



# Digital Necrobacillosis in Norwegian Wild Tundra Reindeer (*Rangifer tarandus tarandus*)

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## Summary

Outbreaks of digital necrobacillosis in Norwegian wild tundra reindeer (*Rangifer tarandus tarandus*) are described. The outbreaks occurred in late summer and autumn 2007 and 2008, subsequent to periods with an unusually high number of days with precipitation and high air temperature. Lesions were generally restricted to one foot and the disease incidence was highest in calves. Single limbs from 20 animals and six whole carcasses were submitted for laboratory examination. Gross lesions were characterized by swelling of the fetlock to coronary band area and cutaneous sinus tracts with sparse discharge of pus. Subcutaneous tissue was inflamed and oedematous with focal necrosis. Tendons, tendon sheaths, joints and periosteum of the digital bones were often affected. Animals shot during winter showed severe chronic periostitis and osteomyelitis and necrotizing deforming arthritis. Microscopically, skin lesions were characterized by deep ulcers with centrally located necrotic tissue, bordered by a zone of oedema and intense inflammation with granulation tissue and fibrosis. Necrosis, suppurative inflammation and oedema were found in the synovial membranes, tendons and tendon sheaths. Digital bone lesions were characterized by necrosis, fibrosis and extensive bone proliferation. Vasculitis and thrombosis were common in all lesions. Elongate filamentous gram-negative bacteria in necrotic lesions from all animals were identified as *Fusobacterium necrophorum* by fluorescence in-situ hybridization. *F. necrophorum* was cultured from the foot lesions of six animals. Five of these isolates were examined by 16S rRNA sequencing. The sequences were identical and differed from all other strains listed in GenBank. These results are consistent with circulation of a reindeer-adapted pathogenic strain of *F. necrophorum* in the wild reindeer population, causing outbreaks of digital necrobacillosis following warm and humid summers.

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**Keywords:** deer; disease ecology; *Fusobacterium necrophorum*; wildlife

## Introduction

Necrobacillosis, caused by *Fusobacterium necrophorum*, occurs worldwide in farm animals, especially ruminants. The disease can be traced back to the 1800s (Langworth, 1977); however, due to the anaerobic nature of this bacterium, early investigators were hampered by the inability to culture the organism and descriptions were based on its fusiform morphology. Establishing a laboratory diagnosis was not

possible until stable anaerobic culture methods became available. *F. necrophorum* frequently occurs in combination with other facultative pathogens, which may also hamper isolation. Today, the bacterium can be identified by in-situ hybridization and polymerase chain reaction (PCR) (Boye *et al.*, 2006; Agerholm *et al.*, 2007).

Various manifestations of necrobacillosis have been recognized in domestic ruminants including digital necrobacillosis, liver abscesses and necrotizing stomatitis in cattle and foot abscesses and abortion in sheep. *F. necrophorum* may also be isolated from the lesions of

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ovine foot rot, although the primary agent in this disease is *Dichelobacter nodosus* (Radostits *et al.*, 2007). Strains of *F. necrophorum* are regarded as normal inhabitants of the alimentary tract, especially the rumen, and are shed into the environment. Pathogenic strains may be part of this microbiota. Pathogenicity is linked to the production of various toxins, with leukotoxin and endotoxin being considered major virulence factors (Langworth, 1977; Tan *et al.*, 1996; Tadepalli *et al.*, 2008). Leukotoxin inhibits migration and causes destruction of leucocytes, whereas endotoxin induces thrombosis, oedema and necrosis. Bovine digital necrobacillosis is associated with wet or muddy ground, which favours bacterial survival and transmission. Digital trauma due to stony ground, lanes filled with sharp gravel and pasturing on coarse stubble are also factors predisposing to infection (Radostits *et al.*, 2007). Pathogenic strains presumably gain entrance and establish infection through minor abrasions in the macerated skin. In cattle, digital necrobacillosis occurs as a sporadic disease, but under favourable conditions as many as 25% of a group may be affected at one time. An increased incidence of disease is seen in wet summer and autumn months (Monrad *et al.*, 1983).

Necrobacillosis has also been reported in many wild animal species (Leighton, 2001) including the following deer species: mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus wapiti*) and caribou (*Rangifer tarandus caribou*) in North America; red deer (*Cervus elphus elaphus*) in New Zealand; sambar (*Cervus unicolor*) in India; and semi-domesticated tundra reindeer (*Rangifer tarandus tarandus*) in northern Scandinavia and Russia. Digital necrobacillosis was the most serious health problem seen in north Norwegian reindeer herding in the 1800s and early 1900s (Horne, 1897; Qvigstad, 1941). The disease was linked to the intensive reindeer herding practised at that time, with small flocks that were frequently gathered in small areas for milking and other purposes. This regular crowding in small muddy and faeces-contaminated areas apparently functioned as a good environment for the bacterium and an effective site of transmission between animals. Since changing to more extensive reindeer herding in the second half of the 1900s, this disease has more or less disappeared (Skjenneberg and Slagsvold, 1968). There is a lack of satisfactory identification of the causative agent in the reported disease outbreaks in semi-domesticated reindeer and necrobacillosis in wild reindeer populations has not been reported previously.

