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Evidence of Three New Members of Malignant Catarrhal Fever Virus Group in Muskox (*Ovibos moschatus*), Nubian ibex (*Capra nubiana*), and Gemsbok (*Oryx gazella*)

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ABSTRACT: Six members of the malignant catarrhal fever (MCF) virus group of ruminant rhadinoviruses have been identified to date. Four of these viruses are clearly associated with clinical disease: alcelaphine herpesvirus 1 (AlHV-1) carried by wildebeest (*Connochaetes* spp.); ovine herpesvirus 2 (OvHV-2), ubiquitous in domestic sheep; caprine herpesvirus 2 (CpHV-2), endemic in domestic goats; and the virus of unknown origin found causing classic MCF in white-tailed deer (*Odocoileus virginianus*; MCFV-WTD). Using serology and polymerase chain reaction with degenerate primers targeting a portion of the herpesviral DNA polymerase gene, evidence of three previously unrecognized rhadinoviruses in the MCF virus group was found in muskox (*Ovibos moschatus*), Nubian ibex (*Capra nubiana*), and gemsbok (South African oryx, *Oryx gazella*), respectively. Based on sequence alignment, the viral sequence in the muskox is most closely related to MCFV-WTD (81.5% sequence identity) and that in the Nubian ibex is closest to CpHV-2 (89.3% identity). The viral sequence in the gemsbok is most closely related to AlHV-1 (85.1% identity). No evidence of disease association with these viruses has been found.

Key words: Base sequence, gemsbok, herpesvirus, malignant catarrhal fever, muskox, Nubian ibex, oryx, phylogeny, rhadinovirus.

Malignant catarrhal fever (MCF), a herpesviral disease syndrome affecting principally ruminant species, is caused by a group of ruminant rhadinoviruses (Plowright, 1990). The MCF virus group has been tentatively defined by the presence of the 15-A antigenic epitope and an appropriate degree of base similarity in conserved regions of the DNA polymerase gene (Li et al., 2001a). At least six ruminant rhadinoviruses have so far been identified within the MCF virus group, four of which are clearly pathogenic. The first MCF virus was identified and isolated in vitro from wildebeest (*Connochaetes* spp.) by Plowright et al. (1960). This virus is classified as a rhadinovirus in the *gammaherpesvirinae* subfamily and termed alcelaphine herpesvirus 1 (AlHV-1) in reference to its principal reservoir host, the wildebeest (Roizmann et al., 1992). The disease induced by this virus, restricted to Africa and zoological collections where wildebeest are present, has been known as wildebeest-associated or 'African form' MCF. Domestic sheep are the worldwide source of the other major MCF virus (Reid and Buxton, 1984). The sheep-associated MCF agent, named ovine herpesvirus-2 (OvHV-2) on the basis of its DNA homology with AlHV-1 (Roizmann et al., 1992), has never been isolated in vitro. Other members of the MCF virus group were isolated from hartebeest (*Alcelaphus buselaphus)* and topi (*Damaliscus lunatus*) (Mushi et al., 1981) and from a roan antelope (*Hippotragus equinus*) (Reid and Bridgen, 1991), and termed alcelaphine herpesvirus 2 (AlHV-2) and hippotragine herpesvirus-1 (HiHV-1), respectively. These viruses have not been reported to cause clinical disease in nature. However, recognition of an AlHV-2-like MCF virus in diseased Barbary red deer (*Cervus elaphus barbarus*) (Klieforth et al., 2002) suggests that these viruses may be pathogenic for some species under certain circumstances.

Recently, two previously unrecognized pathogenic rhadinoviruses were reported within the MCF virus group. One, tenta-

tively termed MCF virus in white-tailed deer (*Odocoileus virginianus*; MCFV-WTD) was found causing the classic MCF syndrome in white-tailed deer in North America (Li et al., 2000). The carrier species for this virus has not yet been identified. The other, provisionally called caprine herpesvirus 2 (CpHV-2), is endemic in domestic goats (Li et al., 2001a) and was associated with alopecia, chronic weight loss, and dermatitis in two species of deer (Crawford et al., 2002; Li et al., 2003). In this short communication, we describe antigenic and DNA sequence evidence for three previously unrecognized rhadinoviruses belonging to the MCF virus group in muskox (*Ovibos moschatus*), Nubian ibex (*Capra nubiana*), and gemsbok (South African oryx, *Oryx gazella*).

