A severe outbreak of contagious ecthyma (orf) in a free-ranging musk ox (Ovibos moschatus) population in Norway

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Received 11 May 2007; received in revised form 23 July 2007; accepted 24 July 2007

Abstract

During July–October 2004, 19 (18 calves, 1 yearling) free-ranging musk oxen (Ovibos moschatus) at Dovre, Norway, were observed with contagious ecthyma-like lesions, and 16 of them were euthanized. Six musk oxen were subjected to necropsy, histopathological and microbiological examinations. All euthanized animals had lesions consistent with contagious ecthyma presenting as wart-like, scabby lesions on the muzzle, lips, oral mucosa and limbs to a variable extent. The histopathological examination showed pustular dermatitis characterized by epidermal proliferation, reticular degeneration, degenerating keratinocytes with intracytoplasmic eosinophilic inclusion bodies, vesicopustules, microabscesses and multifocal ulcerations in the epidermis which was covered by a serocellular crust. Pathology and bacteriology showed evidence of secondary infections in the skin and draining lymph nodes. Electron microscopy (negative staining) of lesions from four animals detected parapoxvirus with the typical arrangement of the outer protein filaments. Parapoxvirus DNA was detected in tissue samples from two examined animals by polymerase chain reaction (PCR) with primers from the B2L-gene. A DNA sequence of 326 nucleotides from the amplicon was compared with similar DNA sequences from parapoxvirus isolated from sheep, reindeer, musk ox and cattle. The outbreak was caused by a virus similar to other circulating orf virus variants in Norway. Antibodies against parapoxvirus were detected with a virus neutralization test in 3 of 35 musk oxen (8.6%) sampled at Dovre between 2004 and 2006. This is the first report of a severe outbreak of contagious ecthyma in free-ranging musk oxen.

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Keywords: Contagious ecthyma; Musk ox; Orf; Ovibos moschatus; Parapoxvirus; Pustular dermatitis
1. Introduction

Contagious ecthyma (contagious pustular dermatitis; orf) is a common viral skin disease of farmed sheep and goats with a worldwide distribution. It is caused by the orf virus, a species within the genus *Parapoxvirus* (family *Poxviridae*) (Robinson and Balassu, 1981; Haig and Mercer, 1998). The disease has also been described in a broad range of wild ruminant species (Robinson and Kerr, 2001) as well as in humans where it represents an occupational hazard among people who handle infected animals (Falk, 1978; Robinson and Balassu, 1981). The epitheliotropic orf virus infects damaged skin and replicates in epidermal keratinocytes (McKeever et al., 1988). Clinically, the skin infection typically progresses from erythema via vesicle formation to pustule and then to scab. Characteristic for the disease is the formation of pustular, scabby lesions in the skin around the mouth and nostrils. Lesions may also develop in the skin or mucosa on other parts of the body; for example the limbs, udder, and oral mucosa. The lesions can be highly proliferative, developing into wart-like papilomas. Primary lesions usually resolve within 4–6 weeks. Morbidity may be very high in small ruminants, particularly in young animals, but mortality is usually low. Secondary bacterial and fungal infections are common and often involved in fatal cases (Robinson and Balassu, 1981; Haig and Mercer, 1998).

Contagious ecthyma is a common and widely distributed disease in farmed sheep and goats in Norway. The disease has also been described in Norwegian semi-domesticated reindeer (*Rangifer tarandus*), both in experimental animals (Kummeneje and Krogsrud, 1979) and under normal herding conditions (Tryland et al., 2001). Severe outbreaks of contagious ecthyma have been reported in herds of captive musk ox (*Ovibos moschatus*) in Norway (Kummeneje and Krogsrud, 1978), Alaska (Dieterich et al., 1981), and Minnesota (Guo et al., 2004). In the single captive musk ox herd in Norway, situated in the northern part of the country and based on animals imported from Greenland in 1969, contagious ecthyma has been endemic since the first outbreak in 1974/75 (Kummeneje and Krogsrud, 1978; Mathiesen et al., 1985; Moens et al., 1990). The disease has also been diagnosed twice as single cases in a free-ranging musk ox population at Dovre, Norway (see below, Gundersen et al., 2005), and there has been serological evidence for the presence of infection in free-ranging musk ox in Alaska (Zarnke et al., 1983).

