

Short Communication

A novel subgroup of rhadinoviruses in ruminants

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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.

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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* (AIHV-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 AIHV-1. Early indications are that CpHV-2 may be moderately less virulent than OvHV-2 or AIHV-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.

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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* (AIHV-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 1. Newly recognized rhadinoviruses in ruminants

Ruminant carrier (species)	Tentative name	No. animals	Locations	Identity* (%) to BLHV		No. animals positive for MCFV antibody
				Nucleic acid	Amino acid	
Bison (<i>Bison bison</i>)	Bison-LHV	13	WY, USA	94·3	91·4	6
Domestic goats† (<i>Capra hircus</i>)	Domestic goat-LHV	6	WA, USA; Alberta, Canada	91·4	93·1	5
Domestic sheep (<i>Ovis aries</i>)	Domestic sheep-LHV	2	ID, USA; WA, USA	75·3	76·8	2
Bighorn sheep (<i>Ovis musimon</i>)	Bighorn sheep-LHV	2	WA, USA	71·8	75·0	2
Oryx†‡ (<i>Oryx dammah</i> ; <i>Oryx gazella</i>)	Oryx-LHV	7	NM, USA; MI, USA	64·4	72·4	7
Addax (<i>Addax nasomaculatus</i>)	Addax-LHV	2	MI, USA	64·9	75·9	0
Black-tailed deer (<i>Odocoileus hemionus</i>)	Black-tailed deer-LHV	8	WA, USA	47·7	53·4	0
Mule deer (<i>Odocoileus hemionus</i>)	Mule deer-LHV	3	WA, USA	47·7	53·4	0
Fallow deer (<i>Dama dama</i>)	Fallow deer-LHV	8	NC, USA	51·7	58·6	4
Elk (<i>Cervus elaphus</i>)	Elk-LHV	6	WA, USA; CA, USA	50·0	53·4	0
Aoudad (<i>Ammotragus lervia</i>)	Aoudad-MCFV	1	NC, USA	52·9§	51·7	1

*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 dammah*) and South African oryx (gemsbok, *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 AIHV-1.

||Amino acid identity of Aoudad-MCFV is 74·6% to CpHV-2, 72·9% to OvHV-2 and 69·0% to AIHV-1.

suggesting a new rhadinovirus was also amplified from 13 bison in this study. This sequence was identical to the sequence found in bison and deposited in GenBank (accession no. AY057986) by Kleiboeker and colleagues in 2001. MCF viral antibody was detected in six of 13 bison, two of two domestic sheep, two of two bighorn sheep, four of eight fallow deer, seven of seven oryx and one of one aoudad. None of the black-tailed deer, mule deer, elk or addax from which the novel rhadinovirus DNA sequences were identified was antibody-positive by cELISA (Table 1). OvHV-2 DNA was detected in all antibody-positive domestic sheep and bighorn sheep.

Alignments and phylogenetic 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) (Rovnak *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 only about 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 Hippotraginae.

In total, 11 rhadinoviruses have been identified within the MCF subgroup from different ruminant species, 10 of which were reported previously (Plowright, 1990; Li *et al.*, 2000, 2001a, 2003b). The newly recognized rhadinovirus within the MCF subgroup in this study was found in an aoudad from a game farm in North Carolina, USA. Among 14 aoudad examined, two were found to be MCF viral antibody-positive by cELISA, four were OvHV-2-specific PCR-positive, and one had a sequence that was distinct from, but related closely to, those of existing members of the MCF subgroup (Fig. 1a). The data suggest that the aoudad was infected with at least two rhadinoviruses, both of which are members of the MCF subgroup. Although HiHV-1 has been previously isolated from a roan antelope (Reid & Bridgen, 1991), no sequence information has been derived from the virus. In order to perform a complete phylogenetic analysis of all existing rhadinoviruses within the MCF subgroup, HiHV-1 DNA was extracted from an infected

rabbit cell line (Reid & Bridgen, 1991). A portion of the herpesviral DNA polymerase gene from HiHV-1 was amplified and sequenced. Interestingly, the sequence from HiHV-1 was identical to the sequence of MCF virus identified in oryx (Li *et al.*, 2003b), indicating that both oryx and roan antelope carry the same or a very closely related virus.

