon et al., 2002). However, these studies were restricted to material collected from the slaughter of cohorts of animals on only a few occasions during the year, involving variable numbers of animals of different ages and sexes. Apart from these studies on Svalbard, only a few cursory reports have been made on gastrointestinal helminths of caribou (the term used for wild *R. tarandus* in the Nearctic region) in North America (Fruetel and Lankester, 1989; Dieterich and Craigmill, 1990), and reindeer in Norway (Bye et al., 1987), Sweden (Rehbinder and Christensson, 1977; Rehbinder and von Szokolay, 1978), Finland (Oksanen et al., 1990; Oksanen, 1999), and South Georgia (Leader-Williams, 1980). These latter studies have involved point-in-time sampling of only a few animals due to logistical and fieldwork constraints, or the temporal availability of material obtained from hunters or slaughterhouses.

The objective of this investigation was to determine, by regular monitoring, the seasonal dynamics of nematode parasite populations in reindeer found in their natural environment, but managed semi-domestically for the reindeer husbandry of Fennoscandia.

2. Materials and methods

2.1. Study animals and area

This study was conducted at the Kutuharju Experimental Reindeer Station (69°N, 26°E) approximately 300 km north of the Arctic Circle in the Muotkatunturi-Muddusjärvi Reindeer Herding districts in Lapland, Finland. Reindeer are the only ungulate livestock on this research station and in the surrounding reindeer herding co-operatives. The Kutuharju reindeer herd consisted of approximately 100 adult females, 80 male and female calves, and 20 mixed age classes of bulls and castrated reindeer. Animals were individually marked with a numbered ear tag and coloured plastic collar. The total grazing area was 42 km² sub-divided into summer and winter pastures on which the animals freely ranged. Vegetation was dominated by pine (*Pinus sylvestris*) and birch (*Betula pubescens*) with an understorey of willows (*Salix* spp.), and low-lying shrubs [lingonberry (*Vaccinium vitis-idaea*), blueberry (*Vaccinium myrtillus*), crowberry (*Empetrum nigrum*), Labrador tea (*Ledum palustre*), cloudberry (*Rubus chamaemorus*)], lichen (*Cladina* spp.), and moss (*Pleurozium* spp., *Dicranum* spp., *Polyrihum* spp.). For the past 16 years, the herd had a history of being dewormed once per year in November or December with ivermectin (Ivomec SC® 10 mg/ml vet. inj. Merial) at the manufacturer's recommended dose rate (200 μg/kg body weight).

2.2. Faecal collections

From June 2002 to September 2004, monthly faecal samples were collected from approximately 15 male and 15 female calves, and 30 adult female reindeer. The herd was gathered and the animals sampled rectally, except in July 2002–2004, August 2002 and 2004, and in October 2003, when fresh faecal samples were collected off pasture from adult females when the herd was followed. No samples were collected in August 2003 and only a few were obtained in July and August 2004 due to logistical constraints.

2.3. Tracer calves

Commencing in January 2003, 2 male and 2 female calves (8 months old) were treated with a subcutaneous formulation of ivermectin at 200 μg/kg body weight, and released to natural pastures with the experimental herd. These calves were culled 7–8 weeks later, and their abomasums, small intestines, and lungs were obtained for the recovery of nematode parasites. This procedure was repeated for 20 consecutive months with a new set of tracer calves on each occasion, ending in August 2004. Tracers ranged in age from approximately 3.5 months in August, being from the current season cohort of calves, to 14 months (born the previous year) in July of each year.

2.4. Meteorology

Snow depth and ambient temperature was measured at the Reindeer Research Station in Kaamanen, 10 km south-east of the experimental site.

2.5. Parasitological procedures

A modified McMaster technique (Gordon and Whitlock, 1939) was used to estimate the density of nematode eggs in faeces, with the minimum detection level of 50 eggs per gram (EPG) faeces. The viscera (abomasum and the proximal 7–10 m of small intestine) of tracer calves were collected and processed according to Donald et al. (1978) and the abomasal mucosa digestion procedures followed Dobson et al. (1990). Four 20 ml replicate sub-samples were taken from each abomasal (3 l) and small intestinal contents (3 l), and mucosal digest (1 l), and preserved by freezing at −18 °C (Dobson et al., 1990). When required for worm counting and species differentiation, the 20 ml sub-samples were thawed on a 30 μm sieve using a gentle stream of warm tap water. All material, including nematodes retained on the sieve, were back-washed into a beaker, stained with Lugols iodine and examined with a stereomicroscope and sub-phase illumination at 16–20×. Early fourth-stage larvae (EL4) were identified at 400× using compound light microscopy. An Olympus digitizer DP50 3.0 and Soft Imaging System AnalySIS 3.1 software was used to distinguish between taxa with the use of scientific keys (Durette-Desset, 1983; Barth, 1991). Replicate worm counts were conducted on all abomasal contents, digests, and small intestine samples.

The lungs of all calves were perfused three times with up to 8 l of water, and the major bronchial airways were opened and thoroughly flushed with warm tap water. The total volume of fluid collected was allowed to settle at 4 °C for approximately 36 h before decanting to a final volume of 50 ml. Samples were preserved by freezing at −18 °C. Total sediment was examined macroscopically for the presence of large lungworms and in addition, 1 ml sub-samples were examined at 400× to determine the presence of larval stages of parasites.

2.6. Statistical procedures

Faecal egg counts and nematode worm count data were examined using quantile–quantile plots to determine the most appropriate transformation to normalize variances via Brodgar 2.60 (Highland Statistics Ltd. Version December 2005, UK). Transformations were determined to be square root or cubic root, for different data sets. Data were plotted as Box plots showing the upper and lower outliers and adjacent values, the 75th percentile, the median, and the 25th percentile (Intercooled Stata 8.0, Texas, USA). Geometric means with standard deviation are presented when describing data in the text. The transformed faecal egg count data were

analysed by the general linear model (ANOVA) with year (2002–2004), month (January–December), reindeer calf gender (male, female), and age class (calf, adult reindeer) as the explanatory variables. The transformed worm burden data were also analysed by the general linear model (ANOVA) with year (2003–2004), month (January–December), and reindeer calf gender (male, female), as the explanatory variables. Between year variation in worm burdens, and faecal egg counts, were analysed with ANOVAs based on equal monthly sampling. Statistical significance was calculated using the F test and significance accepted at the 5% probability level.

3. Results

3.1. Meteorological records

Meteorological data for the period May 2002–December 2004 are summarized in Fig. 1. Snow cover was present from September/October until May–early June, with maximum depths of nearly 45 cm in February–March. Below zero temperatures were common from October until April, with the lowest temperatures in January when it approached −40 °C. Maximum temperatures were recorded in July when it exceeded 20 °C. Twenty-four hours sunlight occurred between mid-May and late July, whenever the sky happened to be clear.

3.2. Nematode faecal egg counts of reindeer calves

Three nematode taxa were identified, namely, the Trichuridae (*Capillaria* sp.)(Fig. 2A), Trichostrongyli-
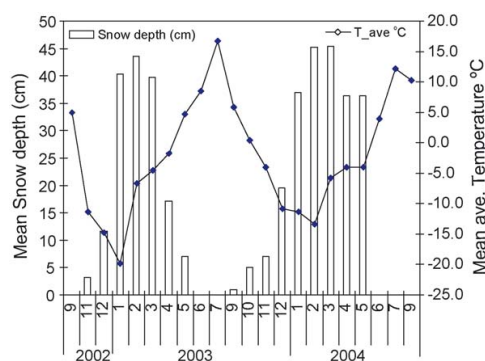
Fig. 1. Meteorological data for the duration of the experimental period. Snow depth represents accumulated snow at the end of each month.

J.T. Hrabok et al./Veterinary Parasitology xxx (2006) xxx–xxx
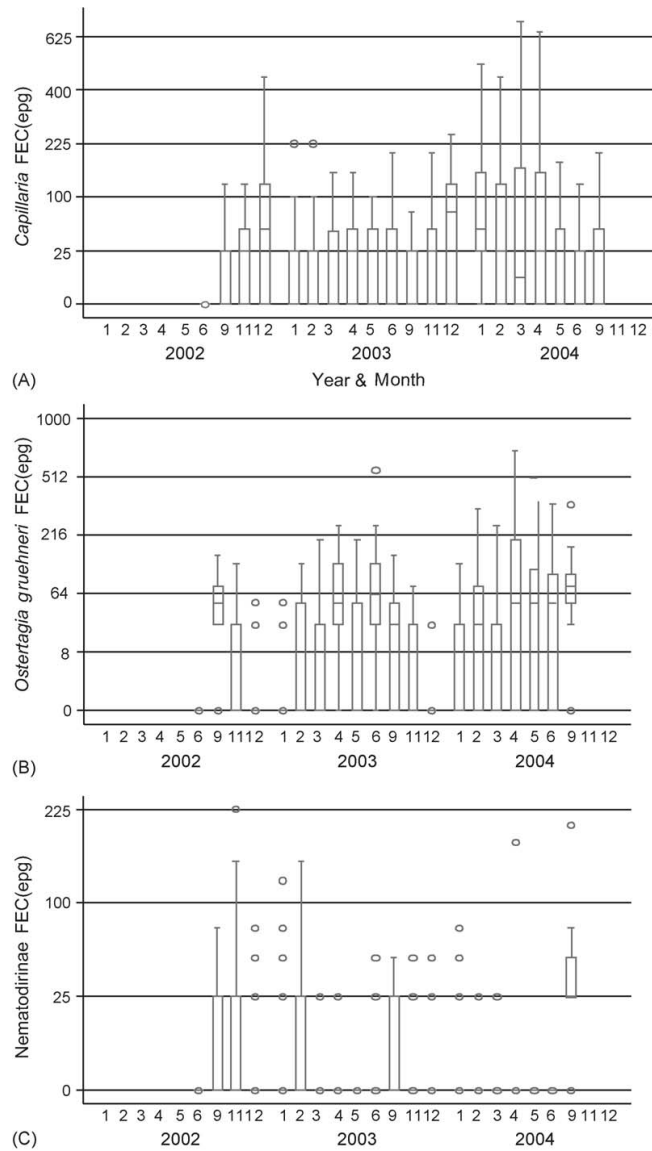
Fig. 2. Box plots of nematode faecal egg counts (eggs per gram) from reindeer calves ($N = 30$ per month): *Capillaria* sp. (A); *Ostertagia gruehneri* (B); Nematodirinae (C). The whole herd was treated with ivermectin after faecal samples were collected in December of each year. Note that the vertical axis is a squared scale for (A and C) and cubed for (B).

dae (*O. gruehneri*) (Fig. 2B), and the Nematodirinae (*Nematodirus tarandi* and *Nematodirella longispicu-lata*) (Fig. 2C), in order of abundance. Nematodirinae eggs were approximately twice as large as other trichostrongylid and *Capillaria* sp. eggs. *Capillaria*

sp. eggs were easily differentiated from the small trichostrongylid eggs by morphology, and for the latter only *O. gruehneri* were recovered from worm counts of tracers (see below), thus it was assumed that all the small trichostrongylid eggs found in faecal samples

J.T. Hrabok et al. / Veterinary Parasitology xxx (2006) xxx–xxx 5

belonged to this species. The majority of the monthly mean faecal egg counts for all species, in each year, were below the detection limit of 50 eggs per gram. However, the abundance of nematode eggs was variable between years and months, and was species specific. *Capillaria* sp. and *O. gruehneri* were most abundant in 2004, whereas the Nematodirinae were most common in calves in 2002. Generally, egg output did not differ among male or female calves, but in 2004, male calves shed higher numbers of *O. gruehneri* eggs than female calves ($p < 0.001$). The most important factor influencing faecal egg count patterns was month, and this was associated with calf age. The abundance of *Capillaria* sp. and the Nematodirinae peaked in the reindeer's first winter (November–December) when calves were between 6 and 7 months old and *O. gruehneri* in the second spring/summer (April–June) when they were 11–12 months of age. Ninety percent of reindeer calves shed *O. gruehneri* eggs in their second spring/summer (April–June) and the prevalence of infection decreased to 17% during the winter months (November–January). *Capillaria* sp. was the dominant winter (November–December) parasite, with approximately 60% of calves shedding eggs. The Nematodirinae FEC also tended to
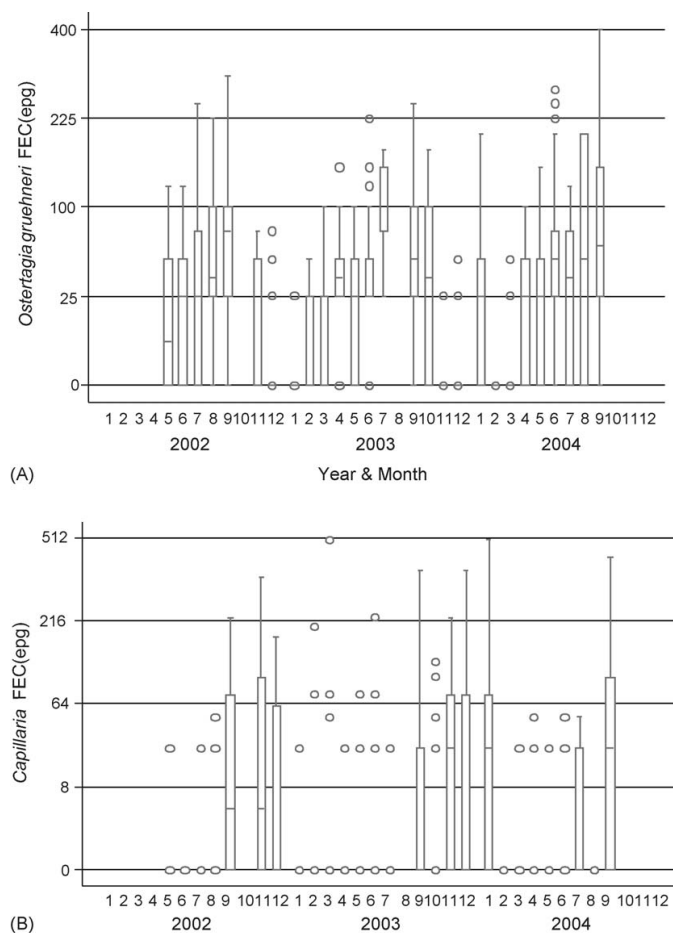
Fig. 3. Box plots of nematode faecal egg counts (eggs per gram) from adult female reindeer ($N = 30$ per month): *O. gruehneri* (A); *Capillaria* sp. (B). The whole herd was treated with ivermectin after faecal samples were collected in December of each year. Note that the vertical axis is a squared scale for (A) and cubed for (B).

increase over the winter, but only 5 or fewer of the 30 ($\leq$17%) reindeer calves sampled each month, shed eggs.

Moniezia sp. eggs and coccidian oocysts were regularly detected in the faecal egg counts, but not recorded here.

3.3. Nematode faecal egg counts of adult female reindeer

O. gruehneri was the most abundant nematode parasite of adult female reindeer in 2002 (May–December), 2003 (January–December), and especially in 2004 (January–September) ($p < 0.001$) (Fig. 3A). *Capillaria* sp. eggs were present in very low numbers each year (Fig. 3B). The monthly trends in *O. gruehneri* eggs were similar to those seen in the reindeer calves, and there was no difference in the numbers of *O. gruehneri* eggs shed between calves and adult female reindeer. Nematodirinae eggs were not detected. Adult female reindeer shed fewer *Capillaria* sp. eggs than reindeer calves ($p < 0.001$), in all years sampled. However, the overall mean monthly abundances of *O. gruehneri* and *Capillaria* sp. eggs were regularly below
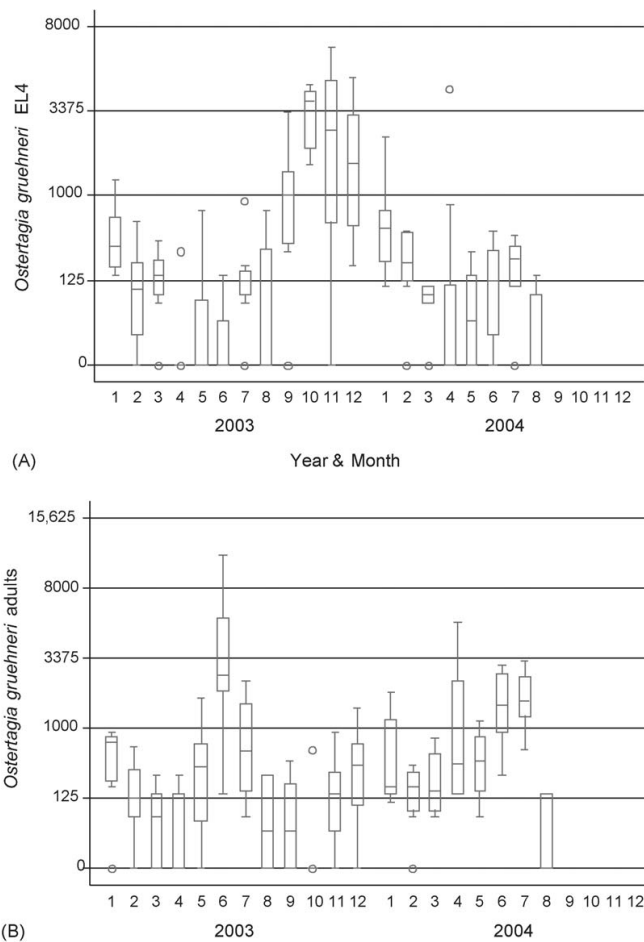
Fig. 4. Box plots of *O. gruehneri* abomasal nematode worm burdens, of 'tracer' reindeer calves ($N = 4$ per month per year) treated with ivermectin 8 weeks before slaughter. The monthly worm burdens represent nematodes newly acquired from pasture up to 5 weeks before culling in 2003 (January–December) and 2004 (January–August): early fourth-stage larvae (A); adult nematodes (B). Note that the vertical axis is cubed for (A) and (B).

the detection limit of 50 eggs per gram irrespective of year sampled, as they were in the calves.

3.4. Tracer worm burdens

The first set of 2 male and 2 female reindeer calves used as tracer animals in January 2003, were approximately 8 months of age. Worm burdens tended to increase with calf age. *Ostertagia gruehneri* was the only species of nematode recovered from the abomasum of tracer animals (Fig. 4). The abundance of adult *O. gruehneri* was not significantly different between male or female reindeer calves between years. Adult *O.*

gruehneri parasites were more abundant from January to July in 2004 than in 2003 (Fig. 4B). The seasonal trend was similar in both years, with the adult parasite winter worm burden significantly lower than the previous and the following summer ($p < 0.001$). The peak in abundance occurred in June of each year, when the tracers were entering their second summer (approximately 12 months of age).

Since *O. gruehneri* was the only species of adult nematode recovered from the abomasum of tracers, immature parasites were assumed to be *O. gruehneri*. In all samples, the overwhelming numbers of larvae were early fourth-stage *O. gruehneri* larvae (EL4).

(A)

(B)
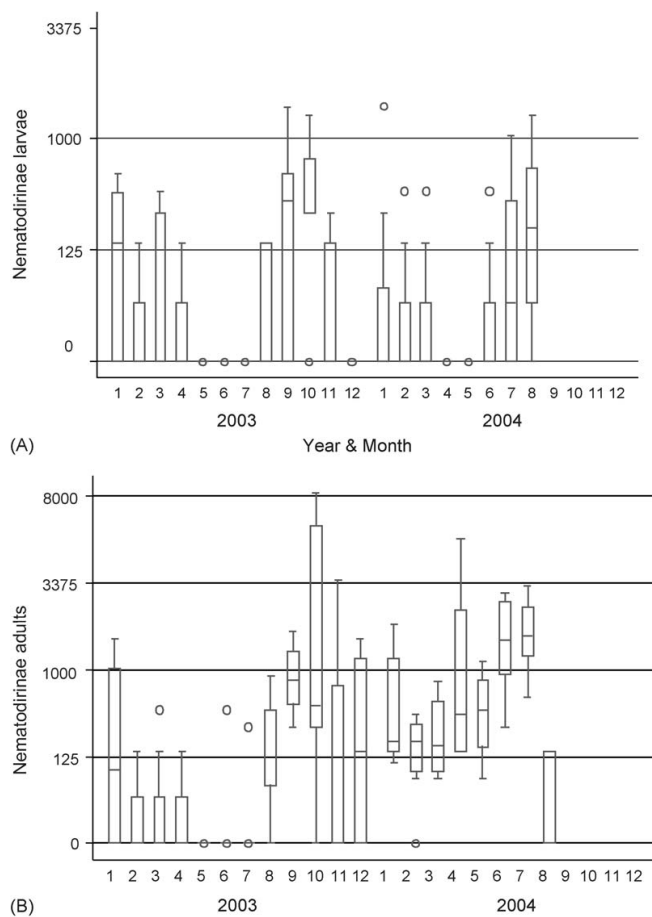
Fig. 5. Box plots of Nematodirinae (*Nematodirus tarandi* and *Nematodirella longispiculata*) nematode worm burdens, from the small intestine of 'tracer' reindeer calves ($N = 4$ per month per year) treated with ivermectin 8 weeks before slaughter. The monthly worm burdens represent nematodes newly acquired from pasture up to 5 weeks before culling in 2003 (January–December) and 2004 (January–August): larvae (A); adult nematodes (B). Note that the vertical axis is cubed for (A) and (B).

The abundance of EL4 did not differ between years and calf gender, but there was a highly significant increase of EL4 in tracers slaughtered during the winter months ($p = 0.002$). Numbers of EL4 noticeably declined in late winter (April) with the onset of warmer weather, and at the same time there was a commensurate increase in the adult *O. gruehneri* (Fig. 4A).

Intestinal nematode infections were all recorded as belonging to the subfamily Nematodirinae (*Nematodirus tarandi* and *Nematodirella longispiculata*). These were not easily differentiated and thus data was analysed at the parasite subfamily level.

The overall abundance of Nematodirinae was significantly higher in 2004 (mean = 780, S.D. 30 worms per tracer calf), than in 2003 (mean = 90, S.D. 40 worms per tracer calf) during the months of January–August ($p < 0.001$). Nematodirinae were recorded in every month, except May 2003, when they were not found. Adult parasites were more abundant than larval stages in every month of the year, with the latter higher in the autumn months (September–October), closely paralleling the trend exhibited by adult stages of these parasites (Fig. 5A). The abundance of Nematodirinae adult nematodes was highly variable between months and years (Fig. 5B). There was no difference in worm burdens between calf gender.

Adult *Moniezia* sp. was found in the small intestine of approximately 25% of the tracer calves. Adult *Dictyocaulus* sp. was recorded in only one of the 80 lungs that were inspected. From the perfusions of these lungs, approximately 70% contained first-stage larvae, some with the characteristic dorsal spine of protostrongylids. Adult stages of these latter parasites may be found in the meninges, the central nervous system, and also in the muscles of reindeer, which were not examined in this study.

