

In ROCKBORN *et al.*, pages 373 - 384, all the figure captions are placed under wrong figures and the figures, consequently, have got wrong numbers.

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The demonstration of a herpesvirus, related to bovine herpesvirus 1, in reindeer with ulcerative and necrotizing lesions of the upper alimentary tract and nose

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**Abstract:** In 11 male reindeer, all exposed to transportation stress, signs of conjunctivitis and later on ulcerative and necrotizing lesions of the mucosa of the nostrils and mouth were recorded. Blood and secretions from the nose were sampled. Antibodies to bovine herpesvirus 1 (BHV-1) were detected in 2 animals. No animal had antibodies to bovine viral diarrhoea virus (BVDV). Virus isolation was negative.

The sampling was repeated 2 weeks later and complemented with biopsies from the mouth lesions, fixed in formalin. At this occasion 3 animals were seropositive to BHV-1 and in biopsies from 2 of these intranuclear herpesvirus-like particles were found by means of electron microscopy. Four animals, 3 of them seropositive, were treated with cortison during 8 days. The size of the ulcers in the mouth increased in all animals. A herpesvirus was isolated from 3 of them at 10 different occasions. The ultrastructural investigation of the virus suspension demonstrated the presence of typical herpesvirus particles. On day 11 all 4 animals suffered from a severe diarrhoea and anorexia. On day 12 one animal died and on day 13 post challenge with cortison two additional animals died. The remaining animal was slaughtered on day 13. Bacteriological investigation revealed growth of *Fusobacterium necrophorum* from the spleen and oral wounds of all 4 animals.

The animals were obviously subjected to an infection with a herpesvirus closely related to BHV-1. Virus could be liberated by cortison treatment. It is possible that infections with the found herpesvirus, and the lesions caused by it, may be the background to earlier recorded severe outbreaks of necrobacillosis of the alimentary tract in reindeer herds.

**Keywords:** *Fusobacterium necrophorum*.

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## Introduction

A disease producing ulcerations in the upper respiratory and oral mucosa (njunne-vikke or njunne-dawda) of reindeer (*Rangifer tarandus* L.) was reported already by Horne (1898) and Turi (1910). Its possible connection with outbreaks of oral and generalized necrobacillosis

has been suggested by Horne (1898), Quigstad (1941) and Skjenneberg and Slagsvold (1968). A similar disease, also reported to be commonly complicated by oral necrobacillosis, is described from the USSR by Nikolaevskii (1961). Serological evidence of the presence of neutrali-

zing antibodies to bovine herpesvirus 1 (BHV-1) in reindeer has been reported by Elazhary *et al.* (1981) and by Dieterich (1981), but none of them reported on connected pathological lesions. In 1982 Ek-Kommonen *et al.* reported on the finding of neutralizing antibodies to BHV-1 in Finnish reindeer. The virus was later on isolated and characterized as a herpesvirus antigenically related to but different from BHV-1 (Ek-Kommonen *et al.*, 1986). The relationship between the reindeer herpesvirus and a herpesvirus isolated from red deer (*Cervus elaphus* L.), which also crossreacts with BHV-1 (Inglis *et al.*, 1983; Rönsholt *et al.*, 1987), is not yet established. Ek-kommonen *et al.* (1982, 1986) did not find any clinical signs of disease in the investigated animals, although in the latter investigation the animals were treated with dexamethasone in order to facilitate the isolation of the virus.

In 1985, Rehbinder *et al.* reported on a suspected virus infection of the oral mucosa in Swedish reindeer exhibiting vesicular and ulcerative lesions. Material for serological investigation was not obtained from autopsied animals but blood samples were obtained from animals at feeding sites where the necropsied animals had died. In some of these blood samples antibodies to BHV-1 were found.

The objective of the present paper is to report on the demonstration of a herpesvirus, related to BHV-1, in reindeer with ulcerative and necrotizing lesions of the upper alimentary tract and nose and exhibiting generalized necrobacillosis.

## Material and methods

### *Case history and clinical specimens*

A number of 13 reindeer (11 males, 2 females) were moved from a grazing herd, by means of a 200 km lorry ride, into a corral where they were given hay and concentrates. The two females were let out but the males were kept in the corral. Three weeks after the transport some of the males showed signs of dullness, puru-

lent conjunctivitis (5 animals) and "wet belly disease" (3 animals). The clinical signs were considered to be caused by the almost grinded hay from which numerous dust particles were found in the conjunctival sacs but also producing the "wet belly disease" (Nordkvist, 1967).

One week later the animals had almost recovered from the initial clinical symptoms but now 6 of the animals had a purulent discharge from the nose. Rounded erosions, 5 mm in diameter were found in the oral mucosa of the lips. Rectal temperatures varied from 39.8 - 40.0°C. Serum samples, mouth and nose swabs (Culturette<sup>R</sup>) were collected. Seventeen days later the sampling was repeated. From 6 animals with erosions of the oral mucosa biopsies were obtained by means of a "Stiefel" biopsy needle with a diameter of 4 mm. The tissues were directly fixed in 10% formalin. Approximately 3 weeks later 4 of the animals (No 84, 86, 88 and 90) were isolated and challenged daily with cortison (0.1 mg dexamethason/kg bwt.) according to Dennett *et al.* (1976). From the 5th day of cortison treatment and onwards serum and swab samples were taken each day, to day 10, on all 4 animals. Additional sampling was performed on some of the animals day 12 and 13.