A small population of free-ranging musk ox, estimated at 170 animals in March 2004, inhabits the Dovre high mountain plateau in Mid-Norway (Bretten, 1990; Gundersen et al., 2005). The population is based on 21 calves and yearlings imported from Eastern Greenland and released during the period 1947–1953. Losses from the musk ox population have been mainly due to euthanization for security reasons and traffic accidents. Contagious ecthyma was diagnosed in one calf in 1987 (August) and one calf in 1994 (March), but no regular outbreaks have previously been reported (Gundersen et al., 2005).

On July 21st 2004, two calves were shot after it had been observed that they had lesions around the mouth, signs of lameness, and that their mothers would not let them suckle. Later the same summer and autumn, 14 musk oxen were euthanized for the same reasons. This article describes the course of the outbreak of contagious ecthyma in the free-ranging musk ox population including descriptions of the pathological findings. The detection and partial characterization of the causative parapoxvirus are presented, as well as a brief comparison with available gene sequences from recent parapoxvirus isolates from sheep, reindeer, musk ox, and cattle. Moreover, a study of the numbers of seropositive reactors for parapoxvirus in the free-ranging musk ox population at Dovre during and after the disease outbreak is reported.

2. Materials and methods

2.1. Clinical cases

The cases reported here originated from a free-ranging musk ox population at Dovre high mountain plateau (centre: 62°20′N, 9°30′E) which has a home range of approximately 340 km² (Anonymous, 2006a). The population is divided in several flocks that vary in size, age and sex composition during the year. The flocks are most stable during the winter when they gather in large groups due to restricted nutritional resources. In the spring and summer the
animals are more dispersed and flock composition varies. The main calving season is in May. During the mating season from the middle of August to the middle of September, a flock typically consists of a dominant male, some cows (1–3), with their calves, and yearlings. An adult male may visit several flocks during the course of the mating season (Bretten, 1990). In recent years, the population size has been estimated by counting animals from the ground once a year in March or April. The population size has increased rapidly during recent years, and had almost doubled from 95 animals in April 1999 to 170 animals in March 2004 (Gundersen et al., 2005). Dovre is a summer grazing area for sheep, and free-ranging wild reindeer also inhabit the Dovre high mountain plateau.

The musk ox population of Dovre was included in the National Health Surveillance Program for cervids (HOP) in Norway from 2004. As a result, the local wildlife management authorities send quarterly reports of all animals found dead, sick, wounded, or shot to the secretariat of HOP at the National Veterinary Institute (NVI) in Oslo and submit samples for laboratory examinations and biobanking.

A total of 19 free-ranging musk oxen from Dovre registered with contagious ecthyma-like lesions by local wildlife management authorities in 2004 were included in this study. Sixteen were shot; six were submitted to NVI for laboratory examinations.

2.2. Pathological and bacteriological examinations

Five musk ox carcasses (Animal Nos. 1–2, 4–6), the skin, the head and distal parts of the limbs of a sixth carcass (No. 3) were submitted to the NVI laboratories and subjected to necropsy, histopathological, and microbiological examinations. Fresh tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin and eosin (H&E) and van Gieson (VG) for histological examination (Culling et al., 1985). Standard bacteriological examination on calf blood agar plates was carried out on samples from five of the animals. Samples were taken from the skin lesions of four animals, the lymph nodes of three animals, the spleen of two animals, and the liver from one of the animals. The plates were incubated aerobically at 37 °C and examined after 24–48 h.

2.3. Transmission electron microscopy

Material for negative staining was obtained by taking skin scrapings or small pieces of skin from affected areas (Animal Nos. 1, 2, 5, and 6) and then homogenizing with sand in distilled water. The supernatant was prepared for negative staining (1% phosphotungstic acid, pH 7.0) and examined on a 400 mesh grid in an EM208S transmission electron microscope (Philips, Eindhoven, The Netherlands) for the presence of virus particles at magnifications of 15,000 or higher (Steffens, 1998). Samples from Animal No. 1 were also examined in a similar way by a different laboratory in a JEM 1010 microscope (JEOL USA Inc., Peabody, MA, USA).

2.4. Virus cultivation

Scab material from one animal (No. 2) was propagated in monolayer cultures of Vero cells (African green monkey (Cercoithecus aethiops) kidney cell line) (Norrby et al., 1970) cultured in modified Eagle medium with Earle’s buffered saline solution (EEMEM), supplemented with Tris-buffer (16.4 mM), L-glutamin (4 mM), and gentamicin (100 μg/ml). The pH was adjusted to 7.6 and all incubations were conducted at 37 °C. All cell culture reagents were from Cambrex, Walkersville, MD, USA. The cultures were inspected daily with an inverted microscope (Leitz) for cytopathogenic effect (CPE).