4. Discussion

This study is the first to investigate the seasonal dynamics of nematode parasitism of reindeer under a management system that is typical for these semi-domesticated ruminants in Fennoscandia. This was achieved by monthly rectal faecal sampling of calves and adult female reindeer, and estimates of infective larval pickup from pasture, over a 2-year period. For the latter procedure, the tracer animal technique was used, employing young reindeer calves previously treated with ivermectin. This anthelmintic has very high levels of efficacy against nematode parasites of reindeer (Oksanen et al., 1992, 1993) and therefore these animals were assumed to be effectively worm-

free when introduced to the common grazing pasture. The persistence of ivermectin in treated animals prevents the establishment of incoming larvae from establishing in sheep and cattle for up to 25 days. However, it has been found that by 4 weeks, ivermectin has a persistent anthelmintic effect against incoming larvae of other livestock species (sheep: LeJambre et al., 1999; cattle: Vercruysse et al., 2000). Therefore, a grazing period of 7–8 weeks was chosen for each set of tracers to enable the acquisition of infective larvae the opportunity to become established, at least in the latter 4 weeks prior to slaughter. Throughout the study, equal numbers of male and female calves of the same age, derived from the existing herd, were used. This ensured that the tracers were already accepted into the herd structure and thus their pickup of parasites from pasture could be considered to represent that for the herd in general, during these successive tracer grazing intervals.

O. gruehneri was the most abundant parasite in tracer calf worm burdens and in faecal egg counts from adult female reindeer, showing distinct seasonal patterns. There was no difference in abundance of *O. gruehneri* egg counts between male and female calves, or between calves and adult female reindeer. The general trend was an increase during the early part of the grazing season to peak levels in mid/late summer, followed by a decrease to negligible numbers in late autumn/early winter for both calves and adults. With egg counts being very low, with many zero estimates, prior to the routine herd treatment with ivermectin, it was not possible to ascribe the continuing low *O. gruehneri* egg counts in the months following treatment (January/February) to drug effect. The reasons for the natural seasonal decline in egg counts are speculative, but they may be due to the change in feed (supplementary winter feed being provided at the onset of late autumn when the first snow arrived), or the change in the parasite population structure with EL4 becoming more abundant, and the numbers of adult parasites declining, or a natural regulation of egg production by adult female parasites during a time when conditions for the development and survival of free-living stages on pasture are inimical.

These results differ somewhat from studies on the wild reindeer populations of Svalbard in which adult animals shed more *O. gruehneri* eggs than young animals (Irvine et al., 2000). These authors also reported substantial between-year variation in the abundance of this parasite and they speculate that *O. gruehneri* plays a significant role in regulating the Svalbard reindeer population (Irvine et al., 2000).

In our study, arrested larval development of *O. gruehneri* was shown to be a consistent feature in tracer calves during the winter months. This phenomenon is well known for the corresponding species in cattle (*Ostertagia ostertagi*) and sheep (*Ostertagia/Teladorsagia circumcincta*) (for review, see Eysker, 1997). Seasonal inhibition of parasites is generally regarded as a mechanism whereby the parasite species may avoid adverse environmental conditions, and at the same time ensure carry-over from one grazing season to the next. It is surprising therefore that Halvorsen and Bye (1999) stated that arrested development did not play a significant role in winter survival of *O. gruehneri* in the wild reindeer of Svalbard.

Capillaria sp. eggs were shed by calves throughout the year and appeared to be most abundant in the coldest winter months, although the range of output was extremely variable. There was an obvious age related difference in egg output for this species, with generally only a few adult female reindeer shedding eggs and at a level that was lower than calves. *Capillaria* sp. egg counts of calves did not show any change during the winter months around the time of ivermectin treatment. This suggests that this species is relatively refractory to this drug, which is in agreement with Oksanen and Nieminen (1998) who showed a similar lack of high efficacy of ivermectin against *Capillaria* sp. in reindeer. No parasitic stages of *Capillaria* sp. were recovered in the tracer animals. This parasite is located in the distal regions of the small intestine, which were not examined in this study where we used methodology that is of standard practice in many parasitology laboratories. It has been recently reported that low numbers of *Capillaria bovis* were recovered from the contents of the entire small intestine of adult reindeer slaughtered in Iceland (Guðmundsdóttir, 2006).

The Nematodirinae were present year-round in very low numbers in calves, although the prevalence was no more than 20% in the faecal egg counts in any month. There were always a few calves shedding high numbers of eggs when the majority shed few or none. At no time was this parasite found in faecal egg counts of adult female reindeer, which conforms to the findings for the population dynamics of *Nematodirus* spp. in cattle and sheep, where these species parasitize only young animals (Urquhart et al., 1996). Tracer worm burdens showed Nematodirinae larvae were acquired throughout the year although at a very low frequency in the late winter/early spring months, which may be partly explained by the typical early spring hatch of *Nematodirus* spp. eggs (Urquhart et al., 1996). Similar to *O. gruehneri*, inhibited populations of these small intestinal parasites showed a tendency to be higher during the winter months.

Our study showed that the acquisition of infective larvae from pasture occurred in all months of the year, even during the winter period from snow covered frozen ground, which is testimony to the foraging abilities of reindeer during these extreme seasonal conditions of the long northern winters. The low intensities of *O. gruehneri* and Nematodirinae in the 3–7 month old calves of our investigation may be due to their low intake of vegetation since calves were predominantly suckling for the first 4 months of age.

In making comparisons with the Svalbard studies, which represent the only other comprehensive investigation into the seasonal dynamics of nematode parasites of reindeer, it is important to consider differences between the two systems. Firstly, an annual treatment with ivermectin is imposed on the entire semi-domesticated herd of reindeer used in our study. This is a common practise amongst the Finnish reindeer herders, with approximately 80% of reindeer treated in early winter (Oksanen, 1999). This treatment is primarily directed against the larval stages of the warble (*Hypoderma tarandi*) and the throat bot fly (*Cephenemyia trompe*), which are recognized as being economically important, production-limiting insects of reindeer (Oksanen, 1999). This treatment has, of course, an additional benefit against nematode parasites. Secondly, grazing density of semi-domesticated herds, including the Kutuharju herd, would generally be higher than in wild populations. In our investigation the population density was approximately 10 reindeer per km^2 during the summer, whereas the stocking rate of the Svalbard herd was approximately 50% less (Irvine et al., 2000). Thirdly, supplementary feed is not available to wild populations of reindeer on Svalbard, which must compete for forage and experience shortages during most winters. Winter pelleted feed is provided daily in feeding troughs for the Kutuharju herd, and during the summer hay is offered as a supplement to natural browse, thus less time and energy is spent grazing and searching for food.

Our investigation showed that although the abundance of nematode parasite infections in these semi-domesticated reindeer was generally low, the prevalence of infection was rather high. This may be due to the fact that certain areas of the forest are preferred by the herd, leading to local concentration for grazing, faecal contamination, and thus increasing their chances for pick-up of infective stages from pasture. Similar findings of the relatively high prevalence and low abundance of nematode parasites of sheep grazing over very extensive areas have been made in Australia (Pullman et al., 1988).

10   *J.T. Hrabok et al. / Veterinary Parasitology xxx (2006) xxx–xxx*

Although these findings cannot necessarily be extrapolated to the situation regarding nematode infections of the semi-domesticated reindeer throughout Fennoscandia, they do indicate that nematode parasitism is unlikely to be a significant economic problem in reindeer herding co-operatives which practice early winter anthelmintic treatment and supplementary feeding, at the current low population densities of 2.3–2.5 animals per km^2.

The diversity of gastrointestinal nematode parasite species we encountered was low. Only four genera, namely, *Ostertagia, Capillaria, Nematodirus* and *Nematodirella* were recorded. Compared with the diverse fauna of nematode parasites generally encountered in livestock in more equable climatic regions, the harsh natural selection pressures for parasite transmission imposed by the environment of the sub-polar regions indicates that fewer parasite genera have found this a suitable ecological niche to occupy. However, reindeer are susceptible to a range of nematode parasites of other ruminants (sheep and cattle), for which they are not the definitive host (Hrabok et al., 2006). Given these adventitious parasites for reindeer (e.g. *Haemonchus contortus*) are reported in sheep and goats near the Arctic Circle in Fennoscandia (Lindqvist et al., 2001), the view of many reindeer herders in the furthest northern husbandry regions is that pastures should be the preserve of reindeer alone, otherwise the nematode parasites of domestic ruminant livestock could become a problem in the future.

Acknowledgements

References

Albon, S.D., Stien, A., Irvine, R.J., Langvatn, R., Ropstad, E., Halvorsen, O., 2002. The role of parasites in the dynamics of a reindeer population. Proc. R. Soc Lond. B 269, 1625–1632.

Barth, D., 1991. Magen-darm-nematoden des rindes, diagnostischer atlas 284 einzelabbildungen. Ferdinand enke verlag stuttgart, 104 pp. (in German)

Bye, K., 1987. Abomasal nematodes from three Norwegian wild reindeer populations Can. J. Zool. 65, 677–680.

Bye, K., Halvorsen, O., 1983. Abomasal nematodes of the Svalbard reindeer (rangifer tarandus platyrhynchus vrolik). J. Wildl. Dis. 19 (2), 101–105.

Bye, K., Halvorsen, O., Nilssen, K., 1987. Immigration and regional distribution of abomasal nematodes of Svalbard reindeer. J. Biogeogr. 14, 451–458.

Dieterich, R.A., Craigmill, A.L., 1990. Safety, efficacy, and tissue residues of ivermectin in reindeer. Rangifer 10, 53–56.

Dobson, R.J., Waller, P.J., Donald, A.D., 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep; the effect of infection rate on the establishment of infective larvae and parasite fecundity. Int. J. Parasitol. 20, 347–352.

Donald, A.D., Morley, F.H.W., Waller, P.J., Axelsen, A., Donnelly, J.R., 1978. Availability to grazing sheep of gastrointestinal nematode infection arising from summer contamination of pastures. Aust. J. Agric. Res. 29, 189–204.

Durette-Desset, M.C., 1983. Keys to genera of the superfamily Trichostrongyloidea, in: Anderson, R.C., Chabaud, A.G. (Eds.), CIH Keys to the Nematode Parasites of Vertebrates. No. 10. Farnham Royal, Bucks, England, Commonwealth Agricultural Bureaux, 86 pp.

Eysker, M., 1997. Some aspects of inhibited development of trichostrongylids in ruminants. Vet. Parasitol. 72, 265–283.

Fruetel, M., Lankester, M.W., 1989. Gastrointestinal helminths of woodland and barren ground caribou (*Rangifer tarandus*) in Canada, with keys to species. Can. J. Zool. 67, 2253–2269.

Gordon, H.M., Whitlock, H.V., 1939. A new technique for counting nematode eggs in sheep faeces. J. Counc. Sci. Ind. Res. 12, 50–52.

Guðmundsdóttir, B., 2006. Parasites of reindeer (*Rangifer tarandus*) in Iceland. Master of Science degree. Faculty of Medicine, University of Iceland, 100 pp. (in Icelandic)

Halvorsen, O., 1986. Epidemiology of reindeer parasites. Parasitol. Today 2 (12), 334–339.

Halvorsen, O., Bye, K., 1999. Parasites, biodiversity, and population dynamics in an ecosystem in the high arctic. Vet. Parasitol. 84, 205–227.

Halvorsen, O., Stien, A., Irvine, J., Langvatn, R., Albon, S., 1999. Evidence for continued transmission of parasitic nematodes in reindeer during Arctic winter. Int. J. Parasitol. 29, 567–579.

Hrabok, J.T., Oksanen, A., Nieminen, M., Rydzik, A., Uggla, A., Waller, P.J., 2006. Reindeer as hosts for nematode parasites of sheep and cattle. Vet. Parasitol. 136, 297–306.

Irvine, R.J., Stien, A., Halvorsen, O., Langvatn, R., Albon, S.D., 2000. Life-history strategies and population dynamics of abomasal nematodes in Svalbard reindeer (*Rangifer tarandus platyrhynchus*). Parasitol. 120, 297–311.

Leader-Williams, N., 1980. Population dynamics and mortality of reindeer introduced into South Georgia. J. Wildl. Mgmt. 44 (3), 640–657.

LeJambre, L.F., Dobson, R.J., Lenane, I.J., Barnes, E.H., 1999. Selection for anthelmintic resistance by macrocyclic lactones in *Haemonchus contortus*. Int. J. Parasitol. 29, 1101–1111.

Lindqvist, Å., Ljungström, B.L., Nilsson, O., Waller, P.J., 2001. The dynamics, prevalence and impact of nematode parasite infections in organically raised sheep in Sweden. Acta Vet. Scand. 42, 377–389.

Nieminen, M., 2005. Reindeer: semi-domesticated. In: Kulonen, U.M., Seurujärvi-Kari, I., Pulkkinen, R. (Eds.), The Saami. A Cultural Encyclopedia. Suomen Kirjallisuuden Seura, Vammala, pp. 295–299.

Oksanen, A., 1999. Endectocide treatment of the reindeer. Rangifer (Special Issue No. 11), 217 pp.

Oksanen, A., Nieminen, M., 1998. Moxidectin as an endectocide in reindeer. Acta Vet. Scand. 39, 483–489.

Oksanen, A., Nieminen, M., Soveri, T., Kumpula, K., 1992. Oral and parenteral administration of ivermectin to reindeer. Vet. Parasitol. 41, 241–247.

Oksanen, A., Nieminen, M., Soveri, T., 1993. A comparison of topical, subcutaneous and oral administrations of ivermectin to reindeer. Vet. Record. 133, 312–314.

Oksanen, A., Nieminen, M., Soveri, T., Kumpula, K., Heiskari, U., Kuloharju, V., 1990. The establishment of parasites in reindeer calves. Rangifer (Special Issue No. 5), pp. 20–21.

Perry, B.D., Randolph, T.F., 1999. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. Vet. Parasitol. 84, 145–168.

Pullman, A.L., Beveridge, I., Martin, R.R., 1988. Epidemiology of nematode infections of weaner sheep in the cereal zone of South Australia. Aust. J. Agric. Res. 39, 691–702.

Rehbinder, C., Christensson, D., 1977. The presence of gastrointestinal parasites in autumn-slaughtered reindeer bulls. Nord. Vet. - Med. 29, 556–557.

Rehbinder, C., von Szokolay, P., 1978. The presence of gastric parasites in winter-slaughtered reindeer. Nord. Veterinaermed. 30, 214–216.

Stien, A., Irvine, R.J., Langvatn, R., Albon, S.D., Halvorsen, O., 2002a. The population dynamics of *Ostertagia gruehneri* in reindeer: a model for the seasonal and intensity dependent variation in nematode fecundity. Int. J. Parasitol. 32, 991–996.

Stien, A., Irvine, R.J., Ropstad, E., Halvorsen, O., Langvatn, R., Albon, S.D., 2002b. The impact of gastrointestinal nematodes on wild reindeer: experimental and cross-sectional studies. J. Animal Ecol. 71, 937–945.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W., 1996. Veterinary Parasitology, 2nd ed. Blackwell Science Ltd., Oxford, England.

Vercruysse, J., Dorny, P., Clarebout, E., Demeulenaere, D., Smets, K., Agneessens, J., 2000. Evaluation of the persistent efficacy of doramectin and ivermectin injectable against *Ostertagia ostertagi* and *Cooperia oncophora* in cattle. Vet. Parasitol. 89 (1–2), 63–69.
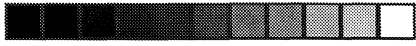
II

Prevalence of gastrointestinal nematodes in winter-slaughtered reindeer of northern Finland

J. T. Hrabok[a*], A. Oksanen[b], M. Nieminen[c], P. J. Waller[a]

[a]Department of Parasitology (SWEPAR), National Veterinary Institute and Swedish University of Agricultural Sciences, SE-751 89 Uppsala, Sweden.
[b]Finnish Food Safety Authority Evira, Oulu Research Unit (FINPAR), P.O. Box 517, FIN-90101, Oulu, Finland.
[c]Finnish Game and Fisheries Research Institute, Reindeer Research Station, FIN-99910 Kaamanen, Finland.

* Corresponding author. Tel.: +46 70454 7032; fax: +46 1867 4304. Email: Jackie.Hrabok@sva.se

Abstract

The objective of this study was to determine the prevalence and intensity of gastrointestinal nematodes in winter-slaughtered reindeer during 2002-2004, from northern reindeer herding cooperatives in Finland. *Ostertagia gruehneri* of the abomasum was prevalent with low levels of infections in 100% of calves (N=53, mean=1300 worms per animal) and in 98% of adults (N=41 of 42, mean=3900 worms per animal). There was no difference in the number of *O. gruehneri* between male and female calves. The proportion of *O. gruehneri* inhibited larvae was significantly higher in calves (81%) than in adult reindeer (39%) ($p =$ 0.005). The intestinal nematodes, *Nematodirus tarandi* and *Nematodirella longissimespiculata,* were detected only in reindeer calves. The numbers of these worms did not differ between male and female calves, but there was a difference in abundance between sites. High prevalence and low intensity of gastrointestinal nematodes characterized the patterns of infection of the reindeer examined in this study. It is assumed that these infections are sub-clinical and would not contribute to productivity losses for reindeer in the northern Finnish reindeer husbandry area, at least not during the study period.

Keywords: semi-domesticated reindeer, parasites, arrested development *Ostertagia gruehneri,* Nematodirinae.

Introduction

Semi-domesticated reindeer represent an important livestock industry in northern Finland. They are likely to be affected by similar productivity constraints as domestic ruminants, such as sheep, goats, and cattle. Many studies have shown that nematode parasitic infections have deleterious effects on animal production, in developing (Perry *et al.,* 2002), as well as developed countries (McLeod, 1995) of the world. Losses due to nematode parasites are generally sub-clinical and primarily result in reduced productivity (Waller, 2005).

There are few areas in Fennoscandia (northern Norway, Sweden, and Finland) where reindeer are free ranging year-round on natural pastures, without overlapping home ranges with domestic ruminants. However, in the extreme north of Finland, reindeer are managed with little or no competition from sheep, goats, or

cattle and they are by far the most abundant ungulate, with only wild moose (*Alces alces*) sharing their environment. In most of the southern reindeer herding cooperatives in Finland, reindeer may be confined in corals during the winter months to receive supplemental feed. During the spring and early summer, it is not uncommon for sheep to occupy the same fenced areas (A. Oksanen & S. Laaksonen personal communications). Hrabok *et al.* (2006a) showed that reindeer are suitable hosts for the common nematodes of sheep (*Haemonchus contortus, Teladorsagia circumcinta*) and cattle (*Ostertagia ostertagi, Cooperia* spp.). Handeland & Sparboe (1991) have also demonstrated that the meningeal nematode of reindeer, *Elapostrongylus rangiferi*, can be naturally transmitted to conventional domestic ruminants, therefore the risk of spread of nematode infection from sheep, goats and/or cattle to reindeer should not be overlooked.

Many northern Sámi reindeer herders utilize traditional methods of herding which vary considerably between cooperatives, dependent upon their size, quality of vegetation, parasite control methods, and risk of predation. One of the most important factors determining the success of the annual reindeer-herding season is climate. Environmental variables such as precipitation, snow depth, insect harassment, temperature, wind, and light/darkness regime directly influence herd management and productivity, however the importance of nematode parasitism is largely unknown.

With the aim of determining the seasonal prevalence of nematode parasites in reindeer of northern Finland, an epidemiological investigation was conducted at the Kutuharju Experimental Reindeer Station, Kaamanen, whereby contamination of pastures was monitored monthly over a 2.5 year period by conducting nematode faecal egg counts on calves and adult female reindeer, as well as assessing pasture infectivity, using the tracer animal technique (Hrabok *et al*., 2006b).

The current study was undertaken to complement the above investigation to determine the prevalence and intensity of naturally occurring gastrointestinal nematode infections in the neighbouring reindeer cooperatives, where the animals are managed according to traditional Sámi herding methods of northern Finland.

Materials and Methods

Study area and animals

This study was conducted in the Sámi Reindeer Husbandry Region in Lapland, Finland, approximately 300-500 km north of the Arctic Circle. Reindeer belonged to 4 discrete herds comprising the Paistunturi, Kaldoaivi, and Muddusjärvi Cooperatives, and the Kutuharju experimental herd (Figure 1). Herds ranged in size from 6,000 - 7,000 reindeer with exception of Kutuharju, which consisted of approximately 200 animals. A post-harvest winter population structure of all herds was approximately 50% adult females, 30% calves, 15% castrated males, and 5% bulls. Grazing areas ranged in size from 2,500 - 3,000 km^2 in the Paistunturi, Kaldoaivi, and Muddusjärvi cooperatives, and 42 km^2 at Kutuharju. Grazing regions were sub-divided into summer and winter pastures, with population densities of 2.3 - 2.5 reindeer per km^2 at Paistunturi, Kaldoaivi, and Muddusjärvi, and 11 reindeer per km^2 at Kutuharju. The herds had a history of being de-wormed

once per year with ivermectin (Ivomec SC® 10 mg/ml vet. inj. Merial; RDR 200 µg/kg, or generic product) between October-December during the winter roundups, for approximately the preceding 7 years. All animals in this study had not received anthelmintics (current season cohort of calves), or were de-wormed the previous winter (~12 months).
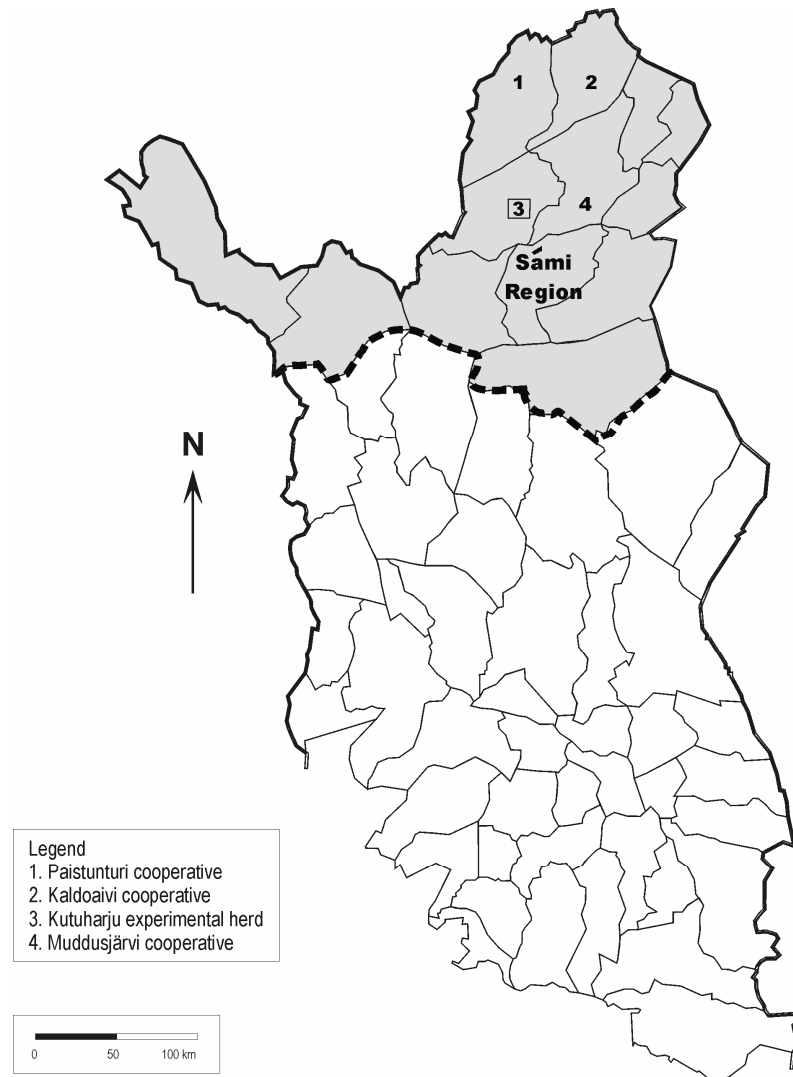
Figure 1. Reindeer herds studied for the prevalence of gastrointestinal nematodes in the Finnish Sámi region. The whole map represents the Finnish reindeer husbandry area divided into cooperatives.