One of the animals died on the 12th day of cortison challenge and two more animals died one day later, and the remaining reindeer was slaughtered on the same day. In connection with the deaths, samples from each animal were taken for:

1. Virological investigation: Blood, nasal mucosa, mouth erosions, conjunctival mucosa, prepuce and fecal samples.
2. Bacteriological investigation: Secretions from mouth and nose by means of Culturette and swabs of the spleen.
3. For histopathology and electronmicroscopy: Pieces from nose, eyelids, oral mucosa, trachea and lungs were fixed in 10% formaldehyde.

### *Cell cultures and virus references strains used*

Primary bovine embryonic turbinate (BT) and primary embryonic dermis (ED) cell were used for isolation and propagation of viruses and serum neutralisation tests (SN). The cell cultures were maintained in Eagle's MEM and supplemented with 1% fetal calf serum.

A Swedish BHV-1 strain isolated from cattle and labeled 96, and one Swedish BHV-1-like isolate from reindeer designated 41, were used in the SN tests.

### *Virus isolation*

If not processed immediately specimens were kept frozen at - 70° C. Swab material was suspended in 0.5 ml of PBS, pH 7.4, and clarified by low speed centrifugation before inoculation into tubes with BT and/or ED cells. Organs were prepared as 10% suspensions (w/v) in PBS. Penicillin and streptomycin were added to the suspensions. BHV-1 isolation: 0.2 ml of the suspension was inoculated into tube cultures, 2 tubes per suspension. After absorption for 1 hour at 37° C, the tubes were rinsed with Eagle's MEM and new medium was added and the tubes further incubated at 37° C. The tubes were checked each day for the presence of CPE for approximately 7 days. If negative after 7 days, 0.1 ml of the cell culture harvest was passaged into new tubes, 2 tubes per material.

Immunofluorescence test for detection of bovine viral diarrhoea virus (BVDV): An indirect immunofluorescence technique was used to detect BVDV in inoculated BT cells. Two tube cultures per material were inoculated with organ or swab suspensions, incubated for 7 days at 37°C and checked for CPE daily. The tubes were freeze-thawed, the medium pooled and clarified by low speed centrifugation. Each pool was inoculated into 2 Leighton tubes with outgrown BT cells. After 4 days of incubation at 37°C the slides were air dried, fixed with acetone and incubated with a 1:50 dilution of a monoclonal antibody (VD-1) directed to a structure of BVDV with a molecular weight of 20,000

(Juntti et al., 1987). A commercially available fluorescein isothiocyanate-labelled rabbit anti-mouse Ig (Dakopatts, Denmark) was used at a dilution of 1:50.

### *SN-test*

Antibody to BHV-1 was detected by a SN-test performed in a microtiter system (Reid et al., 1986). The mixture of serum and virus was incubated for 24 hours at 4 ° C as described by Bitsch (1973).

### *Histopathological investigation*

Tissues were fixed in 10% formalin, embedded in paraffin, cut 5µ and stained with haematoxylin-eosin and periodic-acid-schiff.

### *Electron microscopical investigations*

Selectes areas were cut from paraffin blocks, put in "Histo-clear" (National Diagnostics) for 24 hours, at room temperature, hydrated in graded ethanol series 90% - 70% - 50% - 30% for 10 min each at room temperature, postfixed in Karnovsky solution for 60 - 120 min at 4° C and in 1% osmium tetroxide in 0.1 M phosphate buffer for 60 min at 4°C, dehydrated in ethanol series 30% - 50% - 70% - 90% absolute alcohol for 10 min each at 4°C, put in propyhtanoxide/Epon 1/1 over night at room temperature and embedded in Epon.

After embedding thick sections were cut and stained with toluidine blue for light microscopy and thin sections were prepared with a LKB ultratome and picked up on uncoated copper grids, stained with uranyl acetate and lead citrate and examined in a Philips electron microscope 420 at 60 Kw.

To examine the virus particles ten bottles of TB cells were infected with the reindeer isolate 41. When the cell monolayer showed 80-100% CPE, the cells were freezethawed once and the harvest centrifuged at low speed for 30 min. The supernatant was centrifuged in a Kontron ultracentrifuge rotor TST 28.38 at 100,000 x g for 1 h. The pellet was suspended in 200 µl

of PBS, pH 7.4. Viruspreparations were negatively stained by mixing with an equal volume 2% ammonium molybdate. The samples were applied to 2 % amyloacetate coated collodium film supported on 400 mesh copper grids.