The present study reports outbreaks of digital necrobacillosis in a population of wild tundra reindeer apparently caused by *F. necrophorum* as identified by 16S rRNA gene sequencing and in-situ hybridization.

## Materials and Methods

### Reindeer in Norway

In Norway, there are three groups of reindeer: the semi-domesticated and wild tundra reindeer in mainland Norway and the Svalbard reindeer (*Rangifer tarandus platyrhynchus*) on the high arctic island of Svalbard (Punsvik and Jaren, 2006). The majority of the semi-domesticated reindeer (80%) are held in the northernmost county of Finnmark (69–71°N), consisting of 100,000–200,000 winter animals (WAs). The wild tundra reindeer live in the mountain areas (altitude 800–1,500 m) of southern Norway, comprising a total of 25,000–30,000 WAs. They are dispersed between 23 populations (Fig. 1), between latitudes 59 and 63°N, with a winter population ranging from less than 100 up to 8,000 individuals. The populations are controlled by annual culls that are carried out by licensed hunters between August 20th and September 30th. The hunting is monitored by personnel from the Mountain Inspectorate (MI), who also inspect the populations during the winter.

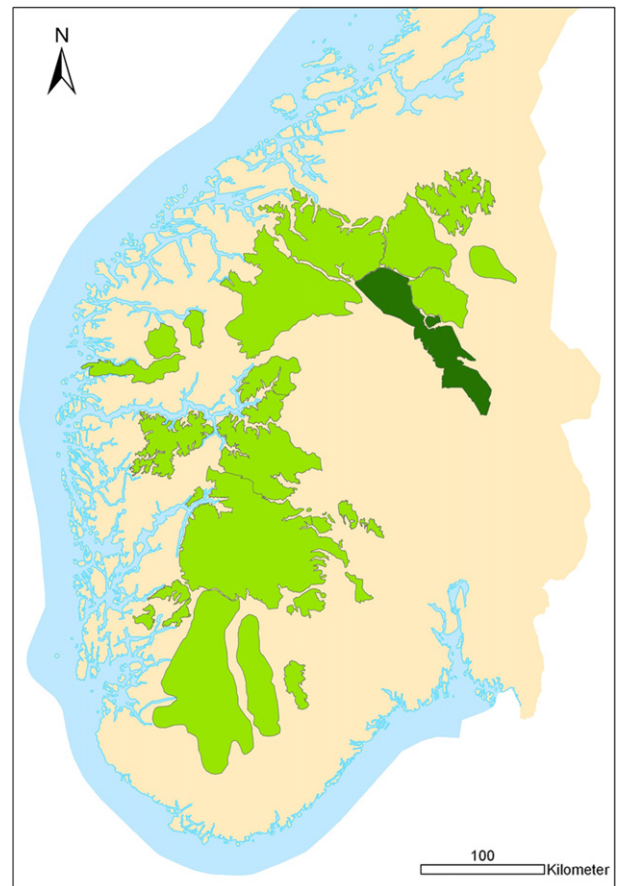


Fig. 1. Map of southern Norway showing the different habitats (green) of wild tundra reindeer. The area of the Rondane population is indicated by dark green. Source: Norwegian Institute for Nature Research, Trondheim, Norway.

*Population History*

This report concerns the wild reindeer population in Rondane, covering a relatively narrow 3,300 km<sup>2</sup> mountain area between the valleys of Østerdalen and Gudbrandsdalen in south-eastern Norway (Fig. 1). The population consists of some 4,000 WAs separated into northern (1,500 WAs; 1,200 km<sup>2</sup>) and southern (2,500 WAs; 2,100 km<sup>2</sup>) subpopulations due to a trunk road, which bisects the region. The annual reproduction rate in the two subpopulations is 20–23%.

Hunters in Rondane South region reported severely lame animals with swollen feet throughout the 2007 hunting season. Affected animals tended to be recumbent and were reluctant to rise when the flock was disturbed or were left behind. Generally only one limb was affected, but, in a few cases, multiple limbs were reported as affected. When standing,

the affected limb was held off the ground (Fig. 2A) and, while moving, the limb was held aloft or only slightly touched the ground. The number of affected animals, as reported by hunters to the MI, was 51. Twenty-eight of these animals were reported shot, whereas seven were found dead. A further nine animals were reported by the MI in October. In total, 60 affected animals were recorded, of which 32 were calves and 28 adults. During the following winter, sporadic cases of lameness continued to be observed in the Rondane South population and some of these animals were shot. No lame animals were recorded from the Rondane North population during the 2007 hunting season.