Pastures

The reindeer husbandry areas of the most northern cooperatives (Paistunturi and Kaldoaivi) have distinguishing biological and ecological characteristics from the herds approximately 100-150 km south (Muddusjärvi and Kutuharju), which may potentially affect the transmission of nematode parasites (Kumpula *et al.*, 2004).
In summer, Paistunturi/Kaldoaivi reindeer graze on a tussock tundra/taiga landscape of open rangelands with rolling mountains and birch interspersed with grasses, sedges, and lichen. Marshland areas are limited and the prevailing winds tend to reduce insect harassment. Muddusjärvi and Kutuharju reindeer graze in a pine-dominated forest with numerous swamps and marshlands, where mosquitoes are a common annoyance to reindeer in the summer causing them to aggregate in large herds in sheltered areas. Muddusjärvi summer pasture area is smaller than Paistunturi/Kaldoaivi, and reindeer density is higher.

In winter, Paistunturi/Kaldoaivi reindeer graze on open landscapes consisting of mostly sedges and grasses. Commercial pelleted feed is given throughout the winter to supplement their dietary needs, as lichen is overgrazed in many areas. A few reindeer herding families manage their individual herds as a common group over the winter.

Muddusjärvi cooperative is adjacent to the grazing range of the Kutuharju experimental herd, being separated by a fence, but sharing a similar pine and birch forest with a relatively level aspect. Muddusjärvi winter pastures are generally in better condition than those of the northern cooperatives, attributed to availability of lichen. Reindeer from Muddusjärvi and Kutuharju graze freely in the winter in the pine-dominated forests. Commercial pelleted feed is given to Muddusjärvi reindeer only if adverse climatic conditions (ice layers over the ground, or deep snow) make it difficult for them to access natural vegetation. Reindeer herders and their families also manage their herds together as one larger unit for ease of monitoring their movements and health. Kutuharju reindeer receive commercial feed once per day during the winter from a centrally located feeding trough.

Management

Calves in the two northern cooperatives (Paistunturi and Kaldoaivi) are born in spring-early summer (late April-mid June) on the tundra/taiga without interference from the owners. Earmarking is performed in September when calves are 4 months old. In Muddusjärvi, pregnant reindeer are gathered into fenced enclosures from early April-late June, and usually supplementary fed in May. Calves are usually earmarked a few hours after birth and are monitored daily. The Kutuharju herd is inspected daily, with monthly gathering for sampling and weighing, as part of a long-term research project. Kutuharju calves are born in a large enclosure on pasture, earmarked within 24 hrs of birth and monitored daily. They remain with their mothers in a fenced area of forest until the autumn, thereafter released to join the entire herd.

4

Slaughter of animals and collection of viscera

The choice of animals for slaughter was based entirely on the owners' decisions. Culling occurred between October 2002 and January 2004. Animals were consigned to the regional reindeer slaughterhouse in Kaamanen, northern Finland. The abomasums and small intestines were obtained from this facility, together with trace-back information (origin) of the animals, as well as their sex and age. Additionally, post-mortem material was obtained from the Muddusjärvi reindeer herding cooperative, Tsiuttajoki station, at the time of winter roundups (Table 1).

| | | Calves | | | | | | Adults | | | | | |
| | | Abomasum | | | Small Intestine | | | Abomasum | | | Small Intestine | | |
Winter[a]	Herd	Male	Female	*Total*	Male	Female	*Total*	Male	Female	*Total*	Male	Female	*Total*
2002-2003	Kaldoaivi	13	11	24	6	4	10	2	1	3	0	0	0
	Paistunturi	4	5	9	4	6	10	0	0	0	0	0	0
	Muddusjärvi	0	0	0	0	0	0	2	0	2	0	0	0
	Kutuharju	6	7	13	6	7	13	3	0	3	0	0	0
2003-2004	Kaldoaivi	0	0	0	0	0	0	14	3	17	0	0	0
	Paistunturi	0	0	0	0	0	0	0	0	0	0	0	0
	Muddusjärvi	0	0	0	0	0	0	10	0	10	9	1	10
	Kutuharju	4	3	7	4	3	7	0	8	8	0	0	0
	Subtotal	27	26	53	20	20	40	31	12	43	9	1	10

[a]October-January 2003, December-January 2004

Table 1. Inventory of biological samples examined for gastrointestinal nematodes from reindeer calves and adults from 4 herd in northern Finalnd

Parasitological procedures

The viscera (abomasum and the proximal 7-10 m of small intestine) were processed according to Donald *et al*., (1978). Two 20 ml (with exception for October and November 2002, in which 30 ml sub-samples were taken) replicate sub-samples were taken from each abomasal (3 L) and small intestinal contents (3 L), and mucosal digest (1 L), and preserved by freezing at -18°C (Dobson *et al*., 1990). When required for worm counting and species differentiation, the 20 ml sub-samples were thawed on a 30μm sieve using a gentle stream of warm tap water. All material, including nematodes retained on the sieve, were back-washed into a beaker, stained with Lugol's iodine and examined with a stereomicroscope and sub-phase illumination at 16-20 X. Early fourth-stage larvae (EL4) were identified at 400 X using compound light microscopy. An Olympus digitizer DP50 3.0 and Soft Imaging System AnalySIS 3.1 software was used to distinguish between nematode taxa with the use of scientific keys (Durette-Desset, 1983; Barth, 1991).

Results

Worm burdens of reindeer calves

Abomasal nematodes were recorded in all calves (N=53) slaughtered (see Table 2). Only adults of one *Trichostrongylus* species were recovered and identified as *Ostertagia gruehneri* based on morphological characteristics, and therefore we assumed that the larvae of the abomasal contents and digests also belonged to this species.

Although *O. gruehneri* was statistically more abundant during the winter of 2002-03 in Kaldoaivi than in the other herds (p<0.001), the difference between means of overall worm burdens was too low to ascribe this as being biologically significant to affect reindeer health. There was no difference in the number of *O. gruehneri* between male and female calves, and the majority of the worm population (~80%) were in the early fourth larval (EL4) stage of development. Similar findings were found in the Kutuharju animals slaughtered in 2003-2004.

Winter[a]	Herd	Calf Gender	Intensity of *O. gruehneri* Larvae	Intensity of *O. gruehneri* Adults	Prevalence	% of early fourth-stage larvae	Density of Reindeer
2002-2003	Kaldoaivi	Male	1010 (400-1933)[b]	217 (50-750)	100 (13/13)[c]	86 (13133/2167)[d]	2,5[e]
		Female	1073(200-1967)	140 (75-267)	100 (11/11)	90 (11808/1258)	
	Paistunturi	Male	633 (367-967)	144 (33-200)	100 (4/4)	85 (2533/433)	2,3
		Female	500 (267-800)	75 (50-100)	100 (5/5)	94 (2500/150)	
	Total for Northern Herds	Male	922 (367-1933)	200 (33-750)	100 (17/17)	86 (15667/2600)	
		Female	894 (200-1967)	128 (50-267)	100 (16/16)	91 (14308/1408)	
		All calves	908 (200-1933)	167 (33-750)	100 (33/33)	88 (29975/4008)	
	Kutuharju	Male	968 (83-1700)	346 (200-917)	100 (6/6)	74 (5808/2075)	11
		Female	555 (33-1450)	212 (100-300)	100 (7/7)	72 (3883/1483)	
		All cavles	746 (33-1700)	160 (100-917)	100 (13/13)	73 (9692/3558)	
2003-2004	Kutuharju	Male	1881 (1400-2775)	863 (325-1525)	100 (4/4)	69 (7525/3450)	11
		Female	1867 (1600-2150)	517 (350-600)	100 (3/3)	78 (5600/1550)	
		All calves	1875 (1400-2775)	714 (325-1525)	100 (7/7)	72 (13125/5000)	

[a]October-January 2003 and December-January 2004
[b]Mean (range)
[c]Percent of population infected (no. of infected animals/animals sampled)
[d]Percent of larvae in sample (larvae/adult)
[e]Animals/km^2

Table 2. Intensity, prevalence, and percentage of larvae, of *Ostertagia grueherni* from the abomasum (contents + lumen) of reindeer calves

Intestinal nematode infections belonged to the family Nematodirinae (*Nematodirus tarandi* and *Nematodirella longissimespiculata),* which could not be easily differentiated into species, thus considered together (see Table 3). Overall, the prevalence of infection during winter 2002-03 was higher in the Kutuharju calves (90%; N = 12 of 13) than the other herds (45%; 9 of 20). There was no significant difference in the abundance of Nematodirinae between male and female reindeer calves, and approximately 30% of this worm burden occurred as EL4. Worm burdens of calves slaughtered in 2003-2004 were similar to the previous year.

Winter[a]	Herd	Calf Gender	Intensity of Nematodirinae		Prevalence	% of early fourth-stage larvae	Density of Reindeer
			Larvae	Adults			
2002-2003	Kaldoaivi	Male	150[b]	300	33 (2/6)[c]	20 (150/600)[d]	2,5[e]
		Female	225 (150-300)	875 (150-1600)	50 (2/4)	20 (450/1750)	
	Paistunturi	Male	100	300	25 (1/4)	25 (100/300)	2,3
		Female	433 (200-600)	425 (200-700)	67 (4/6)	43 (1300/1700)	
	Total for Northern Herds	Male	125 (100-150)	300	30 (3/10)	22 (250/900)	
		Female	350 (150-600)	550 (150-1600)	60 (6/10)	36 (1750/3300)	
		All calves	214 (100-600)	483 (150-1600)	45 (9/20)	26 (1500/4350)	
	Kutuharju	Male	450 (100-1150)	779 (375-1300)	100 (6/6)	32 (2250/4675)	11
		Female	300 (50-650)	917 (550-1850)	86 (6/7)	25 (1800/5500)	
		All calves	368 (50-1150)	848 (375-1850)	92 (12/13)	28 (4050/10175)	
2003-2004	Kutuharju	Male	338 (225-450)	1400 (300-2475)	75 (3/4)	14 (675/4200)	11
		Female	1238 (1200-1275)	1763 (1725-1800)	67 (2/3)	41 (2475/3525)	
		All calves	788 (225-1275)	1545 (300-2475)	71 (5/7)	29 (3150/7725)	

[a]October-January 2003 and December-January 2004
[b]Mean (range)
[c]Percent of population infected (no. of infected animals/animals sampled)
[d]Percent of larvae in sample (larvae/adult)
[e]Animals/km^2

Table 3. Intensity, prevalence, and percentage of larvae, of Nematodirinae from the small intestine of reindeer calves

Worm burdens of adult reindeer

Ostertagia gruehneri was found in all but one animal of the 43 processed. Overall, *O. gruehneri* was statistically more abundant in adult reindeer (mean=3900, N=43) than in calves (mean=1300, N=52) (*p*<0.001), but the percentage of EL4 was significantly less in adult reindeer (mean=40%) than in calves (mean = 80%) (p=0.005) (see Table 4). No parasites were found in the small intestine of adult reindeer.

Winter[a]	Herd	Intensity of *O. gruehneri* Larvae	Adults	Prevalence	% of early fourth-stage larvae	Density of Reindeer
2002-2003	Kaldoaivi	956 (50-1500)[b]	839 (150-1467)	100 (3/3)[cd]	53 (2867/2517)[e]	2,5[f]
	Muddusjärvi	338 (125-550)	2775 (300-5250)	100 (2/2)	11 (675/5550)	2,4
	Kutuharju	3211 (1033-4400)	1278 (500-2000)	100 (3/3)	72 (9633/3833)	11
2003-2004	Kaldoaivi	418 (25-1675)	1855 (75-3975)	94 (16/17)[g]	17 (5850/27825)	2,5
	Muddusjärvi	1808 (725-3625)	4215 (2125-7175)	100 (10/10)[h]	30 (18075/42150)	2,4
	Kutuharju	3031 (1375-5900)	3156 (1425-9050)	100 (8/8)[i]	49 (24250/25250)	11

[a]October-January 2003 and December-January 2004
[b]Mean (range)
[c]Percent of population infected (no. of infected animals/animals sampled).
[d]One of 1 is an infected adult female reindeer
[e]Percent of larvae in sample (larvae/adult)
[f]Animals/km^2
[g]Two of 3 are infected adult female reindeer
[h]One of 1 is an infected adult female reindeer
[i]Eight of 8 are infected adult female reindeer

Table 4. Intensity, prevalence, and percentage of larvae, of *Ostertagia grueherni* from the abomasum (contents + lumen) of adult reindeer

Discussion

This study showed the ubiquitous presence of the abomasal parasite *O. gruehneri* amongst the reindeer herds of northern Finland. All calves (N=52) and all except one adult (N=42) reindeer were infected with this parasite at the time of winter slaughter. Reindeer are normally herded with group sizes of a few hundred individuals, up to occasionally thousands especially during the spring, autumn, and winter roundups. Although they have access to extensive grazing ranges, they are selective feeders, often returning to previously grazed areas where quality of vegetation is high and plentiful. This results in local concentration of dung, containing free-living stages of parasites, and subsequently a greater opportunity for the animals to become initially infected (or re-infected) once they return to such areas. Lankester & Petersen (1996) showed that although the prevalence of *Parelaphostrongylus tenuis* was 79% in wild white-tailed deer fawns (density of 4 animals per km^2), the infective stage of the parasite was prevalent in only 1% of the gastropod intermediate hosts (2.4 snails/slugs per km^2). Based on a daily dry weight food requirement, it would only take 23 days for a white-tailed deer to ingest one infected terrestrial gastropod from pasture. These inferences on food consumption and extremely low density of larvae on pasture could also be extrapolated to the environmental conditions under which reindeer graze, and the chance of them acquiring infective *O. gruehneri* larvae from pasture, with high prevalence and low intensity as in our study. This finding is in accord with Hrabok *et al.* (2006a), who found that previously worm-free 'tracer' calves allowed only 4-6 weeks access to reindeer pastures, together with a free-ranging but more intensively stocked herd, all acquired infections with *O. gruehneri,* irrespective of the season.

8

Inhibited development of *O. gruehneri* at the EL4 stage was also a feature in the worm counts of animals of this study. Inhibition of larval development is a well-described phenomenon for a range of important nematode parasites of livestock (for review see Eysker, 1997) and is generally attributed to an environmental trigger, which enables the parasite to survive within the host at times when conditions for survival in the external environment are unfavourable. Also, by undergoing arrested development, this prevents the development of parasites to egg-laying adult populations during adverse climatic conditions, exemplified by the arctic winter. The trigger for resumption of development is generally attributed to the stress associated with parturition in the adult female animal (O'Sullivan & Donald, 1970; 1979). In this study, the proportion of *O. gruehneri* arrested in development was higher in reindeer calves (approx. 80%) compared to adult animals (approx. 40%), with the overall abundance of *O. gruehneri* populations being statistically higher in adult reindeer than in the calves. The total mean worm burden of *O. gruehneri* in adult reindeer, which were predominantly four to seven year old castrated bulls, was approximately 3900 worms per animal compared to 1300 worms per animal for an equal number of calves. However, these worm burdens are very low for grazing ruminants allowed long-term exposure on pasture, particularly when compared with infections acquired by sheep, goats, or cattle raised under much more intensive livestock conditions (for reviews, see Michel, 1976; Barger, 1982). It is also important to note that the detection limit of our parasitological sampling technique used in this study, equated to one worm recovered from the abomasum contents, representing a total burden of 150 worms. Thus, biologically speaking, there may well be no difference in the abundance of worms found between adult and calf reindeer in our study. Regarding arrested development of *O. gruehneri* larvae, extensive studies on the closely related *Ostertagia/Teladorsagia* species in other ruminant livestock species show that arrested larvae can accumulate to massive numbers in adult cattle (*O. ostertagi:* Eysker, 1993) and in sheep (*T. circumcincta:* Waller *et al.*, 2004). Given that the annual treatment of adult reindeer with ivermectin during the preceding winter would have effectively removed any *O. gruehneri* populations at this time (Oksanen *et al.,* 1992), the worm burdens that were recorded in these animals would have been acquired since the previous winter anthelmintic treatment. Our findings of higher worm burdens in adult reindeer compared to calves support the theories of Halvorsen (1986) and Irvine *et al.* (2000), who claimed that adult reindeer do not appear to mount immunity against this parasite until they are approximately 5 years of age. Thus, *O. gruehneri* populations accumulate with the age of reindeer unless they are eliminated with anthelmintics. If the latter is the case (as is the common management practice in early winter), following on from a short period in which re-infection is prevented due to drug persistence in the animal, infections will be re-acquired in the overwintered reindeer, certainly commencing at a time before calves are born and commence to graze. Higher abomasal worm burdens in adults, rather than in calves, were also seen in wild Norwegian reindeer (Bye, 1987). This is likely due to the fact that calves are not born until considerably later than the time when anthelmintic persistence has disappeared in the treated animals, and that for some months after birth reindeer calves derive most of their nourishment from milk. Nematodirinae (*Nematodirus*

tarandi and *Nematodirella longissimespiculata)* were not detected in adult reindeer. This finding agrees with the dynamics of *Nematodirus* spp. in other ruminant livestock species, which is restricted to young animals that rapidly mount a strong immune response if the infection is of low intensity (Urquhart *et al.*, 1996).

Overall, these findings support the conclusions of Hrabok *et al.* (2006b), that although gastrointestinal nematode parasites of reindeer in northern Finland are common, they are not likely to be of sufficient abundance to have an economic impact on the reindeer industry. Should the current herding management change, e.g. by altering the reindeer population density, quality of pastures, increasing interaction with domestic livestock, time spent in corrals for supplemental winter feeding, and/or winter anthelmintic treatment of herds, then this matter must be re-evaluated.

Acknowledgements

References

Barger, I.A. 1982. Helminth parasites and animal production. *In:* L.E.A. Symons, A.D. Donald and J.K. Dineen (eds). *Biology and Control of Endoparasites.* Academic Press, Sydney, NSW, Australia. pp. 133-155.

Barth, D. 1991. *Magen-darm-nematoden des rindes, diagnostischer atlas 284 einzelabbildungen.* Ferdinand enke verlag stuttgart, 104 pp. (in German).

Dobson, R.J., Waller, P.J., & Donald, A.D. 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep; the effect of infection rate on the establishment of infective larvae and parasite fecundity. *International Journal for Parasitology 20*: 347-352.

Donald, A.D., Morley, F.H.W., Waller, P.J., Axelsen, A., & Donnelly, J.R. 1978. Availability to grazing sheep of gastrointestinal nematode infection arising from summer contamination of pastures. *Australian Journal of Agricultural Research 29*: 189-204.

Durette-Desset, M.C. 1983. Keys to genera of the superfamily Trichostrongyloidea. *In:* Anderson, R.C., Chabaud, A.G. (Eds.), *CIH keys to the nematode parasites of vertebrates.* No. 10. Farnham Royal, Bucks, England, Commonwealth Agricultural Bureaux, 86 pp.

Eysker, M. 1993. The role of inhibited development in the epidemiology of *Ostertagia* infections. *Veterinary Parasitology 46*: 259-269.

Eysker, M. 1997. Some aspects of inhibited development of trichostrongylids in ruminants. *Veterinary Parasitology 72*: 265-283.

Halvorsen, O. 1986. Epidemiology of reindeer parasites. *Parasitology Today* 2: 334-339.

Handeland, K., & Sparboe, O. 1991. Cerebrospinal Elaphostrongylosis in dairy goats in Northern Norway. *Journal of Veterinary Medicine B 38*: 755-763.

Hrabok, J.T., Oksanen, A., Nieminen, M., Rydzik, A., Uggla, A., & Waller, P.J. 2006a. Reindeer as hosts for nematode parasites of sheep and cattle. *Veterinary Parasitology 136*: 297-306.

Hrabok, J.T., Oksanen, A., Nieminen, M., & Waller, P.J. 2006b. Population dynamics of gastrointestinal nematodes of reindeer in the sub-arctic. *Veterinary Parasitology* (in press).

Irvine, R. J., Stien, A., Dallas, J.F., Halvorsen, O., & Langvatn, R. 2001. Contrasting regulation of fecundity in two abomasal nematodes of Svalbard reindeer (*Rangifer tarandus platyrhynchus*). *Parasitology 122*: 673-681.

Kumpula, J., Colpaert, A., Anttonen, M., & Nieminen, M. 2004. The repeated reindeer pasture inventory in the northernmost part (13 districts) of the reindeer management area during 1999-2003. Kala- ja riistaraportteja nro 303. Riistan- ja kalantutkimus, 39 pp. (in Finnish).

Lankester, M.W., & Peterson, W.J. 1996. The potential of wintering yards in the transmission of *Parelaphostrongylus tenuis* to white-tailed deer and moose. *Journal of Wildlife Diseases 32*:31-38.

McLeod, R.S. 1995. Costs of major parasites to the Australian livestock industries. *International Journal for Parasitology 25*:1363-1367.

Michel, J. F. 1976. The hazard of nematode infections as a factor in the management of cattle. *Agricultural Development and Advisory Services 20*: 162-177.

Oksanen, A., Nieminen, M., Soveri, T., & Kumpula, K. 1992. Oral and parenteral administration of ivermectin to reindeer. *Veterinary Parasitology 41*: 241-247.

O'Sullivan, B.M., & Donald, A.D. 1970. A field study of nematode parasite populations in the lactating ewe. *Parasitology 61*: 301-315.

O'Sullivan, B.M., & Donald, A.D: 1973.Responses to infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in ewes of different reproductive status. *International Journal for Parasitology 3*: 521-530.