### *Bacteriology*

The material obtained was subjected to routine bacteriology. For the isolation of *Fusobacterium necroforum* Fastidious Anaerobe Agar was

used. Suspected colonies were inoculated in chopped meat medium for 48 h at 37° C. Standard biochemical test, gas chromatography and microscopy were performed.

### **Results**

#### *Clinical investigations*

During the period when four of the animals were challenged with dexamethason the lesions of the oral mucosa, in these animals, increased considerably (Fig. 1). At the end of the challen-

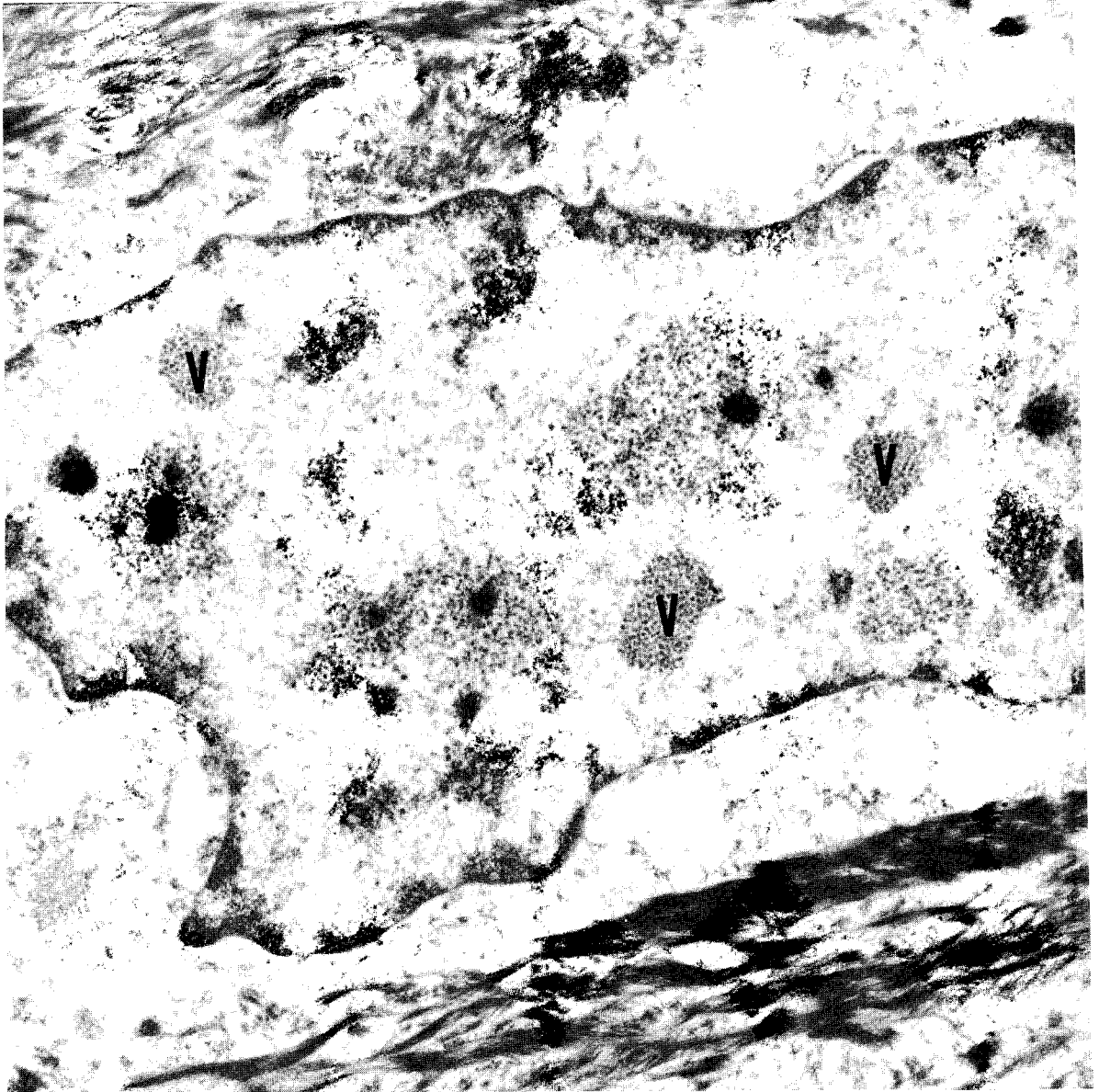


Fig. 1 Ulcers in the oral mucosa of the lip of a reindeer. The ulcers increased in size during the dexamethason treatment.



Fig. 2 Epithelial cell from the oral mucosa. Note structures similar to viral inclusions (V) and scattered virus like particles in the nucleus. Biopsy material x 19200.

ge period all four developed a watery diarrhoea and stopped consuming hay but were still eating lichens. At the same time they presented a serous discharge from the nose and mouth and to a minor extent from the eyes.