At the start of the hunting season in 2008, lame animals with swollen feet were reported in the in the Rondane North population. Nine affected animals from this population were shot during the hunting season and another three animals, with foot lesions,

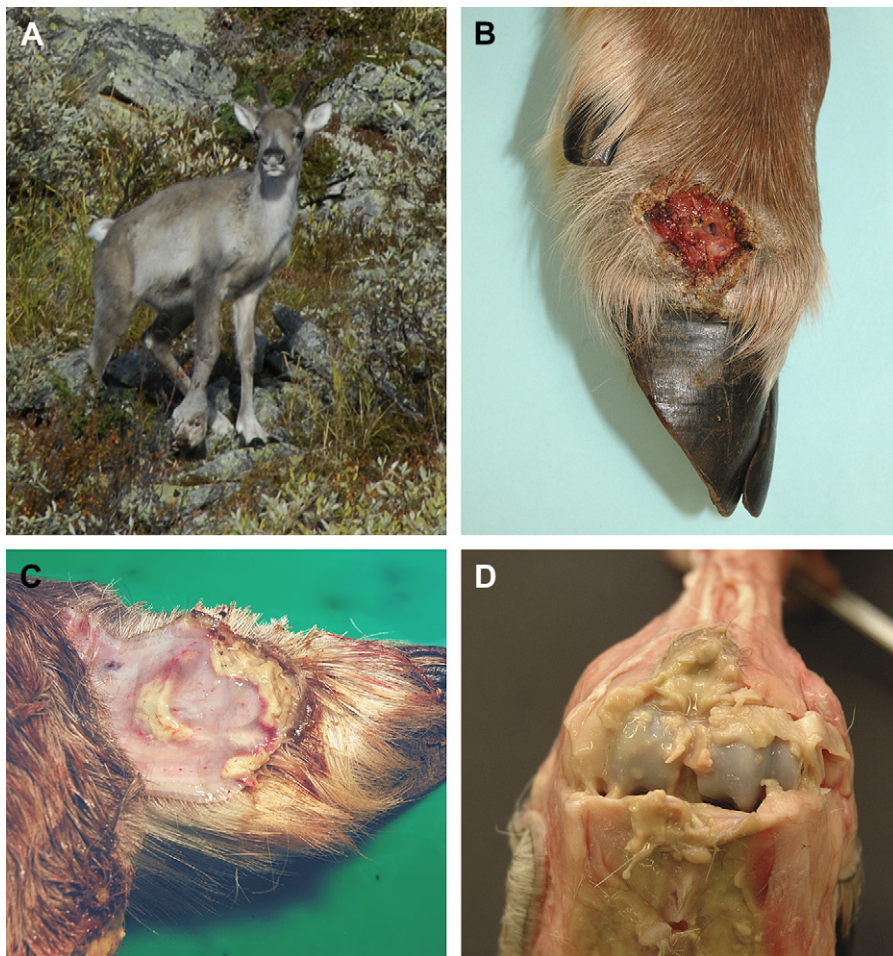


Fig. 2. Lesions of digital necrobacillosis in wild reindeer shot during autumn. (A) The right forelimb is swollen from the carpal joint to the coronary band, affecting both toes. (B) Right hindlimb with an ulcerative wound and sinus tract at the lateral toe. (C) Sagittal section of a claw and interdigital tissue; there are yellow–green foci of necrosis with hyperaemic borders. (D) Forelimb, dorsal view. There is marked accumulation of yellow–green pus in the fetlock joints and along the sheaths of extensor tendons.



were found dead. In the Rondane South population, no lame animals were recorded during the first part of the 2008 hunting season. In the second part, however, five affected animals were shot and another three were humanely destroyed in January 2009.

#### *Pathology, In-situ Hybridization and Immunohistochemistry*

Samples for laboratory examination were submitted from 17 animals from Rondane South. These animals were shot in autumn 2007 ( $n = 11$ ), autumn 2008 ( $n = 2$ ), January 2008 ( $n = 1$ ) and January 2009 ( $n = 3$ ). Samples were also submitted from nine animals shot in Rondane North in autumn 2008. The samples submitted consisted of the distal half of one limb from 20 animals, in addition to six entire carcasses. The four animals killed in January were adults, whereas those killed during autumn included 12 calves and 10 adults. Affected limbs were radiographed prior to gross examination. Multiple tissue specimens were taken from the foot lesions (skin and subcutis) for histological examination from all animals. Samples of affected tendons, tendon sheaths, synovial membranes and digital bones were taken from selected cases. Sampling also included lungs, heart, liver, kidneys and enlarged regional lymph nodes from the carcasses and the tongue lesions identified in one case were also examined. Bones were decalcified in 10% formic acid prior to processing. All samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (3–4  $\mu\text{m}$ ) were stained by haematoxylin and eosin (HE). Additional staining methods applied when considered appropriate included Mallory's phosphotungstic acid haematoxylin (PTAH), Masson's trichrome, van Giesson and Gram (Culling *et al.*, 1985).

