Recognition of another member of the malignant catarrhal fever virus group: an endemic gammaherpesvirus in domestic goats

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A novel gammaherpesvirus in goats that is herein tentatively designated as caprine herpesvirus-2 was identified based on the sequence of a fragment from the herpesvirus DNA polymerase gene. Sequence alignment analysis revealed that the virus sequence isolated from goats was 67% identical to the homologous sequence from alcelaphine herpesvirus-1, 71% identical to ovine herpesvirus-2 and 73% identical to a recently recognized herpesvirus causing malignant catarrhal fever in white-tailed deer. Combined serological and PCR-survey data demonstrated that this virus is endemic in goats and its transmission pattern may be similar to that of ovine herpesvirus-2 in sheep.

Malignant catarrhal fever (MCF) is a disease syndrome primarily affecting ruminant species (Plowright, 1990), though it can affect non-ruminants as well (Loken et al., 1998). It is caused by several distinct but closely related members of the subfamily Gammaherpesvirinae (Plowright, 1990; Crawford et al., 1999). Alcelaphine herpesvirus-1 (AlHV-1), the first identified MCF virus (Plowright et al., 1960) that is endemic in wildebeest as a persistent subclinical infection, is the causative agent of wildebeest-associated MCF (WA-MCF) (Plowright, 1990). Ovine herpesvirus-2 (OvHV-2) is the virus responsible for the worldwide sheep-associated MCF (SA-MCF) (Plowright, 1990). OvHV-2-specific PCR failed to detect viral DNA in a high percentage of seropositive goats (Table 1) prompted this systematic examination of the closely related gammaherpesviruses that naturally occur in goats.

Blood samples from a total of 142 goats, including eight different breeds (Alpine, Angora, Boer, Markhor, Nubian, Pygmy, Saanen and an unidentified breed), were collected from different geographical locations, including the states of Arizona, California, Florida, Minnesota, New York, Ohio, Oregon and Washington and from Alberta in Canada (Table 1). These samples were examined for antibody by a recently reformatted competitive-inhibition (CI) ELISA (Li et al., 1994, 2001) and were also examined by PCR for OvHV-2-specific sequences (Baxter et al., 1993; Li et al., 1995) and for partial

Portions of herpesvirus DNA polymerase genes have been widely used as targets for the detection of new herpesviruses and investigation of their evolutionary relationships (Quackenbush et al., 1998; Rovnak et al., 1998; Springfeld et al., 1998; Ehlers et al., 1999; Richman et al., 1999; Li et al., 2000). In general, DNA polymerase gene sequence alignments from different herpesviruses correlate highly with the subfamily classification of herpesviruses, which is based on a variety of biological properties (McGeoch et al., 1995; Roizman et al., 1995; VanDevanter et al., 1996). In this study, a consensus PCR combined with sequence analysis was used to identify a novel gammaherpesvirus in goats, named here as caprine herpesvirus-2 (CpHV-2). The endemic goat virus was found to be related to, but distinct from, OvHV-2, AlHV-1 and MCFV-WTD.

Traditionally, goats have been considered to be a source of transmission for OvHV-2 (Heuschele, 1988; Wiyono et al., 1994). This concept was based primarily on the fact that the majority of goats, like domestic sheep, possessed antibodies that reacted with determinants of AlHV-1, as measured by polyclonal or monoclonal antibody-based assays (Rossiter, 1981; Li et al., 1996). OvHV-2-specific DNA sequences have also been reported in goats (Wiyono et al., 1994) and a recent study by Wiyono (1999) showed that OvHV-2 sequences could be detected by PCR in 17% of goats surveyed in Indonesia. However, the observation in our laboratory that OvHV-2-specific PCR failed to detect viral DNA in a high percentage of seropositive goats (Table 1) prompted this systematic examination of the closely related gammaherpesviruses.
sequences of the herpesvirus DNA polymerase gene (Li et al., 2000).

Of 142 goats examined, 124 (87%) were seropositive and 11 (9%) of these seropositive goats were PCR-positive for OvHV-2 DNA. Of the 11 OvHV-2-positive goats, nine originated from the same source: a petting zoo environment. Of the 14 animals tested from this facility, nine were OvHV-2-positive. Inquiry revealed that these goats had been housed with sheep for several years.

