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ON CONTAGIOUS ECTHYMA AND ITS TREATMENT IN MUSKOXEN (OVIBOS MOSCHATUS)

By

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MATHIESEN, S. D., T. JØRGENSEN, T. TRAAVIK AND A. S. BLIX: *On contagious ecthyma and its treatment in muskoxen (Ovibos moschatus)*. Acta vet. scand. 1985, *26*, 120—126. — Contagious ecthyma (CE) has been a frequently occurring disease in captive Norwegian muskoxen (Ovibos moschatus), inflicting heavy losses among calves and adult males; adult females, however, have been little affected.

Parapox virus particles from papilloma tissue were observed by transmission electron microscopy. Papilloma tissue excerted a typical cytopathic effect on human continuous lumar cell line. Sera from infected muskoxen contained antibodies reacting with virus antigen from muskoxen papilloma tissue in a complement fixation test.

In animals already affected, papilloma tissue was surgically removed at intervals and the lesions injected with active papilloma tissue homogenate emulsified in Freund's complete adjuvant (FCA). Serum antibody titers against CE virus increased 3 times in response to this treatment which reduced papilloma growth, but recovery was slow in adults and all but 1 calf succumbed when offered this treatment only.

Isolated purified X-ray inactivated CE virus in FCA injected s.c. 4 weeks post partum was first attempted as a vaccine against CE. This treatment increased serum CE-antibody level, but did not prevent CE in calves experimentally injected with live CE virus. The incubation time of CE in this experiment was 20 days.

Adequate protection was, however, obtained with a vaccine consisting of homogenated, glutaraldehyde inactivated, muskox papilloma tissue in FCA injected s.c. 2 weeks post partum. It is assumed that this protection was due to activation of both humoral and cellular immune mechanisms.

parapoxvirus; vaccine; immune system.

Contagious ecthyma (CE), sore mouth or orf, is a common disease in domestic sheep (Ovis aries) and goats (Capra hircus). This disease, first described by *Steeb* in 1787 (cited by *Schmidt* 1967) and later by *Hansen* (1879) is caused by a parapox virus, which according to *Livingston & Hardy* (1960) is viable and capable of producing typical lesions in lambs after storage in the

Figure 1. Photograph of a muskox calf of the 1980 cohort taken 20 days after outbreak of CE. The calf survived after treatment as described (see *Treatment of CE affected animals*).

