Department of Veterinary Biosciences  
Faculty of Veterinary Medicine  
University of Helsinki  
Finland  

and  

Fish and Wildlife Health Research Unit  
Finnish Food Safety Authority Evira  
Oulu  
Finland  

Setaria tundra, an emerging parasite of reindeer,  
and an outbreak it caused in Finland in 2003-2006  

Sauli Laaksonen  

ACADEMIC DISSERTATION  
To be presented, with the permission of  
the Faculty of Veterinary Medicine, University of Helsinki  
for public examination  
in Walter Hall,  
Agnes Sjöbergin katu 2, Helsinki,  
on 12th February, 2010, at 12 noon.  

HELSINKI 2010
CONTENTS

AUTHOR’S NOTE 7
ABSTRACT 8
LIST OF ORIGINAL PUBLICATIONS 10
ABBREVIATIONS AND DEFINITIONS 10

1. INTRODUCTION 12

2. LITERATURE REVIEW 14
   2.1. The Superfamily Filarioidea 14
   2.2. Filaroid nematodes in reindeer 14
      2.2.1. Setaria in reindeer 14
   2.3. Setaria tundra in Scandinavian reindeer 15
      2.3.1. The life history of S. tundra in Fennoscandia 15
   2.4. Setaria-associated pathological changes in reindeer and impact on meat hygiene 15
   2.5. Setaria in wild cervids 16
      2.5.1. Setaria in roe deer (Capreolus capreolus) 16
      2.5.2. Setaria in moose (elk) (Alces alces) 16
      2.5.3. Setaria in deer 16
      2.5.4 The pathogenity of Setaria infection in wild cervids 17
   2.6. Setaria microfilaria (smf) in cervids 17
   2.7. Prevalence of Setaria in relation to host age 18
   2.8. Setaria infections in aberrant hosts 18
      2.8.1. Prenatal infection 19
      2.8.2. Possible zoonotic character 19
   2.9. Transmission 19
   2.10. Vectors of Setaria spp. 20
   2.11. The development of Setaria larvae 20
      2.11.1. Life cycle in the definitive host 20
      2.11.2. Development in arthropod vectors 20
      2.11.3. Vector/intermediate host - parasite interaction 21
   2.12. Prevention of setariosis; antiparasitic treatment and prophylaxis 22
      2.12.1 Antiparasitic treatment with ivermectin 22
      2.12.2 Prophylaxis 22
   2.13. Climate change 23
3. AIMS OF THE STUDY

4. MATERIALS AND METHODS

4.1. Study design

4.2. Free-ranging reindeer at slaughter
    4.2.1. Pathology and meat hygiene
    4.2.2. Parasite collection

4.3. Captive reindeer

4.4. Wild cervids
    4.4.1. Pathology
    4.4.2. Parasite collection

4.5. Arthropods (IV)
    4.5.1. Developmental studies in mosquitoes
    4.5.2. Setaria in wild arthropods
    4.5.3. Questionnaire

4.6. Antiparasitic prevention (V)
    4.6.1. Autumn ivermectin trial
    4.6.2. Winter ivermectin trial
    4.6.3. Summer ivermectin and deltamethrin trial
    4.6.4. Questionnaire

4.7. Climate
    4.7.1. The behavioural response of reindeer to weather
    4.7.2. Climate data

4.8. Laboratory
    4.8.1. Histopathology
    4.8.2. Bacteriology
    4.8.3. Meat hygiene
    4.8.4. Parasitology
        4.8.4.1. Adult Setaria
        4.8.4.2. Setaria microfilaria
        4.8.4.3. Setaria in arthropods
    4.8.5. Arthropods
    4.8.6. Genetics

4.9. Statistics
5. RESULTS

5.1. Peritonitis, disease
   5.1.1. Reindeer at slaughter
   5.1.2. Wild cervids
   5.1.3. Observations in non-slaughtered reindeer

5.2. *Setaria tundra*, the causative agent
   5.2.1. Adult *S. tundra*
      5.2.1.1. Morphology
      5.2.1.2. Genetics
   5.2.2. *S. tundra* microfilaria
      5.2.2.1. Morphology
      5.2.2.2. Genetics
   5.2.3. Third-stage larvae
      5.2.3.1. Morphology
      5.2.3.2. Genetics

5.3. Epidemiology of *S. tundra* microfilariae in cervid blood
   5.3.1. *S. tundra* microfilariae in free-ranging reindeer
   5.3.2. *S. tundra* microfilariae in captive reindeer (III)
   5.3.3. *S. tundra* microfilariae in wild cervids (III)

5.4. *S. tundra* in arthropods, transmission
   5.4.1. The development of *S. tundra* in mosquitoes
      5.4.1.1. Outdoor conditions (IV)
      5.4.1.2. Laboratory conditions
         5.4.1.2.1 *Aedes* mosquitoes (IV)
         5.4.1.2.2. *Anopheles* (IV)
      5.4.1.3 Melanisation (IV)
   5.4.2. *S. tundra* in wild insects
   5.4.3. Questionnaire survey
      5.4.3.1. Mosquito harassment during the summers of 2003, 2004 and 2005
      5.4.3.2. Activity of Culicidae
      5.4.3.3. “Räkkä” (insect harassment) effect on reindeer behaviour

5.5. The antiparasitic treatment and prevention regimes against setariosis
   5.5.1. Autumn ivermectin trial
   5.5.2. Winter ivermectin trial
   5.5.3. Summer ivermectin and deltamethrin trial
   5.5.4. The status of antiparasitic treatment
AUTHOR’S NOTE

I had worked as a reindeer meat inspector as well as a practising veterinarian for over twenty years in Kuusamo, while holding the position of municipal veterinarian. During a few years after 2001, an increasing number of reindeer viscera, especially livers, had been condemned in Kuusamo, mainly due to parasitic lesions. The situation developed slowly but noticeably worsened, until autumn 2003, when an outbreak of peritonitis in reindeer calves occurred. Subsequently, the outbreak was discovered and live *Setaria* sp. parasites were detected for the first time in the Kuusamo area. I was encouraged by Professor Antti Oksanen to pursue a research career in the Finnish Food Safety Authority Evira (earlier the National Veterinary and Food Research Institute, EELA), and to become acquainted with this previously unexperienced phenomenon. From a researcher’s point of view, the situation was particularly fascinating and challenging. I was able to ride on the back of an emerging, vector-borne, parasitic outbreak among sub-arctic cervids in a huge wilderness, and what an adventure it was to become in the years that followed.
ABSTRACT

Recent Finnish studies have revealed an array of filarioid nematodes and associated diseases that appear to be emerging in northern ungulates. All filarioid species produce microfilariae that are present in the host blood, and known vectors are haematophagous arthropods.

Infections attributable to a species of the genus *Setaria* appear to have emerged in Scandinavian reindeer in 1973. The infections were associated with an outbreak of peritonitis. In the same year, tens of thousands of reindeer died in the northern part of the reindeer herding area of Finland. Severe peritonitis and large numbers of *Setaria* sp. worms were common findings. However, the prevalence of *Setaria* sp. in Scandinavian reindeer subsequently diminished.

In Finland, the latest outbreak of peritonitis in reindeer started in 2003 in the southern and middle parts of the reindeer herding area. The proportion of reindeer viscera condemned due to parasitic lesions identified during meat inspections increased dramatically. These increases caused substantial economic losses and increased the workload associated with meat processing. The focus of the outbreak moved northward by approximately 100 km/yr, and by 2005 only the reindeer in Upper Lapland were free of lesions. During the same period, the peritonitis outbreak was apparently fading away in the southern area. The causative, agent based on morphological and molecular data, was identified as *Setaria tundra*.

Reindeer calves with heavy infections of *S. tundra* expressed decreased thriftiness, poor body condition, and an undeveloped winter coat. Meat/post mortem inspection of diseased reindeer carcasses revealed ascites fluid, green fibrin deposits, adhesions, and live and dead *S. tundra* nematodes. Histopathology indicated granulomatous peritonitis with lymphoplasmacytic and eosinophilic infiltration. No specific bacterial growth was found. No significant impact on pH values of meat or on the organoleptic evaluation of meat was found. There was a significant positive correlation between worm counts and the degree of peritonitis, and a negative correlation between the degree of peritonitis and the back-fat layer. Based on the evidence in both ante and post mortem inspections and histological examinations, present studies and historical data indicate that *S. tundra* can act as a significant pathogen in reindeer.

The prevalence and density of *Setaria* microfilariae (smf) were higher in reindeer calves than in adults; the overall prevalence was 42%. In order to monitor the dynamics of *S. tundra* in nature, wild cervids also were sampled. The overall smf prevalences for moose, wild forest reindeer and roe deer were 1.4-1.8%, 23% and 44%, respectively. The focus of microfilaremia in reindeer moved north while simultaneously declining in the south as the observed peritonitis outbreak decreased. Experimentally, in reindeer calves infected in their first summer of life the peak microfilaremia was recorded in their second summer. Captive reindeer were smf positive throughout the year, but smf disappeared from the blood after 2 years. The prepatent period of *S. tundra* was estimated to be about 4 months, with a life span of at least 14 months.
Moose are apparently not a suitable reservoir host for the *S. tundra* haplotype occurring in reindeer. The previous report of a peritonitis outbreak in moose associated with *Setaria* sp. nematodes in Finnish Lapland in 1989 was caused by another *S. tundra* haplotype.

It may well be that among other factors, the high percentage of wild forest reindeer with signs of peritonitis caused by *S. tundra* may also have contributed to a substantial population decline for this herd in Kainuu (1700 to 1000 in 2001-2005). Although *S. tundra* is at present maintained primarily in the reindeer population, roe deer seem to be a suitable host and asymptomatic carrier.

Mosquitoes, particularly *Aedes* spp. and to a lesser extent *Anopheles* spp., play an important role in the transmission of *S. tundra* in reindeer herding areas in Finland. The prevalence of *S. tundra* larvae in naturally infected Finnish mosquitoes varied from 0.5-2.5%. The rate of development in mosquitoes is temperature-dependent.

Ivermectin has good efficacy against adult *S. tundra* nematodes and circulating smf, and therefore there is an obligation to treat heavily infected reindeer calves with ivermectin by injection for animal welfare reasons. At the population level, massive antiparasitic treatment with ivermectin can reduce the number of carriers among reindeer population. The fact that this could not prevent the emergence of the *S. tundra* outbreak in new areas in the North indicates that the transmission dynamics of *S. tundra* are efficient.

The 1973 outbreak of *S. tundra* in Sweden was associated with unusually warm weather and abnormally high numbers of mosquitoes and gnats. The summers of 1972 and 1973 in Finland were also very warm, as were those in 2002 and 2003. Warm summers apparently promote transmission and the genesis of disease outbreaks by favouring the development of *S. tundra* in its mosquito vectors, by improving the rate of mosquito development and reducing their mortality from frost, and finally, by forcing reindeer to stay in herds on mosquito-rich wetlands.

Mosquito-borne diseases are among those most sensitive to weather and obviously will be influenced by climate change. Thus, I predict that global climate change will promote the further emergence of filarioid nematodes and diseases caused by them in the subarctic ecosystem. Moreover, I believe that future outbreaks can be predicted based on the mean temperatures of two consecutive summers.

This study indicated that *S. tundra* probably has an important impact on boreal ecosystems. It also revealed the absence of baseline knowledge concerning temporal parasitic biodiversity in cervids at high latitudes. Therefore, it is important to gain knowledge about these parasites, their ecology, transmission dynamics, and their impact on human and animal health. The putative relationship between climate change and a vector-borne disease identified in this thesis indicates the potential and obvious threats to the individual and population health of arctic ungulates.
LIST OF ORIGINAL PUBLICATIONS


ABBREVIATIONS AND DEFINITIONS

b.w. Body weight. The weight of an animal’s body.

CNS Central nervous system: The central nervous system is the part of the nervous system that consists of the brain and spinal cord.

DIC Differential interference contrast microscopy, based on the gradient of the optical path length (rate of change in wavefront shear).

DNA Deoxyribonucleic acid. One of two types of molecules that encode genetic information.

mf Microfilaria. The prelarval form of any filarial worm. Certain blood-sucking insects ingest these forms from an infected vertebrate host.
MGG  May-Grünwald-Giemsa staining.

PCR  Polymerase chain reaction, a technique in molecular genetics that permits the amplification of short sequences of DNA

per os, p.o.  Peroral, by way of the mouth, as in the administration of medication.

RNA  Ribonucleic acid, a nucleic acid molecule similar to DNA but containing ribose rather than deoxyribose.

Räkkä  Insect harassment. In Finnish.

s.c.  Subcutaneous: just under the skin.

SEM  Scanning electron microscope. An electron microscope that forms a three-dimensional image on a cathode-ray tube by moving a beam of focused electrons across an object and reading both the electrons scattered by the object and the secondary electrons produced by it.

smf  *Setaria* microfilaria. The prelarval form of any *Setaria* worm.

**Filarioidea**: A large superfamily of nematodes of the order Spirurida that comprises the medically important filarial worms and related forms having a slender thread-like body, a simple anterior end with inconspicuous oral lips, a cylindrical esophagus lacking a bulbus, and often unequal and dissimilar copulatory spicules in the male. They are carried and transmitted by mosquitoes and other invertebrates

**Filaria (Plural: filariae)**: Any parasitic nematode worms of the superfamily Filarioidea that live in the blood and tissues of vertebrate animals.

**Filariosis (synonym: filariasis)**: Disease caused by nematodes of the superfamily Filarioidea that invade the tissues and lymphatics of mammals producing reactions varying from acute inflammation to chronic scarring.

**Filaroid, filarial**: Adjective of, relating to, infested with, transmitting, or caused by filariae parasitic worms.

**Filaroid**: A common misspelling of the previous.

**Insecticidal**: Capable of killing insects or controlling their growth.

**Setariosis (synonym: setariosis)**: Infection with nematodes of the genus *Setaria*. 
1. INTRODUCTION

Reindeer (*Rangifer tarandus*) are circumpolar cervids belonging to the Order Artiodactyla, Suborder Ruminantia. The global reindeer herding area extends from the coast of Norway to the Bering Strait and, in the south, to Lake Baikal and Mongolia. The total number of semi-domesticated reindeer (*Rangifer tarandus tarandus*) is about two million (1.2 million in Russia, about 700,000 in Fennoscandia, with minimal populations in other countries including Alaska and Canada). Semi-domesticated reindeer in Fennoscandia are derived from Eurasian tundra/mountain reindeer (Kemppainen et al. 2003); populations in North America were derived from translocation and introduction from either Siberia or Fennoscandia, beginning in the late 1800s.

The reindeer husbandry area (the area where reindeer are allowed to range free) in Finland covers almost the entire area of the Province of Lapland and also a part of the Province of Oulu, being in total one third (114,000 km²) of the area of Finland (Fig. 1). The estimated autumn number of reindeer is 270,000 – 300,000, about one third of which are slaughtered yearly. More than two thirds of slaughter animals are calves (5 to 9 months). The yearly production of reindeer meat is about 2.5 million kg (value ~ EUR 5 million). (Kemppainen et al. 2003).

Figure 1.

The Finnish reindeer husbandry area (black) divided into four areas for analysis of the spatial development of the *Setaria tundra* associated peritonitis outbreak. Grey lines are the borders of Reindeer Herding Cooperatives. Dotted areas mark the northern (Kainuu area) and the southern (Suomenselkä area) populations of wild forest reindeer.
The slaughter season in Finland usually starts in October and continues through early winter, with the last reindeer being slaughtered in February. The reindeer are slaughtered in 19 EU-approved slaughterhouses in which the meat inspection is performed by veterinarians working under the State Provincial Office of Lapland. Smaller amounts (~ 20%) are slaughtered by traditional methods in the field for private consumption or direct marketing, mostly without official meat inspection.

In addition to reindeer herding being an important source of income (meat, by-products, tourism) in the Sami homeland and in marginal and remote Finnish regions, it has great social and cultural importance for northern people. In the last few decades, reindeer herding has faced dramatic changes. The inhabitant depopulation of the provinces, the ageing of reindeer owners and the disappearance of the reindeer herding tradition have created major problems. Moreover, conflicts with forestry, agriculture, mining, road building and tourism have also resulted in trouble for traditional herding and pastures. Large predators have caused increasing losses to reindeer herding, especially in the southern part of the reindeer herding area (Norberg and Nieminen 2007). In spite of this, the total number of reindeer has increased and in many areas caused overgrazing and damage (Kumpula 2001). All of these factors have led to increasing supplementary feeding and corralling of reindeer in winter months in the southern parts of the reindeer husbandry area (Nieminen 2006) and, sometimes, pasturing in unwanted places and areas. In these situations, reindeer are in contact with farm animals, and when they are free ranging they are in contact with wild cervid populations also undergoing population expansion. The increased mobility of people, animals and forage sources is expected to promote the transmission of emerging and re-emerging diseases and foreign pathogens. Changes in the ecological balance caused by climate change and pollution fall out (see Kutz et al. 2004, Hoberg et al. 2008) have also caused concern.