From the 124 seropositive goats, 93 were randomly selected for examination by PCR using degenerate primers (Li et al., 2000). Of these 93 samples, 52 (56%) yielded amplification products. Amplified products from 27 randomly selected goats from diverse geographical locations were cloned and sequenced (Li et al., 2000). The sequences amplified from 24 of these goats were 98% to 100% identical to each other, but only 71% identical to OvHV-2. 67% to AlHV-1 and 73% to MCFV-WTD (Fig. 1). The virus thus identified in goats is a new member of the MCF group of viruses and is tentatively designated herein as CpHV-2. This categorization is based upon the sequence data and upon its possession of the 15-A antigenic epitope, which is conserved among all the MCF viruses known to exist at this time (Li et al., 1994, 2000).

A different sequence was also identified from the remaining three goats. They were 100% identical to each other and 91% identical to the recently reported bovine lymphotropic herpesvirus (BLHV) (Rovnak et al., 1998). Because of its sequence relatedness to BLHV, this gammaherpesvirus in goats was therefore tentatively termed caprine lymphotropic herpesvirus (CpLHV). This result (3 of 27) is not a reliable indicator of the prevalence of CpLHV in goats. A more accurate estimate of its prevalence would require reagent development and application that was beyond the scope of this study. Notably, one goat had both CpHV-2 and CpLHV sequences and one other goat had both CpHV-2 and OvHV-2 sequences.

Since degenerate primer-based PCR amplified DNA fragments from only 56% of the seropositive goats, PCR reactions with primers designed more specifically for the CpHV-2 DNA polymerase gene were developed in order to increase the efficiency of amplification. Both the degenerate upstream primer (CON-EX, 5’ CAYAAATGCTACTCCAC 3’) for the primary reaction and the upstream primer in the secondary reaction (CONS, 5’ TGGCCCTCGGGCATGCTGC 3’) were derived from highly conserved sequences in the homologous regions of OvHV-2, AlHV-1 and MCFV-WTD. The same downstream primer (GOT, 5’ CCGTAAATAGGGGTCTCT 3’) was used in both primary and secondary reactions. It was derived from the sequence amplified from the peripheral blood lymphocyte (PBL) DNA of a seropositive goat. The amplification conditions were as described previously (Li et al., 2000).

DNA samples from PBL of 124 seropositive and 18 seronegative goats were subjected to PCR amplification with primers designed specifically for the CpHV-2 DNA polymerase sequence. Of these, 104 samples (84%) from 124 seropositive animals yielded visible bands. None of the

Table 1. Prevalence of CpHV-2 and OvHV-2 among domestic goats in North America

<table>
<thead>
<tr>
<th>Breed</th>
<th>Location</th>
<th>CI-ELISA</th>
<th>OvHV-2 PCR</th>
<th>Degenerate PCR</th>
<th>CpHV-2 sequence PCR</th>
<th>Dotblot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpine</td>
<td>OR</td>
<td>37/37</td>
<td>0/37</td>
<td>8/11</td>
<td>34/37</td>
<td>1/1</td>
</tr>
<tr>
<td>Angora</td>
<td>OR</td>
<td>1/1</td>
<td>0/1</td>
<td>ND</td>
<td>1/1</td>
<td>ND</td>
</tr>
<tr>
<td>Boer</td>
<td>Alberta (Canada)</td>
<td>10/18</td>
<td>1/10</td>
<td>8/18</td>
<td>10/10</td>
<td>2/3</td>
</tr>
<tr>
<td>Markhor</td>
<td>NY</td>
<td>2/2</td>
<td>0/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Nubian</td>
<td>CA, OH, OR</td>
<td>37/38</td>
<td>1/37</td>
<td>9/19</td>
<td>28/37</td>
<td>4/4</td>
</tr>
<tr>
<td>Pygmy</td>
<td>AZ, CA</td>
<td>12/14</td>
<td>3/12*</td>
<td>10/14</td>
<td>11/12</td>
<td>4/4</td>
</tr>
<tr>
<td>Saanen</td>
<td>OR, WA</td>
<td>13/14</td>
<td>0/13</td>
<td>5/11</td>
<td>8/13</td>
<td>3/5</td>
</tr>
<tr>
<td>Unidentified</td>
<td>Alberta, CA, FL, MN, NY</td>
<td>12/18</td>
<td>6/12*</td>
<td>10/18</td>
<td>10/12</td>
<td>8/8</td>
</tr>
<tr>
<td>Total (%)</td>
<td></td>
<td>124/142 (87)</td>
<td>11/124 (9)</td>
<td>52/93 (56)</td>
<td>104/124 (84)</td>
<td>24/27† (89)</td>
</tr>
</tbody>
</table>

