Detection and Multigenic Characterization of a Herpesvirus Associated with Malignant Catarrhal Fever in White-Tailed Deer (Odocoileus virginianus) from Missouri

Steven B. Kleiboeker,* Margaret A. Miller, Susan K. Schommer, Jose A. Ramos-Vara, Magalie Boucher, and Susan E. Turnquist

Veterinary Medical Diagnostic Laboratory and Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, Missouri 65211

Received 28 September 2001/Returned for modification 30 December 2001/Accepted 19 January 2002

Between 1998 and 2001, tissues from four captive white-tailed deer were observed to have histologic lesions of systemic lymphocytic vasculitis. These lesions suggested malignant catarrhal fever, although epizootic hemorrhagic disease and bluetongue were included in the differential diagnosis. Initial diagnostic efforts, including virus isolation and reverse transcription-PCR for epizootic hemorrhagic disease virus and bluetongue virus, failed to identify an etiologic agent. However, consensus primer PCR targeted to the herpesvirus DNA polymerase gene detected viral genomic DNA in each of these four cases. Nucleotide sequence analysis of the amplified product demonstrated that the detected virus was identical over the compared region to the recently described malignant catarrhal fever virus of white-tailed deer (H. Li, N. Dyer, J. Keller, and T. B. Crawford, J. Clin. Microbiol. 38:1313–1318, 2000). Additional nucleotide sequencing of both the DNA polymerase gene and DNA packaging gene followed by phylogenetic analysis solidified this newly recognized herpesvirus as a member of the Gammaherpesvirinae and suggests that this virus, along with ovine herpesvirus 2, alcelaphine herpesvirus 1, alcelaphine herpesvirus 2 and caprine herpesvirus 2, may be part of a separate clade within this subfamily.

Malignant catarrhal fever (MCF) affects a broad range of domestic and exotic ruminants (12, 20), including at least 13 species of deer (22). The distribution of MCF is worldwide, and the clinical course is typically severe and frequently fatal (20), although inapparent infections do occur in susceptible hosts (13) and cattle have been reported to be chronically infected with and to recover from MCF (18). MCF is usually sporadic, with limited morbidity in a population, although severe epidemics with high morbidity and mortality have been reported (3, 10, 27). Two gammaherpesviruses, alcelaphine herpesvirus 1 (AHV1) and ovine herpesvirus 2 (OHV2) (previously referred to as sheep-associated MCF virus), are considered to be the principal etiologic agents of MCF. Both AHV1 and OHV2 are apathogenic in their natural hosts, i.e., wildebeest and sheep, respectively. Transmission to susceptible ruminants can usually be associated with close contact between carrier sheep or wildebeest calves. However, spread by contact between infected cattle, and presumably other susceptible ruminants as well, does not readily occur (5, 20). While the reason for the lack of transmission between susceptible animals has not been proven, it is likely that the clinically susceptible hosts do not shed sufficient levels of cell-free, infectious virus to infect herdmates.

Recently, a third gammaherpesvirus was detected from the tissues of white-tailed deer with lesions that were characteristic of MCF (14). A combination of molecular and virological testing demonstrated that the affected deer were negative for OHV2 and AHV1 as well as a number of other potential etiologic agents. Genetically, this newly identified herpesvirus was found to be closely related to the other viruses known to cause MCF, with 82 and 71% identity to OHV2 and AHV1, respectively, in the amplified region of the DNA polymerase gene. In this epizootic five of six white-tailed deer on the premises died, and the sixth was euthanized. Tissues from all six deer were found to be positive for this newly recognized virus. These deer were part of a small zoo collection that contained approximately 200 animals, including several non-ruminant species plus a variety of exotic and domestic ruminants.