2.5. Polymerase chain reaction (PCR)

Tissue samples were collected during necropsy from the lesions on lips and legs of two animals (Nos. 1 and 2). Sub-samples of 25 mg were mechanically homogenized in PBS with a pellet pestle device and DNA was extracted using the QIAamp tissue kit (QUIAGEN, GmbH, Düsseldorf, Germany). The PCR primers PPP-1 and PPP-4 for the B2L-gene of orf virus (strain NZ2) were used (Inoshima et al., 2000). DNA extracted from contagious ecthyma lesions of a goat and a reindeer were used as positive controls (Tryland et al., 2001), whereas water and DNA extracted from a
feline cowpox lesion (Tryland et al., 1998), representing another genus of the family *Poxviridae*, were used as negative controls. The PCR protocol was run in a Gene Amp PCR system 9700 (Perkin Elmer Corp., Norwalk, Connecticut, USA) as described previously (Tryland et al., 2001). Amplicons were analyzed by gel electrophoresis (Gibco BRL Horizontal Gel Electrophoresis, Horizon™ 11-14; Life Technologies™, Paisly, Scotland) in a 2% agarose gel (Ultra pure agarose gel; Life Technologies) using TAE buffer (0.04 M Tris-acetate, 1.0 mM ethylenediamine tetraacetic acid) with ethidium bromide for DNA staining.

2.6. Sequencing of viral DNA

The PCR amplicons were prepared for sequencing by removing primers and dNTP by incubating 5 μl PCR product with 1 μl of ExoSapIT reagent (Amersham Pharmacia; Uppsala, Sweden) for 45 min at 37 °C, followed by 20 min at 80 °C for enzyme inactivation. Cycle sequencing was conducted in both directions using Big Dye 3.1 reagents (ABI BigDye™ Terminator Version 3.1, Applied Biosystems, Oslo, Norway). A total of 2 μl 125 mM EDTA, 2 μl 3 M sodium acetate, and 50 μl ethanol were added to the 20 μl sequencing product. The cycle sequencing extension products were subjected to electrophoresis in an ABI Prism™ 377 DNA Analyzer (Applied Biosystems). Raw sequence data were edited by Chromas software (Version 2.21; Technelysium Pty Ltd., Tewantin, Qld., Australia). The PCR amplicon DNA consensus sequence obtained (326 nucleotides) was submitted to EMBL Nucelotide Sequence Database (accession number: DQ028478; musk ox parapoxvirus putative virion envelope antigen gene). The DNA sequence was subjected to a nucleotide sequence alignment search (NCBI blast—quick search for highly similar sequences; Altschul et al., 1997).

2.7. Serology

Serum samples from 44 animals were collected from the musk ox population at Dovre in 2004 (n = 23), 2005 (n = 10), and 2006 (n = 11), and examined for antibodies against parapoxvirus in a virus neutralization test (VNT). Fourteen of the 23 individuals sampled in 2004 had contagious ecthyma-like lesions, including the 5 of the animals that were necropsied (Nos. 1–3, 5–6). Cell cultures inoculated with parapoxvirus isolated from animal No. 2 were stored at −70 °C prior to the VNT (Appel and Robson, 1973). They were subsequently thawed and, after centrifugation (1400 × g, 5 min), a 10-fold dilution series of the supernatant was added to a 96-well cell culture plate (Corning Costar®, Corning, NY, USA) with Vero cells. The plate was incubated and inspected daily for definition of the tissue culture infectious dose with 50% lysis of the cells (TCID50). In the VNT, dilution series of each serum sample was preincubated for 1 h together with the virus-containing supernatant (100 units of TCID50). Thereafter, 50 μl of each dilution was added in duplicates to a cell culture microtitre plate containing freshly prepared Vero cells in 150 μl EMEM added heat inactivated and γ-irrigated bovine foetal serum (100 μl/ml; Cambrex). Serum (titre 1:32) obtained from a lamb scarified and experimentally infected with orf virus (NVI Path. No. 2630/70) was used as the positive control serum. The plates were incubated for up to 10 days and the highest serum dilution with visible CPE was considered as the titre of the sample. Serum samples with titres ≥ 1:4 were considered as seropositive.