Perry, B.D., Randolph, T.F., Mc Dermott, J.J., Sones, K.R., & Thornton, P.K. 2002. Investing in Animal Health Research to Alleviate Poverty. ILRI (International Livestock Research Institute), Nairobi, Kenya, 148 pp.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., & Jennings, F.W. 1996. *Veterinary Parasitology*. Blackwell Science, Oxford, 1996, 307 pp.

Waller, P.J. 2005. Domestication of ruminant livestock and the impact of nematode parasites: possible implications for the reindeer industry. *Rangifer 25*: 39-50.

Waller, P.J., Rudby-Martin, L., Ljungström, B.L., & Rydzik, A. 2004. The epidemiology of abomasal nematodes of sheep in Sweden, with particular reference to overwinter survival strategies. *Veterinary Parasitology 122*: 207-220.

III
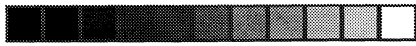
ELSEVIER

Reindeer as hosts for nematode parasites of sheep and cattle

J.T. Hrabok [a,*], A. Oksanen [b], M. Nieminen [c], A. Rydzik [a],
A. Uggla [a], P.J. Waller [a]

[a] Department of Parasitology (SWEPAR), National Veterinary Institute and Swedish University of Agricultural Sciences,
SE-751 89 Uppsala, Sweden
[b] National Veterinary and Food Research Institute, Oulu Regional Unit, P.O. Box 517, FIN-90101, Oulu, Finland
[c] Finnish Game and Fisheries Research Institute, Reindeer Research Station, FIN-99910 Kaamanen, Finland

Abstract

The reindeer husbandry range of Scandinavia overlaps with sheep, goat, and cattle pastures. The aim of this study was to determine whether reindeer are suitable hosts for ovine or bovine nematode parasites, and thus may spread these parasites into the reindeer husbandry regions. To render worm-free, twelve 4-month-old male reindeer calves, six lambs, and six bovine calves were given ivermectin at 200 μg/kg body weight. Five weeks post-treatment, six reindeer calves were each artificially dosed with 10,000 third-stage larvae (L3) of gastrointestinal nematodes derived from sheep, and an additional six reindeer with L3 derived from cattle. Lambs and bovine calves received the same dose of ovine and bovine larvae as reindeer, from the same larval source, respectively. Faecal samples collected on five occasions after the larval dosing revealed that by the fourth week, all reindeer calves, lambs, and bovine calves were infected. Animals were slaughtered on days 40 (reindeer) or 47 (lambs and bovine calves) after the larval dosing. Reindeer calves were most susceptible to L3 derived from sheep. The overall mean intensity of *Haemochus contortus*, *Trichostrongylus axei*, and *Teladorsagia circumcincta*, did not differ between reindeer and sheep; however, early fourth-stage larvae of *H. contortus* were more abundant in reindeer ($p = 0.002$). The establishment of bovine-derived *Ostertagia ostertagi* was similar in reindeer (62%) and bovine calves (57%), but larval inhibition was much higher in reindeer (91%, $p < 0.001$) than in cattle (31%). Very poor establishment of bovine derived *Cooperia oncophora* was recorded in reindeer calves (2%) compared with bovine calves (59%). These results show that young reindeer are susceptible hosts to the important gastrointestinal parasites of sheep (*T. circumcincta*, *H. contortus*) and cattle (*O. ostertagi*), as well as being a suitable host for *T. axei*.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Reindeer; Cattle; Sheep; Reservoir hosts; Nematodes

1. Introduction

The reindeer genus *Rangifer* consists of only a single species (*Rangifer tarandus*), which is separated into eight subspecies encompassing the wild caribou of the Nearctic region (North America and Greenland)

---

* Corresponding author. Tel.: +46 70 454 7032;
fax: +46 18 67 4304.
E-mail address: Jackie.Hrabok@sva.se (J.T. Hrabok).

and reindeer of the Palearctic region (Eurasia) of the Northern Hemisphere (Roed, 2005). The world *Rangifer* population is estimated at 8 million; half of them semi-domesticated and approximately 20% of these are located in Fennoscandia (northern Finland, Norway and Sweden) and 75% in Russia (Oksanen, 1999). Within Scandinavia, many foraging ranges of reindeer overlap with conventional ruminant live-stock, such as sheep, goat, and cattle. Being by nature a gregarious and semi-migratory animal, the stocking rate of reindeer can intermittently exceed the carrying capacity of pastures in specific localities used by conventional livestock (Kumpula et al., 2002; Moen and Danell, 2003). These facts indicate that the opportunity for transmission of pasture-borne para-sites between domestic ruminant species and reindeer is possible.

Limited information is available on the cross-transmission potential of nematode parasites of reindeer to domestic ruminant species, or vice-versa. Most work has focused on the protostrongylid nematodes (*Elaphostrongylus* spp.) responsible for cerebrospinal elaphostrongylosis in wild cervids, reindeer, sheep, goats, and cattle (for review see: Steen et al., 1998; Handeland and Sparboe, 1991; Handeland and Slettbakk, 1995). Literature concern-ing gastro-intestinal nematodes has been limited to artificially infecting sheep and cattle with infective nematode larvae derived from reindeer (Borgsteede, 1982), and studies where viscera were examined from wild reindeer or caribou which had grazed on areas shared with sheep in northern Norway (Bye, 1987), and with sheep and goats in eastern Canada (Ball, 2000). There have been no studies, which examine the infectivity potential of sheep and cattle nematode parasites in reindeer, in relation to their establishment in their definitive hosts. This study was designed to investigate these issues.

2. Materials and methods

2.1. Animals

Six Gotland breed male sheep aged approximately 6 months, and six Swedish black and white male bovine calves aged approximately 4 months, were used in this study. Animals were obtained from commercial sources and transported to the Swedish University of Agricultural Sciences (SLU), Uppsala, September 2004. Sheep and cattle were treated with ivermectin (Ivomec® Merial (200 μg/kg; oral—sheep, subcutaneous injection—cattle) on 24 and 30 September, respectively. Animals were maintained on concrete floored group pens to preclude the possibility of re-infection, and fed concentrates and hay ad libitum from the day of arrival at SLU.

Twelve male reindeer calves, born mid-May 2004, were obtained from the Finnish Reindeer Herders' Association experimental herd, Kaamanen, northern Finland. Animals were treated with the recommended dose of injectable ivermectin (200 μg/kg) for reindeer 20 September, and transferred to the zoological garden at Oulu University, Finland, 24 September 2004. Reindeer were held in two outdoor group enclosures with a gravel substrate base and fed commercially manufactured reindeer fodder.

Prior to ivermectin treatment on 20 September, the 4–5-month-old reindeer calves were free-ranging on 40 km² fenced alpine forest with approximately 200 other mixed age–sex classes of reindeer. Of the gastrointestinal nematodes, calves could potentially have been exposed to natural pasture-borne infections of *Ostertagia gruehneri*, *Nematodirus*, and *Capillaria*. Sheep and cattle livestock had never occupied the land, but wild moose *Alces alces* were occasionally sighted.

2.2. Source and administration of infective larvae

Ovine infective larvae (L3) were obtained from bulk cultures of faeces derived from several sheep farms located in central and southern Sweden during the summer of 2004. Larvae were stored in tap water in tissue culture flasks at 5 °C. Water was replaced every second week, and on the day of dosing >95% motility was observed for the L3s. Morphological differentia-tion of the L3s showed that the species composition was approximately 42% *Haemonchus contortus*, 48% *Trichostrongylus* spp./*Teladorsagia circumcincta*, and 10% *Nematodirus* sp.

Bovine L3 were obtained from bulk-cultured cattle faeces derived from a commercial beef farm located in Södermanland, Sweden during the spring and summer of 2004. Larvae were treated in a similar fashion as the sheep L3 (above), and when required for the

experiment, >90% motility was observed. Based on morphometrics, it was estimated that the population consisted of 45% *Ostertagia ostertagi* and 55% *Cooperia* sp.

From each source, the number of L3 were estimated in a bulk aqueous suspension, and the volume containing 5000 L3 was withdrawn and placed into individual plastic tubes, and stored at 5 °C. Larval doses destined for administering to the reindeer were transported by plane from SLU to Oulu University. Dosing took place on two occasions, 2 days apart (27 and 29 October for reindeer, and 26 and 28 October for sheep and cattle), with the first dose given 5 weeks after the ivermectin treatment. Dosing was achieved by means of a syringe with an oral dosing attachment designed for young ruminant livestock. Each animal received a total of 10,000 L3 (5000 L3 on each of the two dosing occasions). Two operators were used in each procedure, one to restrain the animal and the other responsible for the dosing. Thus in total, six sheep were dosed with sheep-derived L3; six reindeer with sheep-derived L3; six cattle with cattle-derived L3; and six reindeer with cattle-derived L3.

2.3. Parasitological procedures

To assess anthelmintic treatment efficacy, faecal samples were collected from reindeer 17 and 39 days after ivermectin treatment, and 10 and 12 days, respectively, from sheep and cattle. Faecal samples were collected on six occasions (five for reindeer) from all animals commencing 3 weeks after the first day of larval dosing, for individual nematode faecal egg counts. A modified McMaster technique (Gordon and Whitlock, 1939) was used to estimate the density of nematode eggs in faeces with the minimum detection level of 50 eggs/g (EPG) faeces for sheep and cattle, and 25 EPG for reindeer.

All animals were consigned to local slaughter-houses on days 40 (reindeer) and 47 (sheep and cattle) post-L3 dosing. Their viscera (abomasum and small intestine) were collected and processed according to Donald et al. (1978) and the abomasal mucosa digestion procedures followed Dobson et al. (1990). Total gut contents, abomasum and small intestine, were each adjusted to volumes of 2, 3, and 4 L for sheep, reindeer, and cattle, respectively. For abomasal digests, total volumes were 2 L for sheep and cattle,

and 1 L for reindeer. Four 20 mL replicate sub-samples were taken from each abomasal and small intestinal contents, and mucosal digest, and preserved by freezing at −18 °C (Dobson et al., 1990). A gross examination of the caecum was performed only on reindeer viscera, and all contents were washed through a 2.5 mm sieve. When required for worm counting and species differentiation, 20 mL sub-samples were thawed and sifted through a 30 μm sieve using a gentle stream of warm tap water. All nematodes retained on the sieve were stained with Lugols iodine and examined with sub-phase illumination at 16–20× with a stereomicroscope. Representative adult and larval nematodes were mounted in polyvinyl lacto-phenol (PVL) and identified at 1000× with oil immersion using compound light microscopy. The keys used for adult parasite identification were according to Durette-Desset (1983) and Barth (1991) and for EL4 Anonymous (1986). Infective larvae were differentiated using the keys of Borg-steede and Hendriks (1974) and van Wyk et al. (2004). Replicate worm counts were conducted on all abomasal contents, digests, and small intestine samples. Adult *O. ostertagi* recovered from sub-samples from cattle and reindeer abomasums infected with bovine L3, were identified with light phase and fluorescence microscopy, and measured using an Olympus digitizer DP50 3.0 and Soft Imaging System AnalySIS 3.1 software.

2.4. Statistical procedures

Differences in mean intensity of nematodes per host were tested using a Student's *t*-test on logarithmically transformed data corrected for small sample sizes and the assumption of unequal population variances. A probability level of <0.05 was considered significant for all statistical tests.

3. Results

3.1. Faecal egg counts

Faecal samples collected 17 and 39 days after ivermectin treatment showed the presence of *Capillaria* sp. eggs in two of 12 reindeer and *Nematodirus* sp. in three animals. Trichostrongylid eggs were not

detected in reindeer on these two sampling occasions. Analysis of faecal samples from cattle and sheep collected on days 10 and 12 following ivermectin treatment showed no presence of nematode eggs in any of the animals.

All cattle recorded positive nematode faecal egg counts 25 days after dosing with bovine L3, with a mean egg count of 175 with range 50–300 eggs/g (EPG) faeces. Subsequently, the egg counts remained positive and increased, but no more than 500 EPG were detected on any occasion (Fig. 1A). During the 40-day period following experimental transmission of bovine L3 to reindeer, all reindeer excreted trichostrongylid eggs in low quantities (25–100 EPG), on at least one faecal sampling occasion (Fig. 1B). Trichostrongylid eggs were first detected in one reindeer (25 EPG) 24 days after the dosing

with bovine L3. At 40 days after larval dosing, five of the six reindeer shed trichostrongylid eggs immediately prior to slaughter. *Capillaria* sp. was the most abundant species and was present on every sampling date in at least 50% of the reindeer. All reindeer shed *Capillaria* sp. eggs with a mean of 71 (25–250) EPG, on multiple occasions during the 40-day period after dosing with bovine L3. Eggs of *Nematodirus* sp. were detected in two reindeer, with one animal shedding eggs on two occasions. Overall, mean nematode egg counts were approximately 3-fold less in reindeer compared with cattle ($\bar{x}$ = 78 versus 268 EPG).

All sheep receiving ovine derived L3 had positive faecal egg counts 25 days post-L3 dosing ($\bar{x}$ = 1617 (400–3350) EPG). On the subsequent five sampling occasions, all animals remained positive with egg
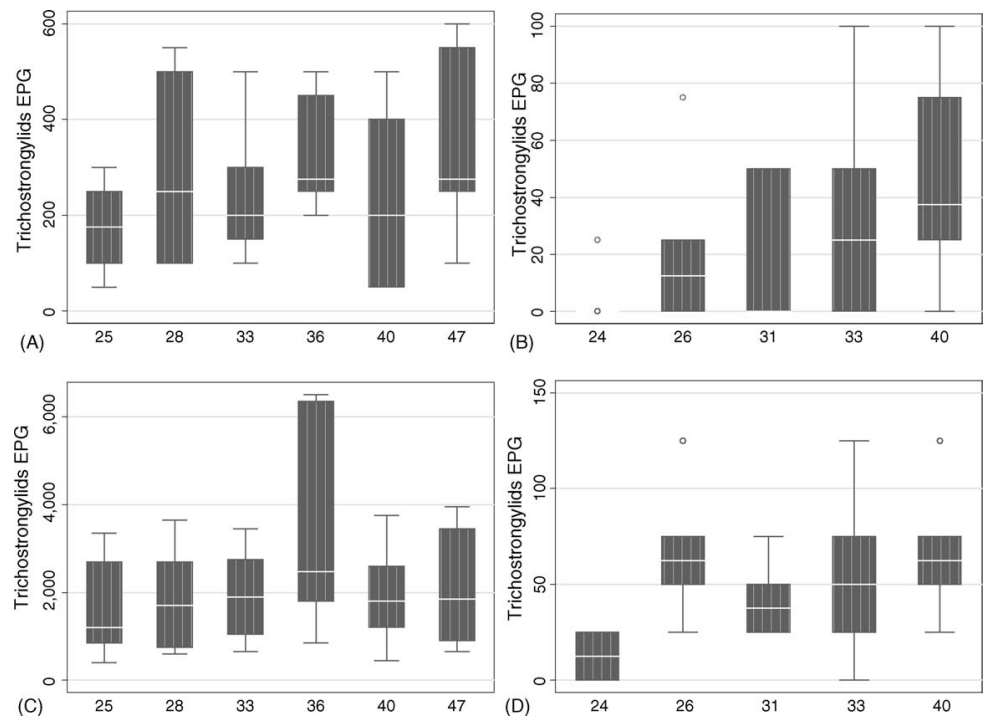
Fig. 1. Trichostrongylid nematode faecal egg counts from cattle, sheep, and reindeer artificially infected with either ovine or bovine infective larvae (L3). The *x*-axis represent days after larval dosing. The boxplots represent the 25th percentile, median, 75th percentile, the upper and lower adjacent values. Outliers (outside values) are designated with a dot. (A) Cattle (*N* = 6) infected with bovine larvae. (B) Reindeer (*N* = 6) infected with bovine larvae. (C) Sheep (*N* = 6) infected with ovine larvae. (D) Reindeer (*N* = 6) infected with ovine larvae.

counts relatively constant, with the exception of two sheep on day-36 post-L3 dosing, when faecal egg counts were considerably higher (6350 and 6500 EPG) than the group mean (3408 EPG) (Fig. 1C). Fifty percent of reindeer had a positive trichostrongylid faecal egg count ($\bar{x} = 25$ EPG) 24 days following dosing with L3 of ovine origin. Subsequently, all animals had positive trichostrongylid egg counts ($\bar{x} = 47$ (25–125) EPG) for the remainder of the study, except for one reindeer, 33 days after L3 dosing (Fig. 1D). The presence of *Capillaria* sp. eggs was recorded on all occasions and was detected in each reindeer at least once within the 40-day period following the ovine L3 dosing. The egg count of *Capillaria* sp. peaked 40 days after L3 dosing, with all six reindeer shedding eggs ($\bar{x} = 138$ (50–375) EPG). One reindeer passed eggs of *Nematodirus* sp. on three occasions in very low numbers ($\bar{x} = 25$ EPG). Overall, the mean nematode egg counts were approximately 10-fold less in reindeer compared with sheep ($\bar{x} = 98$ versus 1241 EPG).

3.2. Nematode burdens

The worm burdens from cattle and reindeer infected with bovine L3 are shown in Table 1. The most abundant nematode species found were *Ostertagia ostertagi* and *Cooperia oncophora*, with mean burdens in cattle of 2566 and 3233, respectively. This represents 57 and 59% establishment of the L3 in the infecting dose. Sixty-nine percent of *O. ostertagi* were present as adult parasites with the remainder arrested in development as EL4. Estimation of *C. oncophora* infections in cattle showed 58% as adult nematodes and 42% as EL4. The intensity of *O. ostertagi* was not significantly different in reindeer than in cattle, but the number of EL4 was significantly higher in reindeer ($p < 0.001$) (Table 1). Only 2% of *C. oncophora* established in reindeer. *Nematodirus* sp. was found in low numbers in the small intestine of two reindeer ($\bar{x} = 75$), and *Trichuris* sp. was recovered from the caeca of all reindeer with a maximum of seven worms in one animal (data not included in Table 1); *Capillaria* was not found.

The worm burdens of sheep and reindeer infected with ovine L3 are shown in Table 2. The most abundant nematode species recovered from sheep and

Table 1
Mean worm burdens from cattle ($N = 6$) and reindeer ($N = 6$) infected with third-stage larvae of bovine origin, followed by ranges in parentheses, and *p*-value for significant effects between animal species

	Cattle	Reindeer	*p*
Abomasum			
O. ostertagi			
Adults	1783 (1400–2400)	242 (150–350)	<0.001
Larvae	783 (300–1150)	2542 (1925–3450)	<0.001
Total	2566 (1700–3550)	2784 (2075–3800)	
Small intestine			
Nematodirus sp.			
Adults	0	13 (0–75)	
Larvae	0	13 (0–75)	
Total	0	26 (0–150)	
Cooperia sp.			
Adults	1883 (1400–2400)	0	<0.001
Larvae	1350 (600–1800)	88 (0–525)	0.002
Total	3233 (2000–4200)	88 (0–525)	<0.001
Small intestine: all species			
Adults	1883 (1400–2400)	13 (0–75)	<0.001
Larvae	1350 (600–1800)	101 (0–600)	0.005
Total	3233 (2000–4200)	114 (0–675)	0.005
Grand total worms: abomasum + small intestine			
Adults	3666 (2800–4800)	255 (150–425)	<0.001
Larvae	2133 (900–2950)	2643 (1925–4050)	
Grand total	5799 (3700–7750)	2898 (2075–4475)	0.018

reindeer, in order of abundance, were *Haemonchus contortus*, *Trichostrongylus axei*, *Teladorsagia circumcincta*, and *Trichostrongylus vitrinus*. *Haemonchus contortus* was present predominately as EL4; 84% in sheep and 100% in reindeer. The estimated establishment of *H. contortus* was 27% in sheep and 43% in reindeer. The overall establishment of *Trichostrongylus* spp. and *Teladorsagia circumcincta*, based on the distribution in the L3 infection doses, was 38% in sheep and 45% in reindeer. In sheep, 99% of *T. axei* were adults, whereas 73% of *T. circumcincta* were EL4 and 22% of *T. vitrinus* were L3/EL4 (Table 2). In reindeer, 95% of *T. axei* and 92% of *T. vitrinus* were adult worms. There was no significant difference in total numbers of worms found in reindeer compared with sheep; however, there were significantly more EL4 *H. contortus* in

Table 2

Mean worm burdens from sheep ($N = 6$) and reindeer ($N = 6$) infected with third-stage larvae of ovine origin, followed by range in parentheses, and *p*-value for significant effects between animal species

	Sheep	Reindeer	*p*
Abomasum			
H. contortus			
Adults	225 (100–350)	0	<0.001
Larvae	1142 (850–1550)	1788 (1500–2175)	0.002
Total	1367 (950–1900)	1788 (1500–2175)	0.059
T. circumcincta			
Adults	133 (0–300)	0	0.005
Larvae	358 (50–500)	825 (150–1700)	
Total	491 (50–800)	825 (150–1700)	
T. axei			
Adults	1084 (750–1450)	1125 (575–1950)	
Larvae	8 (0–50)	63 (0–225)	
Total	1092 (750–1500)	1188 (575–2175)	
Abomasum: all species			
Adults	1442 (850–2100)	1125 (575–1950)	
Larvae	1508 (900–2100)	2676 (1650–4100)	0.005
Total	2950 (1750–4200)	3801 (2225–6050)	
Small intestine			
Nematodirus sp.			
Adults	0	50 (0–150)	
Larvae	0	0	
Total	0	50 (0–150)	
T. vitrinus			
Adults	200 (0–450)	150 (75–375)	
Larvae	58 (50–100)	13 (0–75)	0.005
Total	258 (50–550)	163 (75–450)	
Small intestine: all species			
Adults	200 (0–450)	200 (75–525)	
Larvae	58 (50–100)	13 (0–75)	0.002
Total	258 (50–550)	213 (75–600)	
Grand total worms: abomasum + small intestine			
Adults	1642 (850–2100)	1325 (650–2475)	
Larvae	1566 (900–2100)	2689 (1650–4175)	
Grand total	3208 (1750–4200)	4014 (2300–6650)	

reindeer ($p = 0.002$) (see Table 2). Overall, 40% of the ovine and 29% of the bovine derived L3 experimentally administered to reindeer successfully established within the gastrointestinal tract of the reindeer calves.

3.3. Nematode morphology

The body length of adult *O. ostertagi* was measured from all worms recovered in the sub-samples collected from the abomasums of cattle and reindeer. The total numbers of specimens recovered from the sub-samples were 21 male and 23 female *O. ostertagi* from cattle, and 10 and 22 respectively, from reindeer. Adult male *O. ostertagi* were significantly longer in reindeer ($p = 0.022$) than in cattle (8.08 mm versus 7.36 mm) (Fig. 2). The mean length of adult female *O. ostertagi* was 10.28 mm in reindeer and 9.97 mm in cattle, and was not significantly different. All female *O. ostertagi* recovered from cattle and reindeer had eggs in their uteri.

