In the course of investigating the malignant catarrhal fever (MCF) subgroup of rhadinoviruses, seven novel rhadinoviruses were identified in a variety of ruminants, including domestic sheep, bighorn sheep, bison, black-tailed deer, mule deer, fallow deer, elk and addax. Based on the DNA polymerase gene sequences, these newly recognized viruses clustered into a second distinct subgroup in ruminants with three members identified previously in cattle, goats and oryx. Phylogenetic analysis revealed that the currently known ruminant rhadinoviruses appear to comprise three distinct genetic lineages: (i) the MCF subgroup, defined by sequence identity and the presence of the 15A antigenic epitope; (ii) a second distinct subgroup, devoid of the 15A epitope, which contains the previously reported bovine lymphotropic herpesvirus and related viruses; and (iii) a third distinct subgroup represented by *Bovine herpesvirus 4*. Comparison of phylogenetic trees between the rhadinoviruses and their corresponding hosts further supports the gammaherpesvirus and host co-evolution theory.

The subfamily *Gammaherpesvirinae*, which is expanding rapidly as novel species are discovered, contains two genera of lymphotropic viruses, *Lymphocryptovirus* and *Rhadinovirus*, members of which are receiving increasing recognition as significant pathogens in humans, non-human primates and other animal species (Moore *et al.*, 1996). The viruses associated with the clinical syndrome known as malignant catarrhal fever (MCF) are within one recently defined subgroup of the ruminant rhadinoviruses (RuRVs) (Crawford *et al.*, 2002). The MCF subgroup of rhadinoviruses contains at least nine members, four of which are clearly associated with the disease in clinically susceptible species (Plowright, 1990; Li *et al.*, 2000; Crawford *et al.*, 2002). The first identified MCF virus is *Alcelaphine herpesvirus 1* (AlHV-1), which is carried by wildebeest and causes the classic ‘African’ form of MCF, also known as wildebeest-associated MCF (Plowright *et al.*, 1960). A second virus known as *Ovine herpesvirus 2* (OvHV-2) is the major causative agent of MCF worldwide (Reid & Buxton, 1989). This virus is endemic in sheep and causes sheep-associated MCF in various ruminant species as well as in pigs (Loken *et al.*, 1998). Two previously unrecognized pathogenic members of the MCF virus group were reported recently. One, causing the classic MCF syndrome in white-tailed deer (Li *et al.*, 2000; Kleiboeker *et al.*, 2002), is now tentatively called MCFV-WTD (WTD, white-tailed deer). 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). Both MCFV-WTD and CpHV-2 are distinct from, but closely related to, OvHV-2 and AlHV-1. Early indications are that CpHV-2 may be moderately less virulent than OvHV-2 or AlHV-1. To date, CpHV-2 has been reported to cause disease in two species of deer (Crawford *et al.*, 2002; Keel *et al.*, 2003; Li *et al.*, 2003a), but little information is available about this virus in other clinically susceptible species.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are D0083950–D0083952.
Studies from this and other laboratories have found that several other species of ruminants are infected with well-adapted herpesviruses that belong to the same subgroup to which the classic MCF viruses (from sheep and wildebeest) belong. These viruses include Alcelaphine herpesvirus 2 (AlHV-2) in hartebeest and topi (Mushi et al., 1981), Hippotragine herpesvirus 1 (HiHV-1) in roan antelope (Reid & Bridgen, 1991) and three new members recently recognized in musk ox, oryx and ibex, respectively (Li et al., 2003b). These viruses are closely related both genetically and antigenically and have not been reported to cause clinical disease in nature. In this short communication, we report seven novel viruses in a variety of ruminant species that cluster into a second distinct subgroup of RuRVs, and one new member of the MCF subgroup. The genetic relationships of these viruses to other known rhadinoviruses in ruminant species are also described.