In recognition of these transitions, Finnish authorities established the Reindeer Health Care Program. The project was initially funded by the Ministry of Agriculture and Forestry (MAKER). The main purpose of this health care program is the maintenance and the enhancement of health and welfare in order to improve the productivity of reindeer and maintain the rich culture of reindeer herding. Simultaneously with this establishment in autumn 2003, clinical reports from reindeer meat inspecting veterinarians sounded the alarm about an outbreak of peritonitis in reindeer. The outbreak was first noticed in the southern and middle part of the Finnish reindeer herding area. The causative agent was initially recognized as a filarioid nematode representing an unidentified species of Setaria.
2. LITERATURE REVIEW

2.1. The Superfamily Filarioidea
Filaroid nematodes — mosquito-borne thread-like parasitic worms — represent major global health hazards for humans, domestic animals and wildlife (WHO 2007). They are parasites of tissues and body cavities of all classes of vertebrates other than fishes. Two families are recognized, the Filariidae and the Onchocercidae, and all are transmitted by haematophagous arthropods. (Anderson 2000).

The family Onchocercidae includes a diversity of nematodes (70-80 genera in eight subfamilies). Onchocercids have been reported from all the organ systems and most tissues of the vertebrate hosts. Microfilariae (mf) produced by female worms live in the blood or skin, where they are available to blood-feeding arthropod vectors, such as biting midges (Culicoidea), blackflies (Simulidae), horse and deer flies (Tabanidae), mosquitoes (Culicidae), lice, mites and ticks. The mf are taken up in the blood meal of the arthropod, where they develop into the infective stage. When the intermediate host feeds again, larvae break out and enter the tissue of the definitive host. (Anderson 2000).

The genus Setaria (Onchocercidae) includes 43 species that are normally found in the abdominal cavities of artiodactyls (especially Bovidae) and equines. All these species produce mf, which are present in host blood circulation. Known vectors are insects. (Anderson 2000).

2.2. Filaroid nematodes in reindeer
Nikolevskii (1961) and Mitskevich (1967) described the “foot worm” Onchocerca in reindeer in the USSR. Lisitzin (1964) described a subcutaneous nodule containing Onchocerca sp. in the muzzle of a reindeer in northern Finland, and Rehbinder (1973) later reported similar nematodes in subcutaneous nodules. Moreover, Rehbinder et al. (1975) found a high prevalence of Onchocerca sp. in subcutaneous nodules in reindeer of northern Sweden. Specimens of Onchocerca that were found in the metatarsus and metacarpus in reindeer were identified as O. tarsicola by Bain and Schulz-Key (1974) and by Bain et al. (1979). Bylund et al. (1974) and Rehbinder (1990) reported the abundant occurrence of foot worms O. tarsicola in reindeer in Finnish Lapland and Sweden.

2.2.1. Setaria in reindeer
Setaria tundra was the first filarioid nematode documented in reindeer, the “abdominal worm”, described by Rajevski in 1928 from the USSR. Later S. tundra was found in reindeer from Sweden (Rehbinder et al. 1975), Norway (Kummeneje 1980) and also from the Baikal area (Shagraev and Zhaltsanova 1980). Setaria yehi (S. tundra and S. yehi are considered synonymous by some authors (Taylor et al. 2007)) has been reported from Alaskan reindeer (Rangifer t. tarandus) (Dieterich and Luick 1971) and is known from other cervids in North America (Becklund and Walker 1969). S. yehi
was reported to occur in the majority of the reindeer from Alaska (Barret et al. 1981). It has also been reported in reindeer from Canada (Dieterich and Luick 1971, Fruetel and Lankester 1989) and the related *S. labiatopapillosa* in Chinese reindeer (Wang et al. 1989).

### 2.3. *Setaria tundra* in Scandinavian reindeer

*Setaria* infections seem to have emerged in Sweden during the late 1960s (Rehbinder et al. 1975). *Setaria tundra* had not been observed in northern Norway before the autumn and winter of 1973/74, when an explosive peritonitis epidemic occurred (Kummeneje 1980). At the same time in Finland, in 1973, according to personal communications (S Nikander, K Valtonen and V Tervonen 2004), *S. tundra* worms and associated changes were abundantly seen during reindeer slaughter.

#### 2.3.1. The life history of *S. tundra* in Fennoscandia

Following the outbreak in 1973, the incidence of *Setaria* sp. in reindeer from Scandinavia declined. The prevalence of changes in slaughter reindeer from Kautokeino, Norway, was 6.6% in 1976 (Poppe 1977) and 4% in 1978 (Korbi 1982). The statute for the inspection of reindeer meat in Finland was decreed in 1975. In the first reindeer slaughter season, 1975, *Setaria* sp. was reported from 1.3% of reindeer, and in the next season 3.3% (Savonen 1978). In the Finnish meat inspection data from 1980–86, *Setaria* sp. was diagnosed in 0.9% of reindeer annually (range 0.09 – 4.3%), with the parasite present within the whole reindeer husbandry area but most commonly in the southern parts (Rahkio and Korkeala 1989).

### 2.4. *Setaria*-associated pathological changes in reindeer and impact on meat hygiene

Filarioi d nematode parasites are known for their harmful effects on mammalian hosts, but are still relatively poorly studied in the boreal northern hemisphere, which reflects in the published literature on the issue. According to Nelson (1966), the recorded data indicate that filarial infections in wild animals are usually non-pathogenic but “further studies will undoubtedly show that under certain conditions most filarial worms are pathogenic”. However, after over four decades, published information in the literature is still scarce or anecdotal and reflects the limited number of surveys and inventories to determine the prevalence of infection or distribution of disease conditions.

During 1973 in Sweden, peritonitis associated with *S. tundra* was seen in forest herds of reindeer, but at the same time the mountain herds appeared not to have been affected (Rehbinder et al. 1975). As liver lesions and peritonitis seemed to appear at the same time as severe infestations of *S. tundra*, the association was apparent. Rehbinder et al. (1975) and Rehbinder (1990) further reported that the presence of *S. tundra* in the serous cavities of reindeer was usually asymptomatic, except for focal areas of mild chronic peritonitis. According to the findings of Rehbinder, the death of worms...
in the liver may produce a very marked inflammatory granulomatous reaction and serofibrinous peritonitis with tissue containing the remains of nematodes, eosinophilic granulocytes and masses of eosinophilic detritus and a pure culture of corynebacteria. In Norway, due to peritonitis, hepatitis and perihepatitis caused by *Setaria* worms, a high percentage of livers and adjacent tissues had to be discarded during the epidemic in 1973 (Kummeneje, 1980). Parasites were the most important factor causing meat or organ condemnation at that time in Norway (Poppe 1977). In Finland in 1973, tens of thousands of reindeer died in herds from the northern part of the Finnish reindeer husbandry area. Severe peritonitis and *Setaria* worms were commonly recorded, but the association between the reindeer deaths and the parasite was not confirmed (personal communication by S. Nikander, K. Valtonen and V. Tervonen, 2004). *Setaria yehi* has also been associated with chronic peritonitis in Alaskan reindeer (Dieterich and Luick 1979).

2.5. *Setaria* in wild cervids

2.5.1. *Setaria* in roe deer (*Capreolus capreolus*)

*Setaria tundra* has been reported in roe deer from Germany (Buttner 1975, Rehbein 2000), Bulgaria (Yanchev 1973) and Italy (Favia et al. 2003). Moreover, *Setaria capreola* was found in roe deer from Estonia (Yarvis et al. 1983).

2.5.2. *Setaria* in moose (elk) (*Alces alces*)

*Setaria yehi* has been reported from Alaskan moose (Becklund and Walker 1969, Dieterich and Luick 1971). Across Canada, this species of *Setaria* is also known in moose from Alberta (Samuel et al. 1976) and Ontario (Hoeve et al. 1988). An outbreak caused by *Setaria* sp. was reported in Finnish Lapland in 1989 during a non-parasitological study of genital tracts from female moose (Nygren 1990).

2.5.3. *Setaria* in deer

*Setaria yehi* has been found in the abdominal and/or thoracic cavities of 27% of white-tailed deer (*Odocoileus virginianus*) from the south-eastern states of the USA (Prestwood and Pursglove 1977). In Kentucky, the parasite was also found in white-tailed deer, but not in fallow deer (*Dama dama*) (Davidson et al. 1985). Previously, Becklund and Walker (1969) had examined all specimens of *Setaria* in the US National Parasite Collection and reported *S. yehi* from both white-tailed and black-tailed/mule deer, *O. hemionus*, at localities across North America.

*Setaria altaica* has been recorded in maral deer (*Cervus elaphus maral*) from Russia (Kostyaeva and Kostyaev 1969). However, after studying the specimens, descriptions and reports of *Setaria* in *Cervus elaphus sibiricus* and *Cervus nippon hortulorum* from Altai, Shol’ (1972) concluded that *S. altaica* and *S. cervi* are synonyms. *Setaria cervi* has also been found in maral deer from Russia (Shol...
and Drobishchenko 1973). *Setaria labiatopapillosa* has been reported in sika deer (*Cervus nippon yakushinae*) (Hai et al. 1995) and in Taiwan sika deer (*Cervus taionanus*) (Fey et al. 1992).

### 2.5.4. The pathogenity of *Setaria* infection in wild cervids

Little is known about the harmful effects of *Setaria* on their wild cervid hosts. *Setaria yehi* may cause minor lesions in the abdominal cavity of white-tailed deer, as documented for naturally infected hosts. The pathology of infection included a chronic mild peritonitis, the production of 50-150 ml of straw coloured, serous fluid, the formation of fibrous adhesions between the mesenteries and intestines and the deposition of fibrin on the surface of the liver. Lesions were not usually so extensive that condemnation of the carcass for human consumption would have resulted, although some hunters might have objected from an aesthetic standpoint. Infected white-tailed deer generally had few worms, with an average of 2.6 per host, but a maximum of 297 nematodes in one animal (Prestwood and Pursglove 1977). Additionally, a marked fibrinous peritonitis associated with a severe infection of *S. yehi* was evident in a young New Jersey deer (Pursglove Jr 1977). Pathology documented during a *Setaria* outbreak in Finnish moose was connected (Nygren 1990) to granulomatous lesions caused by adult nematodes in the wall of the urinary bladder and uterus; lesions resembled those caused by adult *S. digitata* in the wall of the urinary bladder of cattle (Yoshikawa et al. 1976). *Setaria cervi* seems to have little effect on the health of *Cervus elaphus maral*. The absence of significant pathological changes indicated, according to Shol` and Drobishchenko (1973), that the severity of natural infection may be reduced by providing young deer with an adequate diet, although no data were presented.

### 2.6. *Setaria* microfilaria (smf) in cervids

An adult female filarioid worm produces thousands of larval stages, or microfilariae, daily; for example, the uterus of *S. labiatopapillosa* contains at least 50,000 mf (Nelson 1966). Microfilariae of the subfamily Setariinae are sheathed and occur in the blood circulation of the host, where they are available to arthropod vectors (Anderson, 2000). The occurrence of *Setaria* sp. mf has earlier been reported in reindeer blood from Alaska (Dieterich and Luick 1971), where reindeer were mf-positive (*S. yehi*) year-round. In Sweden, mf have been found in skin samples from reindeer (Rehbinder 1990), but there are no reports of smf in other cervids or of possible harmful effects of microfilaremia on their cervid hosts. In contrast, there are reports that microfilariosis in buffalo caused by larval stages of *Setaria* spp. is a chronic debilitating disease that is clinically manifested by inappetance, purulent discharges from the eyes, a rough and dry skin coat, pale mucous membranes, a reduced milk yield, a stiff gait and swelling of the dependent parts of the body, and clinical liver damage (Sharma et al. 1981, Kumar et al. 1984, Venu 2000).
2.7. Prevalence of *Setaria* in relation to host age

According to the literature, *Setaria* infections appear to be more prevalent and pathogenic in calves and young adults than in adults in both wild (free ranging) and domestic ruminants. An inverse relationship between host age and infection with *S. yehi* was found in black-tailed deer (*Odocoileus hemionus columbianus*). Fawns were commonly infected (at least 67%), and also yearlings (43%), but the infection was relatively scarce in older deer (Weinmann et al. 1973). Infections with *Setaria* were most prevalent among young white-tailed deer in the USA (Prestwood and Pursglove 1977). For example, in some areas of California, 75% of fawns were infected with *S. yehi* (Weinmann and Shoho 1975). The prevalence of *Setaria* sp. in moose from Finland was also highest among young animals less than 2 years old (Nygren 1990). A high prevalence and intensity of *Setaria* infection in young maral deer was also reported by Shol’ and Drobishchenko (1977). Similarly, the prevalence of *S. labiatopapillosa* was significantly correlated with the age of cattle; it was lower in adult cows (6%) than in young animals (17%) (Osipov 1972).

2.8. *Setaria* infections in aberrant hosts

If vectors have broad feeding preferences, infective filarioid larvae can be transmitted to a variety of vertebrates other than those to which they are adapted. Most transmission of this type is probably harmless for the host, because larvae that leave the vector will not invade the tissues if the host is unsuitable. In some instances, however, larvae may develop to a more advanced stage, but eventually become encapsulated and destroyed by the host’s defence mechanisms. (Anderson et al. 2001).

*Setaria* species are commonly found in the peritoneal cavity of bovine ungulates (cattle, zebu and buffalo) and some species, including *S. digitata*, *S. marshalli* and *S. labiatopapillosa*, are very common parasites of cattle in the Far East and Asia in general (Rhee 1994). The major pathogenic effect of *S. digitata*, a parasite of cattle, occurs when filarioid microfilariae are transmitted by arthropod vectors to ungulate species other than their natural definitive hosts. The larvae of *S. digitata* migrate into the central nervous system of abnormal hosts such as sheep, goats and horses. Cerebrospinal nematodosis occurs, when within these marginal hosts, larvae invade the CNS, eyes, liver, heart or lungs. Complete or partial paralysis occurs in the body parts that correspond to the site in the CNS where the lesion is located. The disease in sheep is widely known as “lumbar paralysis” (Innes and Shoho 1953).

Reports of *S. cervi* in the central nervous system of four individuals of red deer (*Cervus elaphus hippelaphus*) with considerable pathological changes in their nervous systems (Blazek 1976) are worth mentioning in view of the neurotropism of *S. digitata* in cattle. In cervids, few descriptions of filarioid cerebrospinal nematodosis exist. In Taiwan, *S. cervi* caused cerebrospinal nematodosis in deer (Wang 1990) and *S. labiatopapillosa* caused the paralysis of the hindquarters of sika deer (*Cervus nippon*) (Hai et al. 1995). No central nervous signs were associated with the presence of


S. tundra in Swedish reindeer (Rehbinder 1990). According to Innes and Shoho (1953), there is inferential evidence that the same neuroparalysis has existed in many other parts of the world, but the etiology has not clearly been defined.

2.8.1. Prenatal infection
Immature or larval Setaria are capable of penetrating the placenta and migrating into the foetus, where the nematodes can complete their development. It has been believed that prenatal infection is the most common type of infection by S. marshalli (Kitano 1994). Congenital S. marshalli infection has been found in calves (Kitano 1994) and in bovine foetuses (Fujii 1995). Setaria digitata was reported in an 8-month-old bovine foetus from China (Mo et al. 1983). In a 31-day-old black-tailed deer fawn, born in captivity, a large (52 mm) immature female S. yehi was found free in the body cavity (Weinmann and Shoho 1975). There is no evidence of prenatal infection in temperate zones. The simple explanation may be the fact that filarioid infection occurs in warm summer seasons when arthropod vectors are active and transmission during the pregnancy of the cervid host in late autumn and winter is not possible because of the lack of vectors.

2.8.2. Possible zoonotic character
There is no doubt that people in Finland are exposed to infective larvae of filarioid nematodes (Setaria spp., Onchocerca spp. and unidentified species) when blood-sucking insects feed. According to Nelson (1966), filarial worms of animals, when they occur as atypical parasites in people, can cause abscesses, lymphadenopathy, eye lesions, tropical pulmonary eosinophilia and allergic reactions when developing abnormally in the subcutaneous tissue, heart, eyes, and lymphatic and central nervous systems. It is probable that almost any filarial nematodes parasitizing animals can, under appropriate circumstances, infect humans and undergo some degree of development (Orihel and Eberhard 1998). On the basis of extensive data reviewed by Innes and Shoho (1953), it may be assumed that neural nematodosis also exists in man, but the diagnosis is very difficult. The development of cross-immunity against filarioid nematodes in man is possible. There are very clear serological test reactions in patients with onchocercosis, even when the antigens have been prepared from other filarioids. The phenomenon referred to as “zooprophylaxis” (Nelson, 1966) against dangerous filariae has also been noticed in areas where the common mosquitoes that feed on people are heavily infected with other filarioids from man and animals (Nelson 1992).

2.9. Transmission
In the reindeer husbandry areas there is often a mass appearance of blood sucking insects and potential vectors during warmer periods: mosquitoes (Culicidae), gnats (Culicoidea), blackflies (Simulidae) and different types of horse flies (Tabanidae). They can follow the reindeer herds like huge clouds and make life a nightmare for man and animals (Rehbinder 1990). Reports exist on reindeer being exposed to hourly attacks of about 8000 mosquitoes, 240 biting flies and 24 Oestrid
flies (Kadnikov 1989). In California, the host-parasite system between S. yehi and white-tailed deer is facilitated by the peak appearance of mosquito vectors following the fawning season of the deer (Prestwood and Pursglove 1977). The high prevalence and intensity of Setaria infection in young maral deer was considered due to the animals being infected before they develop a non-specific immune response at the age of 20 to 25 days (Shol’ and Drobishchenko 1977).