Tissue blocks from all animals and all types of lesion were selected for demonstration of *F. necrophorum* by fluorescence in-situ hybridization (FISH). For FISH, the oligonucleotide probes were a probe specific for *F. necrophorum*, S-S-*F. necrophorum*-0183-a-A-18, 5'-GATTCCTCCATGCGAAAA-3' (Boye *et al.*, 2006), and a general bacterial probe, S-D-Bact-0338-a-A-18, 5'-GCTGCCTCCCGTAGGAGT-3', targeting nearly all members of the bacterial domain (Amann *et al.*, 1990). The oligonucleotide probes (Eurofins MWG Operon, Ebersberg, Germany) were 5' labelled with the isothiocyanate derivative Cy3 or Alexa Fluor 488. The slides were dewaxed in xylene and dehydrated in 100% alcohol for 30 min before hybridization. The hybridization was carried out at 45°C with 40  $\mu\text{l}$  of hybridization buffer (100 mM Tris [pH 7.2], 0.9 M NaCl, 0.1% sodium dodecyl sulphate) and 200 ng of each probe for 16 h in a Sequenza Slide Rack (Thermo Shandon,

Cheshire, UK). Thereafter, the slides were washed and mounted (Boye *et al.*, 2006). An Axioimager M1 epifluorescence microscope equipped for epifluorescence with a 100-W HBO lamp and filter sets 43 and 38 were used to visualize Cy3 and Alexa Fluor 488, respectively. Images were obtained using an AxioCam MRm version 3 FireWiremonochrome camera and the software AxioVision version 4.5 (Carl Zeiss, Oberkochen, Germany). Entire tissue sections were scanned using the GenePix 4200AL scanner with a pixel resolution of 5  $\mu\text{m}$  (Molecular Devices, Sunnyvale, California).

Selected sections were examined for *Arcanobacterium pyogenes* and *Staphylococcus aureus* by immunohistochemistry (IHC). This was done if a comparison between the FISH general bacterial probe and *F. necrophorum*-specific probe indicated the presence of significant numbers of bacteria other than *F. necrophorum*. The immunohistochemical methods were based on the application of polyclonal antibodies raised in rabbits (Leifsson *et al.*, 1995; Kvist *et al.*, 2002).

#### *Bacteriology, PCR and 16S rRNA Gene Sequencing*

Foot lesions of all animals (one to three samples per animal) were examined bacteriologically. The samples were seeded onto calf blood agar plates that were incubated aerobically and anaerobically for 2 days at 37°C. Isolates of *F. necrophorum* were examined by 16S rRNA gene sequencing and compared with published sequences in GenBank (<http://www.ncbi.nlm.nih.gov/>) using a basic local alignment search tool (BLAST) for homology. Prior to PCR, a pure culture of the isolates was lysed by boiling in water. The 16S rDNA was amplified using 10 mM Tris/HCL pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.1 mM dNTP and 0.5 units of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, California), 2  $\mu\text{l}$  boiling lysate and 0.5  $\mu\text{l}$  (130  $\mu\text{g}/\text{ml}$ ) of each of the primers 10Fx 5'-AGAGTTTGATCCTGGCTNAG -3' and 1509R 5'-GTTACCTTGTTACGACTTAC -3' (DNA-Technology, Risskov, Denmark) in a final volume of 50  $\mu\text{l}$ . PCR was performed using a T3 Biometra Thermal Cycler (Biometra, Göttingen, Germany) using the following programme: 3 min of initial denaturation at 94°C followed by 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 55°C) and extension (1.5 min at 72°C) with a final extension at 72°C for 5 min. Amplified PCR products were analyzed by electrophoresis in 1.5% agarose gels. The amplicons were purified with the High Pure PCR Product Purification Kit (Roche, Mannheim, Germany) and sequenced by cycle sequencing on a 3130 Genetic Analyzer DNA Sequencer (Applied

Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instruction, and 5 pmol of the same primers as used for PCR as well as the internal primers 804Fx 5'GGATTAGATACCCNGGTAGTC 3' and 1054Rx 5'ACGAGCTGACGACRRCCATG 3'. All primers were universal (Weissburg *et al.*, 1991), allowing sequencing of both strands. The numbering of the primers refers to the numbering of *Escherichia coli* 16S rDNA (Brosius *et al.*, 1978). Sequences were read with an automatic sequence analyzer and aligned to published sequences using the ARB sequence environment (<http://www.arb-home.de>) and the 16S rRNA greengenes database (<http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>). Material from the foot lesions of six animals was also tested by PCR for *D. nodosus* (Moore *et al.*, 2005).

#### Meteorology

Precipitation data were obtained from the meteorological station of Atnsjøen for the period 1957–2008 and temperature data from the meteorological station of Venabu for the period 1982–2008. Both stations are close to Rondane. Days of rainfall and days of maximum temperature above 15°C were calculated for the summer months (June–August) in 2007 and 2008 and compared with the mean throughout the respective 50 and 25 year periods of registration.

