Cervid Herpesvirus 2 is endemic in Alaskan caribou and reindeer

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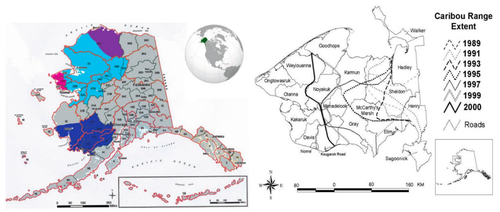
Introduction:

In Rangifer (*R. tarandus*), alphaherpesviruses antigenically similar to Bovine herpesvirus 1 (BoHV1), causing Infectious Bovine Rhinotracheitis (IBR), have been found to be endemic in Norway (Stuen et al., 1993), Sweden (Rehbinder et al., 1992), Finland (Nettleton et al., 1988), Canada (Elazhary et al., 1981) and Alaska (Dieterich, 1981). A recent study showed that CvHV2 is endemic in Norwegian reindeer, with a mean seroprevalence across northern Norway of 48% (1502/3062) (das Neves et al., 2009a).

Persistent viral infections such as those caused by cervid herpesviruses may affect calf mortality and fitness, and potentially result in abortions and weak offspring. Recently, CvHV2 was identified as the primary cause of an outbreak of infectious keratoconjunctivitis (IKC) in Norwegian semi-domesticated reindeer (Tryland et al., 2009). It is unknown if IKC, which is also frequently reported in Alaskan reindeer, is related to a herpesvirus infection (Evans et al., 2008).

We sought to determine if the same virus, or similar herpesviruses, were circulating in Alaskan reindeer and caribou.



a. b.

Figure 1. Map of Alaska (a) showing the range of the Mulchatna (dark blue), Teshekpuk (purple), the Western Arctic Caribou Herd (light blue), and semidomesticated reindeer (pink). Map of the Seward Peninsula (b) showing the progressive expansion of the range of the western arctic caribou herd. Alaska reindeer samples were taken from the Davis herd (northwest of Nome) and from the Ongtowasruk herd (Wales) (Figure a. adapted from Alaska Department of Fish and Game, Figure b. Finstad et al., 2006).

Methods:

Sampling. Serum from 292 reindeer were collected during 1988 through 2005 from a reindeer herd Sampling. Serum from 292 reindeer were collected during 1988 through 2005 from the Davis reindeer herd near Nome, Alaska (Figure 1). Swab samples were collected from the eyes of 20 calves in the Davis herd and the eyes and noses of 30 apparently health calves from a handling at Wales, Alaska (July 2007). Plasma and white blood cells (buffy coat) were collected from three Alaskan caribou herds, the Mulchatna (n=24, April 2009), Teshekpuk (n=34, June 2009) and the Western Arctic (WACH, n=83, September 2009).

Serology. Samples were tested using a commercial BoHV1 blocking ELISA (LSI, Lissieu, France), which is capable of detecting alphaherpesviruses antigenically related to BoHV1 such as CvHV2 (Neves et al., 2009a)

Virus neutralization. A virus neutralization test (VNT), including the viruses CvHV2, Cervid Herpes Virus 1 (CvHV1), Elk Herpes Virus (ElkHV) and BoHV1, was performed on samples from 10 caribou and 10 reindeer to determine which virus the animals were exposed to and had produced antibodies against. The neutralizing titres were calculated as the serum dilution necessary to neutralize the cytopathic effect (CPE) in 50% of the wells (effective dose 50%; ED50).

PCR. DNA was extracted from swab samples collected during the summer of 2007 from 30 apparently healthy calves in Wales, Alaska and buffy coats from 36 seropositive caribou in the WACH herd. Extracted DNA was amplified by a nested pan-alphaherpesvirs PCR (Ros and Belak. 1999) and gel electrophoresis was used to separate the products. For amplicons of the expected size (294 base pairs), removal of primers and deoxynucleoside triphosphates were conducted by ExoSAP-IT (Amersham Pharmacia, Uppsala, Sweden). Amplicons were sequenced (ABI BigDye), and sequences were aligned and compared to corresponding sequences of alphaherpesviruses (GenBank).

