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Short communication

Malignant catarrhal fever in American bison (*Bison bison*) experimentally infected with alcelaphine herpesvirus 2

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ABSTRACT

Malignant catarrhal fever (MCF), due to ovine herpesvirus 2 (OvHV-2), causes appreciable death loss in ranched bison (*Bison bison*) throughout North America. No vaccine exists to protect animals from disease. Since OvHV-2 has not been propagated *in vitro*, one strategy to develop a modified live vaccine is to use a closely related, non-pathogenic member of the malignant catarrhal fever virus family as a vector expressing potentially protective OvHV-2 epitopes. To date, no controlled experimental challenge studies with alcelaphine herpesvirus 2 (AlHV-2) derived from topi (*Damaliscus lunatus jimela*) have been reported The unique or light DNA segment of the AlHV-2 genome was sequenced and annotated and the virus was tested for its ability to infect and induce disease in American bison. Yearling bison were inoculated intranasally ($n = 4$) or intramuscularly ($n = 3$) with $2 \times 10^{-4.7}$ $TCID_{50}$ of AlHV-2, and monitored for infection and the development of disease. Six inoculated bison became infected with AlHV-2. Two of the six animals developed clinical signs and had gross and histological lesions consistent with terminal MCF, which differed in distribution from those in bison with MCF due to OvHV-2. One other animal developed minor clinical signs and had gross and histological pulmonary lesions consistent with early (pre-clinical) stages of MCF. Unmodified low cell culture passage AlHV-2 derived from topi is an unsuitable vaccine vector for the prevention of MCF. However, the annotated genome might be useful in identifying genes which could be deleted to potentially attenuate the virus for bison.

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1. Introduction

Malignant catarrhal fever (MCF) is a generally fatal disease, caused by some viruses in the genus *Macavirus*, subfamily *Gammaherpesvirinae*, affecting many ruminant species including domestic cattle, most cervid species, and North American bison (*Bison bison*). Deaths due to MCF caused by ovine herpesvirus 2 (OvHV-2), which is carried by most domestic sheep (*Ovis aries*), result in significant losses in the ranched bison (*B. bison*) industry in the United States and Canada. The development of an effective vaccine against MCF should improve control of the disease in susceptible species. An impediment to vaccine development is the inability to propagate OvHV-2 *in vitro*. An

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alternative approach is to use a closely related, non-pathogenic MCF virus as a vector for genes encoding key antigens of OvHV-2.

Alcelaphine herpesvirus 2 (AlHV-2) isolated from topi (*Damaliscus lunatus korrigum*) is considered non-pathogenic based on limited experimental studies involving parenteral inoculation of cattle and rabbits (Mushi et al., 1981). AlHV-2 derived from hartebeest (*Alcelaphus buselaphus*), another African antelope species, induced MCF when inoculated into cattle and rabbits (Reid and Rowe, 1973) and an AlHV-2-like virus thought to have originated in hartebeest caused MCF in Barbary red deer (Klieforth et al., 2002). It may be appropriate to consider AlHV-2 derived from topi and hartebeest as two distinct members of the *Macavirus* genus, and to use the designations topi-AlHV-2 and hartebeest-AlHV-2 until their relationship is clarified.

Topi-AlHV-2 has been propagated in embryonic topi and fetal aoudad (*Ammotragus lervia*) cell cultures (Mushi et al., 1981; Seal et al., 1989), and could be manipulated to generate a recombinant virus carrying desired OvHV-2 genes. The infectivity of the virus for bison was determined and the unique or light DNA segment of the topi-AlHV-2 genome was sequenced and annotated.

2. Materials and methods

2.1. Virus culture and genome sequencing

The AlHV-2 isolate 840412, originally isolated from a topi (*Damaliscus lunatus* ssp. *jimela*) at the Zoological Society of San Diego Zoo (Seal et al., 1989), was propagated in fetal mouflon sheep (*Ovis aries orientalis*) kidney cells (FMSK) maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS, penicillin (100 units/ml), streptomycin (100 μg/ml), and Fungizone (250 ng/ml). AlHV-2 inoculum was prepared by infecting FMSK cells, lysing cells by three freeze-thaw cycles once the cytopathic effect reached 80%, and clarifying the lysate by centrifugation for 25 min at 1159 × *g*. Aliquots were stored at −80 °C until use. The tissue culture infectious dose ($TCID_{50}$) of the inoculum was determined on FMSK cells.