3. Results

3.1. Clinical cases

During the period July 21st to October 11th 2004, a total of 16 musk oxen were shot due to severely affected general condition and lesions typical of contagious ecthyma (Fig. 1). Half of the animals were euthanized in the period of August 9th to the 23rd (Fig. 1). The entire home range of the musk ox was represented among the euthanized animals (Fig. 2). All individuals were calves of that year, except one animal which was 1 year old (yearling). The sex distribution was 11 male and 4 female calves and a female yearling (Fig. 1). All euthanized animals had lesions consistent with contagious ecthyma presenting as wart-like, scabby lesions on the muzzle, lips, oral mucosa, and limbs to a variable degrees (Fig. 3). In addition to the 16 euthanized animals, 3 calves in good general condition were observed in September at a
distance, as having contagious ecthyma-like lesions of limited extent on the head or on one of their limbs. During the subsequent winter and during the winter count in March 2005, no observations were made of animals with ecthyma-like lesions.

3.2. Pathological and bacteriological examinations

The six euthanized musk oxen (Nos. 1–6) examined at the laboratory were calves, five males and one female (Fig. 1). Four of the five carcasses were in normal condition with body weights ranging from 43 (the female) to 63 kg, whereas one animal was small and in poor condition with a body weight of 28 kg (No. 6). All six musk oxen had multiple gross lesions on their heads and on two (No. 6) or all four legs consistent with contagious ecthyma: wart-like, scabby lesions with marked proliferation of epidermis covered by a thick, brown-black, greasy crust (Fig. 3A–C). On some lesions the scabs had sloughed off, revealing ulcerated, proliferated skin. The lesions were of variable size from a few mm up to 10 cm in diameter/length, multifocal to coalescing, and some had a papilloma-like appearance. The areas most affected on the head were the skin around the mouth, the muzzle and the lips; often including the mucocutaneous junction, and the gingiva (Fig. 3B). One musk ox also had small papilloma-like lesions on the tongue. Prominent skin lesions were found on the distal parts of the legs mainly in the region from the coronet to the carpus/tarsus (Fig. 3C). Some of the affected legs had large lesions between the hooves.
The lymph nodes on the head and affected limbs were enlarged and had a wet surface when cut. One calf (No. 6) with prominent ecthyma lesions on the distal right pelvic limb, suffered from a suppurative cellulitis extending from the right thigh to the hoof, associated with purulent lymphadenitis in the right popliteal lymph node. In this animal, the lesions on the legs had been invaded by fly larvae. Another calf (No. 3), with large ecthyma lesions on the head, had purulent lymphadenitis in the retropharyngeal lymph nodes.

Histopathological examination of the skin lesions revealed marked epidermal proliferation with elongated rete ridges covered by a thick crust. The epidermis showed parakeratotic and orthokeratotic hyperkeratosis, prominent hyperplasia (acanthosis and hypergranulosis), vacuolation and swelling of the keratinocytes in the stratum spinosum, reticular degeneration, and intraepidermal vesicle formation (Fig. 4). Similar changes were found in the outer root sheath of the upper parts of the hair follicles. Intracytoplasmic eosinophilic inclusion bodies were occasionally seen in degenerating keratinocytes (Fig. 5). The affected epidermis showed multifocal ulcerations, intraepidermal and subepidermal vesicopustules and microabscesses, and was covered by a serocellular crust which, in some of the lesions, contained bacterial colonies. In the superficial dermis abundant dilated capillaries, often with large endothelial cells, were seen, and were particularly prominent in the subepidermal area (Fig. 5). Additionally, hyperemia, areas of oedema and diffuse infiltration of lymphocytes, plasma cells, and variable amounts of neutrophils, often forming microabscesses of variable size, were found. The deep dermis showed perivascular and perifollicular infiltrations of mononuclear cells, predominantly lymphocytes. Similar perivascular infiltrates were seen in the subcutis. In ulcerated lesions, there were heavy infiltrations of neutrophils and haemorrhages in the superficial dermis as well as a diffuse mixed inflammatory cell infiltration in the deep

Fig. 3. (A) This male musk ox calf was euthanized 5th of September 2004 due to a severely affected general condition and lesions typical of contagious ecthyma. Multiple, dark, papillomatous lesions are seen on the limbs (black arrows) as well as large, coalescing lesions on the muzzle and lips (white arrows). (B) Photograph of the rostral part of the head of the musk oxen shown in (A). On the lips and (interdigital cleft).
dermis which was also occasionally found in the subcutis.