2.10. Vectors of Setaria spp.
To date there is only scant information on the transmission and specific vectors of S. tundra in Fennoscandia. Among the related Setaria spp., insect vectors include at least the following: Anopheles hyrcanus, Anopheles sinensis, Armigers obtirban and Aedes togoi (Innes and Shoho 1953, Hagiwara 1992), Aedes caspius (Pietrobelli 1998), Aedes aegypti, (Wajihullah 1981 and 2001), Aedes canadensis (LeBrun 1984), Hematobia irritans and Hematobia stimulans (Shol’ and Drobischenko 1972, Shol’ and Drobischenko 1973, Chuvatina-Shmytova and Khromova 1974). In California, the mosquito Aedes sierrensis serves as a vector for S. yehi (Prestwood and Pursglove 1977). According to Rehbinder (1990), mosquitoes (species of Anopheles, Aedes and Culex) are considered vectors for S. tundra, but no strong empirical evidence was presented to support this assumption.

2.11. The development of Setaria larvae

2.11.1. Life cycle in the definitive host
Rather little is known about the life cycle and routes of migration to the abdominal cavity for Setaria spp. in the definitive host (Anderson 2000), and the life cycle of S. tundra in Northern Europe is poorly understood. The prepatent time after experimental infection of maral deer (Cervus elaphus maral) with 96 S. cervi larvae was 224 days (Shol’ and Drobishchenko 1973). The life span of S. marshalli is approximately one year after the prenatal infection (Fujii et al. 1996), and the life span of adult S. labiatopapillosa is apparently about 16 months (Osipov 1972).

2.11.2. Development in arthropod vectors
Microfilariae are taken up in the blood by the insect vectors, where they exsheath, penetrate the gut wall, migrate to the haemocoel and develop into an infective third stage larva in a certain tissue (Bain and Babayan 2003). Species of the same genus generally develop in similar locations in the arthropod host, such as the fat body, flight muscles, haemocoel and malphigian tubules. In the intermediate host, microfilariae of most species shorten and thicken into the so-called sausage stage. After the first moult, the second stage larva becomes long and slender and eventually mouls to the infective third stage. The third stage larva continues to grow and then migrates along the haemocoel to the head and mouthparts of the vector. Setaria spp. larvae develop in the thoracic muscles of mosquitoes. (Anderson 2000).
For example, the microfilaria of *S. cervi* (280 μm in length and 8 μm in width) must accomplish a number of changes in the thoracic muscles of the mosquito host (*Aedes aegypti*) within about 10 days to complete the transition to the infective stage. After exsheathment, the larva migrates to the thoracic muscles of the mosquito and differentiates into a short and thick larva measuring 215 x 9 μm and 171 x 11 μm after 24 and 48 hours of development. Respectively, by the third day, the larva becomes sausage shaped, measuring 120 x 20 μm, and then increases up to 230 x 37 μm in the late first stage. After the first moult in second stage, *S. cervi* larvae show a spectacular increase in size (from 232 to 1764 x 42 μm) and in the length of the glandular oesophagus and intestine (i.e. 65 to 654 μm and 112 to 935 μm). Following the 2nd cuticular ecdysis, the third stage larva occurs on the 9th day, following transformation of the 2nd stage larva into the infective third stage. The glandular oesophagus becomes so large that it occupies 2/3 of the total body length and forces the intestine to restrict to 448 μm. Infective larvae are 2318 x 39 μm in size. On the 11th day the majority of infective larvae accumulate in the head and proboscis of the mosquito. (Wajihullah 2001).

*Setaria digitata* microfilariae mature in mosquitoes in about two weeks, reaching the salivary glands as infective larvae (Innes and Shoho 1953). According to Pietrobelli (1998), it takes 6-14 days for *S. labiatapopillosa* to develop in *Aedes caspius*, depending on the ambient temperature and relative humidity.

### 2.11.3. Vector/intermediate host - parasite interaction

The interaction between the intermediate host and the filarioid nematode depends on the morphological, physiological and biochemical compatibility of the mosquito vector (Bartholomay and Chiristensen 2002). The microfilariae are usually relatively harmless to their vectors, unless they are present in overwhelming numbers migrating in the tissues of their arthropod hosts (Nelson 1964, Bain and Babayan 2003). Worms can inflict damage on the vector at any stage of development, for example when traversing the midgut or while migrating and developing in the thoracic muscles, disabling or even killing the mosquito host (Bartholomay and Chiristensen 2002). Nevertheless, mosquitoes are able to control and reduce the filarial infection by destroying or preventing the development of the microfilariae (Serrão 2001). The mosquito avoids an excess filarioid burden by regulating the penetration of the stomach wall and by melanotic encapsulation in the haemocoel. These are considered important mechanisms for vector survival and thus also for parasite survival. These mechanisms provide a temporal buffer for mosquitoes to survive long enough to allow the remaining nematodes to develop to the infective stage and for transmission of the parasite to the definitive host (Nelson 1964, Poinar 1974, Chen and Laurence 1985, Kobayashi 1986, Nayar 1989, Serrão 2001, Bartholomay and Christensen 2002, Bain and Babayan 2003). In addition, other factors influencing the vectorial competence of mosquitoes include ambient temperature and relative humidity, feeding behaviour (Nelson 1964) and genetic features (Serrão 2001).
2.12. Prevention of setariosis; antiparasitic treatment and prophylaxis

2.12.1. Antiparasitic treatment with ivermectin

Ivermectin belongs to the avermectins, a group of broad-spectrum antiparasitic compounds chemically modified from abamectin produced by *Streptomyces avermitilis*. It affects endo- and ectoparasites by preventing the conduction of nerve impulses, eventually causing paralysis. As ivermectin cannot cross the blood-brain barrier, it is regarded as harmless to vertebrates (Burg et al. 1979).

Ivermectin has been reported to have a broad spectrum of antiparasitic activity against gastrointestinal nematodes, lungworms, warble flies, mange mites and other nematodes and arthropods in cattle and also in reindeer (Nordkvist et al. 1983, Oksanen 1999). There are some reports on the efficacy of ivermectin for the eradication of setariosis in domestic species (Klei et al. 1980), but no reports of its efficacy against *Setaria* spp. in reindeer, although the drug has been widely and routinely used in Finnish reindeer husbandry since 1982 (Oksanen et al. 1998). Ivermectin treatment was originally targeted against warbles (*Hypoderma tarandi*) and throat bots (*Cephenemyia trompe*), and the spectrum of routine antiparasitic treatment has later been broadened to control the possibly harmful gastrointestinal nematodes.

A single dose of ivermectin at 200 μg/kg body weight had 99.3% efficacy in 4 weeks post injection and 100% in 16 weeks against *S. digitata* mf in calves (Shirasaka et al. 1994). The efficacy of ivermectin at a dose of 200 μg/kg was assessed in 13 buffalo from India. The drug proved to be highly effective against microfilariosis caused by *Setaria* spp. (Sharma 1991). Buffalo treated with 200 μg/kg b.w. sc ivermectin had 100% therapeutic efficacy against *Setaria* mf after 14 days (Singh et al. 1999). The efficacy of ivermectin at 200 μg/kg b.w. against adult *S. equina* in ponies was 80% and at 500 μg/kg, 88%. Furthermore, surviving worms showed reduced motility (Klei et al. 1980). A single dose of ivermectin could kill, on average, 92.4% of mf and 84.2% of adult *S. digitata* worms in experimentally infected lambs (Sharma and Siddiqui 1996).

2.12.2. Prophylaxis

Insecticides have played a central role in controlling the major insect vectors of infectious diseases such as malaria, filariosis and haemorrhagic fever since the early 20th century. Pyrethroids are known to possess high activity against a broad spectrum of insect pests, both adults and larvae, as well as low acute toxicity against mammals and a lack of persistence in the environment (Papadopoulou-Markidou 1983, Zerba 1988, Vijveberg and Van den Bercken 1990).

Deltamethrin is a synthetic pyrethroid that has a strong insecticidic effect. Deltamethrin has a good molecular stability against adverse environmental conditions such as sunlight and rainfall. It has a fast knock-down effect and is neurotoxic to insects (Leak and Walker 1980, Narahashi 1985). Small proportions of deltamethrin penetrating the skin are rapidly metabolised and it is not regarded as a hazard for consumers (WHO 2005).
Deltamethrin is commonly used in cattle to control ticks, lice and flies (Pergram et al. 1989). It is also widely used in Northern Finland in cattle to control lice and flies inside cow houses, and to prevent disturbances and a decrease in milk production due to mosquitoes and biting flies on summer pastures. Farmers generally think that the latter is a useful protective measure for dairy cattle, because they are more peaceful on the pasture and can concentrate on feeding (personal observations). It has been noted that pyrethroids cause a blood-feeding inhibition effect (Hougard 2003) and that deltamethrin provokes an excito-repellency reaction in mosquitoes (Chareonviriyaphap 2004).

The selection for pyrethroid resistance is a potential concern. Resistance has been recorded in some Asian, African and South American countries (Takken 2002). Signs indicating resistance have also been noticed in cow houses in Northern Finland; according to farmers, the drug seems to lose its insecticidal effect against flies after some years of usage.

2.13. Climate change

One of the climate change scenarios concerns how rising temperatures will affect the invasiveness and expansion of infectious diseases; of these, mosquito-borne diseases are the most climate-sensitive maladies (Patz et al. 1996). Filaroid nematodes are transmitted by haematophagous arthropods such as mosquitoes (Culicidae) and have temperature-dependent development (Anderson 2000). The previous *S. tundra* outbreak in Scandinavia 1973 was associated with unusually warm weather and the appearance of exceptionally high numbers of mosquitoes and gnats (Rehbinder 1990). The Arctic climate is changing, leading to an inevitable perturbation in temperature and hydrological processes (Lemke et al. 2007). Thus, in the future, conditions for various disease vectors and intermediate hosts of parasites in many areas are expected or predicted to be modified, which may alter the ecological balance between vectors and hosts. For example, in certain areas, suitable habitats for mosquitoes are increasingly provided by melting permafrost and increased rains, which could directly affect pathogen transmission and the distribution of disease by shifting the vector’s geographical range, and by increasing the vector’s longevity and reproduction. Increasing temperatures may also increase the vector’s biting rates and shorten the pathogen’s incubation period (Patz 2000). Although the potential for substantial ecological perturbation has been identified, an evidence-based process is still lacking to demonstrate a clear link between climate change and the emergence of filarioid nematodes at Northern latitudes. In general, empirical links between climate change (temperature and humidity), altered patterns of occurrence of pathogens (and complex host-parasite assemblages) and the emergence of disease conditions among domestic and free-ranging ungulates across the Arctic remain to elucidated, and have thus far been evident in only a limited number of systems (Kutz et al. 2004, 2005, Hoberg et al. 2008).
3. AIMS OF THE STUDY

A recent review by Hoberg et al. (2008) highlighted the impact of pathogenic parasites on keystone mammalian wildlife species in the Circumpolar North and emphasized the need to monitor these parasites and shifts in host-pathogen relationships, and to demonstrate links between different drivers of emerging pathogens and disease.

A peritonitis outbreak in semi-domesticated reindeer was first noticed during the autumn of 2003 in the south-eastern part of the Finnish reindeer-herding area, and was associated with substantial economic losses and an increased workload for the meat processing industry. The severity of the outbreak prompted the Reindeer Herders’ Association and individual cooperatives to urge immediate research and action in order to avoid further economic losses. The resources of the Finnish Reindeer Health Care Program were directed to initiating a research programme to investigate the outbreak and its impact on the health and wellbeing of reindeer. The study had a “first aid treatment” or response-based character. The majority of the experiments were carried out during normal veterinary practice and sampling was primarily conducted during reindeer slaughter. This provided a rare opportunity to follow an emerging outbreak of a vector-borne parasitic disease under natural conditions in the sub-Arctic. However, the practices of reindeer herding restricted and challenged the experimental planning, demanding considerable voluntary and patient work by herders during round-ups, ear-marking and the busy slaughter period. The reindeer owners involved were highly motivated to facilitate this work because of the serious character of the peritonitis outbreak.

Filaroid nematode parasites are known for their harmful effects on mammalian hosts, but are still relatively poorly studied in the boreal northern hemisphere. The rapid data collection during the emerging outbreak of *S. tundra* during 2003-2006 provided a unique opportunity to contribute to this base of knowledge. Thus, the main aim of this study was to explore and understand the process of emergence, the dynamics of the outbreak and the interactions of vector-borne *S. tundra* in reindeer and wild cervids under natural conditions in the sub-Arctic. Achieving these goals represented a multidisciplinary process involving a synthesis of research in veterinary pathology, epidemiology, parasitology, entomology and climatic meteorology. In order to fulfil these aims the following sub-aims were defined:

1. To elucidate the impact and pathogenic effect of *S. tundra* infection on the health of reindeer and wild cervids, along with the primary costs for meat hygiene and the economy. These factors would be explored by obtaining epidemiological data from reindeer slaughter (and historical meat-inspection data) and through data derived from hunted or road-killed cervids. (I)
2. To describe the causative *S. tundra* (haplotype) involved in the present outbreak, to characterize its larvae morphologically and
genetically and to elucidate basic biology and ecology, including life cycle parameters such as development, the prepatent period and the life span of the parasite, and to obtain new tools for diagnostics. (I,II,III,IV)

3. To collect an extensive spatial and temporal data set on the prevalence and distribution of *S. tundra* larval stages in cervids and in arthropods that would serve as a reliable basis for future monitoring (and prediction) and to develop new tools for data collection. (I,III,IV)

4. To learn to understand and describe the factors related to the ecological adaptation, or possible host switching of *S. tundra*, which enabled the emergence of known outbreaks; to describe the patterns of transmission, reservoirs and carriers, intermediate hosts/vectors and interacting environmental and climatic factors. (I,III,IV)

5. To study the necessity and possible means of preventive measures and antiparasitic treatment against *S. tundra* in reindeer management by testing the efficacy of routine yearly ivermectin treatment at the individual and population levels. Further, I aimed to test an unusual timing of filarioid prevention in reindeer calves to reduce economic losses at slaughter by giving the ivermectin prophylaxis or deltamethrin pour-on solution in midsummer during calf earmarking at the beginning of insect vectors’ mass appearance. The latter treatment was given as a repellent against insect vectors. (V)

6. To attempt to demonstrate a possible link between climate change and the emergence of *S. tundra* outbreaks in Finnish cervid populations. (IV,VI)
4. MATERIALS AND METHODS

4.1. Study design
The design of this study, and also the collection of data and specimens, had to be done very quickly, during the emerging outbreak. No previous information was available to estimate the duration or the development of the S. tundra outbreak, or how long the parasite would be available as a basis for data collection in semi- and fully-wild cervid populations and its insect vectors. The various arthropods potentially suitable as vectors in the geographically large outbreak area also made the work challenging and inspiring.

4.2. Free-ranging reindeer at slaughter

4.2.1. Pathology and meat hygiene
Historical data on reindeer meat inspection were collected from the Oulu and Lapland Council Boards and from the archives of the late National Food Agency. More detailed ante and post mortem meat inspection data were collected from the Kuusamo reindeer slaughterhouse during the outbreak in the winters of 2003 to 2004 from 4614 reindeer. These data were used to calculate the prevalence of peritonitis induced by Setaria sp. in reindeer. The relationship between the intensity of S. tundra infection and the severity of peritonitis was assessed from 418 reindeer. To measure the impact of Setaria sp. infection on meat hygiene, meat samples were collected from ten reindeer suffering from peritonitis and from ten apparently healthy reindeer (I). Tissue materials indicating S. tundra-induced peritonitis (I) were collected from 34 reindeer and were stored in formalin or delivered fresh to Evira.

In order to describe the impact of S. tundra infection on the well being of cervids, new health measures for reindeer were created (I, V). These included a body condition score (scale 1-4), a back fat index (the fat layer in millimetres in the middle of a ten-centimetre-long incision in the pelvic subcutaneous fat at a 45 degree angle craniolaterally from the base of the tail) and a measure of the degree of severity of peritonitis and perihepatitis (none (0), moderate (1), severe (2) and very severe (3)).

4.2.2. Parasite collection
A total of 260 adult and pre-adult Setaria sp. nematodes were collected in Kuusamo and Pudasjärvi for morphological and molecular studies in 2004 (I). In Kuusamo in December 2005, all Setaria sp. specimens from the abdominal cavity of 40 reindeer calves (n = 95) were collected manually and by washing the serosal surfaces of the intestines (II).
Blood samples for smf spatial and temporal studies were collected from 1442 reindeer and from 90 unborn fetuses from 25 reindeer herding cooperatives during the period 2004-2006 by local meat inspecting veterinarians. To provide a historical perspective, 251 blood samples collected for other purposes by veterinarians in 1997 were included in the study (III).

### 4.3. Captive reindeer

To study the dynamics and the periodicity of smf in reindeer blood, eight reindeer hosts naturally infected with *S. tundra* were relocated in March 2004 from Kuusamo to the experimental zoo of the University of Oulu. The smf were monitored from jugular vein blood samples taken weekly over one year. Studies on the effect of physical activity on microfilarial density in peripheral blood were performed. The animal handling procedures were accepted by the Experimental Animal Committee of the University of Oulu (license no. 030/04). In September 2004, two of the reindeer (harbouring high smf densities) were killed for parasitological studies.