4. Discussion

To obtain estimates of the potential of infective larvae of sheep and cattle nematode parasites to infect reindeer, efforts were made to reduce the possible sources of variation. For example, young animals of all species were obtained which had limited prior exposure to parasite nematode infections. There is ample evidence to show that immunity to gastro-intestinal parasites induced by naturally acquired infections is very low, or absent in ruminants of approximately 6 months of age (sheep—Dineen and Outteridge, 1984; cattle—Vercruysse and Claerebout, 1997; reindeer—Irvine et al., 2000). Animals were rendered worm-free by the use of ivermectin, a macrocyclic lactone anthelmintic, which has the highest level of efficacy against nematode parasites of sheep, cattle (Benz et al., 1989), and reindeer (Oksanen et al., 1992). Subsequently, all animals were pen-fed to preclude re-infection, until they were artificially infected at a time when all residues of ivermectin were likely to have been eliminated (Fink and Porras, 1989), approximately 5 weeks after treatment. Infective larvae were derived from single sources (ovine and bovine) originating from field infected Swedish sheep and cattle. They were also stored under identical conditions for relatively short periods of time and were in good condition at the time of use. Larval dosing occurred on similar days for reindeer in Finland, and for sheep and cattle in Sweden, so the age of larvae at the time of introduction
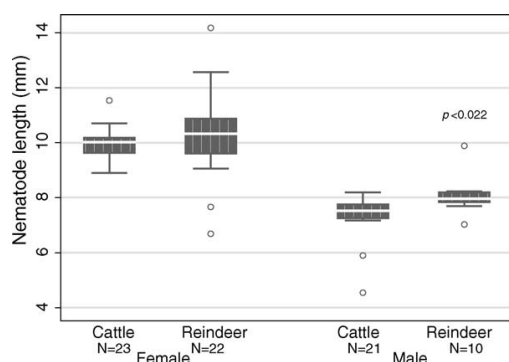
Fig. 2. Length distribution of adult female and male *Ostertagia ostertagi* recovered from the abomasums of cattle ($N = 6$) and reindeer ($N = 6$). The number of nematodes measured for each host is noted above gender of worm. The boxplot represents the 25th percentile, median, 75th percentile, the upper and lower adjacent values. Outliers (outside values) are designated with a dot.

into the animals was very similar. Only moderate doses of larvae were given as it is well known that variation in establishment increase with increasing L3 dosing (Dobson et al., 1990), and the total larval dose was split into two doses, given 2 days apart to enhance the opportunity for establishment to occur. The same personnel conducted post-mortem worm recovery, enumeration, and identification procedures.

This study demonstrated unequivocally that reindeer are highly suitable hosts for both bovine and ovine infective nematode larvae, particularly for the latter. There were no significant differences in the establishment of *H. contortus*, *T. circumcincta*, *T. axei*, and *T. vitrinus* between the natural definitive host (sheep) and reindeer. These species represent the economically most important nematode parasites of sheep and goats throughout the world.

Although all *H. contortus* in reindeer were in the arrested stage of development (EL4), it was also pronounced in sheep (84%). This parasite has a very high propensity to undergo arrested development in sheep in Sweden. Not only does arrested development occur very early in the grazing season (early to mid-summer), it is frequently absolute (100%) even in young grazing lambs (Waller et al., 2004a). Furthermore, high levels of arrested development of Swedish *H. contortus* have been reported previously in artificially infected pen-fed lambs (Waller et al., 2004b). Although it is speculative to assume when these populations detected in reindeer resume development, it is certain that this occurs in peri-parturient

ewes lambing in spring in Sweden (Lindqvist et al., 2001; Waller et al., 2004a). It has been postulated that the way in which *H. contortus* is transmitted from year-to-year in sheep in Sweden is by this process of extended larval inhibition, with resumption of development leading to adult parasites in the lactating ewes, and thus contamination of pastures at turn-out in spring (Waller et al., 2004a). It is well known that the trigger for resumed development of inhibited larval populations is stress (Barger, 1993; Eysker, 1997). This is classically associated with parturition and lactation (Armour, 1978; O'Sullivan and Donald, 1973), and may occur as a response to other stressors, such as transport, seasonal round-ups, feed deprivation, insect harassment, environmental extremes, or calving. All the latter are phenomena to which reindeer are frequently exposed.

Some reindeer herders in the southern cooperatives in Finland keep their herds in corrals from December to approximately February for ease of supplementary feeding in winter. Sheep may occasionally be penned in the same small fenced-in areas during spring lambing or in summer. This study showed that 43% of experimentally infected *H. contortus* established in reindeer as EL4. It is a common practice in Finland that adult female reindeer are gathered in enclosures close to the herder's home during spring calving where the density of animals can reach up to 100 reindeer/ km^2 (Lehtola, personal communication). During this time of parturition and lactation, it is possible that the arrested population of *H. contortus* in reindeer may

resume development, resulting in a significant output of eggs on the localized reindeer calving pastures. In this sub-arctic region, there are 24 h of continuous daylight from mid-May to late July, which may be sufficient to trigger the mass hatching of nematode eggs, thus escalating the potential further spread of this aberrant parasite in reindeer.

Teladorsagia circumcincta is the most prevalent nematode species of sheep throughout the world. Although less pathogenic than *H. contortus* and intestinal *Trichostrongylus* spp. (Symons, 1985), its ubiquity is largely attributed to the hardiness of its free-living stages to environmental extremes (Anderson et al., 1978), capable of surviving in large numbers on frozen and snow covered pasture for extended periods (>5 months) during the Swedish winter (Waller et al., 2004a). Similar to Swedish isolates of *H. contortus*, Swedish *T. circumcincta* also readily undergoes arrested development in artificially infected lambs (Waller et al., 2004b; this study). It is reasonable to assume that *T. circumcincta* is also capable of resumption of development in reindeer, which become infected. *Trichostrongylus axei* was also well represented in the worm burdens of both reindeer and sheep. This finding was expected as this species is well known to parasitize a range of hosts, including some monogastric animals (Urquhart et al., 1996).

With regards to bovine nematodes, this study showed that *O. ostertagi* was capable of infecting reindeer to the same extent as in their definitive hosts, i.e. young, previously worm-free cattle. There appears to be some host-induced effect on the incoming infection with this species, as significantly greater numbers of *O. ostertagi* remained arrested in development in reindeer compared to cattle; however, this parasite also reached patency (egg laying adults) in reindeer. In assumed, unfavorable environments for parasites, such as in hosts with high parasite infections (overcrowding), or in those in which immunity to infection has developed, nematode parasites become less prolific and stunted (Michel et al., 1971; Waller and Thomas, 1978). Thus, it appears that adult *O. ostertagi* in reindeer are not unduly affected by being in these hosts, as the mean lengths of male and female adult worms were marginally longer in reindeer than in their natural definitive host (cattle). In contrast, reindeer appeared to be unsuitable hosts for *C. oncophora*.

Reindeer showed the presence of *Capillaria* sp. in faecal egg counts throughout this study. Little is known about this parasite in reindeer, but it is thought to reside distally in the small intestine (Skirnisson, personal communication). Its presence on all occasions, particularly after de-worming with ivermectin, yet prior to the L3 dosing, clearly indicates that this parasite is refractory to ivermectin. The occasional record of *Nematodirus* sp. eggs in reindeer faeces also indicates that these parasites survived ivermectin in low numbers. This species has been reported as being a dose-limiting parasite at the recommended dose of administration of ivermectin to reindeer (Oksanen, 1999). The small numbers of *Trichuris* sp. found in the caeca of reindeer may have been bovine and/or ovine derived as no examination of caeca of cattle or sheep was conducted in this study.

The implications of this work are important. Reindeer grazing on pastures shared with other ruminants could acquire significant numbers of nematode parasites of either sheep or cattle origin. This is particularly so in such areas in northern Norway, where for example around the Troms region, there is a significant goat dairy industry (Handeland and Sparboe, 1991). It is well known that goats are highly suitable hosts for sheep parasites (Temberley and Hansen, 1996), and it is certain that pastures in this region would be contaminated with ovine nematode parasites, particularly towards the latter part of the grazing season. A common management practice is for reindeer from a particular cooperative to be gathered in large roundups in early winter for annual identification, counting, and slaughtering. More than 80% of reindeer herders in Finland de-worm the overwintering reindeer population with ivermectin, but their main goal is targeted at reducing the warble fly (*Hypoderma tarandi*) and throat bot fly (*Cephenemyia trompe*) infections (Oksanen, 1999). However, acquired ovine (or bovine) parasites would also have met a parasitological dead-end with such a control strategy. If ivermectin treatment is not carried out and reindeer are then moved to spring pastures, populations of parasites that were previously arrested in development could mature and lead to contamination of environments solely utilized by reindeer herds. Thus, introduction of sheep and cattle parasites into traditional reindeer husbandry areas not associated with sheep, goat, or cattle grazing, is a possibility. The

likelihood of reindeer parasites to infect both sheep and cattle was not investigated in this study.

Additionally, there is the potential for ovine or bovine derived nematodes to have a direct effect on the productivity of reindeer. All the nematodes shown to infect reindeer in this study are important production-limiting parasite species in ruminants. There is reason to suspect that similar pathogenicity would occur in reindeer, and this is supported by anecdotal evidence that reindeer appear to be particularly susceptible to parasitic nematode infections when grazing pastures are shared with domestic ruminants and cervids (Larsen and Thamsborg, personal communication). It is speculative whether or not the parasite populations arrested in development in reindeer in this study ultimately become adult parasites, leading to infectivity of pastures and loss of productivity. Further research to elucidate these matters should be an important priority for the reindeer husbandry in Scandinavia.

Acknowledgements

References

Anderson, N., Dash, K.M., Donald, A.D., Southcott, W.H., Waller, P.J., 1978. Epidemiology and control of nematode infections. In: Donald, A.D., Southcott, W.H., Dineen, J.K. (Eds.), The Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia. Academic Press, Sydney, Australia, pp. 23–51.

Anonymous, 1986. Identification of the immature parasitic stages of gastro-intestinal nematodes, Manual of Veterinary Parasitological Laboratory Techniques. Reference book, third ed., vol. 418. Ministry of Agriculture, Fisheries and Food, London, pp. 61–63.

Armour, J., 1978. Arrested development in cattle nematodes with special reference to Ostertagia ostertagi. In: Borgsteede, F.H.M., Armour, J., Jansen, J. (Eds.), Facts and Reflections III. Central Veterinary Institute, Lelystad, The Netherlands, pp. 77–88.

Ball, M.C., 2000. Factors affecting the distribution and transmission of Elaphostrongylus rangiferi in caribou (Rangifer tarandus caribou) of Newfoundland. M.Sc. Thesis. Lakehead University, Ontario, Canada, 64 pp.

Barger, I.A., 1993. Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. Int. J. Parasitol. 23, 463–469.

Barth, D., 1991. Magen-Darm-Nematoden des Rindes, Diagnostischer Atlas 284 Einzelabbildungen. Ferdinand Enke Verlag Stuttgart, p. 104 (in German).

Benz, G.W., Roncalli, R.A., Gross, S.J., 1989. Use of ivermectin in cattle, sheep, goats and swine. In: Campbell, W.C. (Ed.), Ivermectin and Abamectin. Springer-Verlag, New York, pp. 215–229.

Borgsteede, F.H., 1982. The infectivity of some nematode parasites of reindeer (Rangifer tarandus L.) and elk (Alces alces L.) for cattle and sheep. Z. Parasitenkd. 67, 211–215.

Borgsteede, F.H.M., Hendriks, J., 1974. Identification of infective larvae of gastrointestinal nematodes in cattle. Tijdschr Diergeneeskd 99, 103–113.

Bye, K., 1987. Abomasal nematodes from three Norwegian wild reindeer populations. Can. J. Zool. 65, 677–680.

Dineen, J.K., Outteridge, P.M., 1984. Immunogenetic Approaches to the Control of Endoparasites with Particular Reference to Parasites of Sheep. Division of Animal Health, CSIRO Saleable Publications, Australia, p. 169.

Dobson, R.J., Waller, P.J., Donald, A.D., 1990. Population dynamics of Trichostrongylus colubriformis in sheep; the effect of infection rate on the establishment of infective larvae and parasite fecundity. Int. J. Parasitol. 20, 347–352.

Donald, A.D., Morley, F.H.W., Waller, P.J., Axelsen, A., Donnelly, J.R., 1978. Availability to grazing sheep of gastrointestinal nematode infection arising from summer contamination of pastures. Aust. J. Agric. Res. 29, 189–204.

Durette-Desset, M.C., 1983. Keys to genera of the superfamily Trichostrongyloidea. In: Anderson, R.C., Chabaud, A.G. (Eds.), CIH Keys to the Nematode Parasites of Vertebrates. No. 10. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England, p. 86.

Eysker, M., 1997. Some aspects of inhibited development of trichostrongylids in ruminants. Vet. Parasitol. 72, 265–283.

Fink, D.W., Porras, A.G., 1989. Pharmacokinetics of ivermectin in animals and humans. In: Campbell, W.C. (Ed.), Ivermectin and Abamectin. Springer-Verlag, New York, pp. 113–130.

Gordon, H.M., Whitlock, H.V., 1939. A new technique for counting nematode eggs in sheep faeces. J. Counc. Sci. Ind. Res. 12, 50–52.

Handeland, K., Slettbakk, T., 1995. Epidemiological aspects of cerebrospinal elaphostrongylosis in small ruminants in northern Norway. J. Vet. Med. B. 42, 110–117.

Handeland, K., Sparboe, O., 1991. Cerebrospinal elaphostrongylosis in dairy goats in northern Norway. J. Vet. Med. B. 38, 755–763.

Irvine, R.J., Stien, A., Halvorsen, O., Langvatn, R., Albon, S.D., 2000. Life-history strategies and population dynamics of abomasal nematodes in Svalbard reindeer (*Rangifer tarandus platyrhynchus*). Parasitology 120, 297–311.

Kumpula, J., Colpaert, A., Nieminen, M., 2002. Productivity factors of the Finnish semi-domesticated reindeer (*Rangifer t. tarandi*) stock during the 1990s. Rangifer 22, 3–12.

Lindqvist, Å., Ljungström, B.L., Nilsson, O., Waller, P.J., 2001. The dynamics, prevalence and impact of nematode parasite infections in organically raised sheep in Sweden. Acta Vet. Scand. 42, 377–389.

Michel, J.F., Lancaster, M.B., Hong, C., 1971. Host induced effects on the size and body proportions of *Ostertagia ostertagi* (Stiles, 1893) Ransom 1907, a nematode parasite of cattle. J. Parasitol. 57, 1185–1189.

Moen, J., Danell, Ö., 2003. Reindeer in the Swedish mountains: an assessment of grazing impacts. Ambio. 32, 397–402.

Oksanen, A., 1999. Endectocide treatment of the reindeer. Rangifer (special issue no. 11), 217 pp.

Oksanen, A., Nieminen, M., Soveri, T., Kumpula, K., 1992. Oral and parenteral administration of ivermectin to reindeer. Vet. Parasitol. 41, 241–247.

O'Sullivan, B.M., Donald, A.D., 1973. Responses to infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in ewes of different reproductive status. Int. J. Parasitol. 3, 521–530.

Roed, K.H., 2005. Refugial origin and postglacial colonization of holarctic reindeer and caribou. Rangifer 25 (1), 19–30.

Steen, M., Warsame, I., Skorping, A., 1998. Experimental infection of reindeer, sheep, and goats with *Elaphostrongylus* spp. (Nematoda, Protostrongylidae) from moose and reindeer. Rangifer 18 (2), 73–80.

Symons, L.E.A., 1985. Anorexia; occurrence, pathophysiology and possible causes of parasitic infection. Adv. Parasitol. 24, 103–133.

Temberley, S., Hansen, J.W., 1996. Helminth diseases of small ruminants in the tropics: a review of epidemiology and control strategies. In: Le Jambre, L.F., Knox, M.R. (Eds.), Sustainable Parasite Control in Small Ruminants. Proceedings Workshop, Bogor, Indonesia, 22–25 April 1996, ACIAR Proc. 74, 123–127.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W., 1996. Veterinary Parasitology. Blackwell Science, Oxford, UK, p. 307.

van Wyk, J.A., Cabaret, J., Michael, L.M., 2004. Morphological identification of nematode larvae of small ruminants and cattle simplified. Vet. Parasitol. 119, 277–306.

Vercruysse, J., Claerebout, E., 1997. Immunity development against *Ostertagia ostertagi* and other gastrointestinal nematodes in cattle. In: Fourth Ostertagia Workshop: Nematode Parasites of Importance to Ruminant Livestock. Vet. Parasitol. 72, 309–326.

Waller, P.J., Thomas, R.J., 1978. Host-induced effects of the morphology of *Ostertagia circumcincta* in grazing sheep. Int. J. Parasitol. 8, 365–370.

Waller, P.J., Rudby-Martin, L., Ljungström, B.L., Rydzik, A., 2004a. The epidemiology of abomasal nematodes of sheep in Sweden, with particular reference to overwinter survival strategies. Vet. Parasitol. 122, 207–220.

Waller, P.J., Bernes, G., Rudby-Martin, L., Ljungström, B.L., Rydzik, A., 2004b. Evaluation of copper supplementation to control *Haemonchus contortus* infections of sheep in Sweden. Acta Vet. Scand. 45, 149–160.

IV

Taylor & Francis
Taylor & Francis Group
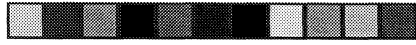
ORIGINAL ARTICLE

Soil nematode populations beneath faeces from reindeer treated with ivermectin

GREGOR W. YEATES[1], JACKIE T. HRABOK [2], ANTTI OKSANEN[3], MAURI NIEMINEN[4] & PETER J. WALLER[2]

[1]*Landcare Research, Palmerston North, New Zealand,* [2]*Department of Parasitology (SWEPAR), National Veterinary Institute and Swedish University of Agricultural Sciences, Uppsala, Sweden,* [3]*National Veterinary and Food Research Institute (EELA), Oulu Regional Unit, Oulu, Finland, and* [4]*Finnish Game and Fisheries Research Institute, Reindeer Research Station, Kaamanen, Finland*

Abstract
The size and composition of the nematode assemblage in soil beneath faecal material derived from reindeer treated with ivermectin oral, or ivermectin subcutaneous formulations in early winter in northern Finland, was studied over a two-year period. This study was performed on both ungrazed and grazed areas that typify the reindeer habitat of the region and comparisons were also made with soil nematodes recovered from soils receiving untreated faecal material. Although significant differences in numbers of soil nematode fauna were observed between treatments on individual occasions, none of the differences occurred consistently with treatment, or with time. These results showed no adverse environmental impact of the faeces of reindeer given either formulation of ivermectin in early winter on soil nematode communities in subsequent years.

Keywords: *Environmental impact, nutrient cycling, parasites, pasture, soil.*

Introduction

The effect of ivermectin excreted in the faeces of treated animals is an issue of continued controversy. There is no doubt that this drug has profound effects on the larval stages of certain coprophilic arthropods (for reviews see Herd et al., 1993; Dardour & Cooke, 1999). Arthropods are only part of the diverse array of organisms that play a role in faeces breakdown and recycling of nutrients contained in it (Yeates et al., 1997). It is also important to note that these arthropods are highly sensitive to the prevailing weather conditions (ideally suited to warm, moist conditions), and egg-laying adults are only attracted to freshly deposited faeces (Waller & Faedo, 1996). To date, very limited information is available on the effects of ivermectin on soil microbiota, particularly microbial-feeding nematodes, in the external environment (Yeates et al., 2002, 2003). These organisms play critical roles in nutrient cycling and have been found to be good indicators of soil processes (Clarholm, 1985; Ingham et al., 1985; Yeates & Pattison, 2006).

A large proportion ($>80\%$ in some locations) of reindeer herders in northern Scandinavia (Norway, Sweden, and Finland), treat their animals with ivermectin once annually, during the winter round-ups between October and February. This treatment recommendation is based on the sound epidemiological understanding that the larval instars of arthropod parasites, notably the warble fly (*Hypoderma tarandi*) and the throat bot fly (*Cephenemyia trompe*), will be targeted before spring pupation. For the treatment of not only these arthropod pests, but also internal parasites of reindeer, the recommended drug of choice and mode of delivery is the subcutaneous formulation of ivermectin. This is based on the prolonged pharmocokinetic profile, high efficacy against the fly larval stages, and nematode faecal egg count reductions of subcutaneously

treated animals, compared with those treated by either the oral or topical formulations of ivermectin (Oksanen et al., 1992, 1993, 1995).

Invariably, faeces of reindeer treated in early winter with ivermectin are deposited on frozen, wind-swept or snow-covered ground. On this basis, it can be assumed that dung beetles and coprophilic flies play insignificant roles in the destruction and disappearance of faeces of reindeer treated with ivermectin in early winter, and breakdown does not occur until the following spring thaw (Nilssen et al., 1999).

No long-term studies have been conducted on the effect on soil nematode communities of ivermectin residues in faeces of reindeer treated in early winter. Soil nematode diversity has been shown to be much less in extreme environments, such as in the arctic region, than in more temperate climates (Boag & Yeates, 1998). This makes extreme environments particularly sensitive to any man-induced changes. On this basis, it is important to conduct environmental impact studies on the effect of any chemical application that may ultimately end up in an arctic environment. This particularly applies to any chemical that has been shown to have detrimental effects on non-target organisms in more equitable environments, such as ivermectin.

Materials and methods

Animals and treatments

In November 2001, eight 25-week-old female reindeer calves, acquired from the Finnish Reindeer Herders' Association Kutuharju herd, were retained indoors on concrete flooring and fed lichen *ad libitum* at the Reindeer Research Station, Kaamanen, Finland. Calves were retained indoors on concrete flooring from 5 to 16 November 2001. Faeces were collected twice daily and stored in plastic bags at $+4°C$ until distributed on experimental plots as control faeces. On 26 November, four calves were given a subcutaneous injection of ivermectin (Ivomec SC® 10 mg/ml vet. inj. Merial) and four received ivermectin orally (Ivomec® 0.8 mg/ml vet. mix. Merial), at the manufacturer's recommended dose rate (200 μg/kg). From 26 November to 4 December, calves continued to be housed indoors and fed lichen *ad libitum*, but in separate group pens for each of the two treatments. Faeces were collected twice daily from each group during the nine days following treatment and stored at $+4°C$ until distributed on the experimental plots. Approximately 800 g of faeces were collected from the control group and 400 g each from the ivermectin injection (IVM SC) and ivermectin oral (IVM O)

treatments, and this material was distributed uniformly over the snow surface of each of the 1 m² experimental plots on 5 December 2001. In November 2002, the feed treatment and de-worming treatment regime was repeated with another set of eight reindeer calves. Approximately 6000 g of faeces was collected from the control group, and 1000 g each from the IVM SC and IVM O treatments. This material was distributed uniformly on each plot on 11 December 2002.