EDTA-anticoagulated blood samples were collected from various ruminant species of interest, namely domestic sheep, bighorn sheep, bison, black-tailed deer, mule deer, elk, fallow deer, addax and aoudad, from zoos, game farms or free ranges in several states of North America, as shown in Table 1. Plasma was examined for MCF viral antibody by using competitive inhibition ELISA (cELISA) (Li et al., 2001b). DNA purified from peripheral blood leukocytes was subjected to consensus PCR, targeting a portion of the herpesviral DNA polymerase gene (VanDevanter et al., 1996; Li et al., 2000). At least two clones from each PCR product were selected for sequencing. The DNA sequences (177 bp), which did not include the primer regions, from the DNA polymerase gene and the translated amino acid sequences were analysed with the CLUSTAL W alignment program (European Bioinformatics Institute, Cambridge, UK), the PAUP* version 4.0 phylogeny program (Sinauer Associates, Inc., Publishers, Sunderland, MA, USA) and the PHYLIP phylogeny program (University of Washington, Seattle, WA, USA). The DNA polymerase gene sequences obtained from this study have been deposited in GenBank; accession numbers are listed in Fig. 1.

Based on the detection and analysis of herpesviral DNA polymerase gene sequences, in total, eight novel rhadinoviruses were identified in nine ruminant species. Specifically, the sequences of these novel RuRVs were identified from two domestic sheep, two bighorn sheep, eight black-tailed deer, three mule deer, six elk, eight fallow deer, two addax and one aoudad, as shown in Table 1, and a sequence

<table>
<thead>
<tr>
<th>Ruminant carrier (species)</th>
<th>Tentative name</th>
<th>No. animals</th>
<th>Locations</th>
<th>Identity* (%) to BLHV</th>
<th>No. animals positive for MCFV antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bison (Bison bison)</strong></td>
<td>Bison-LHV</td>
<td>13</td>
<td>WY, USA</td>
<td>94-3</td>
<td>6</td>
</tr>
<tr>
<td>Domestic goats† (Capra hircus)</td>
<td>Domestic goat-LHV</td>
<td>6</td>
<td>WA, USA; Alberta, Canada</td>
<td>91-4</td>
<td>5</td>
</tr>
<tr>
<td>Domestic sheep (Ovis aries)</td>
<td>Domestic sheep-LHV</td>
<td>2</td>
<td>ID, USA; WA, USA</td>
<td>76-8</td>
<td>2</td>
</tr>
<tr>
<td>Bighorn sheep (Ovis musimon)</td>
<td>Bighorn sheep-LHV</td>
<td>2</td>
<td>WA, USA</td>
<td>75-3</td>
<td>2</td>
</tr>
<tr>
<td>Oryx†‡ (Oryx dama; Oryx gazella)</td>
<td>Oryx-LHV</td>
<td>7</td>
<td>NM, USA; MI, USA</td>
<td>64-4</td>
<td>7</td>
</tr>
<tr>
<td>Addax (Addax nasomaculatus)</td>
<td>Addax-LHV</td>
<td>2</td>
<td>MI, USA</td>
<td>64-9</td>
<td>0</td>
</tr>
<tr>
<td>Black-tailed deer (Odocoileus hemionus)</td>
<td>Black-tailed deer-LHV</td>
<td>8</td>
<td>WA, USA</td>
<td>47-7</td>
<td>0</td>
</tr>
<tr>
<td><strong>Mule deer (Odocoileus hemionus)</strong></td>
<td>Mule deer-LHV</td>
<td>3</td>
<td>WA, USA</td>
<td>47-7</td>
<td>0</td>
</tr>
<tr>
<td><strong>Fallow deer (Dama dama)</strong></td>
<td>Fallow deer-LHV</td>
<td>8</td>
<td>NC, USA</td>
<td>51-7</td>
<td>4</td>
</tr>
<tr>
<td>Elk (Cervus elaphus)</td>
<td>Elk-LHV</td>
<td>6</td>
<td>WA, USA</td>
<td>50-0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Aoudad (Ammotragus lervia)</strong></td>
<td>Aoudad-MCFV</td>
<td>1</td>
<td>NC, USA</td>
<td>52-9§</td>
<td>1</td>
</tr>
</tbody>
</table>