## Results

#### Pathology, In-situ Hybridization and Immunohistochemistry

Gross lesions were present in one limb each from five of the carcasses and in all of the 20 single limb specimens. The sixth carcass had lesions affecting two limbs. All carcasses were in a poor, but not emaciated, bodily condition and showed muscle atrophy and enlargement of regional lymph nodes (cervical, popliteal, inguinal and ileofemoral). Hindlimb lesions were more prevalent than forelimb lesions ( $n = 17$  versus  $n = 10$ ). Limb lesions were characterized by swelling of the foot, which variably comprised an area from above the fetlock to the coronary band of one or both toes and a draining skin wound (sinus tract) with discharge of a small quantity of thick yellow–green pus. The swelling of the foot was significantly more pronounced in the animals killed during winter compared with those killed during autumn. The skin lesions were located both interdigitally and in other sites on the toe (Fig. 2B). The subcutaneous tissue was thickened due to inflammation and oedema and contained areas of necrosis (Fig. 2C). Tendon sheets and digital joints were often involved (Fig. 2D) and joint lesions sometimes

included necrosis and loss of the articular cartilage and subchondral bone. The soft tissue inflammation often affected the surfaces of the digital bones, causing periostitis. The marked swelling of the foot (club-shape) seen in all of the four animals killed during winter was due to pronounced connective tissue and bony proliferation associated with severe periostitis, osteomyelitis and necrotizing deforming arthritis (Fig. 3A, B). The deforming arthritis had resulted in an abnormal position of the affected toe. One of the six carcasses showed tongue ulcerations, whereas no distinct gross lesions were observed in the lungs, heart, liver and kidneys of the animals.

Microscopically, skin lesions were characterized by ulceration with underlying necrosis penetrating deep into the dermis and subcutis. The epithelium bordering the ulcers displayed necrosis, exocytosis of inflammatory cells and formation of pustules and in certain areas also spongiosis and ballooning degeneration. The cutaneous ulcers generally consisted of centrally located necrotic tissue bordered by a zone of oedema and intense inflammatory infiltration of neutrophils, macrophages and lymphocytes, in turn surrounded by inflamed connective or granulation tissue

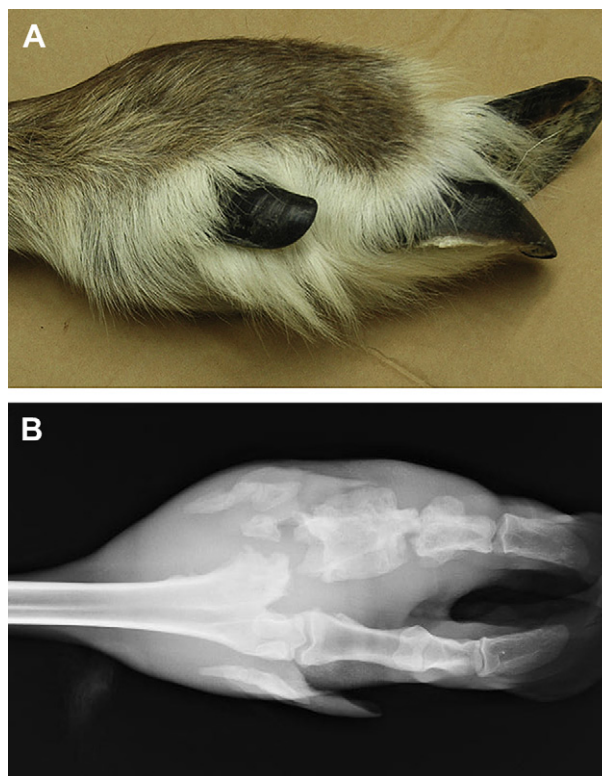


Fig. 3. Lesions of digital necrobacillosis in wild reindeer shot during winter. (A) Club-shaped swelling of the right hind foot with abnormal upward position of the medial toe. (B) Radiograph of the same foot showing severe periostitis, deforming arthritis and loss of the medullary cavity due to chronic osteomyelitis.



(Fig. 4A). The degree of peripheral fibrosis varied, but was pronounced in the animals killed during winter. Vasculitis and extensive thrombosis were present in tissues bordering areas of necrosis (Fig. 4B). Synovial membranes showed areas of necrosis surrounded by suppurative inflammation, oedema, lymphatic vessels distended by fluid and neutrophils and thrombosis. The tendon and tendon sheath lesions were similar to those of the synovial membranes. Digital bone lesions were characterized by necrotizing inflammation with extensive fibrosis and marked bony proliferation. The

tongue lesions consisted of ulcers with necrotic margins and peripheral inflammation. Sparse to very large numbers of elongate filamentous, gram-negative bacteria were present at the periphery of the necrotic areas in the skin, subcutaneous tissue and synovial membranes (Fig. 4C), tendons, tendon sheaths, bones and tongue. The enlarged regional lymph nodes had changes consistent with reactive hyperplasia and oedema. Hepatic lesions were found in one case and these were characterized by disseminated foci of peracute liquifactive necrosis with minimal cellular response