Plots and study area

The plots used were located at the Finnish Game and Fisheries Research Institute Reindeer Research Station at Kaamanen, Finland (69°N, 27°E), 300 km north of the Arctic Circle. In December 2001, six plots each 1 × 1 m were established in two separate enclosures. Three were set up on an area (20 m²) which was last grazed by reindeer in 1995 (ungrazed enclosure) and 3 on an area (1000 m²) previously grazed by reindeer in 2001 (grazed enclosure). Four survey stakes delineated the corners of each plot and a 2.5 m high wooden fence prevented reindeer and large wildlife from entering the enclosures. In December 2002, an additional six plots were established in the same enclosures, located 1 to 5 m away from those established in December 2001. Control plots, where untreated faecal material was distributed, were also established on both areas.

Overall, the vegetation of both enclosures was dominated by approximately 100-year-old pine *Pinus sylvestris*. The understorey of the ungrazed enclosure was dominated by reindeer lichen (*Cladina* spp.) and moss (*Pleurozium* spp., *Dicranum* spp., *Polyrihum* spp.). Lingonberry (*Vaccinium vitis*), blueberry (*Vaccinium myrtillus*), Labrador tea (*Ledum palustre*), crowberry (*Empetrum nigrum*), and mushrooms (*Agaricales* spp.) were also present. On the grazed area, a sparse understorey of heath, shrubs, and moss was present, but reindeer lichen was not found.

At the time of faecal deposition in both years, approximately 10–20 cm of snow covered the entire experimental area, so the boundaries of the plots were demarcated and faeces was distributed within these boundaries to ensure, as far as possible, that it would ultimately remain within the specified plot boundaries.

Meteorology

The Finnish Meteorological Institute provided precipitation data from Ivalo International Airport and sunlight hours from the township of Kevo. Both

localities are within 70 km from the experimental site. Snow depth and ambient temperature were measured at the Reindeer Research Station at Kaamanen.

Soil sampling procedure

A 1 m^2 point frame with 10×10 cm grids was used as a guide to locate sampling points previously determined using random numbers. Two 100 cm^2 sub-samples were taken to a depth of 5 cm from each 1 m^2 plot using a homemade stainless steel square soil corer. Samples were located such that the next nearest sample was at least 10 cm away. A given sample may have included reindeer faeces, vegetation, as well as soil.

For the plots set up in December 2001, the first sampling was carried out in June 2002 and subsequent samplings in August and September of the same year. Sampling continued on these plots in June, July, August, and September 2003. Thus, the sampling period spanned 23 to 88 weeks after faecal deposition in December 2001.

With regarding to plots established in December 2002, the first sampling occurred in June 2003 and subsequently in July, August, and September 2003, followed by sampling in June, July, August, and September 2004. The sampling period on these plots was 22 to 77 weeks after faecal deposition in December 2002.

Soil nematode recovery and identification

In 2002, the duplicate sub-samples were pooled in June, August, and September for processing. However, in 2003 and 2004 (June, July, August, September), sub-samples were processed individually.

Total samples were extracted for 12 to 18 h by the tray method described by Yeates and King (1997). After this time, the water in the trays containing nematodes was transferred to 2000 ml graduated cylinders and settled for >4 h before reduction of the total volume to 100 ml. Contents were allowed to settle for an additional 5 h to a final volume of 45 ml. From this volume, duplicate 1 ml aliquots were taken to estimate total numbers of nematodes. The remaining solution settled for 12 h and the supernatant was aspirated to 5 ml. Nematodes were preserved by adding an equal volume of hot ($\geq 85°C$) 4% formaldehyde.

Total nematode populations were expressed per g soil. An average of 109 specimens from each sample was subsequently transferred to temporary mounts and identified to nominal genus. In total, identifications were made on a total of 2828 nematodes from 30 samples collected in 2002; 11,052 nematodes

from 96 samples in 2003; and 4666 nematodes from 48 samples collected in 2004. Genera were grouped into functional groups (bacterial-feeding, fungal-feeding, plant-associated, plant-feeding, omnivorous, predacious) generally following Yeates et al. (1993) and indices calculated following Yeates and Bongers (1999) and Yeates (2003). The proportional contribution of functional groups, genera, and families, and the Shannon-Weiner index of diversity (H') were calculated.

Data analysis

Data were analysed using ANOVA with grazing (ungrazed and grazed) and treatment (control, IVM SC, IVM O) as main effects, with any interaction being determined. ANOVA was carried out on $\log_{10}(n+1)$ transformed data and results are presented as arithmetic means. The sampling dates correspond to faecal material having been exposed through one winter (23–36 weeks after the 2001 deposition, 22–38 weeks after the 2002 deposition), or two winters (approximately 74, 77, 80, and 88 weeks for each of the December 2001 and December 2002 depositions.

Results

Meteorology and plot observations

Meteorological data are summarized in Figure 1. Precipitation showed a bimodal pattern of peaks during the summer (rainfall) and winter (snow). Snow cover was present from late September until early May with a maximum depth of 45 cm in March. Sub-zero temperatures were common from October until April, with the lowest temperatures in January when they approached $-40°C$. Maximum temperatures were recorded in July when they exceeded 20°C. Twenty-four h sunlight was present mid-May to late July.

Visual observation showed that by the time the first sampling took place immediately after spring thaw, faecal material on all plots was moist, pelleted, and did not appear to have been broken down. On some plots, which had sloping gradients, faeces tended to settle towards the lower sectors. With increasing day length and sunshine hours, faeces became dry, hard masses and only gradually disappeared over the sampling period.

Nematology

A total of 30 nominal genera were discriminated, and from their proportions in sub-samples, the abundance per g of soil was calculated. The
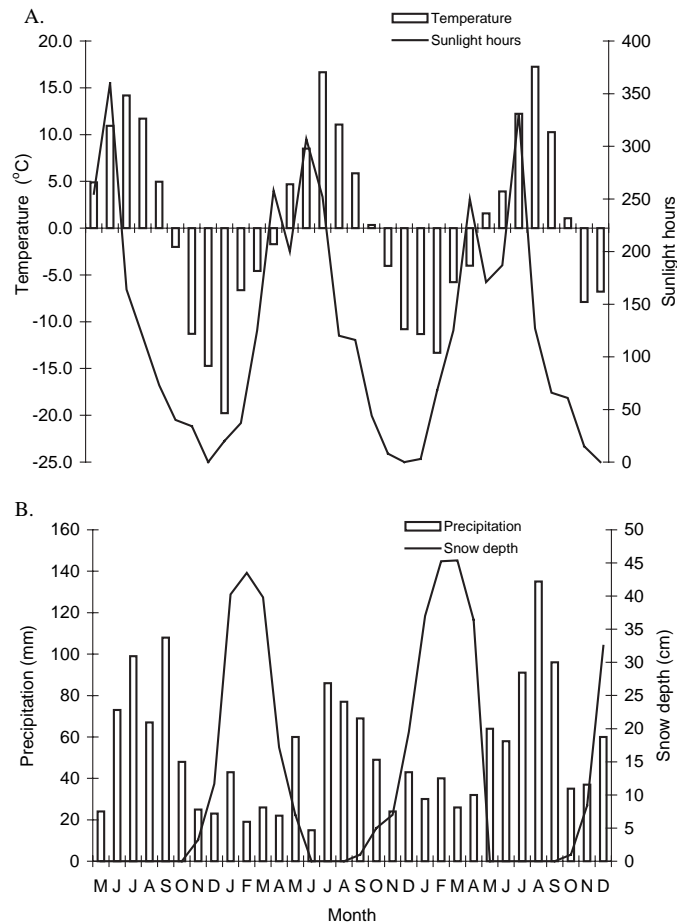
Figure 1. Meterological data in the vicinity of the experimental enclosures in Kaamanen, Finland. A. Mean monthly ambient temperature (°C) and total monthly sunlight (hours). B. Total monthly precipitation (mm) [rain+snow] and monthly snow depth (cm). The x-axis represents consecutive months beginning in May 2002 and ending in December 2004.

following were the more abundant genera whose presence was statistically analysed: *Tylenchus, Aphelenchoides,* Rhabditidae, *Panagrolaimus, Cephalobus, Teratocephalus, Euteratocephalus, Plectus, Eudorylaimus, Tylencholaimus, Mononchus,* and juveniles of zooparasites.

First year observations

December 2001 deposition. ANOVA was conducted on the control and the two IVM treatments. Table I shows that over three initial samplings in 2002, which were 23–36 weeks after the faecal deposition, total nematode abundance did not differ between grazed and ungrazed treatments, or between the control, IVM SC, and IVM O. The abundance of three genera and three feeding groups, however,

showed statistically significant differences between grazed and ungrazed enclosures (plant-associated, omnivorous nematodes, and Dorylaimidae). Only one of these groups (plant-associated nematodes) showed an ivermectin effect, being most abundant in the soil that had received faeces from the IVM O animals (Table I).

None of the indices of the nematode assemblage showed significant effects and there were no significant interactions between pasture and mode of ivermectin delivery.

December 2002 deposition

Samples collected in 2003, 22–38 weeks after the December 2002 deposition, showed no treatment

Table I. Mean abundance of total nematodes and nematode taxa per g soil beneath reindeer faeces that showed significant effects (untreated control; ivermectin oral; ivermectin subcutaneous injection) collected 23 to 36 weeks after faeces were deposited in December 2001.

Group	Weeks	Pasture			Ivermectin delivery			
		Grazed	Ungrazed	p	Control	Oral	Subcutaneous	p
Rhabditidae	All	18.3	1.8	0.007	12.5	5.9	6.8	–
Euteratocephalus	All	1.78	3.81	0.023	2.46	3.85	2.76	–
Eudorylaimus	All	2.53	6.16	0.006	4.45	5.32	3.05	–
Plant-associated nematodes	All	3.1	10.1	0.009	5.8	12.2	3.5	0.034
Omnivorous nematodes	All	2.66	6.16	0.007	4.45	5.66	3.05	–
Dorylaimidae	All	2.60	6.16	0.007	4.45	5.51	3.05	–
Total nematodes	All	74.6	59.0	0.371	66.3	74.8	60.3	0.719

effect on total nematodes (Table II). There were four genera with significant effects, three with grazing effects, and bacterial-feeding *Euteratocephalus* and *Plectus* showing differences between IVM SC and IVM O. Four feeding groups showed significant differences between grazing treatments, but only bacterial-feeding nematodes showed a significant ivermectin treatment (i.e., IVM SC vs. IVM O) effect, being more abundant with IVM O faeces. Several indices showed significant effects, five with grazing and four with ivermectin treatment. All the significant effects among indices showed the same trend (control >IVM O >IVM SC) and all were effectively derived from the same data, namely the proportional contribution of genera to total nematodes. There were no significant interactions.

When first-year data are considered on a sampling time basis (Table III), significant effects were restricted to indices and most were associated with grazing history of the plots, rather than ivermectin treatments. However, there was no consistency with these observations, as none of the significant effects was found on all four sampling occasions.

Second year observations

Samples collected after faecal material had been exposed to two winters showed some significant effects, with nine reflecting grazing treatments and five ivermectin treatment (Table IV). Two genera showed ivermectin effects, both being highest in control plots. While all means are presented for total nematodes/g and Shannon-Weiner diversity (H'), no

Table II. Mean abundance of total nematodes and nematode taxa per g soil and indices of the nematode assemblage beneath reindeer faeces that showed significant effects (untreated control; ivermectin oral; ivermectin subcutaneous injection) collected 22 to 38 weeks after faeces were deposited in December 2002.

Group	Pasture			Ivermectin delivery			
	Grazed	Ungrazed	p	Control	Oral	Subcutaneous	p
Abundance							
Total nematodes	44.7	46.6	0.860	47.8	47.9	41.3	0.843
Euteratocephalus	0.73	0.42	–	1.06	0.65	0.01	0.026
Plectus	9.3	22.0	0.005	17.5	23.6	5.8	<0.001
Tylencholaimus	0	3.33	0.016	2.85	0	2.14	–
Mononchidae	21.5	0	<0.001	10.3	5.3	16.7	–
Bacterial-feeding nematodes	13.2	23.7	0.019	21.3	27.8	6.3	<0.001
Fungal-feeding nematodes	1.0	5.8	0.006	5.0	2.7	2.5	–
Predacious nematodes	21.5	0.0	<0.001	10.3	5.3	16.7	–
Plant-associated nematodes	5.6	1.0	<0.001	2.9	3.6	3.5	–
Tylenchidae	5.6	1.0	<0.001	2.9	3.6	3.5	–
Indices							
Shannon-Weiner diversity (H')	0.98	0.71	0.017	1.03	0.96	0.55	0.001
Species Richness (SR)	0.81	0.78	–	0.95	0.92	0.52	0.001
Evenness (J')	0.62	0.48	0.014	0.61	0.60	0.43	0.016
NCR	0.97	0.84	0.016	0.83	0.93	0.95	–
ΣMI	3.05	2.50	0.006	2.68	2.55	3.09	–
Bacterial-feeding nematodes (proportion)	0.35	0.65	<0.001	0.51	0.59	0.40	–
Taxa identified	4.88	4.33	–	5.38	5.00	3.44	0.002

variable showed significant effects at all four sampling occasions.

Discussion

Although this study showed some statistically significant effects in the numbers of nematodes recovered from soils, beneath which dung from untreated and ivermectin treated reindeer treatment was distributed, none of these differences occurred consistently with treatment, or with time. Neither were there any consistent significant differences in soil nematodes recovered from soils beneath dung of reindeer treated with either ivermectin oral, or subcutaneous formulation.

However, it is important to consider these findings in relation to the dynamics of reindeer faeces dispersal that was observed in this study. It was clearly apparent over the time-course (up to 88 weeks) of this investigation, that breakdown of reindeer faeces occurs very slowly when it is deposited on snow-covered ground in early winter. Faeces from reindeer fed lichen tend to be firm pellets and faeces from such animals that are treated in winter with ivermectin would invariably be deposited on snow that is subsequently exposed to temperatures often below $-20°C$. Such conditions would certainly be inimical to biotic degradation. It was observed that at the time of spring thaw, such faecal pellets become hydrated but quickly loose water again to become characteristically very hard and dry with the lengthening daylight and sunshine hours, which at this study site can be continuous from May to July.

Arthropods, particularly coprophiles, were rarely found during the two-year sampling period of this study. This finding is not surprising as hard, dry faeces of reindeer (Nilssen et al., 1999), cattle (Bryan, 1976), or horses (English, 1979) are known to be unattractive to dung beetles. We were unable to ascertain what role soil-dwelling nematodes play in the breakdown of winter deposited reindeer faeces, but it is presumed that their activity, together with that of other microorganisms (e.g., bacteria, fungi and protozoa), to be minor. Therefore we presume that physical, abiotic forces such as rainfall and temperature, resulting in repeated freezing and thawing of the progressively ageing dung, are likely to be the dominant factors in the eventual disappearance of winter-deposited reindeer faeces in the sub-arctic environment.

Although one conclusion from this work is that ivermectin treatment, whether oral or subcutaneous, of reindeer in early winter is of no biological significance with regard to adverse environmental impact on soil nematode populations in sub-arctic environments, further work needs to be conducted. A companion study estimating ivermectin residues in reindeer dung and soil collected in this investigation, has shown ivermectin to be present in faecal material collected even during the second year following deposition (K. Aasbakk et al., unpublished observation). Therefore, the continual presence of faeces at the soil/pasture mat surface and the persistence of ivermectin in this ageing dung implies that the drug has remained sequestered from soil-dwelling organisms (including nematodes) and may still have

Table III. Mean abundance of total nematodes per g soil and indices of the nematode assemblage beneath reindeer faeces that showed significant effects (untreated control; ivermectin oral; ivermectin subcutaneous injection) collected 22, 26, 30, and 38 weeks after faeces were deposited in December 2002.

Group	Weeks	Pasture			Ivermectin delivery				Interaction
		Grazed	Ungrazed	*p*	Control	Oral	Subcutaneous	*p*	
Abundance									
Total nematodes	22	43.1	23.5	0.095	42.8	33.4	23.6	0.377	–
	26	40.0	61.0	0.448	49.0	81.0	20.0	0.125	–
	30	73.0	89.0	0.551	81.0	52.0	110.0	0.269	–
	38	23.1	13.5	0.202	18.1	25.3	11.5	0.225	–
Indices									
Shannon-Weiner diversity (H')	38	0.90	0.26	0.010	0.63	0.65	0.46	–	–
Species richness (SR)	22	1.34	1.15	–	1.55	1.40	0.78	0.034	–
Evenness (J')	26	0.59	0.36	0.008	0.56	0.53	0.35	0.049	–
	38	0.62	0.27	0.013	0.48	0.48	0.38	–	–
ΣMI	26	2.90	2.20	0.029	2.54	2.26	2.84	–	–
	30	3.55	2.88	0.044	2.52	3.37	3.75	0.026	0.039
	38	3.08	2.11	0.008	2.63	2.38	2.79	–	–
Bacterial-feeding (proportion)	26	0.50	0.86	0.044	0.73	0.78	0.53	–	–
	38	0.40	0.94	0.004	0.63	0.77	0.61	–	–
Taxa identified	38	4.17	2.67	0.040	3.50	3.75	3.00	–	–

Table IV. Mean abundance of nematode taxa and feeding groups per g soil beneath reindeer faeces and indices of the nematode assemblage (untreated control; ivermectin oral; ivermectin subcutaneous injection) collected in the second year following faecal deposition in either 2001 or 2002. Weeks denotes the elapsed time between faecal deposition and soil sampling.

| Variable | Weeks | Pasture | | | Ivermectin delivery | | | | Interaction |
		Grazed	Ungrazed	p	Control	Oral	Subcutaneous	p	
Abundance									
Total nematodes	73	87.5	67.7	–	89.8	92.0	51.0	–	–
	77	69.6	36.2	–	68.9	55.8	34.0	–	–
	80	78.0	102.0	–	145.0	55.0	69.0	0.049	–
	88	29.9	25.0	–	18.7	31.9	31.7	–	–
Euteratocephalus	88	0.99	0.08	0.002	0.63	0.48	0.49	–	–
Plectus	80	10.4	39.4	–	43.9	22.2	8.7	0.012	–
Tylencholaimus	73	2.42	4.45	–	9.75	0.42	0.14	0.001	–
Fungal-feeding nematodes	73	11.3	5.1	–	16.0	2.7	5.9	0.028	–
Indices									
Shannon-Weiner diversity (H')	73	1.26	0.89	(0.076)	1.17	1.05	1.01	–	–
	77	1.07	0.78	–	1.00	0.92	0.85	–	–
	80	1.09	1.05	–	1.13	1.20	0.88	–	–
	88	1.19	0.67	0.020	0.90	0.85	1.04	–	–
Species Richness (SR)	88	1.13	0.76	0.040	0.85	0.98	1.01	–	–
Evenness (J')	73	0.75	0.55	0.005	0.70	0.64	0.61	–	0.040
	88	0.65	0.47	0.039	0.54	0.51	0.62	–	–
NCR	73	0.92	0.83	–	0.77	0.97	0.89	0.036	–
	88	0.99	0.95	0.039	0.98	0.96	0.97	–	–
ΣMI	80	3.10	2.33	0.007	2.63	2.47	3.04	–	–
Bacterial-feeding nematodes (proportion)	88	0.45	0.78	0.008	0.55	0.62	0.68	–	–
Taxa identified	88	6.17	4.25	0.024	5.00	5.25	5.38	–	–

an effect once it finally reaches this ultimate destination.

Additionally, it is obvious that these results are only applicable to the impact of ivermectin on soil nematode communities, and in turn the rate of dung disappearance, of reindeer treated in early winter. Dung of reindeer deposited in the summer months tends to be moist (Nilssen et al., 1999), which is likely to be much more attractive to coprophilic organisms. However, summer anthelmintic treatment of reindeer is managerially difficult (animals are free ranging over extensive areas) and epidemiologically inappropriate (for warble and throat bot fly control), and thus not commonly practised.

The differing vegetation in our grazed and ungrazed areas reflects, in part, the impact of previous selective grazing by reindeer (Den Herder et al., 2003). Furthermore, van der Wal et al. (2004) found plant growth tends not to respond until the third year after addition of reindeer faeces. Thus, we would not expect a marked response to the faeces applied in our trial in soil nematodes or vegetation during the period of our sampling. Indeed, in our study the abundance of soil nematodes showed no significant difference between environments either previously grazed or ungrazed by reindeer.

These findings add to others in ascertaining the environmental consequences of ivermectin treatment of livestock to control internal and external parasites (for review see Herd et al., 1993). In relation to the objective of this study to evaluate the impact of ivermectin, administered as either the oral or subcutaneous formulation, at the commonly practised time of early winter by reindeer herders, these results indicate that this drug had no detectable negative effects on the soil nematode communities, for up to two years following faecal deposition. However, this study also highlights the fact that such dung largely remains intact for a lengthy time, thus highlighting the need for future studies on this issue to be continued for longer periods.

References

Boag, B., & Yeates, G.W. (1998). Soil nematode biodiversity in terrestrial ecosystems. *Biodiversity and Conservation*, *7*, 617–630.

Bryan, R.P. (1976). The effect of the dung beetle, *Onthophagus gazella*, on the ecology of the infective larvae of gastrointestinal nematodes of cattle. *Australian Journal of Agricultural Research*, *24*, 567–574.

Clarholm, M. (1985). Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. *Soil Biology and Biochemistry*, *17*, 181–187.

Dardour, I.R., & Cooke, D.F. (1999). Avermectins and pasture pollution: a solution for dung beetles. In JN Matthiessen (Ed.). *Australasian Grassland Invertebrate Ecology Conference*, *7*, 146–158.

Den Herder, M., Kytoviita, M.M., & Niemela, P. (2003). Growth of reindeer lichens and effects of reindeer grazing on ground cover vegetation in a Scots pine forest and a sub-arctic heathland in Finnish Lapland. *Ecography*, *26*, 3–12.

English, A.W. (1979). The effects of dung beetles (*Coleoptera and Scarabaeinae*) on the free-living stages of strongylid nematodes of the horse. *Australian Veterinary Journal*, *55*, 315–321.

Herd, R., Strong, L., & Wardhaugh, K., eds. (1993). Environmental impact of avermectin usage in livestock. *Veterinary Parasitology*, *48*, 1–343.

Ingham, R.E., Trofymow, J.A., Ingham, E.R., & Coleman, D.C. (1985). Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecological Monographs*, *55*, 119–140.

Nilssen, A.C., Åsbakk, K., Haugerud, RF., Hemmingsen, W., & Oksanen, A. (1999). Treatment of reindeer with ivermectin effect on insect dung fauna. *Rangifer*, *19*, 61–69.

Oksanen, A., Nieminen, M., Soveri, T., & Kumpula, K. (1992). Oral and parenteral administration of ivermectin to reindeer. *Veterinary Parasitology*, *41*, 241–247.

