Outbreak of parasitic peritonitis in reindeer in Finland

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In 2003, there was an outbreak of peritonitis in reindeer in the southern and middle part of the Finnish reindeer herding area caused by the filarioid nematode Setaria species. In the province of Oulu, the proportion of reindeer viscera condemned owing to parasitic lesions increased from 4·9 per cent in 2001 to 40·1 per cent in 2003. In 2004, the focus of the outbreak moved approximately 100 km north. A total of 260 adult and pre-adult Setaria species nematodes were collected for morphological and molecular studies. The parasite was indistinguishable in terms of morphology and molecular biology from Setaria tundra. Samples of parasites were also collected from wild cervids. In elk, only a few cases of pre-adult encapsulated S tundra nematodes were detected on the surface of the liver but there was no peritonitis. Two roe deer had S tundra nematodes in their abdomen but no peritonitis. Of 34 wild forest reindeer, 21 had changes associated with S tundra. At meat inspection of the affected reindeer carcasses, the changes observed included ascites, green fibrinous deposits, adhesions, and live and dead S tundra nematodes. There were histological lesions of granulomatous peritonitis with lymphoplasmacytic and eosinophilic infiltrations. No specific bacterial growth was found. The parasitic infection had no significant effects on the pH or the organoleptic quality of the meat. There was a significant positive correlation between the worm count 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).

However, when the worms died they produced a marked inflammatory granulomatous reaction and serofibrinous peritonitis, and a pure culture of corynebacteria was recovered from many of the lesions (Rehbinder and others 1975, Rehbinder 1990). In 1973, S tundra was observed for the first time in northern Norway, where there was an outbreak of peritonitis and pericarditis in reindeer and a high percentage of their livers and adjacent tissues were condemned (Kummenje 1980). Also in 1973, tens of thousands of reindeer died in the northern part of the Finnish reindeer husbandry area. Severe peritonitis and large numbers of Setaria species worms were commonly found at postmortem examinations, and they were regarded as a contributing factor to the deaths (S. Nikander, V. Tervonen, personal communication). Later, the prevalence of Setaria species in reindeer in Scandinavia decreased. The prevalence of changes in slaughter reindeer in Kautokeino, Norway, was 6·6 per cent in 1976 (Poppe 1977) and 4 per cent in 1978 (Korbi 1982). The statute for the inspection of reindeer meat in Finland was decreed in 1975. In that year, Setaria species were reported in 1·3 per cent of reindeer, and in the next season in 3·3 per cent (Savonen 1978). Between 1980 and 1986, meat inspection data recorded Setaria species in an average of 0·9 per cent of reindeer, with a range from 0·09 to 4·3 per cent, with the parasite present throughout the reindeer husbandry area but most common in the southern parts (Rahkko and Korkeala 1989). The purpose of this study was to investigate the outbreak of S tundra in reindeer in Finland in 2003, how it spread, its impact on the health and welfare of reindeer and on meat hygiene, and its possible interactions with wild cervids.

MATERIALS AND METHODS

Historical data available for reindeer meat inspection were collected from the Oulu and Lapland Council Boards and the National Food Agency to calculate the prevalence of peritonitis induced by Setaria species in reindeer (Tables 1, 2, 3, Fig 1). The total numbers of reindeer carcasses inspected annually from 2000 to 2004 in the southern part (the province of Oulu) and the northern part (the province of Lapland) of the reindeer herding area are given in Table 1. Preliminary data for 2005 were collected until November 11.
Samples of tissue showing signs of peritonitis induced by *S. tundra* were collected and delivered fresh or stored in formalin for further studies at the National Veterinary and Food Research Institute (EELA) in Oulu. The samples were fixed in 10 per cent neutral buffered formalin, embedded in paraffin, cut in 4 \( \mu \)m sections and stained with haematoxylin and eosin.