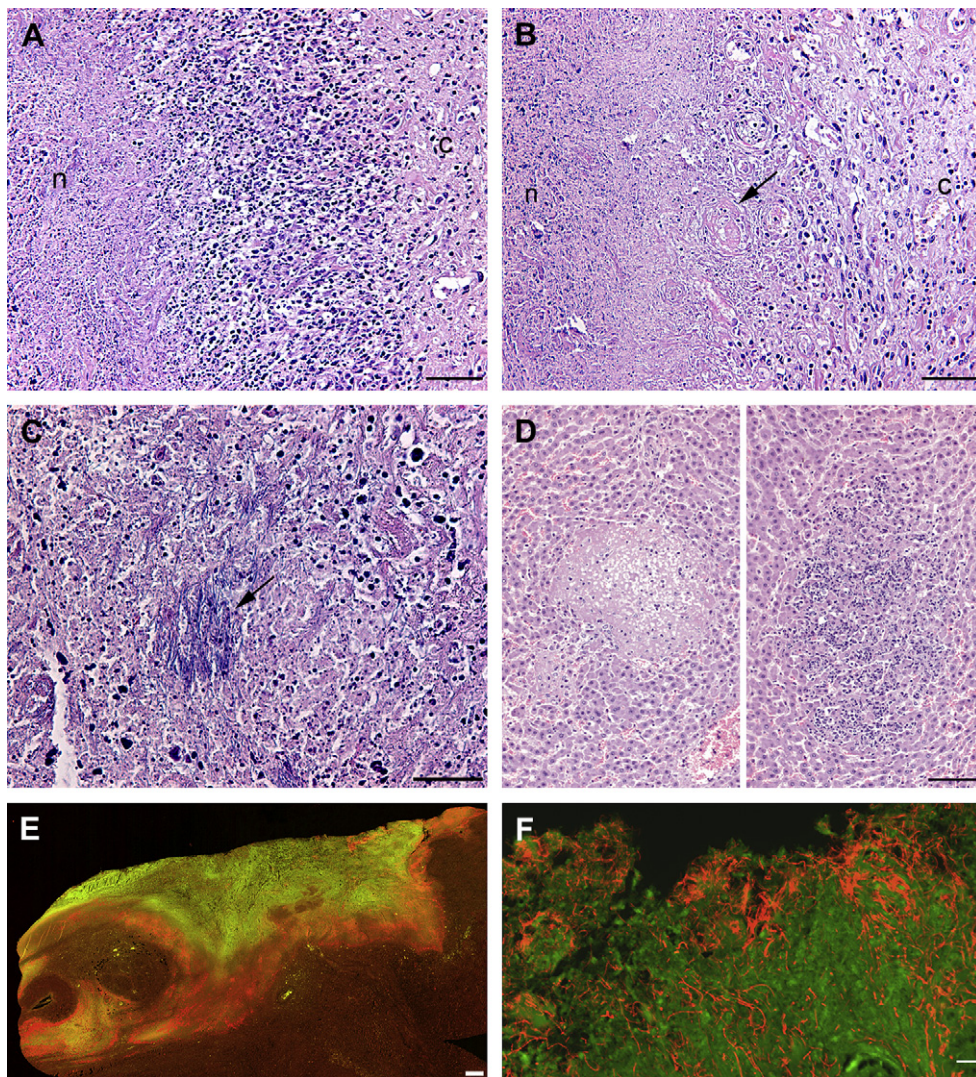


Fig. 4. Necrobacillosis in wild reindeer. (A) Digital skin. Necrotic tissue (n) is separated from the adjacent connective tissue (c) by a band of intense inflammation. HE. Bar, 50  $\mu$ m. (B) Digital skin. Vasculitis and thrombosis (arrow) is present at the periphery of the necrotic tissue (n). Relatively sparse inflammation is seen in the adjacent connective tissue (c). HE. Bar, 50  $\mu$ m. (C) Digital synovial membrane showing necrotic tissue with multiple filamentous bacteria present as colonies (arrow) or spread throughout the debris. HE. Bar, 30  $\mu$ m. (D) Liver showing peracute liquifactive necrosis (left) and acute suppurative and necrotizing hepatitis (right). HE. Bar, 100  $\mu$ m. (E–F) FISH of necrotic digital skin lesions. The specific probe for *F. necrophorum* is labelled with Cy3 (red). In E, multiple microcolonies of *F. necrophorum* (red fluorescence) are present peripherally in the lesion and invade the surrounding connective tissue (brown colour). Debris with only a few *F. necrophorum* is characterized by green autofluorescence. Bar, 400  $\mu$ m. In F, numerous *F. necrophorum* are seen throughout the tissue as long filamentous bacteria displaying red fluorescence. Bar, 10  $\mu$ m.

and foci of acute suppurative and necrotizing hepatitis (Fig. 4D). In addition, scattered thrombosed vessels were seen. Microscopical changes were not found in other internal organs.

FISH demonstrated a very large and almost pure population of *F. necrophorum* in most skin lesions and in synovial membranes. The bacteria were mainly located at the periphery or base of the lesions as a dense band of organisms invading the surrounding viable tissue, while bacteria were less prevalent within the central areas of necrosis (Fig. 4E, F). *F. necrophorum* was also demonstrated in specimens of tendons and tendon sheaths, bones and in the tongue ulcers, but not in the hyperplastic lymph nodes. In the liver with multifocal necrosis, a single colony of *F. necrophorum* was identified. The number of lesional bacteria other than *F. necrophorum* varied; however, it was notable that *F. necrophorum* bordered the base of the lesions, while other bacteria inhabited the external surface or the superficial areas of the necrotic tissue.