### 4.4. Wild cervids

In order to monitor spatial and temporal frequencies and possible sylvatic reservoirs for *Setaria* sp., blood, tissue and parasite samples were collected from wild cervids during the outbreak of 2003-2004 with the help of hunters.

#### 4.4.1. Pathology

In Kuusamo, about 300 moose were inspected post mortem. Fourteen white-tailed deer and 15 roe deer were examined at Evira in Oulu and two roe deer in the field. Hunters were informed about *Setaria* sp. and asked to report any changes and findings in moose and roe deer in the reindeer herding area. A total of 56 moose tissue samples were delivered by hunters to Evira. Tissue samples from thirty-four wild forest reindeer (*Rangifer tarandus fennicus*) were collected by a research project on these cervids in Kainuu and by hunters in Suomenselkä (I) (Fig. 1).

#### 4.4.2. Parasite collection

Adult *Setaria* sp. samples for PCR studies were collected from reindeer and moose in Kuusamo, from roe deer in Kemijärvi in 2004 and from roe deer in southern Finland in 2005. One adult *Setaria* sp was also examined from moose in 1989, by courtesy of T. Nygren. Blood samples for mf detection were collected in 2004–2005 by hunters and from road-killed animals over one year old; 324 moose, 92 wild forest reindeer, 17 roe deer and 9 white-tailed deer (III).
4.5. Arthropods (IV)

4.5.1. Developmental studies in mosquitoes
Evaluation of the larval development of smf in its vectors involved two developmental studies in mosquitoes after an infective blood meal. The experiments were carried out in Oulu during the summer of 2004, one in outdoor conditions (34 mosquitoes) and the other under laboratory conditions in a warm insectary (104 mosquitoes).

4.5.2. Setaria in wild arthropods
Estimation of the prevalence of *S. tundra* larvae in arthropod populations from different areas involved the collection of insect samples from six locations (Fig. 1). Arthropods were sampled by netting when the insects were attacking humans or during hibernation in winter caves adjacent to pastures with a high reindeer density. In total, 2211 mosquitoes (Culicidae) (95 hibernating), 805 black flies (Simulidae), 1267 biting midges (Culicoidea) and 213 head flies (*Hydrotaea* spp.) were captured and dissected. All developing larvae found were measured and photomicrographed for morphological evaluation, and extracted for PCR studies.

4.5.3. Questionnaire
A questionnaire was addressed to all Chiefs of District of the 56 reindeer herding cooperatives in 2006 (IV). Observations were accumulated to assess the timing and severity of the mass appearance of blood sucking insects during the *S. tundra* outbreak in the summers of 2003, 2004 and 2005, and also to describe the behavioural response of reindeer to the resulting insect harassment.

4.6. Antiparasitic prevention (V)
Three medical experiments (V) were carried out in a highly *S. tundra* endemic area in Kuusamo: (1) the treatment of (presumably infected) calves in early autumn with ivermectin injection; (2) ivermectin treatment of breeding reindeer in winter; and (3) the treatment of calves in midsummer, during routine calf ear marking, with ivermectin injection prophylaxis or deltamethrin pour-on solution as a repellent against insect vectors. Trials were planned to be compatible with the annual rhythm of reindeer management; reindeer are driven to large herds in midsummer by the plague of blood-sucking insects and in autumn by the rutting season. In these periods the reindeer are rounded up into summer, autumn or winter corrals for various tasks such as ear marking, counting, transportation and slaughter.

4.6.1. Autumn ivermectin trial
In the first trial, 22 reindeer calves were captured during an autumn round-up in 2003, of which 11
received ivermectin injection (200 μg / kg b.w. s.c.), and another eleven formed the untreated control group. The calves were returned to range free until slaughter (12 calves 35 days and 10 calves 62 days post treatment) and examination.

4.6.2. Winter ivermectin trial
In trial 2, 200 breeding reindeer in winter corrals during 2004 were used to determine the efficacy of the standard antiparasitic treatment regime with ivermectin against mf in blood circulation. Reindeer (158 adults, 42 calves) were allocated into six corrals according to animal ownership. The reindeer were randomly divided into two groups, 100 in each, and treated on 18 January 2004. The study group received an ivermectin injection (200 μg / kg b.w) and the control group was untreated. Blood samples for mf analysis were collected 44 days post treatment.

4.6.3. Summer ivermectin and deltamethrin trial
In trial 3, *S. tundra* prophylaxis in reindeer calves was tested by giving ivermectin by injection (200 μg / kg b.w. s.c.) as an anthelmintic or deltamethrin pour-on solution (250 mg) on the skin as an insect vector repellent in midsummer 2004, during the calf ear marking and at the onset of the mass appearance of insect vectors. The calves were allocated into three groups, 175 animals each: an ivermectin group, deltamethrin group and untreated control group. The calves (109 from the ivermectin group, 108 from the deltamethrin group and 115 from the control group) were slaughtered in seven slaughter batches from 4 November 2004 until 7 February 2005. The results were assessed using the post mortem inspection data and *S. tundra* detection. To control for the potential impact of other parasites on the results, faecal samples were collected from the rectum and examined fresh for the eggs of Trichostrongyloidea spp., *Nematodirus* spp., *Capillaria* spp. and *Moniezia* spp. and oocysts of *Eimeria* spp. by a modified McMaster method.

4.6.4. Questionnaire
The antiparasitic treatment status in all 56 cooperatives of the Finnish reindeer herding area in 2002, 2003 and 2004 was assessed by a questionnaire addressed to the chiefs of the cooperatives. The survey was performed using a standardized form that was delivered by mail and in some cases completed by phone call. In order to estimate the efficacy of the annual ivermectin treatment against *S. tundra* at the population level, the reindeer-herding area was divided into four sub-areas (Fig. 1)

4.7. Climate (IV)

4.7.1. The behavioural response of reindeer to weather
In order to examine the effect of weather conditions on the emergence of *Setaria* during the outbreak years among the semi-domesticated reindeer in Finland, a questionnaire was administered to all Chiefs of District of the 56 reindeer herding cooperatives. Respondents were asked to assess the
behavioural response of reindeer to the prevailing weather conditions. They were also asked about experiences concerning outbreaks of disease in the area associated with \textit{S. tundra}.

4.7.2. Climate data
In order to assess the effect of climate conditions on the emergence of \textit{Setaria} sp. in the past among the semi-domesticated reindeer and wild moose populations in Finland, historical weather data were collected from the Finnish Meteorological Institute across three meteorological stations in northern Finland representing the reindeer herding area: Kuusamo, Sodankylä and Kevo from the years 1961 to 2004.

4.8. Laboratory
Most of the laboratory studies and analyses described in this thesis were conducted at Evira, in the Fish and Wildlife Health Research Department in Oulu (earlier EELA). The morphological description of \textit{S. tundra} (I, II, III and IV) was partly carried out at the University of Helsinki, Faculty of Veterinary Medicine, and some of the arthropods were identified at the University of Oulu, Department of Biology. Meat hygiene analyses (I) were conducted according to the Finnish meat hygiene legislation at the accredited Food and Environmental Laboratory of the Oulu Region.

4.8.1. Histopathology
Tissue materials indicating \textit{S. tundra} -induced peritonitis (I) were collected and delivered fresh or formalin fixed to Evira. Samples were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 4 μm sections and stained with haematoxylin and eosin.

4.8.2. Bacteriology
Bacteriological samples (parasite lesions and granulomas) were cultured in both soymeal-peptone (aerobic) as well as bromthymol blue lactose (anaerobic) agar for two days (I).

4.8.3. Meat hygiene
Evaluation and analyses of the impact of \textit{Setaria} sp. infection on meat hygiene were carried out according to the Finnish meat hygiene legislation (MMM no 12/EEO/1999 and 1/EEO/2000) at an accredited environmental and food laboratory (I).

4.8.4. Parasitology

4.8.4.1 Adult \textit{Setaria}
Adult \textit{Setaria} sp. nematodes, collected from the abdomen of reindeer calves in 2004 at the Kuusamo reindeer slaughterhouse (I, II) for morphological studies, were stored in 70% alcohol or in 10% buffered formalin. Ten female and 10 male specimens stored in alcohol were dehydrated and cleared
in lactophenol for morphometric measurements (II). Several measurements were taken, including the lengths of the body, oesophagus and tail, distance from the nerve ring to the vulva, widths at the vulva and anus, and morphology of the spicules.

In the same slaughterhouse, in December 2005, parasite specimens were collected manually from the abdominal cavity of 40 reindeer calves. In addition, the viscera were rinsed with tap water into a steel container, and the worms were collected and counted. All nematodes collected from the abdomen of reindeer in 2005 were washed in tepid physiological saline, and then fixed in a hot mixture of 10% formalin. Thirty females and thirteen males were dehydrated and cleared for morphometry (II). For the SEM study, five females and five males were dehydrated using a critical point dryer, mounted on aluminium stubs, coated with platinum and examined under a Zeiss DSM 926 scanning electron microscope. Results were compared with previous descriptions and certain morphological features of Setaria nematodes.

In order to estimate the fecundity of S. tundra, two female nematodes collected in February 2004 were dissected fresh under a stereo microscope, uteri were removed and larvae were counted. (II).

4.8.4.2. *Setaria microfilaria*

To analyze the spatial and temporal variation in the prevalence and density of smf during the course of the peritonitis outbreak, a total of 1290 reindeer blood samples were collected from 25 reindeer herding cooperatives covering all regions of the Finnish reindeer herding area. In the winter of 2003-04, 627 animals were sampled and in the winter of 2006, 491 animals. To compare the current prevalence of Setaria infection with that of the previous decade, 251 blood samples collected 1997 were included to the study.

To estimate the prepatent period of S. tundra infection, blood samples were collected at the Kuusamo reindeer slaughterhouse (Fig. 1) from 119 adults and 205 calves from eight slaughter batches between October and January 2004–05. To determine the ability of smf to penetrate the placenta, blood samples from 90 unborn fetuses (5th to 6th month of gestation) were collected.

From wild cervids, 442 blood samples were collected to examine the interactions and possible reservoirs of S. tundra in the wild. Samples were collected by hunters and from road-killed animals. A total of 324 moose, 92 wild forest reindeer, 17 roe deer and 9 white-tailed deer blood samples were collected.

Blood samples were examined for the presence of smf by the modified Knott’s technique and the smf were counted in temporary wet mounts (III, V). The frozen blood samples from the year 1997 were examined for the presence of smf by using heparin to dissolve the clotted blood. Repeatability tests were also performed (III).
To confirm the identity of the parasite in cervid blood circulation (III, V), the mf were compared with smf isolated from the uterus of adult *S. tundra* nematodes. The mf were measured and studied under a light microscope after staining with haematoxylin or MGG methods. Unstained smf were also studied with the aid of a microscope equipped with DIC (III).

### 4.8.4.3. *Setaria* in arthropods

To estimate the prevalence of *S. tundra* larvae in arthropod populations in different *S. tundra* endemic areas, insect samples were collected from various locations (Fig. 1). The samples were taken by netting insects attacking captive reindeer or humans. A total of 4429 mosquitoes were collected, of which 998 were randomly examined for *S. tundra* larvae. Other collected and examined insects were: 805 black flies (Culicidae), 1267 biting midges (Culicoidea) and 213 head flies (*Hydrotaea* spp.).

To examine the possibility of *S. tundra* larvae surviving in mosquitoes over the winter, hibernating and newly emerged *Anopheles* sp. (n = 25) and *Culiseta* sp. (n = 95) mosquitoes were collected from winter caves in pastures in Kuusamo grazed by reindeer with a high *S. tundra* prevalence.

All developing larvae found in arthropods were measured (length, width), photographed and subjected to morphological studies under a light microscope (IV). Six larvae were collected from six mosquitoes: one from a wild *Aedes* mosquito from Kuusamo and five from *Aedes* mosquitoes from the development trial under insectary conditions.

### 4.8.5. Arthropods

In the developmental study (V), mosquitoes were periodically euthanized in a deep freezer (-80 °C, 30 s) and dissected fresh. Field-netted insects were euthanized with ether and stored frozen before dissection. Insects were identified to the genus level and when possible to the species level. The body was divided into three parts, the head, thorax and abdomen, which were examined separately mounted in a drop of *Aedes* Ringer solution (Clark et al. 1998) on a slide. Parts were dissected under a stereomicroscope and then covered and examined under a light microscope (40x magnification). The oviposition times of the mosquitoes were determined, when possible.

### 4.8.6. Genetics

Genetic characterization and identification of *S. tundra* are presented in papers I, III and IV. Samples for PCR studies from adult *Setaria* spp. were collected from reindeer and moose in Kuusamo and from roe deer in Kemijärvi (I), and one adult *Setaria* sp. specimen from a roe deer in southern Finland. One adult *Setaria* sp. specimen from a 1989 parasitized moose in northern Finland (III),
preserved in alcohol, was included in the study.
The specimens were stored in 94% ethanol. The DNA was extracted using a DNeasy™ Tissue Kit (Qiagen, Germany). The four new primers were designed based on a comparison of Filarioidea sequences retrieved from GenBank. The conditions and the primers are described in papers I and III.

Identification of mf by molecular biological methods (III) involved the collection of RNA obtained from 100 μl of blood from six reindeer that harboured only smf (in addition to smf, other species of mf were discovered from reindeer and other cervid species (unpubl. data)). Then, 100 μl of blood was diluted with 2 ml sterile distilled water to lyse erythrocytes and other blood cells and filtered through medium fast filter papers. The filter papers were placed in a 1.5 ml microfuge tube, 500 μl 5% re-suspended Chelex 100 resin mixtures (Bio-Rad) were added and the mixtures were then vortexed. After that, the samples were incubated for 20 min at 56 °C, vortexed briefly and kept at 99 °C for 10 min. Before use in PCR, the Chelex mixture was centrifuged for 2 min and 3 μl liquid from the uppermost supernatant was used for each PCR reaction. The specific S. tundra primers and PCR conditions are described in paper I.

Six third-stage larvae were collected from six Aedes mosquitoes (IV). The larvae were digested in 10 μl of a solution containing 0.45% Tween 20 (Merck, Germany) and 0.45% Igepal CA-630 (Sigma-Aldrich, Germany), PCR buffer (10 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl and 0.1% Triton X-100) (Finnzymes, Finland) and 500 μg/ml Proteinase K (Finnzymes, Finland). Samples were incubated at 65 °C for 30 min followed by 10 min at 95 °C. For one PCR reaction, 2-5 μl of digestion solution was used. The presence of S. tundra was demonstrated by comparing mitochondrial DNA sequences with all known sequences of S. tundra, as well as other Filarioidea species. The primers StCoI 616L and StCoI 1321H were used to amplify part of the CoxI gene. The specific S. tundra primers and PCR conditions were as previously described in paper I and III. The 18S rRNA gene was also amplified using the primers described in paper III.

The presence of S. tundra was demonstrated by sequencing mitochondrial DNA (I, III, IV) and nuclear ribosomal DNA sequences (II, IV) and by comparisons with all the known sequences of S. tundra, as well as the other filarioid species published in the Gene Bank database.

4.9. Statistics
Statistical analyses were performed with Stata 9 (StataCorp LP, USA) software in papers I, III and V and using R (Project for Statistical Computing) in paper VI. In paper I, the slaughter weights were compared using the Student’s t-test, the correlations between the worm count and the degree of peritonitis and between the degree of peritonitis and the fat layer were analysed using Pearson’s chi-
squared test, and meat hygiene analysis results were compared using Wilcoxon signed rank test.

In paper III, the reindeer husbandry area was divided into four areas for analysis of the spatial development of the outbreak (Fig. 1). The areas were used in models as a hierarchical dummy variable, and as a result, differences between areas next to each other were evaluated. The smf counts in blood for Poisson models were divided into 5 groups (0, 1–10, 11–100, 101–300 and >300 smf/ml). The overall effect of the age group and year on the prevalence and density of smf in blood was analyzed using logistic regression and a Poisson model, respectively. The analyses of prevalence among wild cervids were conducted using Pearson’s chi-squared test and density analyses by the Poisson model.

In paper V and in trials 1 and 2, Pearson’s chi-squared test was used to analyse the effect of ivermectin treatment on the prevalence of \( S. \ tundra \) and smf. In trial 3, logistic regression was used to analyse the medication effect on the degree of changes associated with \( S. \ tundra \), the prevalence of \( S. \ tundra \) and the prevalence of other parasites. Logistic regression was also used to study the associations of peritonitis and perihepatitis with the prevalence of \( S. \ tundra \). The Pearson goodness-of-fit test was used to validate all logistic regression models. Health indicator differences between treatment and control groups were tested using linear regression models.

In paper VI, the effects of ambient summer temperatures on the known outbreaks of \( S. \ tundra \) in populations of Finnish cervids, based on historical weather data (1961-2004) and the relationship between the occurrence of epidemic \( Setaria \) and mean temperatures during the current and preceding summers of each outbreak were investigated by fitting a generalized linear mixed model with binomial errors to the data. The fitted model was then reduced by removing non-significant terms starting from the interaction term and using likelihood ratio tests (\( p < 0.05 \)).