At Kuusamo slaughterhouse in 2004, tissue samples for histological studies were collected from 19 reindeer calves with peritonitis; the samples included the abdominal wall or diaphragm with the peritoneal surface from five calves, liver from six, spleen from two, mesenteric lymph nodes from two, ruminal wall from two, digestive tract from one, and the kidneys from one calf. Samples of fresh peritoneal fluid were collected with a sterile syringe from eight randomly chosen reindeer calves with very severe peritonitis for bacteriological culture; they were cultured on casein-peptone soymeal-peptone agar (CASO; Merck) containing 5 per cent bovine blood, on bromothymol blue lactose agar (BBRLAC; Merck) and on fastidious anaerobe agar (FAA) (Labema). The FAA plates were incubated anaerobically and the other plates in aerobic conditions at 37\( \pm \)1\(^\circ\)C. The plates were observed for bacterial growth after one and two days.

At Pudasjärvi slaughterhouse, parasitic lesions and granuloma samples were collected for histological and bacteriological studies from 15 randomly chosen reindeer calves. To assess the impact of *Setaria* species infection on meat hygiene, samples of meat were collected from 10 randomly chosen reindeer with very severe peritonitis and from 10 apparently healthy reindeer. Organoleptic evaluations (boiling test), pH measurements and bacteriological cultures were made according to the Finnish meat hygiene legislation (MMM 12/EEO/1999 and 1EEO/2000) at an accredited environmental and food laboratory.

To monitor the prevalence of sylvatic *Setaria* species, samples were collected from wild cervids. In the autumn of 2003 and 2004, approximately 300 elk/moose (*Alces alces*) shot by hunters in the Kuusamo area were inspected by local veterinarians during meat inspection. Fourteen white-tailed deer (*Odocoileus virginianus*), an introduced species in Finland, and 15 roe deer were examined at EELA in Oulu, and two roe deer were examined by EELA workers in the field in Lohja in southern Finland and in Kemiäjärvi in the southern part of Lapland, 120 km north of Kuusamo.

Hunters were informed about *Setaria* species and asked to report any changes and findings in elk and roe deer during 2004, especially in the reindeer herding area and areas on its southern border. In this area, 26,716 elk were hunted, and 56 tissue samples were delivered to EELA.

Samples of viscera and peritoneum were collected from 31 wild forest reindeer (*Rangifer tarandus fennicus*) shot by hunters in Kainuu, where there were approximately 1000 free-ranging reindeer, and three samples were collected from another population of 1000 individuals in Suomenselkä, further from the reindeer herding area. The Kainuu population is separated from the reindeer herding area by a fence to maintain the genetic purity of the wild forest reindeer.

A total of 190 *Setaria* species nematodes were collected at Kuusamo reindeer slaughterhouse from 44 randomly chosen calves, and 70 were collected at Pudasjärvi from 25 calves, and stored in 75 per cent alcohol or in 10 per cent buffered formalin for subsequent morphological studies.

Samples for PCR studies were made from *Setaria* species parasites collected from reindeer and elk in Kuusamo and from roe deer in Kemiäjärvi; the samples were stored in 94 per cent ethanol. The parasites were cut into 1 cm pieces and the DNA was extracted with the DNeasy Tissue Kit (Qiagen), following the manufacturer’s instructions. Four new primers were designed on the basis of comparisons with Filarioidea sequences retrieved from GenBank. The primers sNnD4L (5′-TGTATGGTGTGATAGCACGTC-3′) and sCdG
The outbreak of serofibrinous peritonitis was noticed by meat-inspecting veterinarians that a large number of peritoneal and abdominal muscles had to be removed or cleaned and live worms were seen. Sometimes entire slaughter batches, consisting of up to 100 animals, were ordered to be heat-treated. In 2004, the focus of the outbreak moved approximately 100 km north, and the preliminary data indicate that the outbreak spread so that in 2005 only the areas covered by the three northernmost slaughterhouses were free of animals with peritonitis (Fig 1, Table 2).