The present study describes detection of the newly recognized MCF-associated virus of deer (deer herpesvirus [DHV]) in necropsy samples from four captive white-tailed deer with lesions characteristic of MCF. A majority of the cases were examined retrospectively, using DNA extracted from formalin-fixed, paraffin-embedded tissues as the template for PCR analysis (4, 5, 8, 9, 16, 25). Affected deer originated from three unrelated premises, and the clinical history did not include a high morbidity or mortality rate within the source herds. Furthermore, contact with exotic ruminants was reported in only one of the cases examined, suggesting a domestic rather than an exotic reservoir host. A survey of multiple tissues from captive and wild white-tailed deer without lesions characteristic of MCF demonstrated that DHV was not detected in any of these deer and thus is probably not widespread within the deer population of Missouri. Additional nucleotide sequence and phylogenetic analysis of both the DNA polymerase and the DNA packaging genes solidify identification of this virus as a...
member of the Gammaherpesvirinae and its relationship to AHV1 and OHV2.

MATERIALS AND METHODS

Cases. Between 1998 and 2001, four white-tailed deer from three separate, unrelated captive herds in Missouri were necropsied by veterinary practitioners and various tissues were submitted to the University of Missouri’s Veterinary Medical Diagnostic Laboratory. Two of the cases were submitted in the month of December, and two were submitted in February. Each of these deer came from a captive herd, and contact with other ruminants was generally limited to cattle, sheep, elk, and/or goats. None of the farms had exotic ruminants on the premises when the deer died or became clinically ill, although contact with exotic species likely occurred in the past for one of the deer.

Thirty-nine control cases were selected from the archives of the University of Missouri’s Veterinary Medical Diagnostic Laboratory. The control cases were submitted between 1998 and 2001, and none had lesions characteristic of MCF. A combination of tissues from both captive and wild white-tailed deer were examined.

Nucleic acid extraction and PCR amplification. For one case with lesions characteristic of MCF, spleen and lymph nodes collected at necropsy were homogenized in phosphate-buffered saline (pH 7.4) (2 to 4 g of tissue per 5 mL). One milliliter of the homogenate was centrifuged at 12,000 × g for 1 min, and the cell pellet was used for DNA extraction. For all other cases (and a majority of control cases), formalin-fixed, paraffin-embedded tissue sections of spleen, kidney, heart, lung, and/or skeletal muscles (two sections per paraffin block; each section 20 μm thick) were deparaffinized by two extractions with xylene, followed by centrifugation. Tissue pellets were then washed twice with 100% ethanol and dried for 10 min at room temperature. For DNA extraction, both fresh and fixed tissue samples were digested with proteinase K (Qiagen, Inc., Valencia, Calif.) in buffer ATL (Qiagen, Inc.) for a minimum of 12 h at 55°C. DNA extractions were performed using the QIAamp DNA minikit (Qiagen, Inc.) according to the manufacturer’s instructions. For each of the DNA extraction steps, strict protocols were followed to avoid cross-contamination of samples. Samples were stored at −80°C until used as templates for amplification.

Amplification of a region of the herpesvirus DNA polymerase gene was performed with nested, degenerate primers targeted to highly conserved regions (26). First-round amplification was performed using two upstream primers (DFA and ILK) and one downstream primer (KG1), each at a final concentration of 0.6 μM with 1.0 U of HotStarTaq (Qiagen, Inc.) in the manufacturer’s buffer containing 2.0 mM MgCl2 and 0.2 mM (each) deoxynucleoside triphosphates in a final reaction volume of 25 μL. Thermocycling conditions were as follows: 95°C (12 min), followed by 10 cycles of denaturation (95°C, 30 s), annealing (70°C, 30 s), and extension (72°C, 90 s) with the annealing temperature in these cycles reduced by 2°C each cycle. An additional 40 cycles of denaturation (94°C, 30 s), annealing (48°C, 30 s), and extension (72°C, 90 s) were performed, followed by a final extension at 72°C for 7 min. Amplification of the DHV-specific primers yielded a product of 197 bp.

Amplification products were visualized in a 2% agarose–1× Tri-aceate–EDTA gel by ethidium bromide staining and UV transillumination (24). Products of the appropriate size were excised from the gel and purified with a QiAex II gel extraction kit (Qiagen, Inc.). Purified products were either sequenced directly using the herpesvirus PCR primers or cloned using the pCR-Blunt TOPO II system (Invitrogen, Inc., Carlsbad, Calif.). When amplified fragments were sequenced directly, a minimum of three sequencing reactions were performed for each product. When amplified fragments were cloned, a minimum of three clones were sequenced. Nucleotide sequencing was performed by the DNA Core Facility, University of Missouri, Columbia. Nucleotide sequence analysis was performed with GeneTool, version 1.0 (BioTools, Inc.).