#### *Pathological investigations*

At the histological investigation of the biopsy material, the oral mucosa of all 4 animals, showed focal vesicle formation, inter- and intracellular oedema, swollen nuclei with degenerative changes and indistinct intranuclear and distinct cytoplasmic inclusion bodies. Areas with sloughed necrotic epithelium and ulcerations were also present. The ulcers were covered with colonies of fungi and bacteria and demarcated by heavy neutrophilic infiltrations. Similar colonies were occasionally found at varying depths in the lamina propria and submucosa. The nucleoli of the epithelial cells at

several instances appeared swollen. Electron microscopy revealed intranuclear structures similar to viral inclusions and scattered virus-like particles with an approximate diameter of 100 nm (Fig. 2) in material from two animals.

At necropsy a catarrhal enteritis, rumen indigestion and enlarged pulpy spleens were found. In the mucosa of the nose and oral cavity, mainly in the rostral parts, erosions and ulcers of the mucosa 5 x 10 mm - 50 x 20 mm were found. Erosions and ulcers were not found anywhere else in the alimentary system. The tracheas showed a moderate to severe hyperemia.

The histopathological investigation of the material from the necropsied animals revealed in the oral mucosa almost the same, but more pronounced changes than found in the biopsy material. The surface epithelium was to a large extent lost and necrotic lesions pronounced. Ulcers were demarcated by heavy infiltrates surrounding bacterial colonies, some of them typical for *Fusobacterium necrophorum*. Bacterial colonies surrounded by neutrophilic infiltrates were also found in the lamina propria and submucosa. The mucosa of the nostrils showed less pronounced changes but the epithelium appeared swollen with loss of surface cells. Ulcerations, when present, were smaller and with less inflammatory response.

The nasal mucosa showed a prominent loss of surface epithelium with swollen tilted basal cells. The epithelium of the submucous glands and ducts was partly disintegrated. Goblet cells were numerous. The mucosa of the trachea showed prominent changes. The vessels and capillaries were dilated. The epithelium was characterized by a heavy destruction, loss of large parts of the epithelial lining, the remaining cells being short and without cilia. The surface was covered by cell detritus masses and blood.

The conjunctivae showed an uneven loss of the surface epithelium. Most epithelial cells were swollen some few were shrunken. The Meibom glands presented epithelial degeneration

and were partly filled with detritus masses. Most mucous membranes investigated revealed limited subepithelial infiltrates by mononuclear cells, mainly lymphocytes, except for areas with ulcerations in which generally a heavy purulent inflammatory process was present.

Affected epithelial cells were enlarged with a swollen nucleus in which commonly was present structures similar to swollen nucleoli. Intracytoplasmic inclusion bodies were common.

Electron microscopy again revealed intranuclear structures similar to viral inclusion bodies and scattered virus like particles. (Fig. 3).

#### *Serological investigations*

Two out of the 11 reindeer had antibodies to the BHV-1 strain 96 when sera of the first sampling was tested, and 3 at a second sampling. None of the animals has antibody titers to BVDV and this was not further investigated.