In 2003, the most common finding at the Kuusamo slaughterhouse during ante-mortem inspection was poor body condition (median score 2), especially in calves. The fur was often dry, lifeless and tangled and the winter fur had not fully developed. In many animals the abdomen was slightly distended and the eyes gave an impression of exophthalmos (Fig 2). However, in 2004 the animals’ median body condition score was 3 and their winter fur was well developed.

Postmortem examinations of the calves at the Kuusamo slaughterhouse revealed that in 2003 there was no measurable fat on the back and the animals’ muscle condition was usually poor to moderate, but in 2004 the average back fat thickness was 3.6 mm and their muscle condition was good. In 2003, the corresponding mean slaughter weight of 828 male calves was 23.4 kg and that of 97 females was 20.8 kg, whereas in 2004 the mean slaughter weight of 538 male calves was 24.1 kg (P < 0.001) and that of 381 female calves was 22.5 kg (P < 0.001).

In 2003, there was often excess acetic fluid in the peritoneal cavity of the reindeer, typically 10 to 150 ml of serious reddish or yellowish exudate, often with green fibrinous membranes floating in the fluid (Fig 3). Greyish or greenish grainy fibrinous membranes, sometimes several millimetres thick, covered the peritoneum and visceral organs, especially the rumen and spleen, giving an impression of a purulent process (Fig 4). The livers were typically covered with thin, matt grey, net-like deposits of fibrin. Fibrinous adhesions were present between the mesentry and the intestines, and in severe cases between all the abdominal organs. In the diaphragm there were often petechial haemorrhages.

White, 1 to 8 cm long and 0.1 to 0.3 cm thick nematodes were seen freely moving in the abdominal cavity (Fig 5); they reacted negatively to light and touch. Dead *Setaria* species worms of variable size were often observed, typically encapsulated and calcified, on the peritoneum and diaphragm and on the surface of the liver and spleen. However, the great majority of the reindeer suffering from peritonitis classified as degree 2 or 3 appeared not to have any dead worms. Adult, well developed *Setaria* species nematodes were found in the thoracic cavity of four animals that had mild to very severe pleuritis, and two animals had *Setaria* species nematodes in the pericardiac sac.
In 2003, 53 of 56 reindeer calves had live *Setaria* species nematodes in their abdominal cavities; the mean (sd) number of worms was 8·5 (6·5), with a range from 0 to 30. One calf at the Kuusamo slaughterhouse that did not belong to the randomly selected sample harboured 178 worms. The worms in each reindeer were generally of similar size. In 2004, 76 per cent of 327 reindeer calves harboured live *Setaria* species, but only 5 per cent harboured more than four worms. In 2003, 16 of 35 adults had a mean (sd) of 1·5 (0·84) living worms. In 2004, the situation in the adults appeared to be similar, but they were not formally sampled. There was a significant positive correlation between the worm counts 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) (Table 4).

The degree of peritonitis in the calves was moderate to very severe (score 1 to 3) and its prevalence was 88 per cent (in 56 examined) in 2003 and 26 per cent (in 327 examined) in 2004. In the adults in both years, the degree of peritonitis varied from only a few old marks of fibrin and scars to severe peritonitis (score 1 to 2). Although the overall prevalence was 96 per cent, the prevalence of severe peritonitis, when the peritoneum had to be condemned, was only 1 per cent (in 381 animals examined).

Lesions indicating parasitic infection were observed histopathologically in all of the samples on the surfaces of the liver, spleen, diaphragm, digestive tract and abdominal wall. There was fibrous thickening of the peritoneum, villous inflammatory proliferation of the serosal surface and chronic granulomatous peritonitis with lymphoplasmacytic infiltration and
mild to moderate infiltration with eosinophilic granulocytes (Fig 6). In four of the liver samples there were also granulomatous lesions. In six of the samples, mineralised remnants of nematode parasites or eosinophilic detritus surrounded by giant cells and fibrous capsules were detected (Fig 7). In the samples of spleen and in the lymph nodes there was chronic reactive hyperplasia with small numbers of eosinophilic granulocytes.