The phylogenetic tree (Fig. 1b), based on a portion of the herpesviral DNA polymerase gene, indicates that currently known RuRVs cluster into three distinct genetic lineages: (i) the MCF subgroup, defined by sequence identity and the presence of the 15A antigenic epitope (Li *et al.*, 1994), also referred to as type 1 RuRV; (ii) a second distinct subgroup, devoid of the 15A epitope, 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 epitope, 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 epitope. However, the following evidence supports that the 15A epitope 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 immunofluorescence 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 epitope. 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 AIHV-2-like MCF virus in diseased Barbary red deer (*Cervus elaphus barbarus*) (Klieforth *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

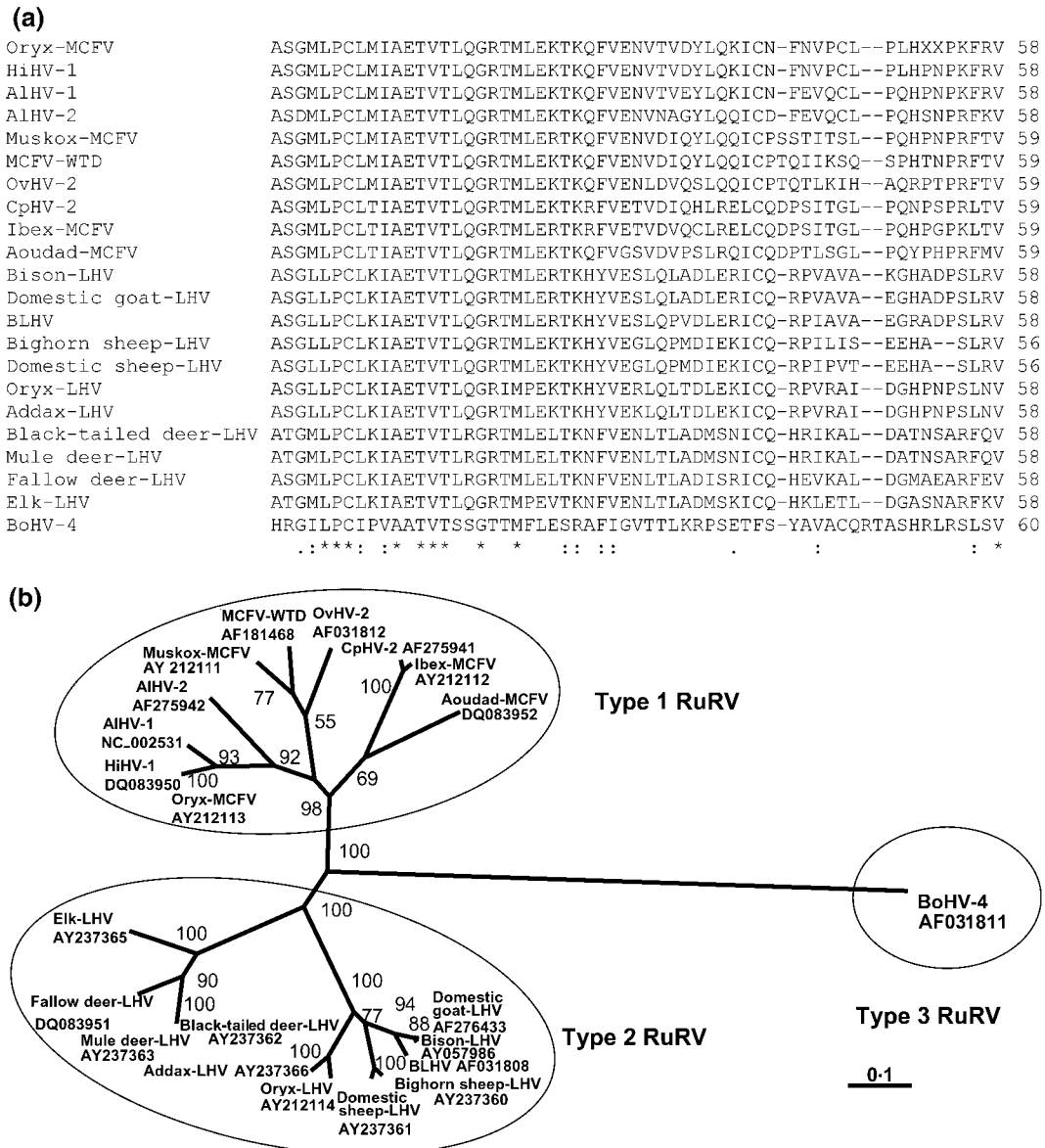


Fig. 1. Alignment (a) and phylogenetic tree (b) of RuRVs based on amino acid sequences of DNA polymerase gene fragments. The sequence alignment was carried out by using the CLUSTAL W alignment program and the phylogenetic tree was constructed by using the PHYLIP program. Bootstrap values are shown at branch points. GenBank accession numbers for the sequences are shown on the tree.