IHC revealed the presence of small numbers of *A. pyogenes* and *S. aureus* in several of the specimens that had an external surface. Large numbers of *A. pyogenes* were occasionally present within subcutaneous abscesses.

#### Bacteriology, PCR and 16S rRNA Gene Sequencing

*F. necrophorum* was isolated from foot lesions after anaerobic incubation from three of the animals from Rondane South, two shot in 2007 and one shot in 2009, and from three animals in Rondane North shot in 2008. Additional facultative bacteria were cultured from the lesions in all animals. The most frequently isolated facultative bacterium was *A. pyogenes*, found in a majority of animals, followed by *S. aureus*. *D. nodosus* was not detected by PCR examination of foot lesions from the six animals examined.

Nearly full-length 16S rDNA sequencing was performed for all isolates of *F. necrophorum*, except for one of the isolates from Rondane South from an animal shot in 2007. The sequence was identical for all five isolates examined and revealed a 99.3% nucleotide homology to the type strain ATCC 25286 for *F. necrophorum* (GenBank accession number AJ867039.1). The highest nucleotide homology (99.6%) was found to *F. necrophorum* as isolated from New Zealand ruminants (AF044948). Four nucleotide positions (base pairs 116, 118, 226 and 800) gave heterogeneity in the sequence for all isolates. The 16S rRNA gene sequence for the five *F. necrophorum* isolates (2007-10-1706, 2008-10-864, 2008-10-886, 2008-10-896 and 2009-10-8) has been submitted to GenBank (<http://www.ncbi.nlm.nih.gov/>; accession number FJ984622).

#### Meteorology

Precipitation data (Atnsjøen) showed total rainfall for the period June–August 2007 (260 mm) and 2008 (247 mm) that was not significantly different from the mean for the 50 year period 1957–2006 (223 mm). However, the number of days with precipitation for the period June–August was significantly higher both in 2007 (57 days) and 2008 (63 days) compared with the mean for the 50 year period 1957–2006 (46 days) and the number of days with precipitation in July 2007 (28 days) and August 2008 (26 days) were new records for these months. Additionally, the number of days with maximum temperature >15°C for the period June–August was higher both in 2007 (52 days) and 2008 (43 days) compared with the mean for the 25 year period 1982–2006 (40 days). An unusually high number of warm days occurred in June 2007 (23 days) and July 2008 (24 days).

### Discussion

The present study is the first to describe necrobacillosis in wild tundra reindeer and the first identification of *F. necrophorum* in reindeer by molecular methods. Culture isolates of the bacterium from both subpopulations of reindeer belonged to a strain not previously reported to GenBank. This strain was most likely the primary cause of disease, as large numbers of bacteria were detected by FISH in close association with deep tissue lesions from all animals examined and there was no evidence of other bacteria, except for secondary invaders. Our findings point to an independent circulation of a virulent strain in the two subpopulations. This strain presumably represents a specific reindeer-adapted strain that could possibly also circulate and cause lameness in other reindeer populations.

*Fusobacterium* spp. constitute an important part of the ruminal microbiota of reindeer (Aagnes *et al.*, 1995) and the demonstrated virulent strain of *F. necrophorum* may be part of the gastrointestinal microbiota of reindeer in Rondane. Faecal excretion is probably the primary source of *F. necrophorum* (Radoszits *et al.*, 2007), although the presence of virulent strains in animal faeces is reported to be rare (Smith and Thornton, 1993a). It has been suggested that disturbances of the gastrointestinal microbiota lead to increased multiplication and faecal excretion of the organism, with contamination of the body surface and risk of necrobacillosis (Smith and Thornton, 1993a). Disturbance of the normal gut microbiota through oral administration of antibiotics has been shown to encourage proliferation and faecal excretion of *F. necrophorum* (Smith and Thornton, 1993b). This



may also be of some relevance in wild reindeer. In a bacteriological study of faecal samples from wild cervids in Norway, a high rate of antibiotic-resistant *E. coli* was documented in reindeer (24%), but not in moose (*Alces alces*), red deer (*Cervus elphahus*) or roe deer (*Capreolus capreolus*) (2%) (Lillehaug *et al.*, 2005). The cause for this is not known, but one could speculate that the reindeer are exposed to naturally occurring antibiotics or similar substances through their feed intake.

The occurrence of digital necrobacillosis in reindeer in late summer and early autumn subsequent to humid weather conditions is in accordance with observations made in domestic ruminants (Monrad *et al.*, 1983; Radostits *et al.*, 2007). The continuously moistened pastures, following an unusual high number of days with precipitation in July and August, presumably improved the survival of the bacterium in the environment and predisposed to digital skin infection. Moistened skin is vulnerable to minor abrasions, creating suitable entrance sites for the bacterium. The flocking behaviour of reindeer may predispose to transmission of a pathogenic strain through discharges from the lesions of affected animals. Additionally, the unusually high number of days with an air temperature above 15°C during the preceding summers may have contributed to the disease outbreaks. High temperature during early summer leads to rapid melting of snow and moistened ground can enhance bacterial activity.