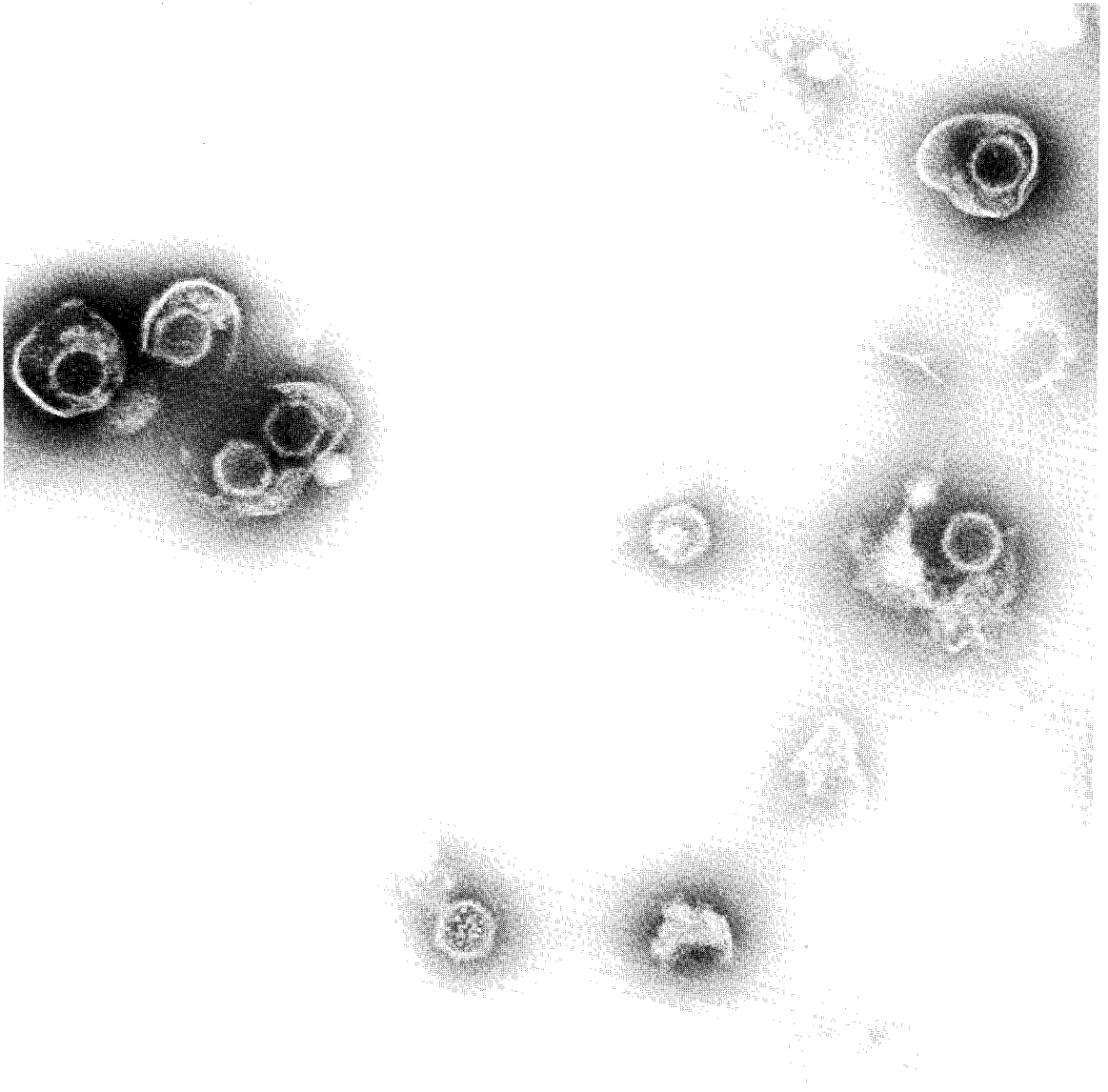


Fig. 3. Epithelial cell from the oral mucosa. Note virus like particles (arrows) and unspecific inclusion body (U.I) Necropsy material x 12500.

These three animals and one additional seronegative animal were isolated, challenged with cortison and repeatedly sampled as described in Material and Methods. The serum samples were tested for the presence of antibodies to BHV-1 isolates 96 and 41. The amount of anti-

bodies detected increased in 3 of the animals and the antibody level was dependent on the virus isolate used in the SN-test (Table 1).

#### Virus isolation

From the two initial samplings of swabs neither BVDV or BHV-1 were isolated. No further

Table 1. Neutralizing (SN-) antibody titers in serum from reindeer to BHV-1 isolate 96 from cattle and BHV-1 isolate 41 from reindeer.

| Day of sampling <sup>a</sup> | SN-titer and reindeer No |    |    |    |          |    |      |    |
|------------------------------|--------------------------|----|----|----|----------|----|------|----|
|                              | BHV-1 96                 |    |    |    | BHV-1 41 |    |      |    |
|                              | 84                       | 86 | 88 | 90 | 84       | 86 | 88   | 90 |
| -38                          | <1 <sup>b</sup>          | <1 | 22 | 4  | 11       | 8  | 90   | 22 |
| -21                          | <1                       | 4  | 8  | 6  | 11       | 11 | 64   | 16 |
| 5                            | <1                       | <1 | 4  | <1 | 6        | 8  | 32   | 16 |
| 6                            | <1                       | <1 | 6  | 2  | 8        | 8  | 32   | 11 |
| 7                            | <1                       | <1 | 4  | 4  | 11       | 6  | 45   | 11 |
| 8                            | <1                       | <1 | 6  | 4  | 8        | 8  | 45   | 16 |
| 9                            | 6                        | <1 | 16 | 8  | 16       | 16 | 178  | 45 |
| 10                           | 22                       | <1 | 45 | 11 | 90       | 11 | >256 | 32 |
| 12 <sup>c</sup>              | NT <sup>e</sup>          | 2  | NT | NT | NT       | 8  | NT   | NT |
| 13 <sup>d</sup>              | 22                       | —  | 45 | 11 | 90       | —  | >256 | 64 |

<sup>a</sup> The reindeer were challenged with cortison Day 0.

<sup>b</sup> Reciprocal of serum dilution.

<sup>c</sup> Reindeer No 86 died.

<sup>d</sup> Reindeer Nos 84 and 88 died; No 90 was slaughtered.

<sup>e</sup> NT=not tested.

Table 2. Recovery of BHV-1-like isolates from reindeer after cortison treatment

| Day of sampling post challenge with cortison | Swab or organ material sampled and labeling of animals <sup>a</sup> |    |    |    |               |    |    |                 |
|--|---|----|----|----|---------------|----|----|-----------------|
|  | Nose  |    |    |    | Mouth erosion |    |    |                 |
|  | 84  | 86 | 88 | 90 | 84            | 86 | 88 | 90              |
| 5  | — <sup>b</sup>  | —  | —  | —  | —             | —  | —  | NT <sup>c</sup> |
| 6  | —   | —  | —  | —  | —             | —  | —  | NT              |
| 7  | —   | —  | —  | —  | —             | —  | —  | NT              |
| 8  | +   | —  | +  | —  | —             | —  | —  | NT              |
| 9  | +   | —  | +  | +  | +             | —  | —  | NT              |
| 10   | —   | —  | +  | —  | —             | —  | —  | NT              |
| 13   | +   | —  | +  | +  | —             | —  | —  | NT              |

<sup>a</sup> Sporadically, samples were taken from the conjunctiva (86, 90) prepuce (88, 90), and faeces (84, 86, 90); all these materials were investigated with negative results.