*Identity was based on a portion of the herpesviral DNA polymerase gene by using sequence pairwise distances from CLUSTAL W by the DNAStar program.
†Parts of the data were reported previously (Li et al., 2001, 2003b).
‡Both scimitar-horned oryx (Oryx dama; Oryx gazella) are carriers for Oryx-LHV.
§Nucleic acid identity of Aoudad-MCFV is 74-6% to CpHV-2, 68-9% to OvHV-2 and 70-1% to AlHV-1.
‖Amino acid identity of Aoudad-MCFV is 74-6% to CpHV-2, 72-9% to OvHV-2 and 69-0% to AlHV-1.
HERPESVIRUS (BLHV) (ROVNACK ET AL., 1995) ISOLATED FROM CATTLE WAS TERMED BOVINE LYMPHOTROPIC HERPESVIRUS 2 (OvHV-2). THE VIRUS PREVIOUSLY IDENTIFIED IN CATTLE, DOMESTIC GOATS AND ORYX (BOTH GEMSBOK AND SCIMITAR-HORNED ORYX). THE VIRUS PREVIOUSLY IDENTIFIED AND ISOLATED FROM CATTLE WAS TERMED BOVINE LYMPHOTROPIC HERPESVIRUS (BLHV) (ROVNACK ET AL., 1998) AND USED AS THE PROTOTYPE FOR THIS SUBGROUP. WITHIN THIS SUBGROUP, THERE APPEARED TO BE TWO GENETIC SUBLINEAGES HOSTED BY THE SPECIES IN THE FAMILIES BOVIDAE OR CERVIDAE. THE BASE SEQUENCES OF THE HERPESVIRAL POLYMERASE GENE FRAGMENTS DERIVED FROM BISON AND GOATS WERE HIGHLY SIMILAR TO THAT OF BLHV, WITH OVER 90 % IDENTITY. ALTHOUGH SEQUENCES FOR BIGHORN SHEEP AND DOMESTIC SHEEP WERE OVER 90 % IDENTICAL TO EACH OTHER, THERE WAS ONLY 71–75 % IDENTITY TO THAT OF BLHV.

ALIGNMENTS AND PHYLGENETIC ANALYSES OF THE SEQUENCES REVEALED THAT THE RHADINOVIRUSES FROM THE DOMESTIC SHEEP, BIGHORN SHEEP, BLACK-TAILED DEER, MULE DEER, FALLOW DEER, ELK AND ADDAX IN THIS STUDY CLUSTERED INTO A DISTINCT SUBGROUP THAT IS RELATED CLOSELY TO THE RHADINOVIRUSES IDENTIFIED PREVIOUSLY IN CATTLE, DOMESTIC GOATS AND ORYX (BOTH GEMSBOK AND SCIMITAR-HORNED ORYX). THE VIRUS PREVIOUSLY IDENTIFIED AND ISOLATED FROM CATTLE WAS TERMED BOVINE LYMPHOTROPIC HERPESVIRUS (BLHV) (ROVNACK ET AL., 1998) AND USED AS THE PROTOTYPE FOR THIS SUBGROUP. WITHIN THIS SUBGROUP, THERE APPEARED TO BE TWO GENETIC SUBLINEAGES HOSTED BY THE SPECIES IN THE FAMILIES BOVIDAE OR CERVIDAE. THE BASE SEQUENCES OF THE HERPESVIRAL POLYMERASE GENE FRAGMENTS DERIVED FROM BISON AND GOATS WERE HIGHLY SIMILAR TO THAT OF BLHV, WITH OVER 90 % IDENTITY. ALTHOUGH SEQUENCES FOR BIGHORN SHEEP AND DOMESTIC SHEEP WERE OVER 90 % IDENTICAL TO EACH OTHER, THERE WAS ONLY 71–75 % IDENTITY TO THAT OF BLHV. THE SEQUENCES FOR BLACK-TAILED DEER AND MULE DEER WERE IDENTICAL EXCEPT FOR ONE MISMATCH. THIS IS NOT SURPRISING, AS THE BLACK-TAILED DEER IS A SUBSPECIES OF THE MULE DEER. VIRAL SEQUENCES FROM ELK AND FALLOW DEER WERE 82–8 AND 77 % IDENTICAL TO THAT OF MULE DEER, WHEREAS THE SEQUENCES BETWEEN THE VIRUSES FROM THE FOUR CERVIDS AND BLHV WERE OVER 50 % IDENTICAL. ONLY FOUR NUCLEOTIDE MISMATCHES (97.7 % IDENTITY) WERE FOUND BETWEEN SEQUENCES FOR THE ORYX AND ADDAX, BOTH OF WHICH ARE IN THE SUBFAMILY HIPPOPOTRAMINAE.