* All of the OvHV-2-positive animals in these two groups originated from the same source: a zoo wherein they had been housed with sheep for several years.
† The three remaining goats had only CpLHV sequence cloned.
ND, Not determined.
seronegative goat samples yielded amplicons. Therefore, sensitivity with the CpHV-2-specific primers increased from the initial 56% with the degenerate primers to 84% with the CpHV-2-specific primers. The specificity of amplified products was also evaluated by dot blot hybridization with a digoxigenin-labelled 108 bp DNA probe. The probe was synthesized by the PCR amplification of a clone containing a partial CpHV-2 DNA polymerase gene, using the primer pair 5’ CCTGCCTCACCATG 3’ and 5’ GGCATAGCTCCTC-TCA 3’. The probe hybridized to 103 of 104 (99%) of the products amplified by the CpHV-2-specific PCR (Table 1), but did not hybridize with sequences amplified by degenerate primers from OvHV-2, AlHV-1, AlHV-2 (a low-virulence strain from hartebeest; Reid & Rowe, 1973), MCFV-WTD or BLHV (data not shown).

An earlier study from this laboratory, examining random samples without age information, found similar overall seroprevalences in sheep and goats (Li et al., 1996). The age-specific prevalence and the kinetics of development of antibody in sheep were subsequently described by additional studies (Li et

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Fig. 1. (a) Comparison of the consensus nucleotide sequences of a region of the herpesvirus DNA polymerase gene derived from CpHV-2 and CpLHV in goats with the homologous regions of OvHV-2, AlHV-1, MCFV-WTD and BLHV. Black boxes represent the nucleotides that vary from the consensus sequence. (b) Comparison of the translated amino acid sequences of the same gene region shown in (a). Light grey shading represents conservative substitutions, dark grey shading represents somewhat similar residues, and black boxes represent dissimilar residues. The OvHV-2, AlHV-1, MCFV-WTD and BLHV DNA polymerase sequences used herein were obtained from GenBank. The accession numbers are: OvHV-2, AF031812; AlHV-1, AF031809; MCFV-WTD, AF181468; and BLHV, AF031808. The multiple alignments were accomplished using the GCG package version 10 (Genetics Computer Group, Madison, WI, USA).
Fig. 2. Phylogram display based on the partial amino acid sequences of the homologous DNA polymerase gene fragment from five members of the MCF group of viruses and other lymphocyte-associated members of the ruminant gammaherpesviruses. The phylogenetic tree was constructed with the NEIGHBOR program of the PHYLIP package (University of Washington, USA), using the amino acid sequences from the following: bovine herpesvirus-4 (BoHV-4, AF031811, serving as the outgroup); BLHV (AF031808); CplHV (AF275433); CpHV-2 (AF275941); MCFV-WTD (AF181468); OvHV-2 (DNA from PBL of MCF cases in cattle, bison, axis deer and moose); OvHV-2 (AF031812); OvHV-2 (DNA from PBL of normal OvHV-2-infected sheep); AlHV-2 (AF275942); AlHV-1 (DNA from the C-500, WC-11, Austria and Minnesota isolates propagated in vitro); and AlHV-1 (AF031809). The sequences without accession numbers were obtained from this study and have not been deposited in GenBank. Bootstrap values shown at branch points were obtained from 100 data sets.

In order to define these same parameters in goats, this study serially examined 15 goat kids born to CpHV-2-infected mothers. The 15 kids were bled twice a month from presuckle until the termination of the experiment at 13 months of age. None of the presuckle kids were seropositive. All of the kids seroconverted following nursing and the maternal antibody declined as expected, becoming undetectable by about 3 months of age. Subsequently, the percentage of seropositive kids progressively increased to 40% at about 9 months of age and reached 100% at 12 months of age. The pattern of antibody development in goats was similar to that of domestic sheep (Li et al., 1998).