For this study, a total of six sera and seven blood samples in ethylenediaminetetraacetic acid (EDTA) were collected from a captive herd of muskox in Saskatchewan, Canada (52°12′N, 106°63′W) and 24 lymph nodes were obtained from a free-ranging herd of muskox in Northwest Territory, Canada (71°59′N, 125°14′W). Six EDTA treated blood samples were obtained from clinically normal Nubian ibex: three from a zoo in California (USA) and three from a zoo in New England (USA). Two scimitar-horned oryx (*Oryx dammah*) blood samples were obtained from a Michigan (USA) zoo and one gemsbok sample came from a wildlife park in Ohio (USA). In addition, 45 EDTA blood samples were collected from free-ranging gemsbok on the White Sands Missile Range (~32°50′N, 106°30′W), New Mexico (USA).

Serum or plasma samples were collected to test for MCF viral antibody using a competitive ELISA (Li et al., 2001b). DNA purified from peripheral blood leukocytes (PBL) or frozen tissues were subjected to a consensus polymerase chain reaction (PCR) using a set of degenerate primers directing amplification of a portion of the herpesviral DNA polymerase gene (VanDevanter et al., 1996). Amplification conditions for the consensus PCR were as described previously (Li et al., 2000). Specific PCRs for OvHV-2, AlHV-1, and CpHV-2 were also as described previously (Li et al., 2000, 2001a). The products amplified by the consensus PCR were cloned and sequenced (Li et al., 2000). At least two clones from each PCR product were selected for sequencing. The 177-bp non-primer DNA sequences and the translated amino acid sequences were analyzed with the ClustalW program (European Bioinformatics Institute, Cambridge, UK) and the Phylip program (University of Washington, Seattle, Washington, USA). The portions of the DNA polymerase gene sequences obtained from muskox, Nubian ibex, gemsbok, and scimitar-horned oryx have been deposited in the National Center for Biotechnology Information database (GenBank accession numbers: AY212111, AY212112, AY212113, and AY212114).

As shown in Table 1, all sera or plasma samples from muskox ($n=13$), Nubian ibex ($n=6$), gemsbok ($n=46$), and scimitar-horned oryx ($n=2$) contained antibody against the MCF group of rhadinoviruses except two gemsbok. No DNA samples from muskox ($n=31$), Nubian ibex ($n=6$), gemsbok ($n=46$), and scimitar-horned oryx ($n=2$) yielded a signal on specific PCR for OvHV-2, AlHV-1, or CpHV-2 (Table 1). Consensus PCR amplified 230 bp DNA fragments from 18 of 31 PBL/lymph node DNAs from muskox, six of six from Nubian ibex, two of two from Scimitar-horned oryx, but only six of 46 from gemsbok (Table 1). The amplified PCR products were randomly selected, cloned, and sequenced. Sequence alignment revealed that all sequences from the seven muskox were identical, and closely related to, but distinct from the analogous regions from OvHV-2, AlHV-1, AlHV-2, MCFV-WTD, and CpHV-2 (Figs. 1, 2). The DNA sequence from the muskox was relatively close to OvHV-2 (79.2% identity) and MCFV-WTD (82.5% identity). All sequences from six Nubian ibex were also

TABLE 1. Malignant catarrhal fever viral (MCFV) DNA and antibody in muskox, Nubian ibex, gemsbok, and scimitar-horned oryx.