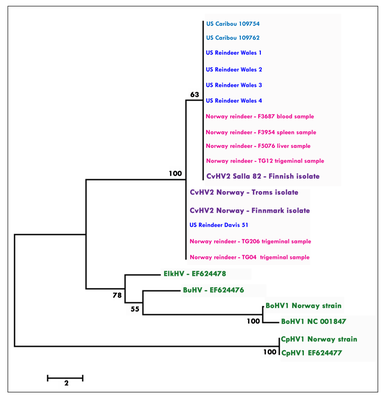


Figure 2 – Phyologenic grouping of known Cervid Herpesvirus 2 isolates and other selected ruminant alphaherpesviruses.

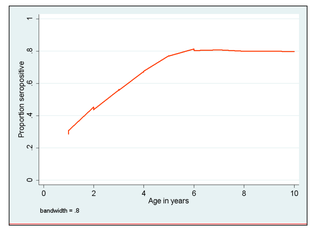


Figure 3. Locally weighted scatterplot smoothing curve of the regression of CvHV2 seroprevalence on age in Alaskan reindeer.

Results:

Reindeer

Seroprevalence was 47% (136/292). Adults were significantly more likely to be seropositive than yearlings (Pearsons chi squared test, chi=29.9209, p= <0.0001). There was no significant difference in seroprevalence by sex.

PCR amplicons of the expected size (294 bp) were obtained from 4 swabs collected from apparently healthy calves at Wales and from 2 swabs from calves in the Davis herd. Sequences from Wales were found to be identical to the CvHV2 Finnish strain (Salla-82AF078727.2), the sequences from the Davis calves were identical except for one synonymous nucleotide mutation at position 188 (Figure 2).

Caribou

The overall seroprevalence was of 60% (87/145) with no significant differences between herds or by sex. The prevalence for each herd is shown in Table 1. Seroprevalence between the caribou and reindeer was not comparable because of differences in sampling structure.

PCR amplicons of the expected size (294 bp) were obtained from 2 of the 36 buffy coats samples. These were identical to the CvHV2 Finnish strain (Salla-82AF078727.2) (Figure 2).

Virus neutralization test (VNT) for both reindeer and caribou:

All samples considered negative by ELISA were negative for all viruses during VNT (2 caribou, 2 reindeer). All samples considered positive by ELISA, except one weak positive, were positive for at least one virus on serum neutralization.

All samples neutralized CvHV2 to a larger titer than any other viruses. After CvHV2, ElkHV was neutralized to the largest extent, followed by CvHV1 and BoHV1.

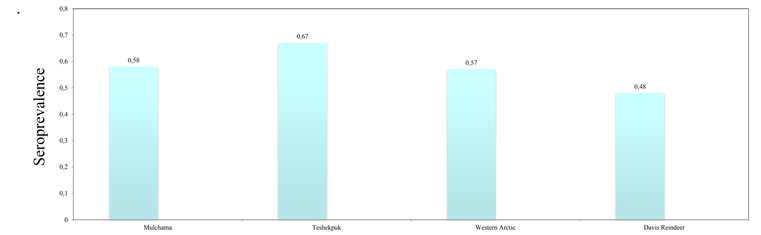


Figure 5. Seroprevalence of alphaherpes virueses in three Alaskan caribou herds and in a reindeer herd near Nome, Alaska.

Conclusions:

The overall seroprevalence of 47% in reindeer over 16 years with no significant change over time indicates that an alphaherpesvirus is endemic in this reindeer population.

As other alphaherpesviruses cross-react serologically to BoHV1, the VNT and PCR amplicon sequencing were required to determine which virus was circulating in Alaskan reindeer and caribou. Both VNT and PCR results indicated that the virus in question is most likely CvHV2, a virus also endemic in reindeer in Norway and Finland.

As the amplicons isolated here were identical to previous Norwegian and Finnish isolates, it leads to questions about whether the virus came from reindeer imported from Siberia or Norway in the 1890s or if the virus was present in caribou populations previous to these imports.

The impact of these viruses on the health of caribou and reindeer in Alaska is unknown. In Norway, CvHV2 has been shown to cause respiratory disease, keratoconjunctivitis and is likely associated with abortions (das Neves et al, 2009b,c).

It is unknown if herpesvirus causes the keratoconjunvitis reported in Alaskan reindeer or if it is a cause of abortions or neonatal mortalities in reindeer or caribou in Alaska.

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