A pure culture of *Mannheimia haemolytica* was isolated from one sample of peritoneal fluid but no bacterial growth was detected in the others. There was seldom any malodour, even in cases of very severe peritonitis. Samples of abdominal muscle and peritoneum and samples of lesions from 15 calves at the Puujärvi slaughterhouse with very severe peritonitis were free from any specific aerobic or anaerobic bacterial growth. In 2004, the prevalence of peritonitis was 53 per cent (in 3867 animals examined).

Organoleptic evaluations by the boiling test, on a scale from 1 to 4 (1 Condemned, 2 Poor quality, 3 Slightly strange odour or taste, 4 Immaculate), of the meat from 10 reindeer with very severe peritonitis, gave mean values for the abdominal muscle (flank) of 3·6 (range 3·2 to 4) and for the shoulder muscle of 3·7 (3·2 to 4). In 10 control reindeer, the values were 3·8 (3·3 to 4) (P=0·06) and 3·9 (3·7 to 4) (P=0·24), respectively. The mean pH value of the affected reindeer meat was 5·7 (range 5·5 to 5·9) and in the control group it was 5·6 (5·5 to 5·7) (P=0·05). There was no bacterial growth in the muscles from either group.

**Observations outside the slaughterhouses**

No increase in the mortality of reindeer was reported in the outbreak areas owing to peritonitis. A total of 26 reindeer more than six months of age, which were found dead or killed because they were diseased, were examined postmortem at EELA in Oulu between January 1, 2004 and February 28, 2005. Peritonitis and/or perihepatitis was diagnosed in 15 of them.

No peritonitis was reported in elk in Kuusamo. Six one-and-a-half-year-old elk had moderate perihepatitis and between one and three encapsulated pre-adult *Setaria* species nematodes on the surface of their livers. Mild peritonitis or perihepatitis was diagnosed at EELA in 18 of 56 samples provided by hunters; in two of them immature *Setaria* species nematodes were detected.

Of the 34 wild forest reindeer shot, 21 were suffering from peritonitis, perihepatitis or granulomas diagnosed as changes associated with *Setaria* species. One roe deer had an adult *Setaria* species worm encapsulated on the surface of its liver, and the two roe deer examined postmortem fresh in the field had two and four live adult *S tundra* in their abdominal cavities, respectively, but no sign of present or previous peritonitis. No changes indicative of *Setaria* species infection were found in the white-tailed deer.

The nematodes were identified morphologically as *S tundra* (Rajewsky 1928). Five specimens from reindeer, two specimens from elk and two specimens from roe deer were identical along the 1389 bp mtDNA sequence. There were six nucleotide substitutions when the sequence was compared...
with the 648 bp sequence of *S tundra* from Italy (GenBank AJ544874) (Casiraghi and others 2004). The difference was regarded as small and justified the identification of the specimens as *S tundra*. The sequence of *S tundra* parasitising reindeer in north Finland was deposited in GenBank under accession number DQ2997309.

**DISCUSSION**

This outbreak of *S tundra*, after 30 years of low prevalence, caused confusion among reindeer herders, the food industry, veterinarians and scientists in north Finland. The outbreak resulted in extra work and additional costs in the herding and slaughter of reindeer. The outbreak started in the southern part of the reindeer herding area in 2003 and spread approximately 100 km north annually, so that in 2005 only the reindeer in the northernmost part of Finland were free of changes caused by *S tundra*. In the same period the outbreak seems to have diminished in the southern area.