<sup>b</sup> - = virus not isolated; + = a BHV-1-like virus isolated.

<sup>c</sup> NT = not tested.

attempts to isolate BVDV were made. On day 8 post challenge BVD-1-like viruses were isolated from nose swabs of 2 of the reindeer. On day 9 virus was also isolated from a mouth erosion of animal No 84. From 3 of the 4 animals BHV-1-like viruses were isolated on 10 different occasions (Table 2). The isolates were identified as closely related to BVH-1 by SN-test with a polyclonal hyperimmune rabbit anti-BVH-1 serum. The isolate designated 41 was used in the SN-tests.

At electron microscopy typical herpesvirus particles were found (Fig. 3 & 4).

#### *Bacteriological investigation*

Spleens and material from the oral mucosa of all the animals produced a moderate to rich growth of *Fusobacterium necrophorum* in a mixed culture.

#### **Discussion**

In 1985, Rehbinder *et al.* reported on a suspec-

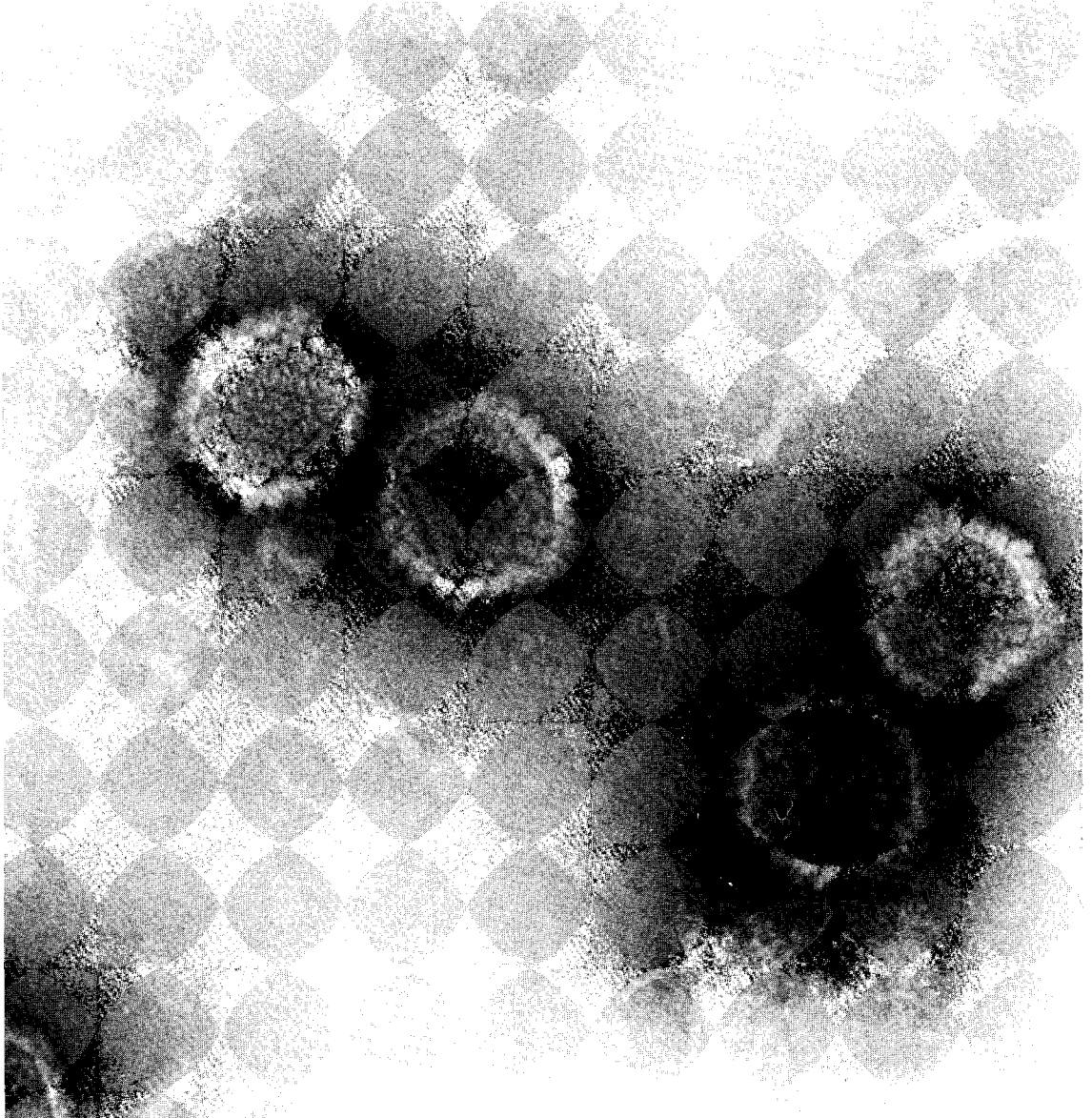


Fig. 4 Virus suspension. Typical herpes virus particles. Note envelope and capsid. Negative stain x 260000.