Alignments and phylogenetic analysis. DNA and deduced amino acid se-quences of amplified herpesvirus PCR products, excluding the primer sequences, were aligned using CLUSTAL W (version 1.74) software. Sequences for comparison were obtained from GenBank and corresponded to AHV1 (GenBank accession no. NC_002531) nucleotide positions 21557 to 21944 for the DNA polymerase gene and 52324 to 52694 for the DNA packaging gene. Phylogenetic analyses of DNA and amino acid alignments were performed using maximum-parsimony methods (DNAPARS or PROTPARS) and distance matrix methods (DNADIST or PROTDIST followed by NEIGHBOR) within the PHYLIP software package (7). Data sets were subjected to bootstrap analysis (6), based on 100 resamplings of the original data set, using the SEQBOOT program to produce a majority-rule consensus tree. Completed tree files were visualized using TreeView 1.5 (19).

Nucleotide sequence accession numbers. The herpesviral sequences obtained in this study have been deposited in the National Center for Biotechnology Information database and assigned GenBank accession numbers AF387516 (DNA polymerase gene fragment) and AY055731 (DNA packaging gene fragment).

RESULTS

Gross lesions and histopathology. Macroscopic lesions were observed in tissue samples from two of the four deer. These lesions were gray-white nodules up to 5 mm in diameter in heart, kidney, or liver. In the heart, nodules were mainly in subendocardial ventricular myocardium; in the kidney, they were concentrated around arcuate vessels at the corticomedullary junction. Histologically, aggregates of small and large lymphocytes with fewer histiocytes and plasma cells comprised these nodules. Smaller to medium-sized muscular arteries were usually at the center of lymphoid nodules. Lymphocytes infiltrated arterial adventitia with focal extension into the tunica media, where inflammation was associated with segmental fibrinoid necrosis. Lymphocytic arteritis and perivascularitis were observed microscopically in the heart, lung, kidney, adrenal gland, spleen, brain, meninges, skeletal muscle, abomasum, and/or intestine in each of the four cases. Lymph node had inconspicuous follicles, but paracortical regions were expanded by lymphocytes. A morphological diagnosis of systemic lymphocytic vasculitis prompted consideration of MCF in the differential diagnosis (16, 17).

PCR amplification and nucleotide sequence analysis. Consensus primer PCR targeted to the herpesvirus DNA polymerase gene amplified specific products from the tissues of each of the four deer with lesions characteristic of MCF. A second round of amplification of the DNA polymerase gene produced two fragments of approximately 225 and 440 bp, with the larger
FIG. 1. Alignment of nucleotide sequences (A) and deduced amino acid sequences (B) from herpesvirus DNA polymerase genes. Only nucleotides or amino acids which differ from the sequence of DHV are shown. Identical bases or amino acids are shown by dots, and gaps are shown by dashes.

A)

<table>
<thead>
<tr>
<th></th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHV</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>G</td>
<td>C</td>
</tr>
<tr>
<td>OHV2</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>AHV1</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>AHV2</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>CPHV2</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>BLHV</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
</tbody>
</table>

B)

<table>
<thead>
<tr>
<th></th>
<th>90</th>
<th>100</th>
<th>110</th>
<th>120</th>
<th>130</th>
<th>140</th>
<th>150</th>
<th>160</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHV</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>G</td>
<td>C</td>
</tr>
<tr>
<td>OHV2</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>AHV1</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>AHV2</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>CPHV2</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>BLHV</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
</tbody>
</table>

FIG. 1. Alignment of nucleotide sequences (A) and deduced amino acid sequences (B) from herpesvirus DNA polymerase genes. Only nucleotides or amino acids which differ from the sequence of DHV are shown. Identical bases or amino acids are shown by dots, and gaps are shown by dashes.
product consistently present in higher concentrations. Nucleotide sequencing demonstrated that the 440-bp fragment was a product of amplification with the TGV and KG1 primers and that the 225-bp fragment was a product of amplification with the IYG and TGV primers and thus was an internal fragment of the larger amplification product. The nucleotide sequence of the smaller fragment has previously been reported (14). For the larger fragment, exclusion of the primer sequences yielded a 388-bp fragment. The sequence of this fragment was determined for amplification products from each of the four PCR-positive deer cases and was found to be 100% identical for all samples from these cases. Pairwise alignment of the nucleotide sequence and deduced amino acid sequence (Fig. 1) demon-