The highest disease incidence was found in the Rondane South population in 2007, affecting about 2% of the animals, with only sporadic cases seen in 2008. However, it should be kept in mind that the reported animals were the most severely affected individuals, with infections penetrating deep into tendon sheaths, joints and bones. The true disease incidence, including minor superficial and transient skin lesions, was probably higher. In the Rondane North population, the opposite situation was seen, with no reported cases in 2007 and a moderate number of affected animals in 2008 (incidence < 1%). This diverging infection pattern may reflect a different immunological status in the two populations. The reduced disease incidence in Rondane South in 2008 may be related to development of immunity in adult animals following the previous year's outbreak. In bovine digital necrobacillosis, naturally acquired immunity inhibits re-infection by the same strain for at least 6 months (Greenough, 2007).

The clinical and pathological findings observed were similar to those reported in cattle (Jubb and Malmo, 1991; Greenough, 2007; Radostits *et al.*, 2007), with lesions most frequently affecting the hindlimbs and normally being restricted to one foot. However, the localized skin lesion, reflecting the site

of traumatic abrasion and entrance of the bacterium, was found in different regions of the toe in reindeer and not primarily in the interdigital skin as for cattle. This may be a result of the typical stony environment of the wild reindeer predisposing them to accidental traumatic damage at all levels of the toe. Significant pain is associated with this disease (Greenough, 2007) and the lesions are often severe and chronic. It is therefore to be expected that affected animals might succumb due to starvation or predation during the winter months. Animals with multiple limb lesions and calves in particular probably have limited chances of survival. The latter hypothesis is supported by the observation that all diseased animals found alive and shot during winter were adults.

The results of the present study suggest that digital necrobacillosis generally remains localized to the foot and that the infection does not often spread via the lymph or blood. However, it may be difficult to detect septicaemic cases that die very quickly, especially in a wild animal population that ranges over vast mountain areas, making it problematic to find carcasses. An outbreak of primary digital necrobacillosis that progressed to fatal septicaemia was observed in a group of captive wild-caught pronghorns (*Antilocapra americana*) in North America (Edwards *et al.*, 2001) and some of the reindeer found dead during the hunting season in the present study may have died due to septicaemia.

The digital lesions seen in the reindeer, characterized by cellulitis and progressive peripheral necrosis linked to vasculitis, thrombosis and ischaemia, were most likely induced by the potent toxins produced by *F. necrophorum* (Langworth, 1977; Tan *et al.*, 1996; Tadepalli *et al.*, 2008). Other commonly isolated facultative bacteria such as *A. pyogenes* and *S. aureus* are known to facilitate the growth of *F. necrophorum* by lowering oxygen tension and the redox potential in tissue (Brook *et al.*, 1984). *D. nodosus*, the primary cause of foot rot in sheep, was not detected in these lesions. The necrotizing stomatitis seen in one animal was probably secondary to foot lesions. Cases of necrotizing stomatitis, either resulting from ingesting bacteria-contaminated food or from licking foot lesions, were also seen during prior outbreaks of digital necrobacillosis in semi-domesticated reindeer (Horne, 1897; Qvigstad, 1941). The multifocal necrotizing hepatitis found in another of the carcasses could be a result of ingested bacteria having established foci of infection in the gastrointestinal (ruminal) wall, with subsequent spread to the liver via the portal circulation. Portal spread of *F. necrophorum* from primary foci of infection in the ruminal wall is regarded as central to the pathogenesis of hepatic necrobacillosis (liver abscesses) in cattle (Radostits *et al.*, 2007).



Based on the apparent link between digital necrobacillosis and the unusually high number of days with precipitation and high air temperature during the preceding summer, an increase in the number of cases in reindeer could be expected in the face of climate change. This hypothesis is supported by Benestad (2005) who carried out empirical–statistical downscaling analyses for monthly mean temperature and precipitation for the 21st century in northern Europe. The author estimated positive trends in both parameters, with the strongest temperature trends for the high mountains in southern Norway and the interior Fennoscandia and the strongest precipitation trends for localized parts of Norway. The occurrence of necrobacillosis in the Rondane and other central wild reindeer populations in southern Norway will be surveyed in the coming years as part of the National Health Surveillance Program for cervids.

### Acknowledgments

We thank T. Vikøren, B. Ytrehus and K. Madslie who carried out the gross examination of eight of the reported cases; M. Opland for assistance during post-mortem examination and sampling; J. Aursjø, M. Sunde, B. Djonne and T. B. Johansen for bacteriological work; I. Asphoug who photographed the live calf shown in Fig. 2; and T. Toldnes and E. Hagen for providing field material from many of the reindeer examined.

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[ Received, May 14th, 2009  
Accepted, December 29th, 2009 ]