ted virusinfection of the oral mucosa in reindeer causing vesicular and ulcerative lesions. In some instances *F. necrophorum* contributed to the death of the animals. Antibodies to a virus related to BHV-1 were found in blood samples obtained from clinically healthy animals from the flocks from which the necropsied animals emanated. Antibodies to BVDV/MD-virus were not present. The importance of the exposure to the BHV-1 related virus was not established.

In reindeer, serological evidence of an expo-

sure to BHV-1 was earlier reported by Elazhary (1979, 1981) and Dieterich (1981). In 1982 Ek-Kommonen *et al.* reported on a virus related to BHV-1 and in 1986 Ek-Kommonen *et al.* described a herpesvirus antigenically related but different from BHV-1. No clinical signs of disease were found to be connected with the presence of the virus. In red deer (*Cervus elaphus* L.) latent herpesvirus infections with a cervidae herpesvirus type 1 (CHV-1) appears to be widely spread (Lawman *et al.*, 1978; Nettleton *et*

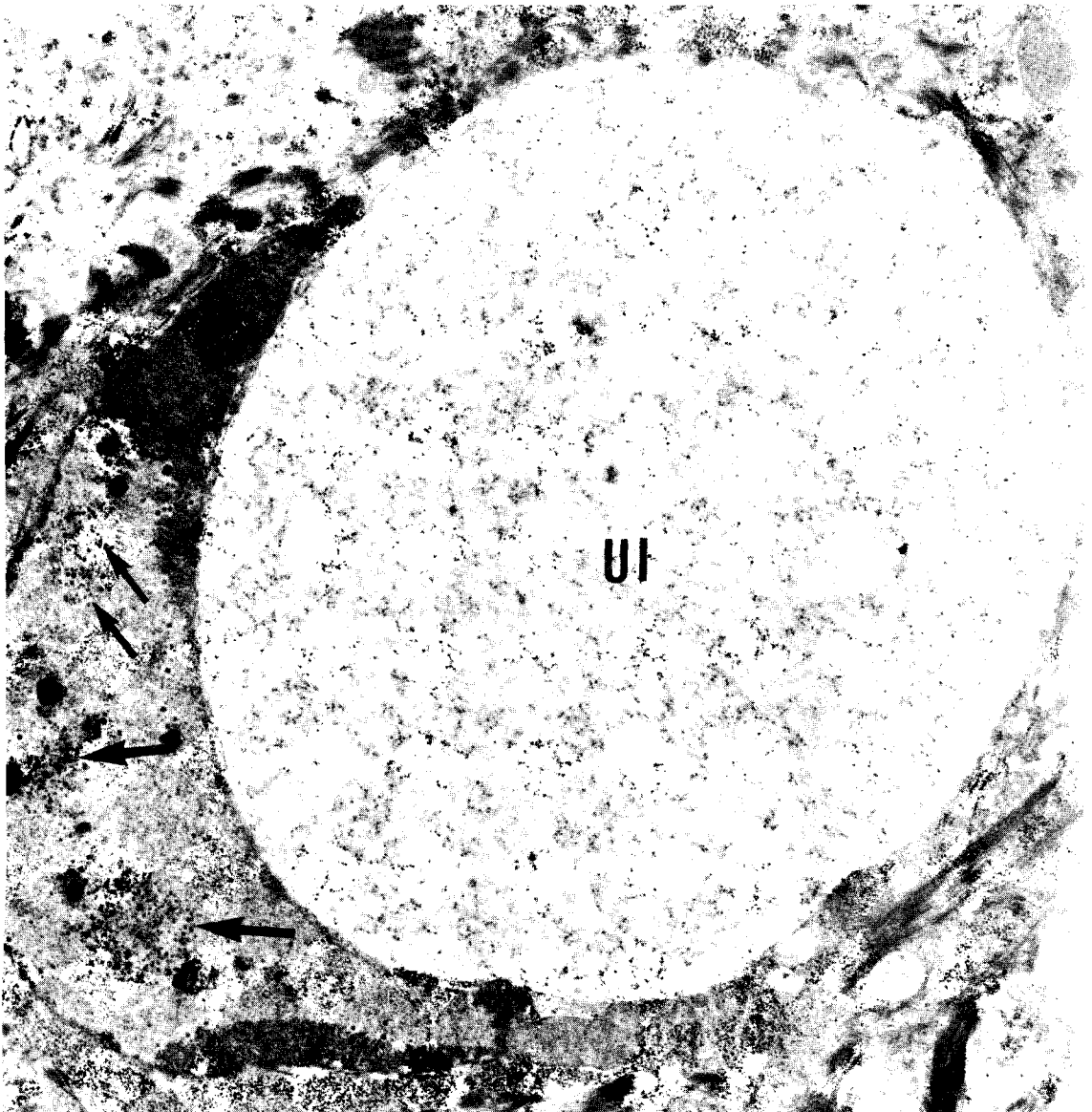


Fig. 5. Virus suspension. Typical herpes virus particles. Note hexagonal shape. Negative stain x 260000.