Oksanen, A., Nieminen, M., & Soveri, T. (1993). A comparison of topical, subcutaneous, and oral administration of ivermectin to reindeer. *Veterinary Record*, *133*, 312–314

Oksanen, A., Norberg, H., Nieminen, M., & Bernstad, S. (1995). Influence of route of administration on the plasma concentrations of ivermectin in reindeer. *Research in Veterinary Science*, *58*, 286–287.

van der Wal, R., Bardgett, R.D., Harrison, K.A., & Stien, A. (2004). Vertebrate herbivores and ecosystem control: cascading effects of faeces in tundra ecosystems. *Ecography*, *27*, 242–252.

Waller, P.J., & Faedo, M. (1996). The prospects for biological control of the free-living stages of nematode parasites of livestock. *International Journal for Parasitology*, *26*, 915–925.

Yeates, G.W. (2003). Nematodes as soil indicators: functional and biodiversity aspects. *Biology and Fertility of Soils*, *37*, 199–210.

Yeates, G.W., & Bongers, T. (1999). Nematode diversity in agroecosystems. *Agriculture, Ecosystems and Environment*, *74*, 113–135.

Yeates, G.W., & King, K.L. (1997). Soil nematodes as indicators of the effect of management of grasslands in the New England Tablelands (NSW): comparison of native and improved grasslands. *Pedobiologia*, *41*, 526–536.

Yeates, G.W., & Pattison, A. B. (2006). Moving up within the food web: protozoa and nematodes. In N Uphoff, AS Ball, E Fernandes, H Herren, O Husson, & Laing, M (Eds.), Chapter 10. Biological approaches to sustainable soil systems. (pp. 149–162). New York: Taylor and Francis, CRC.

Yeates, G.W., Waller, P.J., & King, K.L. (1997). Soil nematodes as indicators of the effect of management of grasslands in the New England Tablelands (NSW): effect of measures for control of parasites of sheep. *Pedobiologia*, *41*, 537–548.

Yeates, G.W., Dimander, S.-O., Waller, P.J., & Höglund, J. (2002). Environmental impact on soil nematodes following the use of the ivermectin sustained-release bolus or the nematophagous fungus *Duddingtonia flagrans* to control nematode parasites of cattle in Sweden. *Acta Agriculturae Scandinavica, Section A, Animal Science*, *52*, 233–242.

Yeates, G.W., Dimander, S.-O., Waller, P.J., & Höglund, J. (2003). Soil nematode populations beneath faecal pats from grazing cattle treated with the ivermectin sustained-release bolus or fed the nematophagous fungus *Duddingtonia flagrans* to control nematode parasites. *Acta Agriculturae Scandinavica, Section A, Animal Science*, *53*, 197–206.

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., & Georgieva, S.S. (1993). Feeding habits in soil nematode families and genera: an outline for soil ecologists. *Journal of Nematology*, *25*, 315–331.

V

Prolonged persistence of faecally excreted ivermectin from reindeer in a sub-Arctic environment

Kjetil Åsbakk[1*], Jackie T. Hrabok[2], Antti Oksanen[3], Mauri Nieminen[4], Peter J. Waller[2]

[1] Section of Arctic Veterinary Medicine (SAV), Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, P.O. Box 6204, NO-9292 Tromsø, Norway.
[2] Department of Parasitology (SWEPAR), National Veterinary Institute and Swedish University of Agricultural Sciences, SE-751 89 Uppsala, Sweden.
[3] Finnish Food Safety Authority Evira, Oulu Research Unit (FINPAR), P.O. Box 517, FIN-90101 Oulu, Finland.
[4] Finnish Game and Fisheries Research Institute, Reindeer Research Station, FIN-99910 Kaamanen, Finland.

Author email addresses: kjetil.aasbakk@veths.no, jackie.hrabok@bvf.slu.se, antti.oksanen@evira.fi, mauri.nieminen@rktl.fi, peter.waller@sva.se

[*]Author to whom correspondence should be addressed, email: kjetil.aasbakk@veths.no, Telephone: +47 77665410, fax: +47 77694911.

ABSTRACT

Faeces from reindeer calves treated with the anthelmintic ivermectin collected for the first nine days following treatment, and faeces from untreated reindeer calves, were distributed on plots established on two types of forested reindeer pasture in northern Finland during the winters of 2001 and 2002. The ungrazed plots were on an enclosure that had been fenced to prevent reindeer access for the last six years. The grazed plots were on an area which had been heavily stocked by reindeer for five years immediately prior to the experiment. Lichens (*Cladina* spp.) were present on the ungrazed but not on the grazed area. After enclosures had been established, reindeer and large wildlife were prevented from entering by a 2.5 m high wooden fence delimiting each enclosure. Topsoil samples (reindeer faeces, vegetation and soil) were collected from the plots monthly during the summers of the following two years, over a time period of from 25 to 95 weeks after deposition of faeces. The samples were analysed for ivermectin using HPLC. Although apparent ivermectin degradation rapidly took place during the first spring, considerable residual ivermectin could be measured throughout the sampling time which spanned more than two summer seasons following treatment, showing that ivermectin in faeces on pasture may not be photodegraded as rapidly as previously believed. The results support the need for further environmental evaluation studies on the use of ivermectin to control parasites of reindeer, particularly in relation to springtails (Collembola), mites (Acari), enchytraeids (Annelida: Oligochaeta: Enchytraeidae) and nematodes (Nematoda) which are particularly important for decomposing of organic matter and nutrient cycling in ecosystems of high latitudes.

INTRODUCTION

Ivermectin (22,23-dihydroavermectin B1) is a broad-spectrum antiparasitic drug in worldwide use for the control of internal and external parasites in production livestock (Vercruysse and Rew 2002), including reindeer (Oksanen et al. 1992). Most of the drug is excreted unaltered in the faeces irrespective of route of administration (Halley et al. 1989; Sommer et al. 1992). Ivermectin is highly lipophilic, with correspondingly low solubility in water (Campbell 1989), binding strongly to particulate material in faeces, with very little leaching or elution induced by rain (Halley et al. 1989; Sommer and Steffansen 1993; Tolls 2001). It is not phytotoxic, antibacterial, or antifungal, is practically immobile in soil, and there is little uptake by plants (Campbell 1989; Halley et al. 1993). When present in water or as thin films on surfaces, ivermectin is rapidly photodegraded to less bioactive compounds (Halley et al. 1993).

After the introduction of ivermectin to the marketplace in 1981, there has been concern about possible impacts of excreted ivermectin on non-target organisms, such as soil or dung dwelling fauna, and thus also pertaining to dung degradation and nutrient cycling (e.g. Strong 1993; Herd 1995). With the lapse in patent protection in the late 1990s, ivermectin is now open to generic manufacture, leading to less expensive products and thus the likelihood of greater use. The environmental impact of the drug remains an issue of continued controversy (e.g. Kryger et al. 2005; Yeates et al. 2006).

Ivermectin treatment of reindeer is targeted at the larval stages of the warble fly (*Hypoderma tarandi*) and throat bot fly (*Cephenemyia trompe*) and various nematode species (Nordkvist et al. 1983; Haugerud et al. 1993). A large proportion (> 80% in some locations) of reindeer in northern areas of Finland, Norway and Sweden is treated with ivermectin once annually, during the winter round-ups between October and February. This early-winter treatment recommendation is based mainly on the understanding that the larvae of the flies will be targeted before they cause excessive harm to the host and at the latest before their spring pupation. Summer antiparasitic treatment is not commonly practiced since it is managerially difficult with the free ranging reindeer herds and also is considered epidemiologically inappropriate (Yeates et al. 2006).

Following treatment of reindeer with ivermectin by subcutaneous injection, the faecal concentration increase to a maximum around day four after treatment, followed by gradual decrease, and residual levels can still be detected more than 30 days after treatment (Nilssen et al. 1999). Faeces from treated reindeer thus leads to high local concentrations of ivermectin in the field.

There are discrepancies concerning the degradation of faecally excreted ivermectin. For instance, Lumaret et al. (1993) reported concentrations of ivermectin in dung from cattle to be reduced to zero about seven days after treatment, whereas Sommer and Steffansen (1993) found no apparent degradation of ivermectin in cattle dung on pasture over a 45-day period of aging under both

temperate and tropical conditions. With the exception of Nilssen et al. (1999), there are no other published reports on the persistence of faecally excreted ivermectin from reindeer on pasture. This study addresses the issue of persistence in reindeer dung on a sub-Arctic natural reindeer pasture.

MATERIALS AND METHODS

Animals and treatments

In November 2001, eight 25-week-old female reindeer calves, obtained from the Finnish Reindeer Herders' Association Field Station herd, Kaamanen, Finland, were retained indoors and fed lichen *ad libitum*. The daily faecal production were collected during a two-week period, pooled, and stored at 4 ^{o}C (control-dung-01). One week later, the calves received ivermectin by s.c. injection (Ivomec® 10 mg/ml vet. inj.; Merial Inc., Haarlem, Holland) or by the oral route (Ivomec® 0.8 mg/ml vet. mixt., Merial). The dose rate was 200 μg ivermectin/kg body mass as recommended by the manufacturer. The daily faecal production from each treatment group was collected during the first nine days following treatment and pooled in two bags (IVM-01 Oral, IVM-01 SC) and stored at 4 ^{o}C for subsequent distribution on experimental plots.

In November 2002, the feed and ivermectin treatment regime was repeated with another set of eight reindeer calves, and faeces were collected resulting in control-dung-02 and ivermectin treatment groups dung (IVM-02 Oral, IVM-02 SC).

Experimental plots and study area

In December 2001, 1 m x 1 m plots were established in two separate enclosures of forested reindeer pasture of the Reindeer Research Station in Kaamanen, Finland (69º N, 27º E); one fenced to prevent reindeer access (ungrazed) since 1995, the other (grazed) on an area which had been heavily stocked by reindeer for five years up until the commencement of the experiment. The plots within each area were located 1 to 5 m away from each other and delimited by a survey stake in each corner. A 2.5 m high wooden fence delimited each of the enclosures, preventing reindeer and large wildlife from entering.

The vegetation of both enclosures was dominated by approximately 100 year-old pine, *Pinus sylvestris*. On the ungrazed enclosure there was reindeer lichen (*Cladina* spp.), moss (*Pleurozium* spp., *Dicranum* spp., *Polyrihum* spp), lingonberry (*Vaccinium vitis-idaéa*), blueberry (*Vaccinium myrtillus*), heath (*Calluna vulgaris*), Labrador tea (*Ledum palustre*), crowberry (*Empetrum nigrum*) and a variety of fungi. The grazed enclosure had a sparse vegetation of heath, shrubs and moss, and reindeer lichen was absent.

Meteorology

The Finnish Meteorological Institute provided the precipitation data from the airport at Ivalo, and temperature and sunlight hours from the township of Kevo. Both localities are within 80 km from Kaamanen. Snow depth and ambient temperature was measured at the Reindeer Research Station in Kaamanen.

Distribution of faeces on plots

On 5 December 2001, 400 g/plot of each of the IVM-01 Oral dung, IVM-01 SC dung and control-dung-01 were distributed on one plot each at the ungrazed and the grazed enclosure. This amount per plot is comparable to what would be deposited on an area with few reindeer roaming during the year. In addition, one plot on each enclosure were designated true controls void of all faeces (no-dung-01 plots). On 11 December 2002, 5 kg/plot of each of the IVM-02 Oral dung, IVM-02 SC dung and control-dung-02 were distributed on one plot each of the ungrazed and grazed enclosures. This amount per plot represents a heavy load per area unit, comparable to what would be deposited on an area densely populated with reindeer, such as on areas where reindeer are gathered in corrals for winter confinement and antiparasitic treatment. Both years, 10 - 20 cm of snow covered the experimental area at the time of faecal deposition. The faeces were evenly distributed between the delimiting survey stakes to ensure, as far as possible, that it would remain within the plot boundaries. Some of the plots had sloping gradients, and observation by subsequent sampling times showed that the faeces tended to settle towards the lower parts.

Soil sampling procedure

A 1 m^2 point frame was used as a guide to locate sampling points previously determined using random numbers. With a 10 cm x 10 cm square stainless steel soil corer, two subsamples were taken to a depth of 5 cm from each plot at each sampling time. Samples were located at least 10 cm away from each other. Each sample included faeces, vegetation and soil. Faeces comprised a minor and varying part of the total weight or volume.

For the plots with dung from 2001, sampling times were in May, July and September 2002, and in June, July, August and October 2003, spanning 25 to 95 weeks after faecal deposition. For the plots with dung deposited in 2002, sampling times were in June, July, August and October 2003, and in June, July, August and October 2004, spanning 25 to 95 weeks after faecal deposition. The samples were stored in plastic bags at –20 ºC for subsequent determination of ivermectin.

Determination of ivermectin

Ivermectin concentration in faeces was determined by HPLC using abamectin as internal standard (Åsbakk et al. 1999). Concentrations in composite (soil corer) samples were determined using the same method, with the only modification being that after thawing at room temperature, 10 g wet weight of the sample was used for analysis instead of 1 g of faeces. Prior to weighing, samples were thoroughly mixed in their plastic bags and pebbles >3–5 mm in size were removed. The heterogeneity of samples was great due to varying amounts of humus and mineral particles, vegetation components (pine needles, lichens, moss, grass etc.) and reindeer faeces.

Calculation of ivermectin concentration was based on linear calibration lines for the concentration ranges 2-100 and 100-500 ng/portion found after analysis of a range of ivermectin standards in samples from no-dung-01 plots. Concentrations were recorded as ng of ivermectin/g of dry weight sample, according to Åsbakk et

4

al. (1999). All samples were analysed in a blind manner, only with random numbers on sample bags and no further information available until after all samples had been analysed. However, after 40 control plot (no-dung and control-dung plots) samples devoid of ivermectin had been analysed, the remaining samples from control plots were identified and removed from the pool of samples to be analysed. For the sampling times in 2002 for plots with dung deposited in 2001, only one of the two subsamples from each plot and time was available for the analysis work.

Concentration of ivermectin in faeces deposited on plots

The mixing of the contents of each of the bags with the composite dung samples consisted of end-over-end shaking by hand. Faecal pellets were mechanically not substantially broken down by this form of mixing. Four subsamples of each of the IVM-02 Oral and the IVM-02 SC dung prior to deposition on plots were analysed. For the IVM-02 Oral dung subsamples concentrations were determined to 119, 179, 1555 and 2335 ng/g dry weight faeces, and for the IVM-02 SC dung subsamples concentrations were 7, 10, 49 and 70 ng/g dry weight faeces. Concentrations for the IVM-01 dung (Oral and SC) before deposition on plots were not obtained.

Statistical calculations

Ivermectin concentrations in groups of subsamples were compared using Student's *t*-test. Pearson's r statistic was used to check for any correlation between time of stay of faeces on experimental plots and residual levels of ivermectin.

RESULTS

Ivermectin analysis

The retention times for the B_{1a} peaks of abamectin and ivermectin (Åsbakk et al. 1999) were approximately 4.8 and 7.2 minutes, respectively, as evidenced from analysis of samples from no-dung-01 plots fortified with ivermectin and abamectin. All chromatogram peaks were well separated, making identification of peaks and concentration calculations unequivocal. The combined analysis results for the control samples showed that there were no components present in the soil or faeces giving peaks interfering to any significant extent with the abamectin or ivermectin peaks (Fig. 1).

Control plots

Six no-dung-01 plot samples and 34 control-dung plot samples (24 from control-dung-01 plots, 10 from control-dung-02 plots, ungrazed and grazed enclosures) were analysed (Table 1). Of these 40, ivermectin was absent in 38, while in the latter two concentrations were determined to 2.1 and 16 ng/g dry weight, respectively. The two were from the same plot of the ungrazed area, from June 2003 (77 weeks after deposition) and October 2003 (95 weeks after deposition).
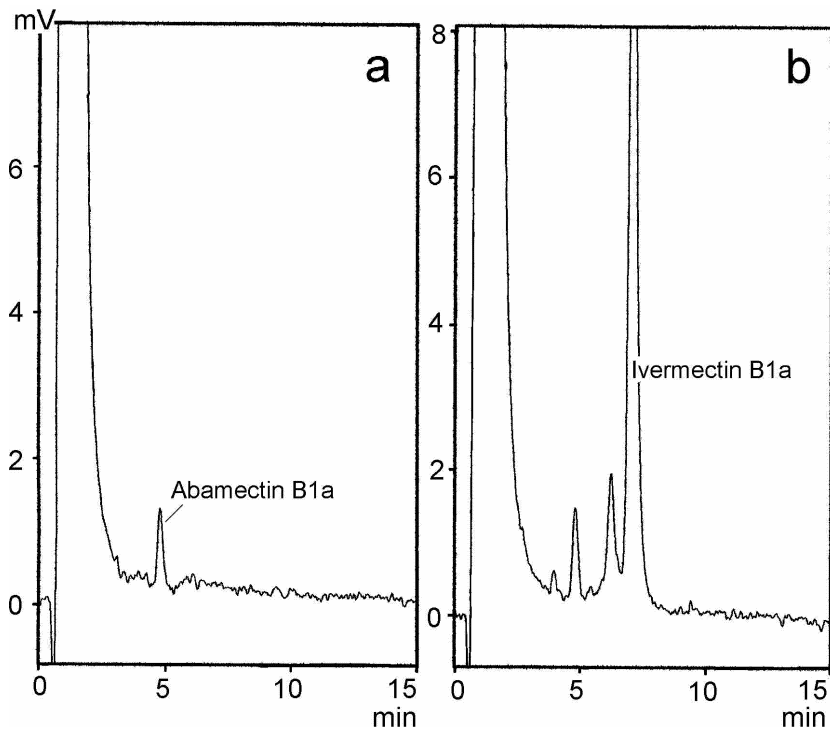
Figure 1. Typical HPLC chromatograms. Sample with no ivermectin, from plot with control dung (a), and sample with relatively low ivermectin concentration, 66 ng/g dry weight (b). Retention time in minutes, detector response in mV. The $B1_a$-peak for each of abamectin and ivermectin are indicated.

Plot	2002			2003				
	May (25)*	July (34)	Sept. (39)	June (77)		July (82)	October (95)	
No-dung-01 ungrazed or grazed	0 (n=2)	0 (n=2)	0 (n=2)					
Control-dung-01 ungrazed or grazed	0 (n=4)	0 (n=4)	0 (n=4)					
Control-dung-02 ungrazed				0 (n=3)	16 (n=1)	0 (n=3)	0 (n=3)	2.1 (n=1)
Control-dung-02 grazed				0 (n=4)		0 (n=3)	0 (n=4)	

*weeks after deposition

Table 1. Ivermectin (ng/g dry weight) in control plot samples.

6

Plots with dung deposited in 2001

The results for the 44 IVM-01 Oral and SC plot samples analysed are shown in Table 2.

Plot		May (25)*	July (34)	Sept. (39)	June (77)		July (82)		August (87)		October (95)	
		2002			2003							
Ungrazed	IVM-01 Oral	3.2	12	1.0	4.0	28	0	0	0	0	4.5	28
	IVM-01 SC	0.4	16	21	12	23	0	9.0	16	0	3.6	14
Grazed	IVM-01 Oral	3.9	10	8.6	0	36	0	0	0	0	3.9	23
	IVM-01 SC	0	6.1	0.4	1.3	3.2	0	0	0	0	0	5.6

*weeks after deposition

Table 2. Ivermectin (ng/g dry weight) in samples from plots with dung deposited in 2001.

Plots with dung deposited in 2002

Tables 3A and 3B gives the results for the 60 IVM-02 Oral and SC plot samples analysed. Student's *t*-test revealed a significant difference between the concentration of the June 2003-subsamples from the ungrazed (Oral and SC) plots as one group and the subsamples from the grazed (Oral and SC) plots as another group ($p = 0.01$). Pearson's r statistic showed that there was no significant reduction ($r = 0.27$) in the mean of the levels for the eight subsamples (ungrazed and grazed, Oral and SC) during the period from July 2003 (time zero in calculation) to August 2004 (13 months in calculation). Fig. 2 shows the mean of the levels for the eight subsamples from June 2003 to August 2004 together with standard deviations.

Plot		June (25)[1]		July (29)		August (34)		October (42)	
		2003							
Un-grazed	IVM-02 Oral	368	557	34	22	139	24	259	56
	IVM-02 SC	515	254	147	22	44	43	282	95
Grazed	IVM-02 Oral	113	143	147	144	86	94	268	70
	IVM-02 SC	66	111	17	60	302	47	213	163

[1]weeks after deposition

Table 3A. Ivermectin (ng/g dry weight) in samples from plots with dung deposited in 2002.

Plot		2004							
		June (80)[1]		July (84)		August (87)		October (95)	
Un-grazed	IVM-02 Oral	153	264	31	167	41	252	88	21
	IVM-02 SC	100	301	39	0	65	250	19	17
Grazed	IVM-02 Oral	22	650	258	154	35	145	n.d.[2]	n.d.
	IVM-02 SC	2.7	0	93	84	64	34	n.d.	n.d.

[1]weeks after deposition
[2]not determined

Table 3B. Ivermectin (ng/g dry weight) in samples from plots with dung deposited in 2002.
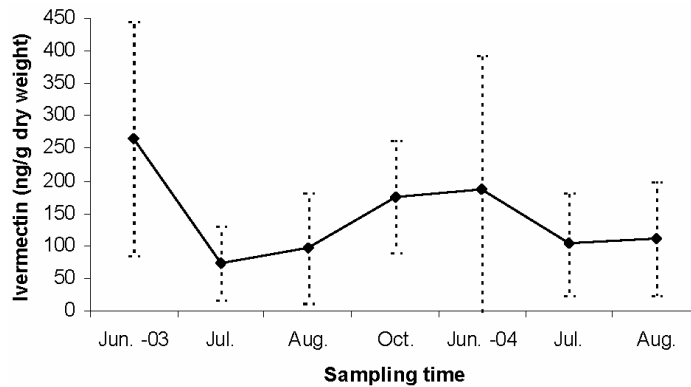
Figure 2. Mean and standard deviation for ivermectin concentrations of the eight subsamples (ungrazed and grazed plots, Oral and SC treatment) by the different sampling times until August 2004 for plots with dung deposited in December 2002.

Meteorology and plot observations

Meteorological data for the period May 2002 – December 2004 are summarised in Fig. 3. Snow cover was present from September - October and until May - early June, with maximum depths of nearly 45 cm in February - March. Below zero temperatures were common from October to April, with lowest temperatures in January (below –40 ^{o}C). Maximum temperatures were in July, exceeding 20 ^{o}C. At this latitude, there is 24 hour sunlight between mid-May and late July.

8

Figure 3. Meteorological data. Precipitation data from the airport at Ivalo, temperature and sunlight hours from Kevo, precipitation and snow depth from the Reindeer Research Station in Kaamanen. A: Mean monthly ambient temperature and total monthly sunlight. B: Total monthly precipitation (rain + snow) and monthly snowdepth.