Species (scientific name)	Location	Number positive/number tested[a]					DNA sequence confirmation
		cELISA	AlHV-1 PCR	OvHV-2 PCR	CpHV-2 PCR	Consensus PCR	
Muskox (*Ovibos moschatus*)	Saskatchewan	13/13	0/31	0/31	0/31	18/31	7 (MCFV-Muskox)[b]
Nubian ibex (*Capra nubiana*)	New England, California	6/6	0/6	0/6	0/6	6/6	6 (MCFV-Ibex)
							6 (MCFV-Oryx)
Gemsbok (*Oryx gazella*)	New Mexico, Ohio[c]	44/46	0/46	0/46	0/46	6/46	1 (LHV-Oryx)[d]
Scimitar-horned oryx (*Oryx dammah*)	Michigan	2/2	0/2	0/2	0/2	2/2	2 (LHV-Oryx)

[a] cELISA=competitive enzyme-linked immunosorbent assay; AlHV-1 PCR=alcelaphine herpesvirus-1 polymerase chain reaction; OvHV-2=ovine herpesvirus-2; CpHV-2=caprine herpesvirus-2.
[b] PCR products from seven muskox DNA samples were cloned and sequenced. All sequences were identical.
[c] Only one gemsbok blood sample was obtained from Ohio and this animal contained MCF viral antibody and MCFV-Oryx sequence.
[d] Both MCFV-Oryx sequence and LHV-Oryx sequences were cloned from the same individual LHV-Oryx=abbreviation for the virus close to bovine lymphotropic herpesvirus recognized in oryx.

identical and closer to CpHV-2 (89.3% identity) than to any other members of the MCF virus group (Figs. 1, 2). The PBL DNA extracted from only six of 44 antibody-positive gemsbok yielded 230 bp amplicons on consensus PCR. The sequences of all six were identical and were 85.1% homologous to AlHV-1 (Figs. 1, 2). However, two distinct sequences were amplified from one gemsbok. One was closely related to AlHV-1 (as above) and the other most resembled the so-called bovine lymphotropic herpesvirus (BLHV) with 66.3% identity (Rovnak et al., 1998). The consensus PCR amplified 230 bp DNA fragments from both of the seropositive Scimitar-horned oryx that were identical to the sequence amplified from the gemsbok, which was more similar to BLHV than the MCF virus group (Fig. 2).

Oryx and muskox have antibodies that cross-react with AlHV-1 antigens (Reid et al., 1975; Heuschele et al., 1984; Li et al., 1996), implying that these species may be the natural hosts for this group of viruses (Plowright, 1986). Combined PCR, sequence, and serology data from this study provide evidence that muskox, Nubian ibex, and gemsbok are infected with previously unrecognized rhadinoviruses that are closely related to existing MCF group viruses.

High seroprevalence to the MCF virus group has been shown in muskox (Li et al., 1996; Zarnke et al., 2002). In the mid-1990s, several cases of muskox with chronic hair and weight loss were submitted to the Washington Animal Disease Diagnostic Laboratory, Pullman, Washington. All five were positive for MCF viral antibody by competitive ELISA (cELISA); however, OvHV-2-specific PCR failed to yield a signal from PBLs or tissues of these animals. This suggested that they were infected not with OvHV-2, but with a closely-related virus that shares the MCF virus group-specific epitope. However, it has not been established whether this virus was responsible for the chronic hair and weight loss and whether it was the same virus as