In all papers, the level of significance was set at 5% (\( p < 0.05 \)).
5. RESULTS

5.1. Peritonitis, disease

5.1.1 Reindeer at slaughter
According to the clinical data (I), the outbreak of peritonitis in reindeer, caused by *S. tundra*, emerged in 2003 in the southern and middle parts of the Finnish reindeer herding area. The proportion of reindeer viscera condemned due to parasitic lesions in meat inspection increased from 4.9% in 2001 to 40.1% in 2003 in the province of Oulu. In 2004 and 2005, the focus of the outbreak moved approximately 200 km north during the next two years, and simultaneously declined in south. Expansion of the outbreak was extensive, and only the reindeer in the northernmost part of Finland appeared to remain free of disease (I), although data on parasite prevalence are lacking. In ante mortem inspection, the fur of infected calves was often dry, lifeless and tangled and the winter fur had not fully developed. The abdomen in many individuals was slightly distended and the eyes gave an impression of exophthalmos (Fig. 2).

Figure 2.

Reindeer calf suffering from heavy *Setaria tundra* infection. Note the poor body condition with poor fur quality and distended abdomen. (With the permission of the British Veterinary Association)

The meat inspection findings of peritonitic reindeer carcasses included perihepatitis (Fig. 3) ascites fluid, green fibrin deposits, adhesions and live and dead *S. tundra* nematodes (Fig. 4). Greyish or greenish grainy fibrin membranes, sometimes several millimetres thick, covered the peritoneum and visceral organs, especially the rumen and spleen, giving an impression of a purulent process (Figure 5).
Figure 3.

*Setaria tundra* specimens on a reindeer liver with severe perihepatitis.

Figure 4.

Ascites fluid (yellow arrow) in the peritoneal cavity of a reindeer calf with green fibrin formation (green arrows) and *Setaria tundra* nematodes (white arrows). (With the permission of the British Veterinary Association)

Figure 5.

Fibrin membranes covering the peritoneum and visceral organs (red arrows) and straw coloured ascites fluid (yellow arrow) giving an impression of a purulent process. (With the permission of the British Veterinary Association)
Histopathologically, changes indicated granulomatous peritonitis with lymphoplasmacytic and eosinophilic infiltration. No specific bacterial growth was found. The degree of peritonitis was much more severe in reindeer calves than in adults. No significant impact on meat pH values or on the organoleptic evaluation of meat was found. There was a significant positive correlation between theworm count in the abdominal cavity and the degree of peritonitis \( (p < 0.001) \), and a negative correlation between the degree of peritonitis and the thickness of the back fat layer \( (p = 0.015) \) (I).

### 5.1.2. Wild cervids

Of the 34 wild forest reindeer, 21 (62%) were suffering from changes in the abdominal cavity associated with \( S. \) \( tundra \) infection. No peritonitis was reported in moose from Kuusamo. Six, 1.5-year-old moose had 1 to 3 encapsulated preadult \( Setaria \) sp. nematodes on the liver surface associated with moderate perihepatitis. In tissue samples from 18 moose of 56 delivered by hunters to Evira, mild peritonitis or perihepatitis was diagnosed. In two cases, immature \( Setaria \) sp. nematodes were detected. One roe deer had an adult \( Setaria \) sp. worm encapsulated on the surface of the liver and two roe deer autopsied fresh in the field had live adult \( S. \) \( tundra \) (2 and 4 worms) in the abdominal cavity, but no sign of peritonitis. In white-tailed deer, no changes indicative of \( Setaria \) sp. infection were observed. (I)

### 5.1.3. Observations in non-slaughtered reindeer

No increased reindeer mortality was reported in the outbreak areas due to peritonitis. Peritonitis/perihepatitis was diagnosed in 15 of 26 reindeer (58%) delivered to Evira for autopsy during the outbreak (I). In the questionnaire (IV) across areas of high \( S. \) \( tundra \) prevalence, some Chiefs of District (11 of 56) reported on their own initiative that the prevalence of disease associated with \( S. \) \( tundra \) (peritonitis detected at slaughter) in reindeer herds varied depending on the site of the herd’s summer pasture.

### 5.2. \( Setaria \) \( tundra \), the causative agent

#### 5.2.1. Adult \( S. \) \( tundra \)

#### 5.2.1.1. Morphology

The worms were diagnosed to belong to the superfamily Filarioidea (unequal spicules) and to the genus \( Setaria \) (peribuccal crown and the morphology of the spicules). The mean length of the female worms was 75 mm and that of males 35 mm. The nematodes were tentatively identified in paper I and in greater detail in paper II as \( S. \) \( tundra \) (Rajevsky 1928). The female/male sex ratio in reindeer was 4.3:1. The uteri of the female parasites contained eggs and developing larvae as well as about 200,000 microfilariae.
5.2.1.2. Genetics

The genetic sequences (1389 bp long mtDNA) of Setaria sp. specimens from reindeer (5 specimens), moose (2 specimens) and roe deer (2 specimens) were identical (I). There were six nucleotide substitutions when compared to an S. tundra sequence (648 bp) from roe deer in Italy. The difference was regarded as small and justified identification as S. tundra. The sequence of S. tundra parasitizing reindeer in North Finland was deposited in GenBank under accession number DQ097309 (I).

The partial mtDNA Cox1 sequence of the one adult Setaria sp. from moose in 1989 (GenBank EF661848) and the one adult Setaria sp. from roe deer in southern Finland in 2005 (GenBank EF661849) were identical along a sequence of 680 bp (III). The sequences confirmed an identification of S. tundra but demonstrated a discrete population in moose separated by eight nucleotide differences (1.18%) from S. tundra in reindeer. Thus, parasites circulating in reindeer, and those that have been demonstrated as the causative agent of the current outbreak, appear to be discrete from those that may be circulating in other cervids; more detailed population-level comparisons appear warranted in documenting the genetic diversity of these parasites.

5.2.2. Setaria tundra microfilaria

5.2.2.1. Morphology

The smf were actively moving, long (mean 331 μm (SD 17 μm) including the sheath) and slender with a blunt anterior and tapering posterior end (III) (Fig. 6).

Figure 6.

Sheathed (arrows) Setaria tundra microfilaria. (With the permission of Parasites & Vectors)

5.2.2.2. Genetics

The partial 18S ribosomal RNA gene sequences of smf in all six reindeer blood samples (III) were similar to the sequence of adult S. tundra parasitizing reindeer in northern Finland and are deposited in the GenBank under accession number EF08134 (III).
5.2.3. Third-stage larvae

5.2.3.1. Morphology
The third-stage larvae were elongated (mean length 1411 μm (SD 207), mean width 28 μm (SD 2)). The anterior end was blunt and bore two liplike structures, while the posterior end was slightly tapering with a prominent terminal papilla. (IV)

5.2.3.2. Genetics
The 680 bp mtDNA sequence from all 6 S. tundra 3rd stage larvae examined were identical to those from adult S. tundra parasitizing reindeer in northern Finland (GenBank DQ097309). The partial 18S ribosomal RNA gene sequences of the S. tundra larvae were also identical to those from smf from the blood of these reindeer (GenBank EF081341). (IV)

5.3. Epidemiology of Setaria tundra microfilariae in cervid blood

5.3.1. Setaria tundra microfilariae in free-ranging reindeer
The mean prevalence of smf for all reindeer examined across the herding area in 2004 was 56% in calves and 35% in adults. The corresponding figures in 2006 were 54% and 28%. The spatial smf prevalence and density in the blood circulation of calves and adult reindeer during the years 2004 and 2006 are presented in Table 1 of paper III. Overall, smf infection was more prevalent (p < 0.001) and intense (p < 0.001) in calves. In 2004, the smf prevalence was higher in southern areas of the herding range. In 2006, smf appeared in reindeer in northernmost Finland, but the prevalence and intensity was still lower than in the southern area (p < 0.001). In historical samples from 1997, smf were present in 4% of the samples from reindeer in the central area, and were not found in other areas.

No smf were detected in reindeer calves at the beginning of the slaughter season in late October in Kuusamo. The first microfilaremic calves were observed at the beginning of November and the prevalence increased to 80% by the end of slaughter season in January 2005. Altogether, 4.8% of adult reindeer were already positive at the beginning of the slaughter season and the prevalence also increased, but only to 20% during the slaughter season. No placental penetration of smf could be detected from 90 unborn fetuses.

5.3.2. Setaria tundra microfilariae in captive reindeer (III)
The peak period of microfilaremia was from the beginning of June until mid-September in the experimental reindeer in Oulu Zoo (Fig. 7). No smf were detected in blood from three reindeer the following January, whereas another three reindeer maintained low microfilaremia at the beginning
of the next summer, but none in the autumn. In the same summer, three of the animals in the group gave birth and all the calves, obviously born non-infected, acquired *S. tundra* infection during the summer from arthropod vectors.

**Figure 7.**

Periodical densities of *Setaria tundra* microfilaria in the blood of the Oulu Zoo group of eight naturally infected reindeer (Thick red line = mean smf/ml blood).

In exercise assessments, moderate movement of reindeer increased the density of smf with an average of 130% (range: 10–447%).

Two experimental reindeer slaughtered during the following September had adult female *S. tundra* nematodes in the abdominal cavity.

### 5.3.3. *Setaria tundra* microfilariae in wild cervids (III)

The prevalence and density of smf in wild cervids are presented in Table 1. There was a significant difference in smf prevalence between the two wild forest reindeer populations (36% of 33 in Kainuu vs. 15% of 59 in Suomenselkä; *p* < 0.05). The density of smf was also higher (*p* < 0.01) in Kainuu (mean in infected animals 144 smf/ml blood, range 1-830; Suomenselkä mean 23 smf/ml, range 1-105). The roe deer originating from North Finland (*n* = 9) and the southern coastal part of Finland (*n* = 8) were similarly infected, with prevalences of 33% and 50%, respectively. (III)
Table 1. *Setaria tundra* prevalence and density in cervids aged more than 8 months between 26 January 2004 and 4 June 2005.

<table>
<thead>
<tr>
<th>Cervid species, area</th>
<th>Number of animals examined</th>
<th>S. <em>tundra</em> microfilaria prevalence (%)</th>
<th>Mean smf/ml (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moose outside reindeer herding area¹</td>
<td>112</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td>Moose within reindeer herding area²</td>
<td>212</td>
<td>1.4</td>
<td>1 (1-3)</td>
</tr>
<tr>
<td>Wild forest reindeer adjacent to the reindeer herding area (Kainuu)²</td>
<td>33</td>
<td>36</td>
<td>144 (1–830)</td>
</tr>
<tr>
<td>Wild forest reindeer without contact with the reindeer herding area (Suomenselkä)²</td>
<td>59</td>
<td>15</td>
<td>23 (1–105)</td>
</tr>
<tr>
<td>Roe deer, different locations</td>
<td>17</td>
<td>44</td>
<td>187 (10–650)</td>
</tr>
<tr>
<td>White-tailed deer, South-West Finland</td>
<td>9</td>
<td>0</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

¹The moose originated from the Province of Oulu
²See Fig. 1

5.4. *Setaria tundra* in arthropods, transmission

5.4.1. The development of *S. tundra* in mosquitoes

5.4.1.1. Outdoor conditions (IV)

Species of *Aedes* included in the study were *Aedes communis* (n = 14), *A. hexodontus* (n = 8); *A. punctor* (n = 4) and unidentified *Aedes* spp. (n = 9). The prevalence of *S. tundra* immediately following the blood meal on captive reindeer known to be infected was 57%, and the number of developing larvae in infected individuals varied from 1 to 25. On day 1 after the blood meal, smf were already exsheathed and were still in the abdomen. During day 2, the larvae migrated into the thorax. Larvae reached the so-called “sausage stage” on days 6 to 9 after the blood meal and remained in the late first stage or early second stage on day 16. No third-stage infective larvae were observed up to day 22 after the blood meal, when the trial ended.
5.4.1.2. Laboratory conditions

5.4.1.2.1. Aedes mosquitoes (IV)

*Aedes* mosquitoes (n = 97) harboured 1 to 51 (mean 8, SD 10.7) larvae. During day 1, the larvae exsheathed and migrated to the thoracic muscles. Larvae reached the sausage stage after days 4 to 6. After days 9 to 10 the larvae were slender and had approximately doubled in length. In these second-stage larvae, the anal plug was visible, but the tail had been lost during the first moult. Approximately two weeks after the blood meal, infective elongated third-stage larvae were present. Approximately 51% of the infective larvae were located in the thorax, 31% in the head or proboscis and 18% in the abdomen. The morphometrics and the development of *S. tundra* are presented in Table 2 and Figure 8.

### Table 2.

The development of *Setaria tundra* larvae in *Aedes* mosquitoes under laboratory insectary conditions (21 °C, SD 2.8, relative humidity 65%, SD 12.4).

<table>
<thead>
<tr>
<th>Days after blood meal</th>
<th>1</th>
<th>2 - 3</th>
<th>4 - 6</th>
<th>7 - 8</th>
<th>9 - 10</th>
<th>11 - 13</th>
<th>14 - 16</th>
<th>17 - 20</th>
<th>21 - 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Mean larvae (SD)</td>
<td></td>
<td>1</td>
<td>1.5</td>
<td>2.8</td>
<td>3.3</td>
<td>11</td>
<td>8.4</td>
<td>5.5</td>
<td>28</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>(0.5)</td>
<td>(1.9)</td>
<td>(1.2)</td>
<td>(8.4)</td>
<td>(6.5)</td>
<td>(2.5)</td>
<td>(18)</td>
<td>(11.2)</td>
</tr>
<tr>
<td>Mean length μm (SD)</td>
<td>266</td>
<td>194</td>
<td>191</td>
<td>295</td>
<td>451</td>
<td>885</td>
<td>1687</td>
<td>1333</td>
<td>1504</td>
</tr>
<tr>
<td>Range</td>
<td>(9.3)</td>
<td>(19)</td>
<td>(42)</td>
<td>(60)</td>
<td>(99)</td>
<td>(388)</td>
<td>(104)</td>
<td>(180)</td>
<td>(155)</td>
</tr>
<tr>
<td>Mean width μm (SD)</td>
<td>257-275</td>
<td>176-220</td>
<td>132-330</td>
<td>165-385</td>
<td>198-803</td>
<td>165-1210</td>
<td>1540-1760</td>
<td>935-1595</td>
<td>1100-1760</td>
</tr>
<tr>
<td>Range</td>
<td>(7.9)</td>
<td>(25)</td>
<td>(23)</td>
<td>(31)</td>
<td>(35)</td>
<td>(38)</td>
<td>(29)</td>
<td>(27.4)</td>
<td>(29)</td>
</tr>
<tr>
<td>Development site</td>
<td>7.9</td>
<td>25</td>
<td>23</td>
<td>31</td>
<td>35</td>
<td>38</td>
<td>29</td>
<td>27.4</td>
<td>29</td>
</tr>
</tbody>
</table>

The data for *Setaria tundra* larvae in *Aedes* mosquitoes under laboratory insectary conditions (21 °C, SD 2.8, relative humidity 65%, SD 12.4).
5.4.1.2.2. *Anopheles* (IV)

Six of the seven *Anopheles* spp. contained *S. tundra* larvae. Three of the mosquitoes examined 6-7 days after the blood meal contained developing larvae (mean burden 2.7 (SD 1.2)). At each examination on days 9-10, 11-13 and 21-22 after the blood meal a single *Anopheles* mosquito was found infected and 4, 3 and 7 larvae, respectively, were recovered. The mean lengths of these larvae were, respectively, 440 μm (SD 95.3), 231.5 μm (SD 9.2) and 1423 μm (SD 68.7), and the mean widths 36.6 μm (SD 0.97), 32.6 μm (SD 5.6) and 29.3 μm (SD 2.1), respectively.

5.4.1.3. Melanisation (IV)

Melanisation of smf was recognized in 11 mosquitoes during the developmental studies. Melanised larvae were mostly microfilariae in the abdomen, but further developed and melanised larvae were also observed in the thorax.

5.4.2. *Setaria tundra* in wild insects

The development of *S. tundra* to the infective stage only occurred in mosquitoes, in several *Aedes* species and also in *Anopheles* mosquitoes. The prevalence of *S. tundra* in wild mosquitoes varied...
from 0.5% to 2.5% in the endemic areas. The oviposition status and number were determined from 166 dissected mosquitoes (Aedes spp.), of which 64 (38.5%) had laid eggs one or more times. All captured blackflies (Simulidae), biting midges (Culicoidea) and headlies (Hydrotaea spp.) were negative for developing S. tundra larvae, although smf were often detected (1-800 /insect) in the guts of these insects after a blood meal.

5.4.3. Questionnaire survey
All Chiefs of District of the 56 reindeer herding cooperatives in the Finnish reindeer herding area (Fig. 1) responded to the questionnaire on mosquito harassment and its effect on reindeer behaviour (IV).

5.4.3.1. Mosquito harassment during the summers of 2003, 2004 and 2005
The results indicate that overall harassment by flying mosquitoes was less severe than the average (69% of respondents for 2003, 77% for 2004 and 46% for 2005) or about average (29%, 22% and 52% of respondents, respectively) in the whole reindeer herding area during the emergence of the S. tundra outbreak.