*Arcanobacterium pyogenes* was isolated from skin lesions of two animals (Nos. 1 and 2), β-toxic *Staphylococcus* sp. from several lymph nodes of another animal (No. 5), and *Streptococcus dysgalactiae equisimilis* from skin lesions and lymph node of the musk oxen with suppurrative cellulitis (No. 6). An overgrowth of *Proteus* sp. was found on the plates cultured from lymph node and skin from animal No. 3.

3.3. Transmission electron microscopy

Negative stained oval-shaped virus particles with the arrangement of the outer protein filaments characteristic for members of the genus *Parapoxvirus* were found in skin lesions of all examined animals (Nos. 1, 2, 5, and 6). In one animal (No. 1), virus particles with equal morphology were found in two laboratories. The size of the virus particles was approximately 260 nm × 130 nm.

3.3. Virus cultivation

After 7 days, approximately 50% CPE was observed in monolayer cultures of Vero cells where skin scab material from animal No. 2 had been inoculated.

3.5. Polymerase chain reaction (PCR)

PCR amplicons of approximately 594 basepairs (bp) were obtained from the DNA extracted from the tissue samples of animal Nos. 1 and 2 (data not shown).

3.6. Sequencing of viral DNA

Conducting NCBI nucleotide blast on the musk ox DNA sequence to search for highly similar DNA sequences revealed 100% homology with an orf virus
strain (Orf-11) and a reindeer parapoxvirus isolate from Finland (Table 1). Also high grades of homology (98–99%) were found with parapoxvirus isolates obtained from musk ox in captivity (Table 1; Norway 1994) and from sheep and reindeer, whereas the percentage of homology with pseudocowpox virus, another species within the genus *Parapoxvirus*, isolated from Norwegian cattle in 1979 and 1992 was only 94 and 95%, respectively.

### 3.7. Serology

Nine of the 44 serum samples were toxic to the Vero cells in the VNT and thus excluded from the study. Antibodies against parapoxvirus were detected in 3 of the 35 tested animals (8.6%). The seropositive animals were two female calves euthanized due to contagious ecthyma-like lesions in 2004 (both titre 1:4), and one adult male musk ox hit by a train and euthanized in 2005 (titre 1:16). Sera from five of the six necropsied animals were among those tested and three were classified as seronegative (Nos. 1, 3, and 6) whereas two were toxic to the test (Nos. 2 and 5).

### 4. Discussion

A severe outbreak of contagious ecthyma was diagnosed in the free-ranging musk ox population at Dovre, Norway, in the summer and autumn of 2004. Cases were registered over the whole home range of the musk ox population, thus the majority of the flocks seemed to have been affected. Although only young animals were found diseased, it is likely that most individuals in each flock were exposed to the virus. The observation of musk ox cows that would not let the calves suckle can suggest that they might have had lesions on the teats or udder that were not visible when observed from a distance. The observed behaviour is also seen in ewes with infected teats which refuse to nurse their youngs (Robinson and Balassu, 1981). The loss of 15 calves in addition to possible fatal, undetected cases can be estimated to around one-third of the calf production that year. The long-term impact on the population, however, does not seem to have been crucial. The population size stagnated from March 2004 to March 2005, 170 animals counted for both years (Gundersen et al., 2005; Anonymous, 2005), however, the population had increased to 213 animals (including 54 calves) by March 2006 (Anonymous, 2006b), the highest ever counted. No clinical cases of contagious ecthyma have been observed during the two following years (2005 and 2006).

Based on the DNA sequence amplified by PCR (B2L-gene), the virus causing the outbreak of contagious ecthyma in musk oxen was similar to recently characterized orf virus isolates obtained from captive musk ox, reindeer, and sheep in Norway, but different from parapoxvirus isolated from cattle (pseudocowpox virus). This conclusion is strengthened by the fact that all these viral isolates were obtained directly from lesions, and not from viruses that have been passaged and adopted to cultured cell lines in the

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laboratory. During such adaptations, viruses often change genetically. The genetic comparison reported here is based on a DNA sequence within the open reading frame B2L which codes for the protein p42K. This is a homologue of the major envelope antigen p37K in vaccinia virus (Sullivan et al., 1994). Sheep mount a strong antibody response against this protein and it also stimulates lymphocytes derived from draining lymph nodes following natural infections with the orf virus strain NZ2 (Sullivan et al., 1994). It is therefore believed that this genetic information is of great importance to the virus and that the encoding gene is highly conserved (Büttner and Rziha, 2002), thus making it highly suitable for genetic comparisons of parapoxvirus isolates and strains (Inoshima et al., 2000; Klein and Tryland, 2005).