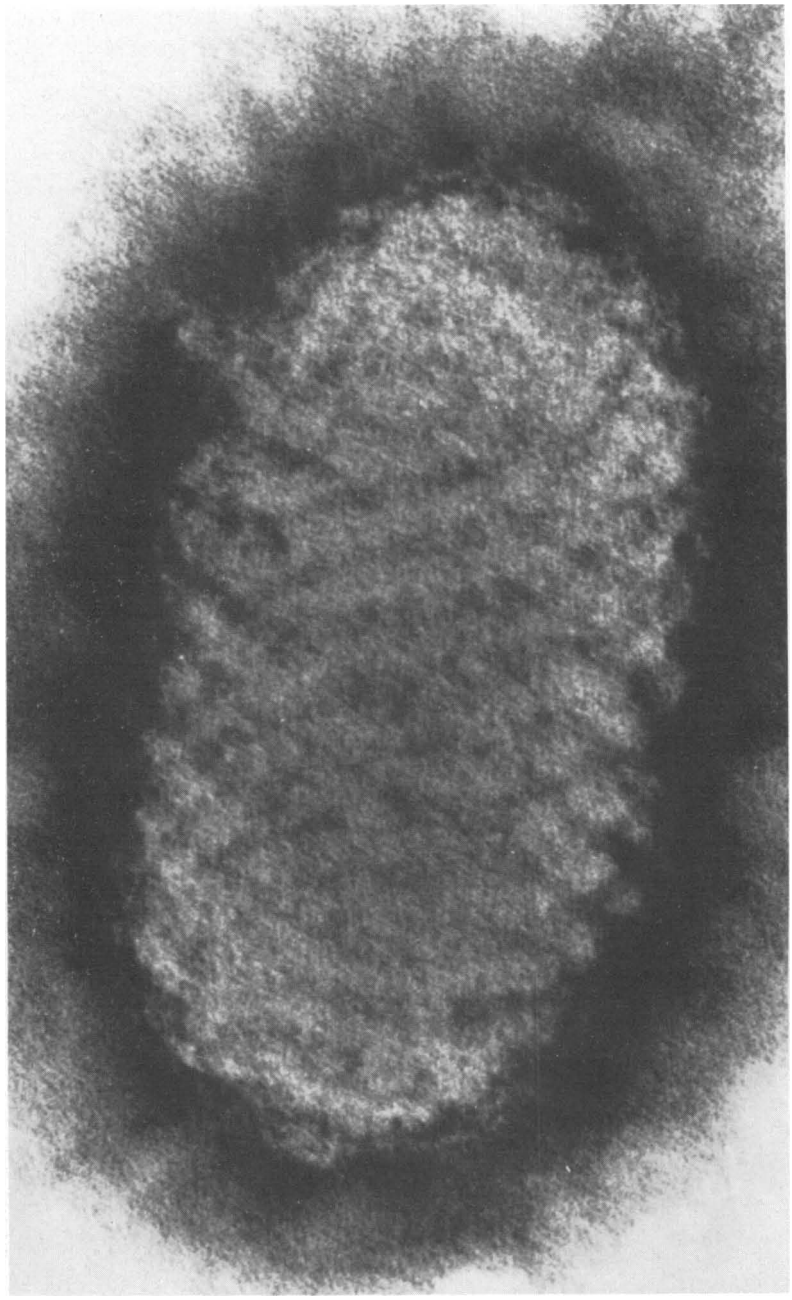

Figure 2. Electron micrograph of a parapox virus like particles found in papilloma tissue taken from the animal seen in Fig. 1. The viron is 300 nm long and 160 nm wide, and demonstrates the pattern of coat protein subunits typical of parapox viruses.

laboratory for more than 22 years. *Beck & Taylor* (1974) indicated that the resistence and persistence of the virus may account for the sporadic occurrence of the disease, usually coinciding with lambing.

In Norway, CE is said to be common in sheep and goats, has been found in reindeer (Rangifer tarandus) (*Kummeneje & Krogsrud* 1979), and has repeatedly haunted a herd of domestic muskoxen (Ovibos moschatus). Papilloma growth in Norwegian muskoxen is often explosive, particularly in calves (Fig. 1) which normally succumb within weeks if untreated. Vaccination of a 3 week old calf with live sheep orf vaccine has been attempted without success (*Kummeneje & Krogsrud* 1978).

In this paper we present the CE case history of the domestic Norwegian muskox herd from its introduction from East-Greenland in 1969 till the present day. We also describe how CE can (in some cases) be treated and we report on a vaccination procedure which appears to give adequate protection against CE in muskoxen.

MATERIAL AND METHODS

Case history

In 1969 twenty-five muskoxen calves aged 4 months were transferred from east Greenland to northern Norway in an attempt to establish a herd of captive muskoxen. Out of these, 8 males and 11 females were alive in December 1974, when the first outbreak of CE occurred. All members of the herd showed signs of infection. Five of the males died, while the females only showed small warts. In the animals surviving this outbreak CE has never reoccurred.

In the summer of 1975, five calves were born. All developed CE at age 4 weeks while suckling their mothers, and died or were sacrificed untreated within a few weeks.

Between 1975 and 1980 the record of the herd is discontinuous, but interviews with the herders indicate that no outbreak of CE occurred in this period, and that the small (unexamined) warts found on a few of the animals in 1977 (*Kummeneje & Krogsrud* 1978) were of another origin.

In 1980 the entire domestic herd of muskoxen in Norway, then consisting of 8 females and 1 male, was donated to the Department of Arctic Biology at the University of Tromsø. That year 7 calves were born. Three were immediately used for research

purposes, while 4 were raised on an artificial milk-mixture from about 2 months of age. All 4 calves developed papilloma like warts at 3 month of age. These warts were highly vascularized and granulated, located on the nostrils, lips, forehead, inside the mouth, on the eyelids, and between the hooves. Both sexes were equally affected and developed papillomas in the same way. The growth of the papillomas was explosive, the result of which can be seen from Fig. 1. All calves were treated as described below (Treatment of CE affected animals), but 3 of the 4 died before the end of the year.