Visual observation showed that by the time the first sampling took place immediately after spring thaw, faecal material on all plots was moist, pelleted, and did not appear to have broken down. With increasing day length and sunshine hours, faeces became dry, hard masses.

DISCUSSION

Top-soil samples from ungrazed and grazed enclosures of reindeer pasture with evenly distributed faeces from reindeer calves treated orally or subcutaneously with ivermectin, or with control faeces from untreated calves, were analysed by HPLC. Although each bulk of faecal material distributed on plots (IVM-02 Oral, IVM-02 SC) were mixed before use, four subsamples of the IVM-02 SC bulk showed ivermectin concentrations of from 7 to 70 (mean 34) ng/g dry faeces, and four subsamples from the IVM-02 Oral bulk showed concentrations of from 119 to 2335 (mean 1047) ng/g dry faeces. The dung consisted of small and loosely packed pellets which mostly remained intact after the mixing, and since it was collected over a nine day period following treatment, there would have been pellets with near

9

zero concentrations (day 1 post treatment, p.t.), and pellets with high concentrations (e.g. day 3-5 p.t.) (Nilssen et al. 1999). The great variation between the subsamples from the same bulk can most likely be explained in terms of the small amount (1 g) analysed. Each 1 g sample consisted of few pellets, each with either low, medium or high concentration. The difference between the IVM-02 Oral and IVM-02 SC bulk results may be explained partly by differences in excretion profiles. Following oral (intraruminal) administration of ivermectin to sheep, most of the dose was not absorbed to the body (Prichard et al. 1985) but rather bound to the ingesta, leading to more rapid excretion than after subcutaneous administration. Similar absorption differences can be seen in the reindeer (Oksanen et al. 1995). According to previously reported concentrations in dung from reindeer treated with ivermectin (Nilssen et al. 1999), and supported by the results presented here for the various plots, it is evident that considerable amounts of dung-excreted ivermectin were distributed on the plots, but distribution in terms of amount of ivermectin have obviously been uneven within each plot and between plots. The uneven distribution within the dung distributed on the plots was evident also by the great variation in concentration between subsamples from each plot at the various sampling times. The heterogeneity of the top-soil samples, each consisting of different amounts of humus, mineral soil, faeces and herbage, obviously also contributed to the variation between subsamples. Also the tendency of settling of faeces towards the lower parts of plots with sloping gradients may have contributed to the differences in measured levels. During the collecting and mixing of dung samples prior to distribution on plots, some pellets would inevitably be mechanically disintegrated, and uneven distribution with respect to pellet degradation on the plots would also contribute to concentration differences since ivermectin in comminuted pellets would be more exposed to photodegradation.

Of the total of 40 samples from control plots analysed, 38 showed zero concentration of ivermectin, demonstrating that there were no components in those samples interfering with the ivermectin peak of the chromatograms. Two, however, showed concentrations of 2.1 and 16 ng/g, respectively. Both were from a plot of the ungrazed area, and contamination of the area with ivermectin-containing faeces prior to or after deposition on plots can therefore be excluded as an explanation for the ivermectin levels. An alternative explanation could be that the faeces had been contaminated with ivermectin-containing pellets during the collection. The explanation is, however, most likely to be found as part of the ivermectin analysis procedure itself. Samples were run on HPLC in groups of five, with intermitting time of sample preparation for each five-sample group between HPLC-runs. The 16 ng-control sample was run as the fifth sample in the first group of five after the HPLC analysis work had started. The sample analysed on the apparatus prior to this one showed a very high level of ivermectin (557 ng/g), and therefore the most likely explanation is that there has been a spill-over from this sample to the subsequent sample due to insufficient washing of the sample injection syringe and needle. Sample 1, 2 and 3 of this first five-sample group all had high levels of ivermectin (254 - 515 ng/g), so insufficient washing resulting in ivermectin residues in the syringe in the range of 16 ng/g would have relatively little effect on results for these samples. However, after analysis of this first group of samples,

10

control HPLC runs where acetonitril alone was injected, showed low levels of remnants in the injection device from the previous sample injected. After this the number of washing cycles was sufficiently extended. Reanalysis would probably have resolved this discussion. The 2.1 ng-control was part of a group of five samples where the peak height for the abamectin internal control was very low compared to the abamectin peak of all other samples analysed. This was most likely due to accidental addition of too little volume of abamectin solution to the samples. The height of the peak corresponding to the ivermectin peak of the chromatogram was very low, virtually not higher than peaks that normally would be interpreted as noise in chromatograms with more normal height of the internal control peak. Thus, normally, this peak would not have been interpreted as ivermectin. The finding that this whole group of five samples showed these low internal control peak heights was not clearly discovered until after the completion of the analysis work, and the samples were unfortunately not reanalysed. The other samples of this five-sample group all showed zero concentrations.

For the plots established in 2002, by June 2003, approximately six months after distribution of faeces, concentrations for the four IVM-02 ungrazed plots (Oral and SC) subsamples ranged between 254 and 557 ng/g dry weight (mean 423), and for the four subsamples representing the IVM-02 grazed plots (Oral and SC) concentrations ranged between 66 and 143 ng/g (mean 108). Student's t-test revealed that concentrations for the ungrazed plot subsamples as one group were significantly higher than concentrations for the grazed plot subsamples as a group. This could have resulted from a higher rate of photodegradation on the grazed area during late winter and early spring due to the more abundant lichen and other vegetation on the ungrazed plots, with dung pellets buried in prostrate vegetation. According to the meteorological data, there could have been some days before the sampling in June where plots were free from snow cover. Another factor contributing to the explanation of the lower degradation on the ungrazed enclosure could be that the presence of light colored reindeer lichen on this enclosure could make the snow cover persist longer than on the generally darker surface of the lichen-free grazed enclosure. A third factor which may also have contributed to the higher rate of degradation on the grazed enclosure could be accelerated weathering and mechanical breakdown of dung pellets due to greater exposure to wind and rain erosion of pellets not buried in vegetation. Weathering, livestock trampling and disturbance by birds contribute to the rate of livestock dung degradation (Halley et al. 1993; McKellar 1997)

By July and August 2003, levels on the ungrazed plots had declined so that the mean of levels were similar for the ungrazed and grazed plot samples. The highest concentration determined, 650 ng/g, was in June 2004, 80 weeks after deposition, in a sample from a grazed plot. During the entire period of approximately 20 months from December 2002 to August 2004, the mean concentration for each set of eight subsamples by each sampling time was higher than 74 ng/g, and mean levels did not decrease significantly from July 2003 until August 2004. The results for the concentrations on the plots established in 2001, with no obvious decrease in concentrations over the time of the study as inferred from the different single sample results, supported the results for the plots established in 2002. The results clearly show that considerable amounts of ivermectin can persist on reindeer

11

pasture for a time exceeding two grazing seasons following treatment, and thus for considerably longer time than shown in any earlier reported study.

It is well documented that ivermectin residues in faeces of livestock may have detrimental effects on several species of dung-dwelling insects such as various species of Diptera and Coleoptera, particularly their larval stages (Wall and Strong 1987; Madsen et al. 1990; Sommer et al. 1992; Barth et al. 1993; Strong 1993; Krüger and Scholtz 1998a, 1998b; Wardhaugh et al. 2001). The effects, which range from being sublethal to lethal (Herd 1995), may result in retarded rates of dung degradation (Wall and Strong 1987; Madsen et al. 1990; Sommer and Bibby 2002; Svendsen et al. 2003). It has, however, also been shown that dung from cattle treated with ivermectin can degrade normally (Jacobs et al. 1988; McKeand et al. 1988). Many studies on the impact of ivermectin on dung fauna and dung degradation were conducted in temperate climatic zones of the northern hemisphere, where earthworms play a major role in the degradation process (Curry 1987; Holter 1979). Several of these studies concluded that following typical usage of ivermectin in cattle, there was no adverse effect on the survival and growth of earthworms (e.g. *Lumbricus terrestris*) (Halley et al. 1989; Madsen et al. 1990; Halley et al. 1993; Barth et al. 1994; Svendsen et al. 2005). Thus, where earthworms are abundant, detrimental effects of faecally excreted ivermectin on the activity of insect larvae in dung pats may be overridden by the effect of earthworms (Svendsen et al. 2003).

Barth et al. (1993) found that numbers of some dung-specific saprophytic nematodes were reduced in pats from cattle treated with ivermectin. They registered no toxic effects on soil nematodes that invaded the pats. Similarly, there were no toxic effects on the soil nematode *Pristionchus maupasi* in naturally excreted concentrations of ivermectin in cattle faeces (Grønvold et al. 2004). Yeates et al. (2002; 2003) and Dimander et al. (2003) found no detrimental effects on total numbers, diversity or functional groups of nematodes from faecally excreted ivermectin from cattle. Results of a companion study to this investigation indicate that faecally excreted ivermectin from reindeer had no detectable negative effects on the soil nematode communities beneath the dung (Yeates et al. 2006).

The arctic soil faunal composition is characterized by reduced species diversity compared to more temperate regions. In the Arctic, earthworms are scarce or absent, and springtails (Collembola), mites (Acari), enchytraeids (Annelida: Oligochaeta: Enchytraeidae) and nematodes (Nematoda) are particularly important in decomposing of organic matter and nutrient cycling (Rusek 1998; Laakso and Setälä 1999; van der Wal 2004; Jänsch et al. 2005; Sjursen et al. 2005). Mites and springtails disperse detritus particles and feed on microorganisms (Swift et al. 1979; Seastedt 1984), and the abundance of springtails may reach up to several million/m^2. The highest biomasses of springtails have been demonstrated on the tundra biome (Rusek 1998), but the biodiversity also of springtails is characteristically low in arctic regions. Enchytraeids are part of the saprophagous fauna of the litter and upper layer of mineral soils (Didden 1993), and also the largest populations of enchytraeids have been found in cold to temperate habitats (Didden 1993). Through their feeding activity and digging ability, they promote a fine-grained structure of surface soil layers which improve aeration and water

drainage. Reduced abundance or activity of enchytraeids may render the soil structure less stable and more compact (Didden 1993; Jänsch et al. 2005), and this could in turn bring about increased soil erosion during snow melt and heavy rain. Also soil nematode diversity is low in the arctic (Boag and Yeates 1998). If one or more species of these few and important organism groups are particularly vulnerable to soil ivermectin residues, the ecological impact may be more pronounced than in ecosystems with higher species diversity.

Since the antiparasitic treatment of reindeer is normally done in early winter, the faeces with ivermectin is usually deposited on frozen ground where presence or activity by most insects is excluded. In addition, egg-laying adults of coprophilic dung beetles and flies are attracted only to freshly deposited dung (Waller and Faedo 1996). Reindeer winter dung consists of small (11-12 mm) dry pellets which appear to be unattractive for most coprophilous beetles and flies in the subsequent warmer seasons. These insects therefore play insignificant roles in degradation of dung from reindeer after winter treatment (Nilssen et al. 1999).

Due to photodegradation, it would be reasonable to assume that faecally excreted ivermectin on pasture would disappear during the subsequent summer under light regimes such as in Kaamanen and other northern sites (24 h daylight during summer months). In contrast to the considerable amount of information available on the impact of faecally excreted ivermectin residues on coprophilic organisms in more environmentally equitable climates, very little is known of possible impacts in relation to springtails, mites and enchytraeids of arctic and sub-arctic ecosystems. Due to the hydrophobicity of ivermectin, the drug will remain bound to the faecal organic matter on pasture (Halley et al. 1989; Tolls 2001). In the opaque reindeer dung pellet ivermectin will be protected from photodegradation, as has been demonstrated under more temperate conditions for ivermectin within pats or in dung stored below ground by dung beetles (Herd 1995). It can thus be speculated that ivermectin in interior parts of intact reindeer dung pellets can remain largely unchanged as long as the dung is not mechanically degraded.

A report on two soil dwelling species, the springtail *Folsomia fimetara* and the enchytraeid *Enchytraeus crypticus* (Jensen et al. 2003) demonstrated a threshold value for toxicity of ivermectin (10 % reduced reproduction or EC10 values) to the springtail of 0.26 mg/kg (260 ng/g) dry soil. The threshold value for the enchytraeid was higher. The value for the springtail is within the range of sample concentrations determined in the present study. Thus, there are strong grounds to support the need for further environmental evaluation studies on the use of ivermectin to control parasites of reindeer.

ACKNOWLEDGEMENTS

REFERENCES

Barth, D.; Heinze-Mutz, E.M.; Roncalli, R.A.; Schlüter, D.; Gross, S.J. The degradation of dung produced by cattle treated with an ivermectin slow-release bolus. *Vet. Parasitol.* 1993, *48*, 215-227.

Barth, D.; Heinze-Mutz, E.M.; Langholff, W.; Roncalli, R.A.; Schlüter, D. Colonisation and degradation of dung pats after subcutaneous treatment of cattle with ivermectin or levamisole. *Appl. Parasitol.* 1994, *35*, 277-293.

Boag, B.; Yeates, G.W. Soil nematode biodiversity in terrestrial ecosystems. *Biodivers. Conserv.* 1998, *7*, 617-630.

Campbell, W.C., Ed. Ivermectin and abamectin. Springer-Verlag, New York, 1989.

Curry, J.P. The invertebrate fauna of grassland and its influence on productivity. 1. The composition of the fauna. *Grass Forage Sci.* 1987, *42*, 103-120.

Didden, W.A.M. Ecology of terrestrial *Enchytraeidae*. *Pedobiologia* 1993, *37*, 2-29.

Dimander, S.O.; Höglund, J.; Waller, P.J. Disintegration of dung pats from cattle treated with the ivermectin bolus, of the biocontrol agent *Duddingtonia flagrans*. *Acta Agricult. Scand. Sect. A, Animal Sci.* 2003, *44*, 171-180.

Grønvold, J.; Svendsen, T.S.; Kraglund, H.-O.; Bresciani, J.; Monrad, J. Effects of the antiparasitic drugs fenbendazole and ivermectin on the soil nematode *Pristionchus maupasi*. *Vet. Parasitol.* 2004, *124*, 91-99.

Halley, B.A.; Jacob, T.A.; Lu, A.Y.H. The environmental impact of the use of ivermectin: environmental effects and fate. *Chemosphere* 1989, *18*, 1543-1563.

Halley, B.A.; VandenHeuvel, W.J.A.; Wislocki, P.G. Environmental effects of the usage of avermectins in livestock. *Vet. Parasitol.* 1993, *48* (1-4), 109-125.

Haugerud, R.E.; Nilssen, A.C.; Rognmo, A. On the efficacy of ivermectin against the reindeer sinus worm *Linguatula arctica* (Pentastomida), with a review on ivermectin treatment in reindeer. *Rangifer* 1993, *13* (3), 157-162.

Herd, R. Endectocidal drugs: ecological risks and counter-measures. *Int. J. Parasitol.* 1995, *25* (8), 875-885.

Holter, P. Effect of dung-beetles (*Aphodius spp.*) and earthworms on the disappearence of cattle dung. *Oikos* 1979, *32*, 393-402.

Jacobs, D.E.; Pilkington, J.G.; Fisher, M.A.; Fox, M.T. Ivermectin and degradation of cattle faeces. *Vet. Rec.* 1988, *123*, 400.

Jänsch, S.; Römbke, J.; Didden, W. The use of enchytraeids in ecological soil classification and assessment concepts. *Ecotox. Environ. Safety* 2005, in press, www.sciencedirect.com.

Jensen, J.; Krogh, P.H.; Sverdrup, L.E. Effects of the antibacterial agents tiamulin, olanquindox and metronidazole and the anthelmintic ivermectin on the soil invertebrate species *Folsomia fimetaria* (Collembola) and *Enchytraeus crypticus* (Enchytraediae). *Chemosphere* 2003, *50*, 437-443.

Krüger, K.; Scholtz, C.H. Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. I. Impact of ivermectin under drough conditions. *Acta Oecol.* 1998a, *19* (5), 425-438.

Krüger, K.; Scholtz, C.H. Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. I. Impact of ivermectin under high-rainfall conditions. *Acta Oecol.* 1998b, *19* (5), 439-451.

Kryger, U.; Deschodt, C.; Scholtz, C.H. Effects of flurazon and ivermectin treatment of cattle on the structure of dung beetle communities. *Agricult. Ecosyst. Environm.* 2005, *105*, 649-656.

Laakso, J.; Setälä, H. Sensitivity of primary production to changes in the architecture of belowground food webs. *Oikos* 1999, *87*, 57-64.

Lumaret, J.P.; Galante, E.; Lumbreras, C.; Mena, J.; Bertrand, M.; Bernal, J.L.; Cooper, J.F.; Kadiri, N.; Crowe, D. Field effects of ivermectin residues on dung beetles. *J. Appl. Ecol.* 1993, *30*, 428-436.

Madsen, M.; Nielsen, B.O.; Holter, P.; Pedersen, O.C.; Jespersen, J.B.; Vagn Jensen, K.-M.; Nansen, P.; Grønvold, J. Treating cattle with ivermectin: effects on the fauna and decomposition of dung pats. *J. Appl. Ecol.* 1990, *27*, 1-15.

McKeand, J.; Bairden, K.; Ibarra-Silva, A.-M. The degradation of bovine faecal pats containing ivermectin. *Vet. Rec.* 1988, *122*, 587-588.

McKellar, Q.A. Ecotoxicology and residues of anthelmintic compounds. *Vet. Parasitol.* 1997, *72*, 413-435.

Nilssen, A.C.; Åsbakk, K.; Haugerud, R.E.; Hemmingsen, W.; Oksanen, A. Treatment of reindeer with ivermectin – effect on dung insect fauna. *Rangifer* 1999, *19* (2), 61-69.

Nordkvist, M.; Rehbinder, C.; Christensson, D.; Rönnbäck, C. A comparative study on the efficacy of four anthelmintics on some important reindeer parasites. *Rangifer* 1983, *3* (2), 19-38.

Oksanen, A.; Nieminen, M.; Soveri,T.; Kumpula, K. Oral and parenteral administration of ivermectin to reindeer. *Vet. Parasitol.* 1992, *41*, 241-247.

Oksanen, A.; Norberg, H.; Nieminen, M.; Bernstad, S. Influence of route of administration on the plasma concentrations of ivermectin in reindeer. *Res. Vet. Sci.* 1995, *58*, 286-287.

Prichard, R.K.; Steel J.W.; Lacey, E.; Hennessy, D.R. Pharmacokinetics of ivermectin in sheep following intravenous, intra-abomasal or intraruminal administration. *J Vet Pharmacol Ther.* 1985, *8,* 88-94.

Rusek, J. Biodiversity of Collembola and their functional role in the ecosystem. *Biodiv. Conserv.* 1998, *7*, 1207-1219.

Seastedt, T.R. The role of microarthropods in decomposition and mineralization processes. *Annu. Rev. Entomol.* 1984, *29*, 25-46.

Sjursen, H.; Michelsen, A.; Holmstrup, M. Effects of freeze-thaw cycles on microarthropods and nutrient availability in a sub-Arctic soil. *Appl. Soil Ecol.* 2005, *28*, 79-93.

Sommer, C.; Steffansen, B.; Nielsen, B.O.; Jensen, V.; Jespersen, K.M.V.; Springborg, J.B.; Nansen, P. Ivermectin excreted in cattle dung after subcutaneous injection or pour-on treatment – concentrations and impact on dung fauna. *Bull. Entomol. Res.* 1992, *82*, 257-264.

Sommer, C.; Bibby, B.M. The influence of veterinary medicines on the decomposition of dung organic matter in soil. *Eur. J. Soil Biol.* 2002, *38*, 155-159.

Sommer, C.; Steffansen, B. Changes with time after treatment in the concentrations of ivermectin in fresh cow dung and in cow pats aged in the field. *Vet. Parasitol.* 1993, *48* (1-4), 67-73.

Strong, L. Overview: the impact of avermectins on pastureland ecology. *Vet. Parasitol.* 1993, *48*, 3-17.

Svendsen, T.S.; Grønvold, J.; Holter, P.; Sommer, C. Field effects of ivermectin and fenbendazole on earthworm populations and the disappearence of dung pats from bolus-treated cattle. *Appl. Soil Ecol.* 2003, *24*, 207-218.

Svendsen, T.S.; Hansen, P.E.; Sommer, C.; Martinussen, T.; Grønvold, J.; Holter, P. Life history characteristics of *Lumbricus terrestris* and effects of the veterinary antiparasitic compounds ivermectin and fenbendazole. *Soil Biol. Biochem.* 2005, 37 (5), 927-936.

Swift, M.J.; Heal, O.W.; Anderson, M.J. Decomposition in terrestrial ecosystems. University of California Press, Berkley, USA, 1979, 372 pp.

Tolls, J. Sorption of veterinary pharmaceuticals in soils: a review. *Environ. Sci. Technol.* 2001, *35*, 3397-3406.

van der Wal, R.; Bardgett, R.D.; Harrison, K.A.; Stien, A. Vertebrate herbivores and ecosystem control: cascading effects of faeces in tundra ecosystems. *Ecography* 2004, *27*, 242-252.

Vercruysse, J.; Rew, R.S. Macrocyclic lactones in antiparasitic therapy. CABI Publishing, Oxon, New York, 2002.

Wall, R.; Strong, L. Environmental consequences of treating cattle with the anti-parasitic drug ivermectin. *Nature* 1987, *327*, 418-421.

Waller, P.J.; Faedo, M. The prospects for biological control of the free-living stages of nematode parasites of livestock. *Int. J. Parasitol.* 1996, *26*, 915-925.

Wardhaugh, K.G.; Holter, P.; Longstaff, B. The development and survival of three species of coprophagous insect after feeding on the faeces of sheep treated with controlled-release formulations of ivermectin or albendazole. *Aust. Vet. J.* 2001, *79* (2), 125-132.

Yeates, G.W.; Dimander, S.-O.; Waller, P.J.; Höglund, J. Environmental impact on soil nematodes following the use of the ivermectin sustained-release bolus or the nematophagous fungus *Duddingtonia flagrans* to control nematode parasites of cattle in Sweden. *Acta Agric. Scand., Sect. A, Animal Sci.* 2002, *52*, 233-242.

Yeates, G.W.; Dimander, S-O.; Waller, P.J.; Höglund, J. Soil nematode populations beneath faecal pats from grazing cattle treated with the ivermectin sustained-release bolus or fed the nematophagous fungus *Duddingtonia flagrans* to control nematode parasites. *Acta Agric. Scand., Sect. A, Animal Sci.* 2003, *53*, 197-206.

Yeates, G.W.; Hrabok, J.T.; Oksanen, A.; Nieminen, M.; Waller, P. Soil nematode populations beneath faeces from reindeer treated with ivermectin. *Acta Agric. Scand., Sect. A, Animal Sci.* 2006, in press.

Åsbakk, K.; Bendiksen, H.R.; Oksanen, A. Ivermectin in reindeer feces: Determination by HPLC. *J. Agric. Food Chem.* 1999, *47*, 999-1003.