*Setaria yehi* has been associated with low-grade chronic peritonitis in Alaskan reindeer (Dieterich and Luick 1971), and *S tundra* with mild to severe peritonitis together with *Corynebacterium* species in Swedish reindeer (Rehbinder and others 1975). The results of the present study revealed that *S tundra* can act as a significant pathogen for reindeer, as evidenced by both antemortem and postmortem inspections and histological examinations. The severity of influence on the health of the reindeer appeared to be dependent on the intensity of infection, that is, the number of adult worms living in the abdominal cavity. In contrast with previous reports (Rehbinder and others 1975), no association was observed between the presence of dead and encapsulated worms and the degree of peritonitis.

The impact of the outbreak on the quality of the meat appeared to have been slight and mostly aesthetic. The peritonitis appeared to be aseptic, and no specific bacterial growth was present in the ascitic fluid, the affected tissues, organs or in the muscles. No corynebacteria were detected in the parasitic lesions, nor any granulomatous lesions like those described by Rehbinder and others (1975). Even a heavy *S tundra* infection had little influence on the pH of the meat or on organoleptic evaluation in autumn and early winter. As a result, there appears to be no reason to limit the normal use of the meat for human consumption or to heat-treat the carcasses as a precautionary measure, if there is no other contributory reason. The removal of the affected parts of the carcass and visceral organs should be adequate measures.

Heavy *S tundra* infections had a pronounced influence on the welfare of the reindeer calves. There is a positive correlation between the survival of reindeer calves through the winter and their bodyweight (Reimers 1984). The slaughter weight, back fat index and body condition of the reindeer calves were lower in Kuusamo during 2003 than in 2004, when the peak of the outbreak had passed. There are many other factors influencing these values (Kumpula and Colpaert 2003), but for the difference between these two years the outbreak was probably the most important; there is no lack of summer pastures in this area (Kumpula and Nieminen 1992). The condition of the heavily infected calves indicated that their welfare had been compromised; their body condition was poor and their winter coat was undeveloped. The lack of reported mortality in the area of the outbreak may have been due to good nutrition and intensive antiparasitic treatment, in which approximately 80 per cent of the winter stock are treated annually with ivermectin (Oksanen 1999). In the southern part of the Finnish reindeer herding area virtually all the reindeer are corralled and receive supplementary feed during the winter months. It has been reported that the severity of natural *Setaria cervi* infection can be reduced by providing young deer with an adequate diet (Shol and Dróbishchenko 1973). The situation may be quite different if the outbreak spreads into the northern parts of the herding area, where supplementary feeding is not commonly practised and survival during the winter is highly dependent on environmental conditions (Kumpula and Colpaert 2003).

The adult reindeer slaughtered in Finland are mostly eight- to 12 year-old females removed from the breeding stock; the data show that they can act as asymptomatic carriers of *S tundra*. It is unlikely that elk act as a reservoir for *S tundra* in reindeer. In 2003 to 2005 the elk population in northern Finland peaked, but no acute peritonitis or living adult *Setaria* species worms were reported and only a few pre-adult worms were found encapsulated on the surface of the animals’ livers. Roe deer are probably a host and asymptomatic carrier for *S tundra*; the fact that the first appearance of *S tundra* in Scandinavia and the invasion of northern Sweden by roe deer occurred simultaneously may be connected. It is not known whether the high percentage of wild forest reindeer shot in Kainuu with signs of peritonitis caused by *S tundra* was connected with the decrease in their population from 1700 individuals in 2001 to 1000 in 2005.

The outbreak of *S tundra* in Sweden in 1973 was associated with unusually warm weather and the appearance of larger than usual numbers of mosquitoes and gnats (Rehbinder and others 1975). In Finland the summers of 1972 and 1973 were also very warm, as were 2002 and 2003 (Finnish Meteorological Institute data). The life cycle of *S tundra* in Scandinavia is poorly understood. Mosquitoes are thought to be vectors, but there is apparently no good evidence. There is no national system for monitoring the populations of mosquitoes in Finland, and the possible role of mosquitoes in the transmission of *S tundra* in this outbreak cannot therefore be assessed.

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