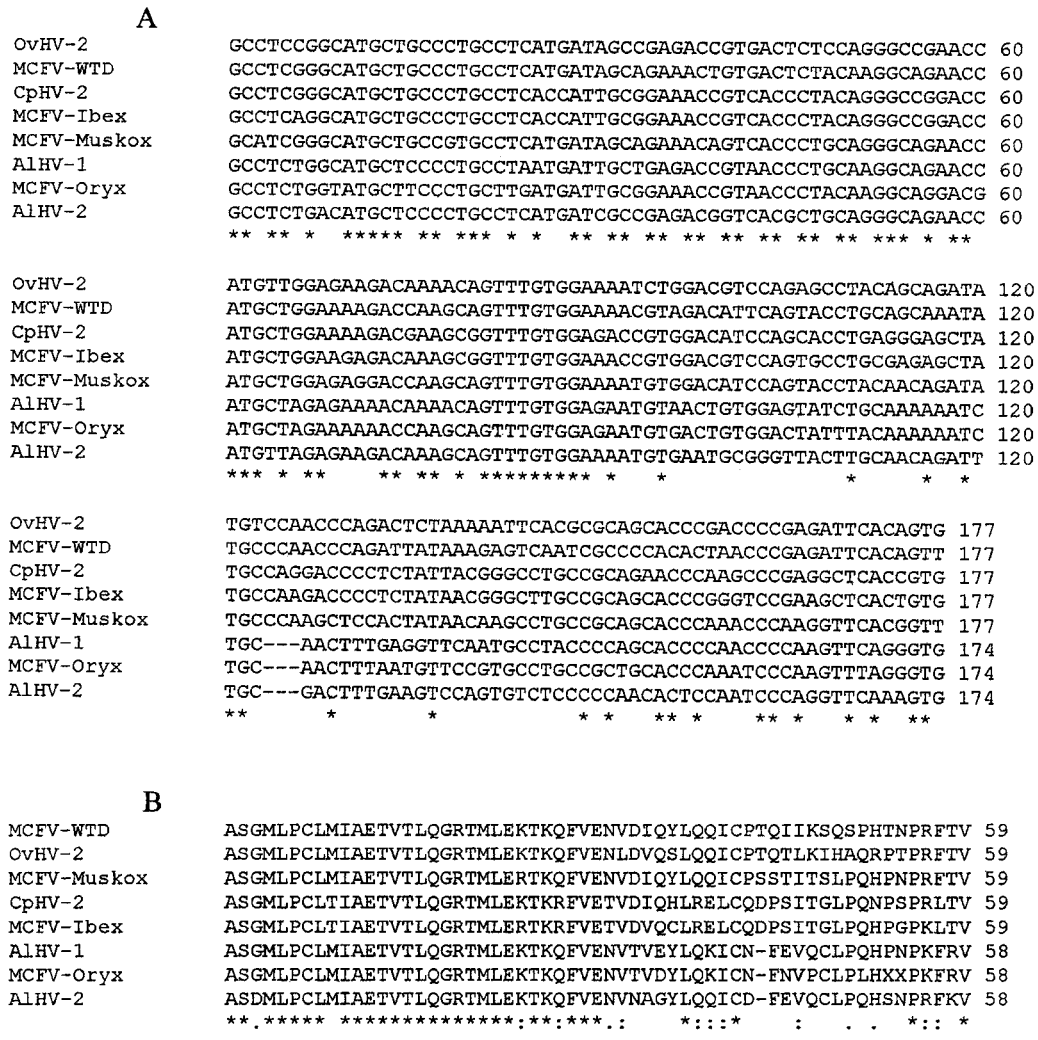

FIGURE 1. (A) Comparison of the nucleotide sequences of a region of the herpesviral DNA polymerase gene derived from newly identified MCF viruses in muskox (MCFV-Muskox), ibex (MCFV-Ibex), and gemsbok (MCFV-Oryx) with the homologous region of OvHV-2, AlHV-1, MCFV-WTD, and CpHV-2. * represents those nucleotides that were identical in all the viruses. (B) Comparison of the translated amino acid sequences of the same gene region shown in (A). *, :, and . represent identical residues, conservative substitutions, and somewhat similar residues, respectively. The OvHV-2, AlHV-1, MCFV-WTD, and CpHV-2 DNA polymerase sequences were obtained from GenBank, the National Center for Biotechnology Information database. The accession numbers are: OvHV-2: AF031812; AlHV-1: AF031809; MCFV-WTD: AF181468; and CpHV-2: AF275941. Multiple alignments were produced by the ClustalW software program from European Bioinformatics.

found in this study. Another MCF group member, CpHV-2, has been linked to chronic wasting and hair loss in sika deer (*Cervus nippon*) and white-tailed deer (Crawford et al., 2002; Li et al., 2003). More studies are needed to definitively answer the question of whether the MCF-group virus in muskox is pathogenic, either for its carrier host or for other ruminants.