THE PHYLOGENETIC TREE (FIG. 1B), BASED ON A PORTION OF THE HERPESVIRAL POLYMERASE GENE, INDICATES THAT CURRENTLY KNOWN RuRVs CLUSTER INTO THREE DISTINCT GENETIC LINES: (I) THE MCF SUBGROUP, DEFINED BY SEQUENCE IDENTITY AND THE PRESENCE OF THE 15A EPITOPIC epitope (LI ET AL., 1994), ALSO REFERRED TO AS TYPE 1 RuRV; (II) A SECOND DISTINCT SUBGROUP, DEVOID OF THE 15A EPITOPIC, WHICH CONTAINS BLHV AND RELATED NON-MCF LYMPHOTROPIC HERPESVIRUSES, REFERRED TO AS TYPE 2 RuRV; AND (III) A THIRD DISTINCT SUBGROUP REPRESENTED BY Bovine herpesvirus 4 (BoHV-4) AND REFERRED TO AS TYPE 3 RuRV. WHETHER ALL TYPE 2 RuRVs ARE DEVOID OF THE 15A EPITOPIC, WHICH IS PRESENT IN ALL MEMBERS OF TYPE 1 RuRVs (MCF SUBGROUP), HAS BEEN A MATTER OF DEBATE. AS MANY SPECIES, INCLUDING DOMESTIC SHEEP, DOMESTIC GOATS, BIGHORN SHEEP, BISON, ORYX AND FALLOW DEER, ARE CO-INFECTED WITH BOTH TYPE 1 AND TYPE 2 RuRVs, IT IS NOT POSSIBLE TO SPECIFY THE VIRUS RESPONSIBLE FOR THE ANTIBODY TO THE 15A EPITOPIC. HOWEVER, THE FOLLOWING EVIDENCE SUGGESTS THAT THE 15A EPITOPIC IS NOT PRESENT IN THE TYPE 2 RuRV: (I) THE 15A mAb USED IN THE cELISA DID NOT REACT WITH BLHV (PENN-47 ISOLATE) (OSORIO ET AL., 1985) IN BLHV-INFECTED CELLS BY INDIRECT IMMUNOFLOUORESCENCE ASSAY; (II) SERUM FROM A COW INFECTED WITH BLHV DID NOT INHIBIT THE 15A mAb IN THE cELISA; (III) A SIMILAR PERCENTAGE OF BISON HAD TYPE 2 RuRV REGARDLESS OF THEIR MCF ANTIBODY STATUS; AND (IV) IN THIS STUDY, ALL BLACK-TAILED DEER, MULE DEER AND ELK DETERMINED TO HAVE TYPE 2 RuRVs WERE NEGATIVE FOR ANTIBODY AGAINST THE 15A EPITOPIC. AS THERE ARE NO SPECIFIC, SENSITIVE ASSAYS AVAILABLE FOR THESE NEWLY RECOGNIZED RuRVs, THE PREVALENCE OF THESE RuRVs IN INDIVIDUAL SPECIES IS NOT KNOWN.

CURRENTLY, NO DISEASE ASSOCIATION HAS BEEN FOUND WITH THESE NEWLY IDENTIFIED RuRVs. HOWEVER, THE RECOGNITION OF AN AHV-2-LIKE MCF VIRUS IN DISEASED BARBARY RED DEER (Cervus elaphus barbarus) (KLEIFORTH ET AL., 2002) RAISES THE QUESTION OF WHETHER THESE VIRUSES MAY ALSO BE PATHOGENIC FOR CERTAIN SPECIES UNDER CERTAIN CONDITIONS. ALTHOUGH ADDITIONAL PATHOGENIC MEMBERS OF THE MCF VIRUS GROUP ARE BEING FOUND REGULARLY, MORE AETIOLOGICAL AND EPIDEMIOLOGICAL STUDIES ARE NEEDED BEFORE CONCLUSIONS CAN BE DRAWN ABOUT THE PATHOGENICITY OF ANY NEWLY IDENTIFIED VIRUSES.