5.4.3.2. Activity of mosquitoes (Culicidae)
Respondents reported that the peak activity of mosquitoes occurs from mid-June to the end of July. The mosquito feeding activity on reindeer was reported to be the highest in the evenings (37% of respondents) or at dawn (18%) and at dusk (10%) or, if warm enough, at night (18%).

5.4.3.3. Räkkä (III,IV) effect on reindeer behaviour
According to 86% of the Chiefs, weather conditions and insect harassment alter reindeer behaviour so that during warm weather reindeer mostly congregate on wetlands, swamps and riversides, and sometimes in forests (18% of respondents), on fells (14%) and in different kinds of open expanses (16%). In cool summers, aggregation behaviour is diminished.

5.5. Antiparasitic treatment and prevention regimes against setariosis

5.5.1. Autumn ivermectin trial
Ivermectin at a dose of 200 μg/kg b.w. s.c. was 100% effective against adult S. tundra worms in trial one. The degree of peritonitis was less also severe in the ivermectin group and the type of peritonitis was organized, while the peritonitis in the control group was wet with 50 to 150 ml of ascites fluid. All the reindeer (n = 11) in the control group and three reindeer of the ivermectin group slaughtered in the latter slaughter batch on 12 January 2004, had smf in the blood circulation (Table 3).
Table 3. The slaughter findings from reindeer calves, treated or non-treated on 11 November 2003 with ivermectin (200 µg/kg b.w. s.c.).

<table>
<thead>
<tr>
<th>No/Group</th>
<th>Slaughter day</th>
<th>Living Setaria tundra</th>
<th>Dead Setaria tundra</th>
<th>Degree of peritonitis</th>
<th>Type of peritonitis</th>
<th>Smf in blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 / Treated</td>
<td>16.12.03</td>
<td>0</td>
<td>many</td>
<td>1</td>
<td>dry</td>
<td>Negative</td>
</tr>
<tr>
<td>2 / Treated</td>
<td>16.12.03</td>
<td>0</td>
<td>many</td>
<td>0</td>
<td>dry</td>
<td>Negative</td>
</tr>
<tr>
<td>3 / Treated</td>
<td>16.12.03</td>
<td>0</td>
<td>many</td>
<td>2</td>
<td>dry</td>
<td>Negative</td>
</tr>
<tr>
<td>4 / Treated</td>
<td>16.12.03</td>
<td>0</td>
<td>many</td>
<td>1</td>
<td>dry</td>
<td>Negative</td>
</tr>
<tr>
<td>5 / Treated</td>
<td>16.12.03</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>dry</td>
<td>Negative</td>
</tr>
<tr>
<td>6 / Treated</td>
<td>16.12.03</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>dry</td>
<td>Negative</td>
</tr>
<tr>
<td>7 / Treated</td>
<td>12.1.04</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>dry</td>
<td>Positive</td>
</tr>
<tr>
<td>8 / Treated</td>
<td>12.1.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>dry</td>
<td>Positive</td>
</tr>
<tr>
<td>9 / Treated</td>
<td>12.1.04</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>dry</td>
<td>Negative</td>
</tr>
<tr>
<td>10 / Treated</td>
<td>12.1.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>dry</td>
<td>Negative</td>
</tr>
<tr>
<td>11 / Treated</td>
<td>12.1.04</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>dry</td>
<td>Positive</td>
</tr>
<tr>
<td>1 / Control</td>
<td>16.12.03</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>2 / Control</td>
<td>16.12.03</td>
<td>16</td>
<td>0</td>
<td>3</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>3 / Control</td>
<td>16.12.03</td>
<td>20</td>
<td>0</td>
<td>3</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>4 / Control</td>
<td>16.12.03</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>5 / Control</td>
<td>16.12.03</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>6 / Control</td>
<td>16.12.03</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>7 / Control</td>
<td>12.1.04</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>8 / Control</td>
<td>12.1.04</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>9 / Control</td>
<td>12.1.04</td>
<td>12</td>
<td>0</td>
<td>2</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>10 / Control</td>
<td>12.1.04</td>
<td>15</td>
<td>0</td>
<td>3</td>
<td>wet</td>
<td>Positive</td>
</tr>
<tr>
<td>11 / Control</td>
<td>12.1.04</td>
<td>2</td>
<td>missing</td>
<td>missing</td>
<td>missing</td>
<td>Positive</td>
</tr>
</tbody>
</table>
5.5.2. Winter ivermectin trial

The prevalence of smf in the ivermectin group was nine times lower than in the control group. The results are presented in Table 4.

Table 4. Number of reindeer and the prevalence of *Setaria tundra* microfilariae in the ivermectin and control groups 44 days after ivermectin injection (200 µg/kg b.w. s.c.) in the winter ivermectin trial.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ivermectin</th>
<th>Control</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves (n)</td>
<td>22</td>
<td>20</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>smf prev.</td>
<td>14%</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td>Adults (n)</td>
<td>78</td>
<td>80</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>smf prev.</td>
<td>1%</td>
<td>34%</td>
<td></td>
</tr>
<tr>
<td>Total (n)</td>
<td>100</td>
<td>100</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>smf prev.</td>
<td>4%</td>
<td>36%</td>
<td></td>
</tr>
</tbody>
</table>

*Difference between treated and control group analyzed with the chi-squared test

5.5.3. Summer ivermectin and deltamethrin trial

There was no significant impact of antiparasitic treatment on the degree of peritonitis, the prevalence of *S. tundra* nematodes or prevalence and density of other parasites between treatment groups. However, significantly fewer animals with perihepatitis (p = 0.018) were recorded in the ivermectin group compared with the control group. The degree of peritonitis was positively associated with finding of *S. tundra* nematodes in the abdomen (p = 0.002) and smf in the blood (p = 0.047). The prevalence of parasites, both adult *S. tundra* (p < 0.001) and smf in the blood circulation (p = 0.003) and also the degree of peritonitis (p < 0.001) were positively associated with the prevalence of perihepatitis.

There were no treatment effects on the daily weight gain or on the slaughter weight of calves. The treatment effect on the thickness of the fat layer was also not significant. However, the difference between the ivermectin and control groups was close to significance (thicker in the ivermectin group: mean 4.7 mm (SD 4.9) and 3.7 mm (SD 4.2), respectively, p = 0.052).
5.5.4. Status of antiparasitic treatment

The prevalence of antiparasitic treatment from 2002-2004 in the whole Finnish reindeer herding area and reindeer population densities are presented in Table 5. The data were obtained from the questionnaire addressed to the Chiefs of District of the reindeer herding cooperatives.

**Table 5. The prevalence of antiparasitic treatment of reindeer with ivermectin during 2002-2004 in separate Finnish reindeer herding areas.**

<table>
<thead>
<tr>
<th>Year</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>Reindeer/km² in summer 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area*</td>
<td>Inject¹ / Paste²</td>
<td>Inject / Paste</td>
<td>Inject / Paste</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>52% / 24%</td>
<td>57% / 23%</td>
<td>68% / 17%</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>82% / 4%</td>
<td>83% / 4%</td>
<td>75% / 2%</td>
<td>2.2</td>
</tr>
<tr>
<td>3</td>
<td>77% / 0.2%</td>
<td>87% / 0.3%</td>
<td>90% / 0.2%</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>59% / 5%</td>
<td>64% / 5%</td>
<td>66% / 5%</td>
<td>3.4</td>
</tr>
<tr>
<td>tot</td>
<td>69% / 7%</td>
<td>73% / 7%</td>
<td>75% / 5%</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*See Figure 1.

¹Treatment with ivermectin (200 μg/kg b.w. s.c.)
²Treatment with ivermectin (equine paste, 200 μg/kg b.w. p.o.)

5.6. Climate

5.6.1. Effect of weather on reindeer behaviour

According to 86% of the Chiefs, warm summer weather conditions and the associated insect harassment altered reindeer behaviour. During warm weather, reindeer mostly congregate in wetlands, swamps and riversides, and sometimes in forests, on fells and in different kinds of open expanses. In cool summers, aggregation behaviour is diminished. (IV)

5.6.2. Climate data

The results of generalized linear modelling indicated that the probability of *Setaria* outbreaks increases rapidly when the summer mean temperature (June-Aug) during the preceding year exceeds 14 °C. The long-term (1964-2003) mean for the region was 12 °C (range 8.8 – 15.1).
The relationship between the mean summer temperature (June-August) during year t-1 and the predicted probability of a *Setaria tundra* epidemic during year t.
6. DISCUSSION

The sudden appearance of a peritonitis outbreak with the observation of a mass appearance of nematodes in the abdominal cavity of reindeer at the beginning of the slaughter season in autumn 2003 confused reindeer meat inspecting veterinarians, reindeer herders and scientists in Northern Finland (I). The outbreak highlighted the lack of baseline knowledge of vector-borne parasites and their detrimental impacts on cervids in the boreal forests of the northern hemisphere.

This thesis has provided evidence of the detrimental effects of parasitic disease caused by *S. tundra* in reindeer and described the causative agent and diagnostics of this nematode in its different developmental stages, as well as the basic biology including life cycle parameters, reservoirs and vectors. It has also produced information on the genesis and development of a disease outbreak in space and time, with recognition of the dynamics of cervid populations and interacting climatological and other drivers. The thesis demonstrates that it may be possible to predict future outbreaks in good time by utilizing temperature data from the previous summer. Finally, the study has elucidated the possibility and the profitability of different treatment and prevention regimes against setariosis in reindeer management.

6.1. The outbreak seen at slaughter

The outbreak of peritonitis in reindeer calves started in the southern parts of the Finnish reindeer herding area. According to meat inspection data, the first signs of the increasing prevalence of peritonitis and perihepatitis were already visible in 2002 when the proportion of condemned reindeer viscera increased from 4.9 to 15.3% in the southern part of Finnish reindeer herding area (I). However, the situation did not come to public or professional attention before the beginning of the slaughter season in autumn 2003, when serofibrinous peritonitis and living nematodes were prevalently seen in the peritoneal cavity of reindeer calves. The situation led at the most to about 50% of viscera being condemned in the area (I). The outbreak was most intense in the southern part of the reindeer herding area in 2003 and 2004, where in some locations, 90% of calves were diseased. The focus of the outbreak moved northwards by approximately 100 km/yr. Only reindeer in the northernmost Upper Lapland were free of lesions (I).

The outbreak led to a mass condemnation of internal organs, especially livers, and the removal of peritoneums, caused a lot of extra work in slaughter houses and reduced the aesthetic value of the meat. These factors resulted in substantial economic losses and increased the workload associated with meat processing. Slaughter was found unpleasant. The lesions were usually extensive, had a purulent character and were previously unseen by meat inspectors. This led in the beginning, before more detailed studies, to an overreaction; the condemnation of carcasses from human consumption
and heat treatment of entire slaughter batches (I).

The sudden onset and consequences of the outbreak for meat hygiene were very similar to those described by Rehbinder et al. (1975), Rehbinder (1990), Poppe (1977) and Kummeneje (1980) during the first reported S. tundra outbreak in Scandinavia, 30 years earlier in 1973 (I). The outbreak created a rare opportunity for researchers to follow an emerging vector-borne parasitic disease outbreak among free-ranging reindeer. It also demonstrated the need for more organized cervid disease surveillance and the importance of data collection, including health indicators, during slaughter and hunting. In addition, it created the foundation for future health care work and for securing meat safety in the slaughter business.

6.2. Parasitic peritonitis

Calves heavily infected with S. tundra expressed decreased welfare, a low body condition and an undeveloped winter coat (I). In post mortem examination, typical pathological changes were initially perihepatitis, and during the course of the infection, also peritonitis (I, V). Histological changes on the serosal membranes also indicated parasitic infection; serofibrinous or granulomatous peritonitis with lymphoplasmacytic and eosinophilic infiltration were present (I). Although these changes gave an impression of a purulent process, neither specific bacterial growth, nor a significant impact on the organoleptic evaluation of the meat was detected (I). A similar histopathological picture with a pure culture of corynebacteria in liver lesions was discovered in Sweden in 1973 (Rehbinder et al. 1975). Thus, according to the results, advice for meat inspection was given to remove affected parts of the carcass and viscera and not to limit the normal use of the meat or to heat treat the carcasses, if there were no other contributory factors.

The disease was most common and severe in calves. The decrease in health indicators (I, V) indicates that systemic disorders may be involved in the pathogenesis of S. tundra. In this study, mild peritonitis was common in adult reindeer, but condemnations were very rare. Also interesting was the observation of perihepatitis and 1 to 3 encapsulated preadult Setaria sp. nematodes on the liver surface of six young moose from a highly S. tundra endemic area. This inverse relationship between host age and setariosis has been reported in many earlier studies (Weinmann et al. 1973, Prestwood and Pursglove 1977, Weinmann and Shoho, 1975, Osipov 1972, Shol’ and Drobishchenko 1977) in which infection and disease was most prevalent in calves and yearlings. As the adult reindeer slaughtered in Finland are mostly old females (8 to 12 years) removed from the breeding stock, relatively few yearlings or other young adult reindeer were examined.

Similar but milder changes, which usually did not lead to condemnations, have earlier been described in the abdominal cavity of white-tailed deer by Prestwood and Pursglove (1977). These changes were caused by an average of 2.6 S. yehi worms, whereas the mean number of S. tundra worms during the present outbreak was 8.6 in the individual reindeer affected. Nevertheless, there is also a report
of a marked fibrinous peritonitis associated with a severe *S. yehi* infection in a young New Jersey white-tailed deer (Pursglove 1977). Unlike previous reports (Rehbinder et al. 1975), no association between dead and encapsulated worms and peritonitis was observed (I,V).

6.3. Microfilaremia

The impact of *S. tundra* microfilaremia on cervid health remains unknown and it is difficult to separate from the impact of adult worms. The captive reindeer in the experimental zoo (with a mean of 950 smf/ml blood (range 62 – 4000)) appeared to suffer, and they had inexplicable symptoms resembling those described in chronic *Setaria* spp. microfilariosis in buffalo (Sharma et al. 1981, Kumar et al. 1984, Kumar and Sharma 1994, Venu 2000). These include anorexia, a purulent discharge from the eyes, pale mucous membranes, a rough and dry skin coat, and a stiff gait. Because these reindeer also harboured adult *S. tundra* and other parasites, it is impossible to fully evaluate the role of smf in their overall health. According to Nelson (1966), the circulating microfilariae cause no apparent damage. The observation by Sharma et al. (1998) also revealed systemic pathological changes after experimental *Setaria* infection: enlargement of the liver, kidney and spleen, haemorrhagia in the brain and spinal cord and emphysema in the lungs. These changes may be caused by the circulation and absorption of the secretions and excretions of the parasite produced during their growth and reproduction, and the disintegration products of dead worms and microfilaria. However, a role for microfilariae in the pathogenesis is suggested by observations involving several species of *Setaria* reported by Mohan (1976), in which multiple nodules containing microfilariae associated with eosinophilia and chronic splenitis, degeneration of the liver with bundles of microfilariae in its blood vessels, focal collections of inflammatory cells associated with microfilariae in the kidneys, focal necrotic nodules containing dead microfilariae near the periphery of lung lobes and necrotic areas in heart muscles resulting from blockage of blood vessels with microfilariae. Although the local changes and their clinical manifestation caused by *S. tundra* and many other filarial parasites are well known, there is a need for further studies on the systemic pathology of setariosis to understand the pathogenesis and the chronology of clinical implications.

6.4. Population health

Heavy *S. tundra* infection has a pronounced influence on the welfare of reindeer calves (I,V). The slaughter weight, back fat index and body condition of reindeer calves were lower in Kuusamo during the outbreak in 2003 than in 2004, when the peak of the outbreak was over (I). There is a proven positive correlation between reindeer calf winter survival and body weight (Reimers 1984). Although there are numerous other factors influencing these values (Kumpula and Colpaert 2003), such as the condition of summer pastures (Kumpula and Nieminen 1992), a heavy *S. tundra* infection obviously impairs the winter survival of calves.
The influence of *S. tundra* infection is dependent on the intensity of infection (I). It is easy to assume that the greater the parasite population, the greater the damage produced by living or dying adult or developing worms and mf at different intervals of time. The degree of peritonitis and perihepatitis were positively associated with finding of *S. tundra* nematodes in the reindeer abdomen and smf in the blood (V), and there was a significant positive correlation between the worm count and the degree of peritonitis (I). The mass mortality of reindeer in 1973 was associated with a massive *S. tundra* outbreak in Finland, which was regarded as a contributing factor to the deaths (I). The lack of reported mass mortality in the present outbreak area may be a consequence of the fact that winter survival is no longer highly dependent on environmental conditions, in contrast to 1973. Good nutrition is ensured when most reindeer receive supplementary feed during the winter months (Nieminen 2006). It has been reported that the severity of a natural *S. cervi* infection may be reduced by providing young deer with an adequate diet (Shol’ and Drobishchenko 1973). The intensive antiparasitic treatment with ivermectin (V), which obviously improves the health of breeding reindeer, may also have improved their winter survival. In addition, the majority of spring-born calves are slaughtered before the most critical time for survival. However, the fact that 58% of a total of 26 autopsied reindeer that were dead or killed due to any disease during the outbreak had peritonitis/perihepatitis in autopsy is indicative of a detrimental disease syndrome.