For sequencing of the viral genome, culture supernatant from infected FMSK cells was harvested when cytopathic effect reached 80%. Virions were collected by ultracentrifugation through a 35% sucrose cushion and further clarified by DNAse digestion (0.5 mg/ml; Roche) to remove unprotected viral and cellular DNA. Viral DNA was released by the addition of proteinase K (0.5 mg/ml; Sigma-Aldrich) and SDS (0.3%), extracted twice with phenol/chloroform/isoamyl alcohol (24:24:1), and precipitated in 95% ethanol. The purified topi-AlHV-2 DNA was sheared with a Hydroshear (Genomic Solutions) and a shotgun library (4–5 kb) constructed using the pSMARTLCKan kit (Lucigen) according to the manufacturer's instructions. Clones (768) were sequenced in the forward and reverse directions using the BigDye Terminator v3.1 cycle sequencing kit on an ABI Prism 3130xl Genetic Analyzer (both from Applied Biosystems/Life Technologies). Sequence assembly was performed with PHRAP (www.phrap.org, Ewing and Green, 1998) and resulting contigs compared to the complete sequence of the AlHV-1 strain C500 as a scaffold to simplify orientation and link contigs. Annotation was performed manually and submitted to GenBank (KF274499).

2.2. Animal inoculation and sampling

Seven yearling bison (five male; two female), were purchased from a commercial producer and maintained at the animal facility at the University of Wyoming, Laramie, WY. The study was done in accordance with a university-approved animal care and use protocol. Before they were purchased, the bison were screened for the presence of OvHV-2 DNA using a semi-nested PCR assay (Li et al., 2004) and for MCF viral (MCFV) antibodies using a competitive inhibition ELISA (cELISA) (Li et al., 2001). None of the 7 had detectable OvHV-2 DNA or MCFV antibodies.

Because the AlHV-2 stock was contaminated with a bovine viral diarrhea virus (BVDV) determined to be a type 1b genotype (data not shown), attempts were made to eliminate BVDV from small aliquots of the stock using goat anti-BVDV (type 1 and type 2) neutralizing antibodies (kindly provided by Dr. Chungwon Chung, VMRD, Pullman, WA). This approach did not eliminate viral contamination so it was decided to use low passage (p7) untreated stock for the study. To mitigate confounding effects of BVDV infection, bison were immunized against BVDV on three occasions before inoculation with topi-AlHV-2, as described below.

The seven bison were vaccinated on three occasions against BVDV using a polyvalent commercial vaccine (Pyramid® 4 + Presponse® SQ; Boehringer Ingelheim Vetmedica, Inc., Fort Dodge, IA 50501). The BVDV component in the vaccine consisted of modified live Singer strain (1a genotype; cytopathic biotype) (Fulton, 2005). The other biological components in the vaccine were modified live bovine parainfluenza 3, bovine respiratory syncytial virus, and a *Mannheimia haemolytica* toxoid. The third and final vaccination was given four weeks before AlHV-2 inoculation.

Four bison were inoculated via intranasal nebulization (IN), as described previously for OvHV-2 (Li et al., 2004), with $2 \times 10^{-4.7}$ $TCID_{50}$ of topi-AlHV-2. Three other bison were inoculated with the same dose of virus intramuscularly (IM) in the thigh. Blood was collected weekly and assayed for topi-AlHV-2 DNA and MCFV antibodies. One of the IN inoculated bison died from intercurrent disease before any data could be obtained. A complete set of tissues was collected from all animals at the time of necropsy and fixed in 10% buffered formalin. Fixed tissues were embedded in paraffin and processed routinely for histological examination.

2.3. Nucleic acid and antibody analysis

DNA was extracted from peripheral blood leukocytes (PBL) and from tissues [liver, tracheobronchial (TB) lymph node, spleen, lung and urinary bladder] collected post-mortem using the Fast DNA kit (QBiogene) according to the manufacturer's directions. A 75 bp fragment of the AlHV-2 A4.5 gene was amplified and detected using the CFX96 Real-Time PCR Detection System (Bio-Rad).