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 rhabdoviruses 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 rhabdoviruses 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 93.1% 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

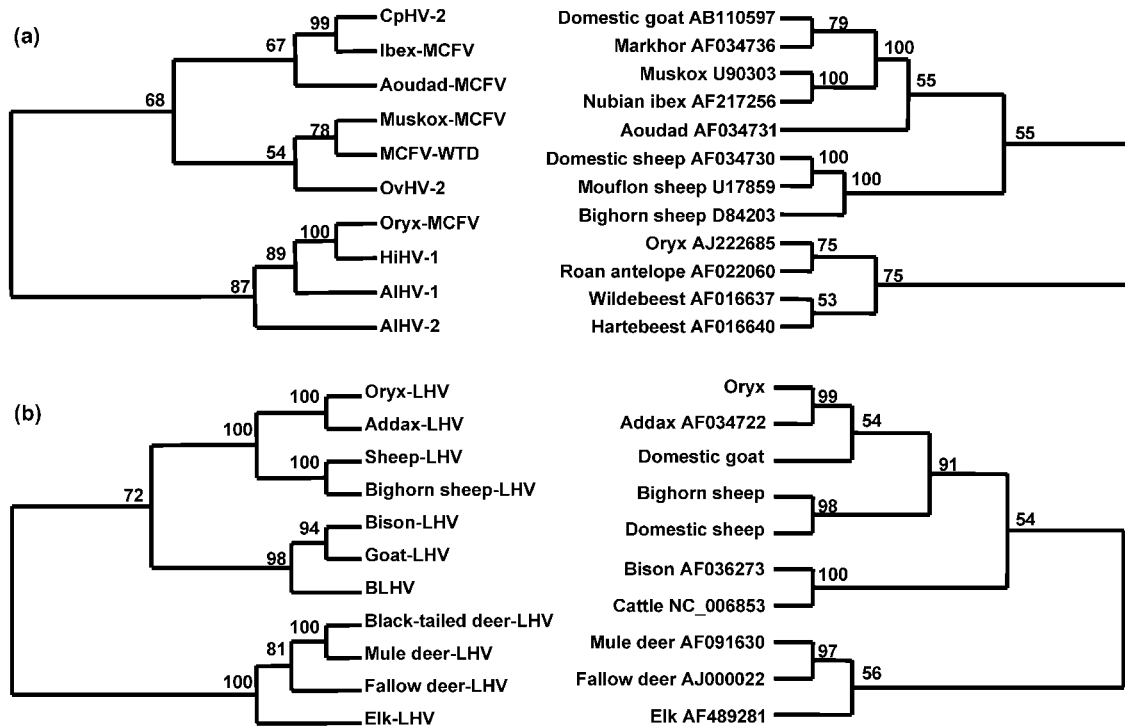


Fig. 2. Comparison of rhadinoviruses and corresponding host trees. (a) Trees for 10 rhadinoviruses in the MCF subgroup, based on the polymerase gene (left), and their corresponding hosts, which are based on mitochondrial cytochrome *b* (right), are shown. (b) Trees for 11 rhadinoviruses in the non-MCF subgroup (left) and their corresponding hosts (right) are shown. The trees were constructed by using the PAUP* program. Bootstrap values are shown at branch points. GenBank accession numbers for the sequences are shown on the tree.

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 97.1% 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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