Several reports have shown neutralizing antibody against AlHV-1 in gemsbok from East Africa and North America (Reid et al., 1975; Heuschele et al., 1984). Moreover, it has been observed at the National

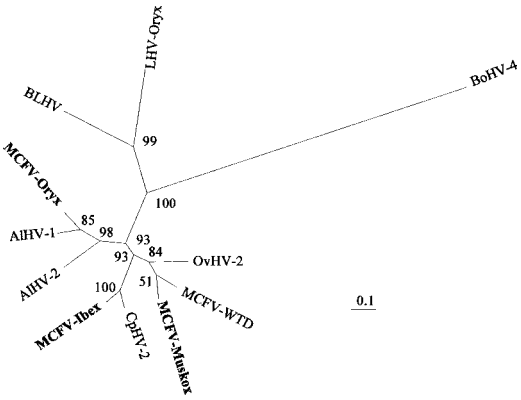

FIGURE 2. Phylogram display based on the DNA sequences of homologous DNA polymerase gene fragments from eight members of the MCF group of viruses and other members of the ruminant rhadinoviruses. The phylogenetic tree was constructed with the NEIGHBOR program of the PHILIP software package (University of Washington), using nucleotide sequences from the following: BoHV-4 (bovine herpesvirus 4, AF 031811); BLHV (AF031808); CpHV-2 (AF275941); MCFV-WTD (AF181468); OvHV-2 (AF031812); AlHV-2 (AF 275942); AlHV-1 (AF031809); MCFV-Muskox (AY212111); MCFV-Ibex (AY212112); and MCFV-Oryx (AY212113). Bootstrap values shown at branch points were obtained from 100 data sets. The branch lengths are proportionate to relative genetic distances as shown by the bar representing a 10% of the distance.

Veterinary Services Laboratories (Ames, Iowa) that gemsbok were negative by AlHV-1 PCR, but seropositive by immunoperoxidase and AlHV-1 neutralization tests (J. Warg, pers. comm.). These same animals were seropositive by cELISA (Li et al., unpubl. data). The present data establish the presence of a new, oryx-associated member of the MCF virus group. Flach et al. (2002), using PCR amplifying different regions of the AlHV-1 and OvHV-2 genomes, support this concept.

The present study provides evidence that oryx are infected with at least two distinct rhadinoviruses: one MCF-group virus closely related to AlHV-1 and the other a non-MCF-group virus related to BLHV in the so-called 'lymphotropic herpesvirus' group.

Currently there is no evidence that either of these viruses is pathogenic in oryx. Recent recognition of an 'AlHV-2-like' MCF-group virus causing disease in Barbary red deer in the San Diego Wildlife Park (Klieforth et al., 2002) highlights the question of the role of the oryx-associated MCF virus in clinically-susceptible hosts. Although additional pathogenic members of the MCF virus group are regularly being found, more data are needed before conclusions can be drawn about the pathogenicity of the oryx virus.

Nine members of the MCF virus group and several members of the non-MCF lymphotropic herpesvirus group have now been recognized. The terms 'MCF virus group', and 'non-MCF lymphotropic herpesvirus group' are quite cumbersome for communication. In order to facilitate communication about these agents until taxonomy and nomenclature are more clearly defined, we have proposed to designate the MCF-group lymphotropic herpesviruses, the non-MCF-group lymphotropic herpesviruses, and the bovine herpesvirus-4-type viruses, as ruminant rhadinoviruses type 1, type 2, and type 3, respectively (Crawford et al., 2002). On an interim basis, these three new members of the MCF virus group could appropriately be referred to as type 1 ruminant rhadinoviruses of the muskox, ibex, and oryx, respectively, and the non-MCF group oryx virus referred to as the type 2 ruminant rhadinovirus of oryx.

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