Whether orf virus is endemic in the Dovre musk ox population or whether the virus is occasionally introduced from local sheep flocks is not known. No outbreaks of contagious ecthyma have been registered among reindeer at Dovre. The disease is endemic in local sheep herds which utilize the same pasture area as the musk ox. Based on the genetic similarity of the PCR amplicons obtained from musk ox and other parapoxvirus isolates, it is therefore likely that orf virus has been transferred from sheep to musk ox whilst at pasture. Orf virus can survive in dry scabs for extended periods of time (Livingston and Hardy, 1960), however virus survival is reduced once exposed to rainfall (McKeever and Reid, 1986). Scabs lying in sheltered locations where sheep and musk ox share pasture or salt licks, act as a likely source for infection of musk oxen. Higher population density may increase the likelihood for a musk ox to come into contact with orf virus from sheep. Once the infection is introduced, a dense population may also facilitate the spread of the virus within and between musk ox flocks. In a free-ranging population of Rocky Mountain bighorn sheep in Canada, visible signs of contagious ecthyma were commonly seen in lambs once the population attained high density levels (L’Heureux et al., 1996). In addition, the considerable increase in the density of the musk ox population during the last years may have lead to raised social and environmental stress levels, particularly during the mating season. Increased stress during the mating season, which overlapped with the period when most of the contagious ecthyma cases appeared, could have lead to increased susceptibility to infection, particularly among the calves. A denser population with higher stress levels, in combination with many calves which were novel to orf virus, may explain why a severe outbreak of contagious ecthyma occurred among free-ranging musk ox this particular season.

The serological response against parapoxvirus, seems to have been weak during and after the outbreak. Some of the blood samples from diseased animals may have been obtained prior to seroconversion. On the other hand, the serology results are consistent with results from ecthyma infection in sheep where neutralizing antibodies are usually only produced with low titres (Haig and Mercer, 1998). Low titres of neutralizing antibodies were found in a small survey of hunted, free-ranging musk oxen in Alaska showing a seroprevalence of 45% (Zarnke et al., 1983).

In conclusion, this is the first report of a severe outbreak of contagious ecthyma in free-ranging musk ox. The gross lesions and histopathological findings registered were in accordance with those previously reported in captive musk ox (Kummeneje and Krogsrud, 1978; Dieterich et al., 1981; Guo et al., 2004). An orf virus encoded vascular endothelial growth factor (VEGF) has been identified which stimulates the proliferation of epidermal cells and blood vessels in the dermis underlying the site of infection (Lyttle et al., 1994; Savory et al., 2000). This is consistent with the histopathological findings in the lesions of the examined musk oxen, showing abundant, dilated capillaries in the superficial part of dermis (Fig. 5) and large haemorrhages in ulcerated lesions. These haemorrhages together with desquamated keratinocytes and inflammatory cells contribute to the formation of thick, black crusts on the skin surface. The crusts are good media for secondary pathogens. As documented in this study, and as reported previously (Kummeneje and Krogsrud, 1978), secondary bacterial infections seem to be a common sequel to orf virus infection in musk ox, causing serious disease and mortality.

In sheep, contagious ecthyma typically has a cyclic course in a herd. This is also the experience with this disease in captive musk ox herds (Mathiesen et al., 1985) and it can be expected that new cases will appear in the free-ranging musk ox population at Dovre in the future. Due to the surveillance
established through the National Health Surveillance Program for cervids (HOP), we anticipate that any new and severe cases of contagious ecthyma in this population will be detected.

Acknowledgements

Financial support was provided by the Directorate for Nature Management through the funding of HOP. We would like to thank Johan Schulze and Kjell Handeland for performing necropsies, Eva Breines for help with the PCR and sequencing of amplicons, Inger-Lise Larsen for cultivating virus and performing serological assays and Attila Tarpai for designing Fig. 2. We are grateful to Ingolf Røtvei, Haldor Haugland, Trond Toldnes, Asgeir Myhrmoen, Hans Olav Arneklev, and Håvard Rønning for surveillance and field work at Dovre.

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