*al.*, 1986; Kokles *et al.*, 1988) and some times connected with disease.

The main clinical and pathological lesions reported are associated with ocular lesions and affection of the upper respiratory tract and oral mucosa (Inglis *et al.*, 1983; Reid *et al.*, 1986; Argyle, 1986; Rönsholt *et al.*, 1987). Although it is prevalently occurring in red deer populations, the CHV-1 does not appear to be a major cause of disease. Almost all outbreaks of CHV-1 related disease seem to have occurred at times of stress (Nettleton *et al.*, 1986). The present investigation shows that, in reindeer, clinical signs of disease affecting the eyes and the mucous membranes of the mouth and nose were observed after a row of stressful events such as handling, transport and changes to a less suitable fodder. In these animals, by means of electron microscopy and virus isolation, an infection with a herpesvirus was proven. The intranuclear presence of virus particles, partly forming intranuclear inclusion bodies, has to be considered typical to herpesviridae. The intracytoplasmic inclusion bodies have to be considered the result of an unspecific degenerative change (Diaz *et al.*, 1989).

The virus was antigenically related to but different from BHV-1 as seen by the results presented in Table 1. It seems likely that the herpes virus isolated in this investigation is the same or closely related to the herpesvirus isolated from Finnish reindeer by Ek-Kommonen *et al.* (1986). The relationship between those two virus isolates and CHV-1 will be genetically determined. The clinical manifestations of CHV-1 in red deer and the herpes virus of this investigation infecting reindeer appears very similar and connected with stressful events.

A herpes virus infection as a possible cause to the disease producing ulcers in the upper respiratory and oral mucosa as reported by Horne (1898) Turi (1910) and Nikolaevskii (1961) seems obvious. The death of the dexamethason treated animals in generalized necrobacillosis, with the entrance port most probably being the

oral ulcers, judged from histopathology and bacteriology, indicates a connection between herpesvirus infections and outbreaks of necrobacillosis as suggested by Horne (1989), Quigstad (1941) and Skjenneberg and Slagsvold (1968). Principally any lesion in the oral mucosa whether caused by trauma, viral or bacteriological agents or parasites give way to *F. neophorum* bacteria to enter the submucosal tissues and to establish their presence (Rehbinder & Nordkvist, 1983). Similar lesions as observed in connection with the presently described herpes virus infection is reported in cattle and sheep in association with BVDV infection. Antibodies to BVDV have been found in reindeer (Elazarhy, 1979; Dieterich, 1981; Rehbinder, unpublished observations). The importance of this agent to reindeer is not known. Red deer are known to have antibodies to BVDV-virus (Nettleton *et al.*, 1980; Derek *et al.*, 1988) and the virus has been isolated in red deer (Nettleton *et al.*, 1980) roe deer (Romvary, 1965) and fallow deer (Díaz *et al.*, in press). In red deer (Anon, 1981) and fallow deer Díaz *et al.*, in press) the virus is reported to produce lesions similar to those seen in cattle. In this investigation and the investigation by Rehbinder *et al.* (1985) antibodies to BVDV were not found.

Kerato-conjunctivitis, oral and cutaneous lesions associated with poxvirus infection in reindeer were reported by Barker *et al.* (1980). This outbreak was confined to a zoo and as poxviridae are easy to diagnose by means of electron microscopy or tissue culture and no further reports are available, the outbreak has to be considered incidental. Kerato-conjunctivitis in reindeer, neither associated with outbreaks of necrobacillosis nor ulcerative lesions of the mouth and upper respiratory tract, but of a multifactorial genesis (trauma, bacterial infections and UV- irradiation ), is common among woodland reindeer (Rehbinder, 1978). The disease ought to be easily distinguished from the presently described ailment.

In addition, malignant catarrhal fever gives

rise to ocular and mucosal lesions. Reindeer kept in close contact with sheep may be affected (Beatson, 1985, Røken, personal communication, 1988). Typical vascular lesions are present, but were not observed in this investigation. As presented in this investigation, the BHV-1-like virus isolated from reindeer has the capacity to cause disease and, most probably, give way to infections with other microorganism, especially *F. necrophorum*.

## Conclusions

Reindeer were obviously subjected to an infection by a herpesvirus closely related to IBRVirus. The relationship will later be genetically determined. Virus could easily be liberated by cortison treatment. It is possible that infections with the found herpes virus, and the lesions caused by it, may be the background to earlier recorded outbreaks of necrobacillosis of the alimentary tract in reindeer herds.

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