In 1981 five calves were born. Of these, 2 were used for research purposes, while the other 3 were vaccinated with Vaccine-I (described below) at the age of 4 weeks. Six weeks thereafter the calves were experimentally infected with a subcutaneous injection of live CE virus. The vaccine (I) did not provide adequate protection against this infection and all calves developed CE as the year before. The calves were immediately isolated and treated as described below (Treatment of CE affected animals). One of the 3 survived.

In December the same year (1981) 2 adults, 1 male and 1 female, which until September had been kept isolated from the rest of the herd, contracted CE from an unidentified source. Both animals had been vaccinated with Vaccine-I the previous year. The female only developed small warts which did not require treatment, while the male developed papillomas similar to those described for the calves. This male survived after treatment as described below (Treatment of CE affected animals).

In 1982 and 1983 two and in 1984 six calves were born. All 10 calves were vaccinated at the age of 2 weeks using Vaccine-II described below, and have not contracted CE, in spite of being born and raised on a range heavily contaminated with CE-virus.

Identification of virus

Papilloma tissue was surgically removed under Rompun® anaesthesia (0.30 mg/kg in calves and 0.16 mg/kg in adults). The tissue was homogenated in phosphate buffer to a 10 % suspension, and sedimented by low speed centrifugation (600 × g, 10 min). The supernatant subsequently underwent ultra centrifugation (45 000 × g) and the pellet stained with phosphotungstic acid (2 %) at pH 7.0, and examined by use of a Hitachi® (HCl-12) transmission electron microscope.

For further characterization of the virus, cell cultures (KB cells, a continuous lumar cell line) were inoculated with 10 % papilloma tissue suspension.

A standard microtitration complement fixation test employing 2 exact units of complement was used to examine the reaction between virus antigen prepared from papilloma tissue, and antibodies in sera from infected muskoxen, as well as reference sera containing virus antibodies.

Treatment of CE affected animals

Papilloma tissue was surgically removed at 3—4 week intervals under Rompun® anaesthesia using the doses given above. Twenty grams of tissue was subsequently disrupted using a Dounce homogenizer, and suspended in 20 ml of 10 mmol/l tris buffer (pH 9.0). Large debris was removed and the remaining suspension subjected to centrifugation at 45 000 × g for 1 h, using a Beckman® SW 27 rotor. The pellet was then dispersed in 10 ml of 10 mmol/l tris buffer (pH 9.0), and emulsified in an equal volume of FCA. Three to five ml of this emulsion was injected into the remaining papilloma tissue, in addition to a subcutaneous injection in the thigh, immediately after surgical removal of the papillomas. The immunization was made in an attempt to influence the competition between papilloma growth and the activation of the immune system, in favour of the immune system of the animal. The papillomas were finally washed at intervals with 3 % H_2O_2, 2 % crystal violet with a 36 % solution of the product from condensation of metakresolsulfonacid and formaldehyde (Nelex®) to avoid secondary infections.

Vaccine-I

Purified CE-virus were produced from papilloma tissue according to *Katz & Moss* (1970) with few modifications: Twenty grams of papilloma tissue was homogenized (Dounce homogenization), 20 ml of 10 mmol/l Tris buffer, pH 9.0 added and all solid sedimented by low speed centrifugation (600 × g), and the supernatant sonicated (amplitude = 20 micron) for 1 min and then sedimented through 36 % (w/v) sucrose gradient in 10 mmol/l Tris buffer (pH 9.0) (Beckman® SW 27 rotor, 45 000 g for 1 h). The pellet was dispersed and sedimented through a 40 to 60 % (w/v) sucrose gradient (Beckman® SW 27 rotor,

75 000 × g, 90 min). The virus band was collected, diluted with 10 mmol/l tris buffer (pH 9), and centrifuged at 45 000 × g for 1 h.

This procedure was repeated once. The purified virus fraction was X-ray inactivated at 750×10^3 rad (Siemens® Stailiphan x-ray machine, without filter) and then diluted in 10 mmol/l tris buffer (pH 9.0) to OD 1.4 at 280 nm. Viability of the virus was tested on cell cultures (KB). Growth of virus was not detected. Purified and inactivated virus material was emulsified in FCA and 2 ml of emulsion injected s.,c., into the thigh of each animal.