HERPESVIRUSES ARE HIGHLY DISSEMINATED IN NATURE AND MOST MAMMALIAN SPECIES CARRY AT LEAST ONE HERPESVIRUS. MANY MORE HERPESVIRUSES, PARTICULARLY MEMBERS OF THE SUBFAMILY Gammaherpesvirinae, HAVE BEEN RECOGNIZED RECENTLY BY USING NEWER MOLECULAR TECHNOLOGY IN A VARIETY OF SPECIES (EHLERS ET AL., 2003). DURING THE COURSE OF INVESTIGATING MCF VIRUSES IN RUMINANT SPECIES, WE HAVE IDENTIFIED SIX RHADINOVIRUSES WITHIN THE MCF SUBGROUP (TYPE 1 RuRV) AND NINE RHADINOVIRUSES BELONGING TO THE NON-MCF SUBGROUP (TYPE 2 RuRV). INTERESTINGLY, THE PHYLOGENETIC TREES OF THESE RuRVs
within the subgroups seem to have certain similarities with the evolutionary relationships of the corresponding hosts. In order to compare the evolutionary similarities between the rhadinoviruses and their corresponding hosts, phylogenetic trees were constructed based on the sequences of mitochondrial cytochrome \( b \) proteins of the corresponding hosts. All mitochondrial cytochrome \( b \) sequences were obtained from GenBank and their accession numbers are listed in Fig. 2. As shown in Fig. 2(a), the phylogenetic tree of rhadinoviruses in the MCF subgroup (type 1 RuRV) has similar branch patterns to their corresponding carrier hosts except for the virus carried by musk ox. Based on the relationship shown in the host tree, the MCF virus in musk ox should be related more closely to CpHV-2 than to OvHV-2. Comparison of the trees for the type 2 RuRV (non-MCF subgroup) and their hosts (Fig. 2b) reveals a great degree of similarity between the two trees, with the exception of the virus carried by domestic goats (Goat-LHV). This virus has 93\% amino acid identity to BLHV, the virus identified in cattle. The reason why this type 2 RuRV in domestic goats is related so closely to BLHV and the virus in bison rather than to any other type 2 RuRV carried by the species in the subfamily Caprinae is not clear. To verify that the exception observed in this study was not due to analysis of the limited segment

![Fig. 1. Alignment (a) and phylogenetic tree (b) of RuRVs based on amino acid sequences of DNA polymerase gene fragments. The sequence alignment was carried out by using the CLUSTAL W alignment program and the phylogenetic tree was constructed by using the PHYLIP program. Bootstrap values are shown at branch points. GenBank accession numbers for the sequences are shown on the tree.](image-url)
of herpesviral DNA polymerase genes, an additional DNA fragment (513 bp) of the herpesviral glycoprotein B gene amplified from both the virus in goat and BLHV showed 97.3% identity in amino acid sequence (data not shown). Although it is not certain whether some of these species from which the virus was identified are the natural or accidental hosts, comparison of the trees between the viruses and corresponding hosts strongly suggests that these species are likely to be the natural hosts for the viruses. Co-evolution of host and virus lineages within the subfamily *Gammaherpesvirinae* has been proposed (McGeoch, 2001). Phylogenetic relationships of selected members of the subfamily have been intensively investigated using a large set of virus genes, indicating that the majority of the major viral lineages arose in a co-evolutionary manner with host lineages (McGeoch et al., 2005). The phylogenetic trees from RuRVs and their corresponding hosts from this study further support the gammaherpesvirus and host co-evolution theory.

Over 20 rhadinoviruses have been identified in a variety of ruminant species. Based on their genetic lineages, these viruses clustered into three distinct subgroups and are provisionally designated type 1, type 2 and type 3 ruminant rhadinovirus, respectively (Crawford et al., 2002). Only a few viruses (AIHV-1, AIHV-2, OvHV-2 and BoHV-4) are formally classified (Minson et al., 2000). In order to facilitate communication about these agents, until formal nomenclature is more clearly defined, we propose to use MCF virus (MCFV) as the common term for type 1 RuRV (MCF subgroup) and lymphotropic herpesvirus (LHV) as the common term for type 2 RuRV (non-MCF subgroup), although the viruses in all subgroups are lymphotropic. Individual viruses in the subgroup are termed by the name of corresponding host plus the common term of the subgroup. For examples, the two rhadinoviruses recognized in oryx are called Oryx-MCFV and Oryx-LHV, respectively.

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**References**