The situation might be quite different if *S. tundra* had spread into the northern mountain regions of the Finnish reindeer herding area, where supplementary feeding is not so intensively practiced and winter survival is still highly dependent on environmental conditions (Kumpula and Colpaert 2003). The same applies for northern wild reindeer/caribou populations. In wild forest reindeer, the prevalence and density of *S. tundra* was similar to semi-domestic reindeer, and the prevalence of peritonitis as well as smf-positive animals was high, especially in the area adjacent to the southern border of the Finnish reindeer herding area (I, III). The association between the coexistent high prevalence of *S. tundra* and the rapid decrease in the population remained unclear in this area, which is strongly influenced by predation by large carnivores (Kojola et al. 2007, 2009). However, the infected animals may be more susceptible to predation (Kutz 2001, Hoberg 1995). The *Setaria* outbreak in Finnish moose was also associated with granulomatous lesions caused by adult *S. tundra* worms in the wall of the urinary bladder and uterus (Nygren 1990) resembling the changes caused by adult *S. digitata* in the wall of the urinary bladder of cattle (Yoshikawa et al. 1976).

The current study clearly indicates that *S. tundra* can have a substantial effect on reindeer. Further, the results suggest a heavy rate of infection by *S. tundra*. *Setaria tundra* may have detrimental effects on *Rangifer* populations and could thus affect entire circumpolar ecosystems, which demonstrates an expanding challenge to food security for subsistence cultures at high latitudes (VI). However, the current declines in global *Rangifer* populations (Vors and Boyce 2009) have not been examined in the context of infectious diseases or parasites (VI).
6.5. Population dynamics

Presumably, after the previous outbreak in Fennoscandia during 1973, *S. tundra* existed in low numbers in the Finnish reindeer population for 30 years. To support this conclusion, smf prevalences and densities in the blood samples from 1997 were low (III), and pathological changes associated with *S. tundra* were rarely reported in reindeer meat inspection records before the peritonitis outbreak in 2003 (I). It is evident that even a low *S. tundra* prevalence and density can maintain the infection in the reindeer population (III). Our data indicate that adult reindeer can act as asymptomatic carriers of *S. tundra* to the next generation (I,III). A low infection prevalence and low smf density can probably exist for years in the population and the infection can be transmitted from dam to calf by efficient insect vectors (III). When the conditions for transmission are favourable, the possibility of an outbreak in calves is clear. According to meat inspection data, the prevalence of *S. tundra* infection had already increased in slaughter reindeer in 2002, one year before the outbreak (I). It is therefore reasonable to assume that the parasite must reach a high enough prevalence in the population before the other conditions enabling the outbreak surpass a threshold (I). This is probably not possible within just one favourable year in subarctic zones, where the transmission of *S. tundra* is only possible in warm summers.

The lower density of *S. tundra* in adult reindeer, as well as milder pathological changes (I) and smf prevalence and density (III), and the decline in the outbreak in the south while emerging in the north (I), may indicate a developing adaptive immunity against *S. tundra* in reindeer populations. This conclusion is supported by the findings that all naturally-infected reindeer cleared their infections within two years, in spite of infection pressure demonstrated by the infection of their calves (III). However, this study did not include serological research and there is no serological evidence of a humoral response, which warrants future studies. Experimental studies have clearly shown that animals can develop immunity against filarial infections, which may result in a reduction in the worm load or in the suppression of microfilarial production (reviewed by Nelson 1966). Another obvious factor influencing the *S. tundra* infection rate in the reindeer population is the massive and routinely applied antiparasitic treatment in the autumn, which can reduce the proportion of carriers for the next summer (V). The impacts of the other changes in reindeer management would also be instructive to explore.

It is also possible that *S. tundra* has been endemic in Finnish reindeer (and other) populations not just over the past 30 years, but further back in time. The 30-year period is of further interest because this coincides with what appears to have been a shift in northern systems to one of accelerated warming (Kutz et al. 2005), with potential impacts on the dynamics and tipping points of parasites (Hoberg et al. 2008). However, in the absence of empirical data (diseases, historical climate dynamics), the estimation of these impacts is difficult.
6.6. Transmission dynamics

6.6.1. Sylvatic reservoirs

Although *S. tundra* can be maintained in reindeer populations by the reindeer itself, the role of other cervids as reservoirs may be significant. Thus, other cervids may have been mediators of a range expansion or introduction of the parasite to the north and a host-switch may have occurred. However, the strain variation in *S. tundra* (given that the difference between for example reindeer and moose is constant (III)) may suggest a longer period of isolation. The strain of the parasite is probably of significance in determining differences in filarial infections in man (Nelson 1966), and most possibly also in other animals, as evidenced by epidemiological differences in infection between reindeer and moose (I, III, IV). In a recent study (Solismaa et al. 2008), no *Setaria* spp. were found in Finnish farm animals.

The role of moose, the most abundant wild cervid in the reindeer herding area, as a reservoir for *S. tundra* in the present outbreak is unlikely. The moose population peaked in 2003-05 in northern Finland and no acute peritonitis or living adult *S. tundra* worms were reported, while only a few pre-adults were found encapsulated on the surface of livers (I). Although, the changes on the livers resembled the changes associated to *S. tundra* in reindeer, the causality was not established. Respectively, in 1989 during the *Setaria* sp. outbreak in northern moose population, which was probably caused by a genetically different *S. tundra* haplotype (III) and took place within the reindeer husbandry area, no concurrent reports of an associated increased morbidity in reindeer exist (I).

The *S. tundra* outbreak in reindeer started from the south, adjacent to the Kainuu wild forest reindeer population. Smf densities and prevalence were similar in these two subspecies. The role of wild forest reindeer as a reservoir of *S. tundra* for reindeer may be significant, but nevertheless, it could also work the other way around (I, III), especially as *S. tundra* prevalence and densities were higher in the northern wild forest reindeer population than in the southern population, which has no contacts with semi-domesticated reindeer.

The roe deer appears to be a universal asymptomatic host (I, III) and may have had a role as a long-distance vector/reservoir of different haplotypes of *S. tundra* in Finland (I, III). The roe deer population has recently spread to northern Finland, and is now present in almost the entire reindeer herding area. Hence, the association between the first appearance of *S. tundra* in Scandinavia in the 1970s and the invasion of the north by roe deer (Haugerud 1989) is interesting. Considering the reservoir host capacity of roe deer and the dynamics of *S. tundra*, it is worth noting that especially young male roe deer can migrate many hundreds of kilometres from their birthplace (Cederlund and Liberg 1995), and thus can be efficient long-distance vectors for *S. tundra*. Furthermore, supporting this theory is the observation that only minor nucleotide differences exist between the reindeer *S. tundra* sequence and that of specimens from roe deer in Italy (Casiraghi et al 2004), so that they can be considered as the same haplotype (I).
Setaria yehi appears to be very common in North American deer (Prestwood and Pursglove 1977). Although roe deer and wild forest reindeer have contacts with the Finnish white-tailed deer population, no Setaria spp. were found in the latter cervids (I, IV).

The final question is whether these are actually separate species of Setaria that are circulating in sympatry in sub-arctic cervids. If the differences (genetic) are constant and these are reproductively isolated, no matter how minimal the genetic divergence, then it is probable that they do not represent conspecifics. Considerably greater sampling would be required to explore this hypothesis, and the relationship across other species recognized in the genus.

6.6.2. Vectors
This study has provided evidence that mosquitoes, particularly Aedes spp. and to a lesser extent Anopheles spp., have an important role in the transmission of S. tundra in the reindeer herding areas of Finland (IV). The Aedes genus was considered the most important vector, because the majority of flying mosquitoes in midsummer in Finland belong to the Aedes group, which also has the greatest species diversity (Utrio 1978). Adult female Aedes spp. are vigorous round-the-clock feeders that can be infected with many infective S. tundra larvae (IV). Heavy infections may conversely increase vector mortality (Bain and Babayan 2003) and thus decrease vector efficiency. Although Anopheles mosquitoes are also present, and can serve as vectors for S. tundra, their epidemiological significance in Finland is likely to be limited because of their life cycle parameters and low numbers compared to Aedes spp. (Utrio 1978). The role of Anopheles mosquitoes may be more important in more temperate areas or may increase in Finland as a consequence of climate change. Our results suggest that S. tundra is not a very vector-specific parasite, and this may enhance its ability to expand its geographical range.

Massive swarms of blood-feeding insects attacking herds of caribou/reindeer are well known (Anderson and Nilssen 2008), and in the study area the reindeer are the main source of blood for the mosquitoes (Natvig 1948). Some estimates suggest that reindeer can be exposed to attacks of approximately 8000 mosquitoes/hour during the räkkä period (Kadnikov 1989).

In highly endemic areas, the prevalence of filarioid infection rarely exceeds 1% of the total mosquito population (Dadaev 1984). In this study, the S. tundra prevalence in different mosquito populations in endemic areas was 0.5–2.5%, the highest prevalence being around the experimental zoo, an urban park-like environment, almost in the city of Oulu (IV). In these conditions, reindeer could be attacked by 40 to 200 mosquitoes carrying S. tundra larvae hourly.
6.6.3. Transmission drivers
There are many non-climatological and climatological factors enhancing the complex life cycle of *S. tundra*, its transmission and survival in the reindeer population. The effect of these drivers on the patterns of parasites, host-pathogen relationships, distribution and finally on the emergence of an outbreak, may be both incremental (cumulative) and extreme (episodic) (I,III,IV,VII). Although Hoberg et al. (2008) discussed some interactions between these drivers related to climate, the present study provides strong empirical evidence to explore the relationship.

6.6.3.1. Non-climatic drivers
The peak period of *S. tundra* microfilaremia in reindeer is from mid-June to the end of August (III), which according to data from the questionnaire suggests a co-occurrence with the peak activity of mosquitoes (IV). These peaks in microfilaremia (associated with the high ability of *S. tundra* to produce mf (II)) and insect abundance and contact with reindeer occur just after the calving season, when reindeer shed their winter hair, leaving their skin relatively exposed to the attacks of mosquitoes. At that time, the passive immunity of calves is at its lowest (Orro et al. 2006, see also Prestwood and Pursglove 1977 and Shol’ and Drobishchenko 1977). This synchrony probably promotes the transmission of *S. tundra* from adult carriers to calves.

The observations suggest that the migrations of reindeer and the characteristics of reindeer pastures can affect the transmission dynamics of *S. tundra*. Behavioural responses to avoid insects are commonly observed in reindeer (Weladj et al. 2003). Reindeer have to keep in constant movement during the period of mass harassment by insects in order to reduce the attacks (Natvig 1948) and to avoid mosquito clouds. For example, *A. punctor* has been reported to fly up to 46 km in still air, and there is evidence that mosquitoes take advantage of the wind on long flights (Clements 1963). In traditional reindeer herding in Fennoscandia, the herds may migrate several hundreds of kilometres towards the coast of the Arctic Ocean and to the higher altitudes to escape the mass appearance of insects. However, this escape in Finland is currently restricted by the management of reindeer herding and legislation dividing the Finnish reindeer herding area into 56 separate cooperatives (Fig. 1).

Reindeer/caribou have been shown to seek relief from insect harassment on snow patches, windswept ridges, mountain tops and coastal areas (Mörchel 1997, Anderson and Nielsen 2008), as well as road sides (Pollard 1996) and sandy patches (Helle and Aspi 1984). In these studies, the harassment effect has generally been aroused by warble flies (*Hypoderma tarandi*), throat bot flies (*Cephenemyia trompe*) (Weladj et al. 2002) and horse flies (Helle et al. 1992) in the daytime. Overall, reindeer/caribou have been shown to respond to disturbances by herd formation (reviewed by Helle et al. 1992). In this study, according to the questionnaire results, warm summers were associated with hundreds of reindeer congregating in dense herds in mosquito-rich wetlands (IV). This behaviour
may be explained by the availability of drinking water and fresh food plants or the benefits of the habitat for thermoregulation (Anderson and Nilssen 2008). However, in these areas the microclimate is also favourable for mosquitoes and it is presumable that in these kinds of highly endemic areas, the infection rate of *S. tundra* in mosquitoes can reach or even exceed the prevalence detected in the experimental zoo (IV). For example, in Uzbekistan, the highest prevalence of *S. labiatopapillosa* in cattle was found in irrigated, marshy and river catchment areas (Dadaev 1984). In this study, in the ancient destination of reindeer summer migration, upper Lapland, neither peritonitis (I) nor mf (III) were found in reindeer blood and *S. tundra*-infected mosquitoes were also absent (IV). Mires constitute about 30 to 40% of the total land areas of the *S. tundra* outbreak area, but only about 12% of upper Lapland (Kumpula et al. 1999), which was believed to be free from *S. tundra* (I, III). This may help explain the numerous observations by reindeer herders of great variation in *S. tundra* prevalence in reindeer herds originating from different summer pastures (IV). This is also explained by the concept of spatial and temporal heterogeneity, and the distribution of a parasite and the disease caused by the parasite being very different, i.e. the concept of mosaics in space and time (Hoberg et al. 2008).

Insect harassment causes stress in reindeer, which can be seen as higher rates of activity, i.e. running, scratching and biting the body (Mörchel and Klein 1997), and may cause exhaustion (Colman 2003), especially in calves (Helle and Tarvainen 1984). Our experiments demonstrate that following acute exercise, the concentration of microfilariae in the blood circulation of reindeer momentarily increases (III).

### 6.6.3.2. Climatic drivers

The Arctic climate is undergoing accelerating change resulting in both cascading and cumulative weather events that influence temperature and hydrological processes. One of the climate change scenarios concerns how increasing temperatures will affect the invasiveness and spread of infectious diseases; of these, mosquito-borne diseases are the most climate-sensitive maladies (Patz 1996). For example, in certain areas, suitable habitats for mosquitoes are increasingly provided by melting permafrost and increased rainfall, which could directly affect disease transmission by shifting the geographical range, longevity and reproductive rate of vectors. Increasing temperatures may also increase the feeding rates for vectors and reduce the incubation and generation time for pathogens (Patz 2000). According to Calado and Navarro-Silva (2002), the haematophagic activities and oviposition of mosquitoes (*Aedes albopictus*) were significantly influenced by temperature.

Our data demonstrate that warmer summers, which may become even warmer as a consequence of climate change, can promote *S. tundra* outbreaks in northern latitudes, as they may have implications for the range expansion of mosquito species to the north (Barry 2001). The transmission of *S. tundra* is highly dependent on the life span of female mosquitoes (IV), with the survival of adult
mosquitoes being partly dependent part on both temperature and humidity (Barry 2001). Within limits, this warmth affects the development, reproduction, longevity and feeding habits of mosquito vectors (Clements 1963, Delatte 2009), and the lowest temperature of the seasonal regime is a limiting factor for mosquito survival (Löwenberg-Neto and Navarro-Silva 2004). There is unfortunately no information on the longevity of mosquito populations in Finland, although in the present study (IV) adult Aedes spp. survived approximately four weeks in a laboratory insectary at room temperature. In the present study, older females comprised a considerable part of the “wild” mosquito population, but the data from the questionnaire suggest that the mosquito densities were low (IV). The S. tundra outbreak in Sweden in 1973 was associated with unusually warm weather and with the appearance of especially large numbers of mosquitoes (Rehbinder et al. 1975). However, the presence and proportion of older female mosquitoes is a more important factor for S. tundra transmission than a high mosquito density as such. Older females can have fed several times and are thus more likely to have become infected with tens of S. tundra larvae.

It has also been demonstrated that the development of S. tundra in mosquitoes to the infective third stage is highly temperature dependent. Warmth decreases the time required for the larval development of S. tundra (IV). Questionnaire results also suggest a linkage between the levels of vector activity in the field, the transmission of S. tundra among reindeer and the association of warm summers with hundreds of reindeer congregating in dense herds in mosquito-rich wetlands (IV).

The S. tundra burden and high larval abundance in Aedes mosquitoes (up to 70, IV) may, however, reduce vector efficiency. Individual mosquitoes can be infected with many 3rd stage S. tundra larvae. This high larval abundance may, however, increase vector mortality and decrease vector efficiency (Bain and Babayan 2003). The maximum number of larvae compatible with insect survival depends on the species of mosquito (Canrini and Gabrielli 2007). Mosquitoes have different defence mechanisms that block larval development and may consequently limit the infective larval load. Melanisation of Setaria larvae in mosquitoes was also observed in the present study (IV). When the parasite load is too heavy in the mosquito, tubule function is compromised and the insect dies (Canrini and Gabrielli 2007).

Further, the results indicate that the probability of Setaria outbreaks rapidly increases when the summer mean temperature during the preceding year exceeds 14 °C. The long-term mean for the region is 12 °C (range 8.8 – 15.1 °C) (VI). This result is congruent with observations during the origin of the recent disease outbreak (I,III); the emergence of disease is not immediate, and is associated with a considerable time lag between the initiation of the spread of infection in the reindeer population and the development of the mass appearance of parasites. It appears that in this system, temperature increases above 14 °C may represent a tipping point for the development and amplification of the parasite (Hoberg et al. 2008) in reindeer populations. The cumulative effect is also demonstrated by the fact that outbreak years have been warm (mean 13.0 °C, range 11.7 – 14.2) (Fig. 10), which is consistent with what we know about the life cycle and transmission of S. tundra (I,II,IV).
Finally, our data demonstrate that climate warming could promote serious outbreaks and the emergence of filarioid parasites across northern latitudes. Future global climate change could also facilitate the translocation and introduction of species of dangerous tropical filarioids into the Northern Hemisphere, leading to detrimental effects on mammalian host populations.