Vaccine-II

Papilloma tissue was homogenized and sedimented as described for Vaccine-I. The pellet was then dispersed in 20 ml 0.1 mol/l phosphate buffer (pH 7.3), containing 0.4 % glutaraldehyde. After 36 h of incubation (room temperature, continuous agitation) the suspension was dialyzed against phosphate buffered saline (pH 7.3) over night, and emulsified in FCA. Two ml of the emulsions was injected s.c. into each animal. Vaccine not used immediately was stored at —70°C.

RESULTS AND DISCUSSION

A particle assumed to be a parapox viron found in papilloma tissue of Norwegian muskoxen is shown in Fig. 2. The pattern of the coat protein bands typical of a parapox virus are easily recognized in this specimen, which is 300 nm long and 160 nm wide.

Inoculation of KB cells with papilloma tissue suspension resulted in a cytopathic effect and parapox virus like particles were observed in the culture medium.

Virus antigen prepared from papilloma tissue and infected cell cultures reacted in the complement fixation test with sera from infected muskoxen and with sera from vaccinated animals which is indicative of production of antibodies against the CE virus.

In employing Vaccine-I a specific antibody response which indicates activation of the humoral immune system of the animal was demonstrated. This vaccine was, however, inadequate in protecting the animals against an experimental infection with live CE-virus. The outbreak of CE in this experiment showed the incubation time of CE in muskoxen calves to be 20 days.

In employing Vaccine-II (2 calves, born in 1982), significant antibody titers were shown even 8 months after vaccination. Vaccine-II was employed again in 2 calves born in 1983 and 6 calves born in 1984.

Neither of the calves born in 1982, 1983 or in 1984 have contracted the disease in spite of being kept on a heavily infected pasture. It therefore appears that Vaccine II gives adequate protection against CE in muskoxen, possibly due to activation of the cellular immune system of these animals. Vaccine I, which probably activated mainly the humoral part of the immune system, failed to give such protection.

Experiments done on other mammals (mice and man) have demonstrated that immunologic responses against virus antigens depend on a dual recognition of the virus antigen and tissue typing antigens (major histocompatibility antigens) by cytotoxic T lymphocytes (*Benacerraf & Germain* 1978, *Zinkernagel & Doherthy* 1975, *Snell* 1978).

According to this idea, Vaccine II, which consists of killed virus plus fragmented cell membranes may present the antigens more efficiently and give a better stimulation of cellular immunity than purified virus particles alone (Vaccine I).

The source of CE in Norwegian muskoxen is at present unknown. *Kummeneje & Krogsrud* (1978) have suggested a transmission of parapox virus from domestic sheep and goats, but recently *Traavik* (unpublished data) found indications of strain differences between the virus isolated from Norwegian muskoxen and that causing CE in sheep.

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SAMMENDRAG

Om Contagious ecthyma og dens behandling på moskus.

Contagious ecthyma (CE) har vært et tilbakevendende problem i forbindelse med domestisering av moskus.

Utbrudd av CE har som oftest resultert i store tap av kalver og voksne okser, mens kuene bare har utviklet små vorter.

Parapox-lignende partikler i papillomvev fra CE-angrepne kalver ble observert i transmisjon electron-mikroskop. Videre utviklet papillomvevet en cytopatisk reaksjon på en kontinuerlig human lumar celle rekke. Det ble også påvist antistoffer i sera fra CE-angrepne moskus som reagerte med virus-antigen fra papillomvevet i en komplement fiksasjons-test. På dyr som allerede var angrepet ble papillomvevet regelmessig fjernet kirurgisk. For hver slik behandling ble dyrene injisert med homogenisert papillomvev blandet med „Freunds complete adjuvant" (FCA). Serum antistoff titer var 3 ganger høyere efter denne behandlingen. Likevel gikk rehabiliteringen sakte hos voksne dyr, og mange av kalvene døde.

Renset CE-virus, røntgen inaktivert og blandet med FCA, ble derefter brukt som vaksine ved injisering i 4 uker gamle kalver. Denne behandlingen økte CE-antistoff nivået i serum, men det var ikke tilstrekkelig til å forhindre utbrudd av sykdommen ved eksperimentell infektion med levende CE-virus i kalver. Inkubasjonstiden for CE var 20 dager i dette eskperimentet.

Tilstrekkelig beskyttelse mot CE-virus ble oppnådd ved injeksjon av homogenisert glutaraldehyd inaktivert papillomvev i FCA 2 uker efter fødselen. Vi antar at dette skyldes aktivering av både de humorale og cellulære immunmekanismene i dyret.

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