The serology, DNA alignment and phylogenetic analysis (Fig. 2) clearly indicate that CpHV-2 is a previously unrecognized member of the MCF group of the gammaherpesvirus subfamily that is related to, but distinct from, other identified MCF viruses. Although little data exist on the pathogenicity of CpHV-2, a disease characterized by chronic dermatitis and weight loss in Sika deer has recently been associated with this virus, using sequence analysis and serology (T. B. Crawford, H. Li, S. Rosenberg & M. M. Garner, unpublished results). However, it is not clear at present whether or not CpHV-2 causes typical MCF. It is apparent that some goats are naturally infected not only with CpHV-2, but also with OvHV-2 or CpLHV, or perhaps even with all three of these viruses. The two goats found to be co-infected with CpHV-2 and OvHV-2 in this study were both from zoo environments where they had been in contact with sheep. It is not known whether the occasional goat that is infected with OvHV-2 is capable of transmitting the virus to clinically susceptible species. Until more is known, it may be prudent to continue to recommend separation of OvHV-2-infected goats from susceptible species.

The similar antibody-acquisition pattern between goats and sheep suggests that the transmission mode of CpHV-2 among goats may be similar to the transmission of OvHV-2 among sheep. A program for production of OvHV-2-free sheep has been described (Li et al., 1999). Determination of whether production of CpHV-2-free goats is possible using a similar strategy, a potentially valuable program for game farms and petting zoos, awaits additional studies which are currently under way.

Since several members of the MCF group of gammaherpesviruses have now been recognized, an examination of the genetic relatedness of their DNA polymerase genes was undertaken. Four isolates of AlHV-1, one isolate of AlHV-2 and samples from four normal domestic sheep and six clinically susceptible ruminants with SA-MCF were examined by degenerate primer PCR and sequence analysis. The sequences from all four AlHV-1 isolates (Minnesota isolate, Austria isolate, WC-11 and C-500) were 100% identical to each other and 80% identical to AlHV-2 (Fig. 2). The data strongly suggest that both the Minnesota isolate, originally derived from a cow involved in a putative SA-MCF outbreak in Minnesota (Hamdy et al., 1978), and the Austria isolate, a putative SA-MCF virus isolate from Austria (Schuller et al., 1990), are actually members of the AlHV-1 group. This suggests that the original reports probably reflected laboratory contamination with an alcelaphine strain of virus.

Alignment of sequences amplified from the four domestic sheep and the six ruminants (two each of cattle and bison, one moose and one deer) with clinical SA-MCF from varied geographical locations including Arizona, Colorado, Idaho, Montana and Washington, and Alberta in Canada, revealed 98% to 100% identity with the sequence of the OvHV-2 DNA polymerase gene fragment in GenBank (Fig. 2). This confirms OvHV-2 as the major agent associated with MCF outbreaks in a spectrum of ruminant species in North America.

The number of viruses associated with the disease syndrome known as ruminant MCF, as well as the scope of disease expressions properly considered within the syndrome, have increased significantly within the past few years. Traditionally, MCF was considered to be an acute highly lethal disease with a short course, few survivors and a fairly characteristic set of signs and lesions. This concept is now changing as new agents (Li et al., 2000), which are close genetic relatives of the
prototypical viruses, and new subacute or chronic disease presentations (O’Toole et al., 1997) are being recognized. It is to be expected that this trend will continue as the more incisive investigative methodologies are applied to this group of ruminant gammaherpesviruses.

The MCF subgroup of the ruminant gammaherpesviruses is rapidly expanding. In addition to the four MCF viruses that have been known for a number of years, AlHV-1, AlHV-2, OvHV-2 and hippopotagrine herpesvirus-1 (HhHV-1) (Reid & Bridgen, 1991), two more have been described in the past year: one of unknown origin causing classical MCF in white-tailed deer and the agent described herein in domestic goats. The appropriate criteria for defining relationships among the emerging group of ruminant rhadinoviruses are still in flux, but genetic sequence comparison of conserved genes and the presence of the 15-A epitope have to date served reliably to help us identify members of the MCF virus group. Among the rhadinoviruses, pathogenicity is highly species-dependent and thus not a reliable criterion. The MCF virus (AlHV-2) described in hartebeest and topi, for example, clearly falls within the group on the basis of base sequence (Fig. 1) and antigens (H. Li & T. B. Crawford, unpublished results), but has not been reported to cause significant disease in nature. The virus described herein in goats was until recently not known to be pathogenic, but we have recently observed it apparently causing chronic disease in Sika deer (T. B. Crawford, H. Li, S. Rosenberg & M. M. Garner, unpublished results). The exclusive use of the presence of known pathogenicity is thus an unreliable basis for classification of these viruses, which exist in such complex interrelationships with their hosts. We herein propose that the MCF subgroup of ruminant gammaherpesviruses can at this time be reliably 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.

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References


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