6.7. *Setaria tundra*, the causative agent

A detailed description, figures, morphometry and genetic data for *S. tundra* in its different stages are provided in papers I, II, III and IV.

The morphology of *S. tundra* correlated relatively well with the reference values given in the original description by Rajewsky (1928), but there were more differences compared to the descriptions of *S. tundra* from US white-tailed deer and mule deer published by Yeh (1959) (II). The synonymity of *S. tundra* in the sense of Yeh (1959), later named as *S. yehi*, and the original *S. tundra* described by Isaichikov and Raevskaia 1928 has been strongly questioned (reviewed and redescribed by Backlund and Walker 1969), which is also supported by our findings. The resolution of this taxonomic question of whether the various Finnish and American isolates represent distinct species or only one highly variable species in different hosts will require an integrated approach in the future, in which the DNA-based and morphological identifications are consistent.

The reindeer, moose and roe deer have been mentioned as hosts for *S. tundra* in Europe (Sonin 1977). In this study, the *S. tundra* specimens from reindeer, moose and roe deer from Finland were identical along the 1389 bp long mtDNA sequence (I, II, III,), and had only six nucleotide substitutions.
when compared to an *S. tundra* sequence originating from roe deer in Italy (I). These differences were regarded as small and justified identification (Ferri 2009) as *S. tundra*. Based on the current morphological and PCR studies, the peritonitis outbreak described in this study was caused by *S. tundra*. The question whether the abundant *S. tundra* in Scandinavia in 1973 was the same as the present haplotype remains open due to the lack of original isolates/material from the outbreak. The parasite has been present, albeit probably infrequent, in reindeer in Finland since 1973 (I,III) and has seldom been associated with peritonitis (I). Although we demonstrated that the pathogenicity of *S. tundra* is dependent on the intensity of the infection (I), it is also possible that the reindeer in Finland at present are infected by different, perhaps more pathogenic strains of *S. tundra* originating from another host (see Nelson 1966), such as the wild forest reindeer or, more likely, the roe deer (I,II,III). There is lack of morphological descriptions and individual gene sequences of *Setaria* spp. for circumpolar ungulates. It is important in the future to be able to accurately detect and identify filarial species and their strains in order to study their distribution and epidemiology.

The life cycle of *S. tundra* in mosquitoes observed in the present study was similar to those previously described for other *Setaria* spp. (Anderson 2000, Nelson 1962, Nelson 1964, Zhong-Xing and Li-rong 1990, Wajihullah 2001). However, there was considerable variation in the morphology of the developing larval stages, as observed earlier for species of the Filarioidea developing in different vectors (Serrão et al. 2001). These variations might make the morphological identification of the larvae difficult beyond the family level, and illustrate the important role of PCR-based methods for species identification. This is also an important contribution to epidemiology; molecular-based methods allow geographically extensive and site-intensive sampling without the need for necropsy (reviewed by Hoberg et al. 2008).

6.7.1. The life cycle of *S. tundra* (Fig 11)

The life cycle of *S. tundra* in reindeer remains partly unresolved. As blood sucking insects play a major role, the infection has to take place during the summer months (III,IV,V). The prepatent period of *S. tundra*, based on the temporal monitoring of batches of reindeer calf at slaughter, is about 4 months (III). This is less than the reported prepatent period (224 days) of *S. cervi* from East European red deer (Shol’ and Drobishchenko 1973). The life span of adult females of *S. tundra* in the definitive host is at least 14 months, and probably longer (III). This is congruent with the life span of *S. marshalli*, which is reported to be one year after infection (Fujii et al. 1995) and that of *S. labiatopapillosa*, about 16 months (Osipov 1972). The absence of male parasites in the two captive reindeer autopsied probably indicates that they are more short-lived and redundant after fertilization. Microfilariae, which are produced by female worms in very large numbers and enter the blood circulation, can also be long-lived and perhaps survive for several years (Nelson 1966), but our data indicate that the vast majority of smf only live for months, at most.
The life cycle of *Setaria tundra*; A: Adult nematodes inhabit the peritoneal cavity of reindeer and produce microfilariae in the host's blood circulation, especially on summer months. The life span of the adult *S. tundra* female is at least 14 months. B: Microfilariae get with the blood meal into the intermediate mosquito (Culicidae) host. C: Microfilariae penetrate the gut of the mosquito and develop through two molts into infective third-stage larvae. The development is temperature dependent and takes about two weeks at 21 °C (mean). D: When the mosquito is feeding again, the third-stage larvae break out and penetrate the skin of the host through mosquito’s puncture wound. Then they develop to the adult stage through two molts in the host and find their way to the abdominal cavity. The prepatent time is approximately 4 months.

6.8. Treatment regimes (V)
The questionnaire confirmed that endectocidic antiparasitic treatment is widely and routinely used in Finnish reindeer management every late autumn to early winter. The overwintering reindeer are treated with ivermectin during autumn round-ups or later in winter corrals where they are gathered for feeding. Originally, treatment with various insecticides was targeted against warbles (*Hypoderma tarandi*) and throat bots (*Cephenemyia trompe*). After the discovery of ivermectin, the first endectocidic macrocyclic lactone, the spectrum of the routine antiparasitic treatment broadened to control potentially harmful gastrointestinal nematodes (Oksanen 1999), and recently also the deer ked (*Lipoptena cervi*) (Kynkäänniemi et al. unpublished).
A major conclusion of the present study is that the treatment of reindeer calves in the autumn, and to a lesser degree in the summer, with injectable ivermectin resulted in a decreased severity of peritonitis and perihepatitis due to setariosis. During a heavy outbreak, early autumn treatment can improve the health and quality of slaughter calves, but it would impair the reputation of reindeer meat as a natural product. On the other hand, mid-summer treatment of calves with ivermectin or deltamethrin during earmarking round-ups is neither efficient nor cost-effective. Ivermectin has good efficacy against adult *S. tundra* nematodes in the abdominal cavity and circulating smf, and therefore there is also an obligation to treat heavily infected reindeer calves with ivermectin by injection (200 μg/kg b.w. s.c.) for animal welfare reasons. The results also show that routine antiparasitic treatment, especially of calves left alive for breeding purposes, is well justified during an outbreak. Overall, the results obtained from the trials were comparable to earlier reports in which a variable efficacy of ivermectin against *Setaria* spp. infection has been demonstrated in domestic animals.

At the population level, massive and routinely applied antiparasitic treatment with ivermectin can reduce the number of carriers among breeding reindeer in the next summer. The fact that this could not prevent the emergence of the *S. tundra* outbreak in new areas in the North (I,III) indicates that the transmission dynamics of *S. tundra* are efficient. On the other hand, the present intensity of antiparasitic treatment may possibly have prevented mass mortality in the winter, such as described earlier, by improving the health of breeding reindeer.

The economic and ethical feasibility of various routine treatment regimes for the reindeer population can be questioned. Chemical mass treatment of reindeer calves to be selected for slaughter could damage the organic reputation of reindeer meat in the foodstuff market (Oksanen 1999). Although there is currently no formal evidence for the development of resistance to any drug used against filariosis, several cases of ‘non- or poor responsiveness’ to treatment of onchocercosis with ivermectin have been reported (Molyneux 2003). The results obtained from this study (V) suggest that development of resistance to ivermectin may also have occurred among other ecto- and endoparasites of reindeer, which warrants further investigation. It is clear, however, that the endectocidic antiparasitic treatment of reindeer in the future should be based on proper health care and clinical and meat inspection findings, which may have considerable spatial and temporal variation.

Although insecticides have played a central role in controlling the major insect vectors of infectious diseases such as malaria, filariosis and haemorrhagic fever since the early 20th century, the prevention of *S. tundra* transmission by the insecticide and mosquito repellent deltamethrin was not successful. It is possible that the effect against mosquitoes does not last very long in the occasionally rainy conditions in Finland, or the timing of the treatment in relation to the transmission of *S. tundra*
(IV) was wrong. In addition, the emergence and spread of insecticide resistance in culicine filarioid vectors, environmental pollution, and unresolved issues pertaining to their toxicity to humans and non-target organisms (reviewed by Scholte et al. 2003) hamper the progressive use and broad acceptance of these tools in Finland.
7. CONCLUSIONS

The current study has provided baseline information to improve our understanding of the ecology and dynamics of *S. tundra* and the disease outbreaks associated with this parasite. It has provided evidence of the detrimental effects of *S. tundra* on reindeer and described the causative agent and diagnostics of the *S. tundra* nematode in its different developmental stages as well as the basic biology, including life cycle parameters, reservoirs and vectors. It has also provided information on the origins and movement of a disease outbreak, its dynamics in cervid populations and the diverse interacting climatological and non-climatological drivers. Finally, the study has elucidated the possibility and profitability of different treatment and prevention regimes against setariosis in reindeer management.

A heavy *S. tundra* infection is likely to be detrimental, at least to semi-domesticated reindeer, and may thus have harmful consequences for arctic ungulate populations. A heavy infection load in reindeer calves leads to substantial economic losses in reindeer management and an increased workload associated with meat processing.

*Setaria tundra* was probably introduced to Fennoscandian reindeer by wild cervids decades ago, but is presently maintained in the reindeer population by the reindeer themselves. The efficient transmission of the parasite by mosquitoes, especially *Aedes* spp., ensures at least a low prevalence, even under unfavourable weather conditions. Many drivers can enhance the genesis of an outbreak. A key factor promoting the transmission of *S. tundra* is a warm ambient summer temperature, which improves the development, longevity and reproduction of vectors and may within certain limits control their feeding possibilities, as well as the larval development of *S. tundra*. Warm summers also alter the behaviour of reindeer, which may increase the infection pressure. Warm summers, which may become even warmer in the boreal northern hemisphere in the future as a consequence of climate change, may facilitate the translocation, geographical expansion and dissemination of pathogenic tropical filarioids into the Northern Hemisphere. This may lead to detrimental effects on mammalian host populations and might then become an even greater threat to arctic ungulates and to food security for subsistence cultures at high latitudes. To understand these processes, we should investigate the emergence of vector-borne parasitic diseases in northern wildlife. The complex interactions between parasites and their arthropod and mammalian hosts could be studied by monitoring these parasites in cervid blood circulation during slaughter and hunting, and also in heamatophagous arthropods, for which tools are described in the present study. Special attention should be paid after warm summers in which temperatures exceed the 14 °C threshold. Humans are obviously exposed to larvae via blood-feeding insects, and further studies together with public health scientists will be of scientific value and interest.

I hope that the information presented in my thesis will be useful in understanding the complex interactions among these parasites, their invertebrate and mammalian hosts and the environment, and finally in predicting, and preventing, future outbreaks.
This study was carried out in the Fish and Wildlife Health Research Unit, Finnish Food Safety Authority Evira (and its predecessor the National Veterinary and Food Research Institute, EELA), at the Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki and at the Experimental Zoo of the University of Oulu. The work was financially supported by the Ministry of Agriculture and Forestry (MAKERA) and the Finnish Food Safety Authority Evira. Financial support from the Finnish Veterinary Foundation, the Walter Ehrström Foundation, the Department of Fisheries and Game of the Ministry of Agriculture and Forestry, the Orion Corporation Research Foundation, Orion Pharma Oy, Vetcare Oy, Algol Oy, Finnair Oy and the University of Helsinki is also gratefully acknowledged.

I thank the heads of the present and former institutes, Director General Jaana Husu-Kallio and Director Tuula Honkanen-Buzalski, for their positive attitude and working facilities during the past years. I am very grateful to my supervising professor, Professor Antti Sukura, and Docent Sven Nikander, for their encouragement and practical words of advice during this work.

I am deeply indebted to my supervisor and Head of the Fish and Wildlife Health Research Unit, Professor Antti Oksanen, for his dynamic contribution, tireless response and constructive criticism when sharing reflections about the fascinating world of parasitism during countless debates. His belief in the principal concept of this thesis was essential for the completion of this work. I also express my gratitude to the personnel and colleagues at Evira in Oulu for their support and patience in training a clumsy veterinary practitioner for the laboratory work, which helped me to grow as a researcher as well as a person. My sincere thanks go to the all staff at the Experimental Zoo of Oulu University for their skilful help, and taking good care of our reindeer.

The study was enabled by multidisciplinary collaboration with other research institutes and concerned occupations. Warm thanks for generous help in this process go to the State Provincial Office of Lapland, the Reindeer Herders’ Association and all the Chiefs of District of the 56 Reindeer Herding Cooperatives for answering repeated questionnaires, and especially to Kallioluoma Reindeer Herding Cooperation and its Chief, Heikki Härmä, and Reindeer Research and Moose Research of the Finnish Game and Fisheries Research Institute and Hunter’s Central Organization, Game Management Districts and Associations.

My special thanks go to my creative and hardy research fellows, co-author Milla Solismaa and Jari Ylönen, the head of the experimental zoo, for their joyful help, which carried us over unbelievable round-the-clock experimental arrangements and realizations during the study.

I wish to offer my warmest thanks to all my numerous friends and hunters in the field for the busy sampling. I especially want to mention Kauko Kilpeläinen, who provided the wild forest reindeer samples.
I express my gratitude to my colleagues in the municipalities who so much helped the study with reindeer sampling, despite their own toilsome obligations. My research roots are still in the field after over 20 years of veterinary practice, and I hope that I have been able to instill something of that attitude in this thesis. I also thank the Municipality of Kuusamo for its liberality and trust when granting me a generous leave of absence at the beginning of this study.

I am grateful to my co-authors Milla Solismaa, MSc, Jussi Kuusela, MSc, Toomas Orro, DVM, PhD, Seppo Saari DVM, Raine Kortet, MSc, PhD, Mauri Nieminen, MSc, PhD, Harri Norberg, MSc, Sven Nikander, DVM, PhD, Antti Oksanen, DVM, PhD, Antti Sukura, DVM, PhD, Jyrki Pusenius, MSc, PhD, Jouko Kumpula, MSc, PhD, Ari Venäläinen MSc, PhD and Eric Hoberg, PhD and anonymous reviewers for their huge effort and contribution to the manuscripts. I am especially grateful to Dr Eric Hoberg, without whom the global world of parasites would be much stranger for me. He is also acknowledged for the superior language revision and encouraging comments when preparing this dissertation. Roy Siddall, PhD, is also acknowledged for linguistic revision making my text more intelligible. I thank Sven Nikander very much for drawing the life cycle illustration (fig 11).

My appreciation also goes to Dr Odile Bain for fruitful discussions about filarioid nematodes, and Dr Juhani Itämies for guidance on the interesting world of mosquitoes.

I also acknowledge Leena Oksanen, DVM, for repeatedly urging me to complete this thesis because she wanted to have some reason to celebrate.

I wish to thank my parents, Aune and Reino, for their support in whatever I was doing. I owe Irene Sirkka, my ex wife and the mother of my sons, a great debt for taking care of the boys during my absence. My deepest gratitude is owed to my beloved wife Ulla and glorious children Janne, Aleksii, Topias and Linda. Thank you for your existence and the journey so far.
9. REFERENCES


Anderson JR, Nilssen A: Do reindeer aggregate on snow patches to reduce harassment by parasitic flies or thermoregulate? Rangifer 2008, 18:3-17.


Bain O, Rehbinder C, Petit G: Occurrence of Onchocerca tarsicola Bain and Schulz-Key, 1974 in reindeer, Ann Parasitol Hum Comp 1979, 54:263-265. [In France with English abstract (author’s transl)]


Chuvatina-Shmytova GYa, Khromova LA: The role of some Diptera as intermediate hosts of nematodes of domestic animals in the Mongolian People’s Republic. Trudy Gel’mintologicheskoi Laboratorii (Ekologia i geografiya gel’mintov) 1974, 24:211-220.


Haugerud RE: Rådyret vandrer mot nord.[Roe deer migrate to North]. *Ottar* 1989, 5:31-36. (in Norwegian)


Innes JRM, Shoho C: Cerebrospinal nematodiasis. Focal encephalomyelomalacia of animals caused by nematodes (*Setaria digitata*); a disease which may occur in man. *Arch Neurol & Physch* 1953, 70:325–349.


Korbi KA: Sykdomsregistrering i reinkjøttkontrollen [Disease recording and meat inspection in reindeer in Norway]. *Norsk Veterinaertidsskrift* 1982, 94:45-49. (In Norwegian, with English abstract)


Kumar B, Joshi HC, Kumar M: Clinico-haematological changes in microfilaria affected buffaloes (*Bubalus bubalis*). *Ind Vet J* 1984, 4:45-47.


Rajevsky SA: Zwei bisher unbekannten Nematoden (Setarien) von *Rangifer tarandus* und von *Cervus canadensis asiaticus*. [Two hitherto unknown nematodes (*Setaria* species) from *Rangifer tarandus* and from *Cervus canadensis asiaticus*] *Z Infekt Krankh Hyg Haustiere* 1928, 35:40-52. (In German)


Savonen E: Kotimaisen poronlihan tarkastuksen toteutuminen. [The implementation of domestic reindeer meat inspection], Suomen Eläinlääkärilehti 1978, 84:651–653. (In Finnish)