FIG. 2. Phylogenetic tree for the DNA polymerase gene proteins of representative alpha-, beta-, and gammaherpesviruses plus the MCF virus of white-tailed deer (DHV). The unrooted tree was generated using the distance matrix program NEIGHBOR with the jumble option evoked. Distance values were calculated using the PROTDIST program with the Dayhoff percent accepted mutation matrix. Bootstrap values of greater than 50 are shown, and the branch lengths represent relative genetic distances. The herpesviruses used for comparison and their accession numbers are as follows: ateline herpesvirus 2 (AtHV2), U63457; AHV1, NC_002531; AHV2, AF275942; asinine herpesvirus 4 (AsHV4), AY054990; AsHV5, AY054993; bovine herpesvirus 1 (BHV1), X89500; BHV2, AF031810; BHV4, AF031811; BLHV, AF031808; canine herpesvirus 1 (CHV1), X89500; CPHV2, AF275941; DHV, AF181468; Epstein-Barr virus (EBV) (human herpesvirus 4 [HHV4]), V01555; equine herpesvirus 1 (EHV1), M86664; EHV2, NC_001650; EHV5, AF141888; gallid herpesvirus 2 (GHV2) (Marek’s disease virus), L40431; human cytomegalovirus (HCMV) (HHV5), M14709; HHV6, X83413; HHV7, U93872; herpes simplex virus type 1 (HSV1), X04771; HSV2, M16321; murine gammaherpesvirus 68 (HV68), U97553; saimiriine herpesvirus 2 (HSV), M31122; OHV2, AF031812; pseudorabies virus (PRV), L24487; retroperitoneal fibromatosis herpesvirus (RFHVMn), AF005478; varicella-zoster virus (VZV) (HHV3), X04370; wildass herpesvirus (WAHV), AF141888; and zebra herpesvirus (ZHV), AF141889.

### TABLE 1. Nucleotide and deduced amino acid sequence identities of the DNA polymerase gene fragment

<table>
<thead>
<tr>
<th>Virus</th>
<th>% Identity with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHV</td>
</tr>
<tr>
<td>DHV</td>
<td>83.2</td>
</tr>
<tr>
<td>OHV2</td>
<td>87.5</td>
</tr>
<tr>
<td>AHV1</td>
<td>77.5</td>
</tr>
<tr>
<td>AHV2</td>
<td>72.8</td>
</tr>
<tr>
<td>CPHV2</td>
<td>72.8</td>
</tr>
<tr>
<td>BLHV</td>
<td>68.2</td>
</tr>
</tbody>
</table>

* Values for deduced amino acid sequence identities are in boldface.

### TABLE 2. Nucleotide and deduced amino acid sequence identities of the DNA packaging gene fragment

<table>
<thead>
<tr>
<th>Virus</th>
<th>% Identity with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHV</td>
</tr>
<tr>
<td>DHV</td>
<td>86.1</td>
</tr>
<tr>
<td>OHV2</td>
<td>91.9</td>
</tr>
<tr>
<td>CPHV2</td>
<td>89.5</td>
</tr>
<tr>
<td>AHV1</td>
<td>77.4</td>
</tr>
</tbody>
</table>

* Values for deduced amino acid sequence identities are in boldface.
strated that the 388-base DNA polymerase gene fragment was 83.2 and 87.5% identical, respectively, to OHV2 (Table 1). Somewhat lower levels of identity were found when this sequence was compared to AHV1, as well as the recently identified AHV2 (15), caprine herpesvirus 2 (CPHV2) (2, 15), and bovine lymphotrophic herpesvirus (BLHV) (23). Phylogenetic analysis of the deduced amino acid sequence from the DNA polymerase gene sequence revealed that the herpesvirus detected from these deer, along with OHV2, AHV1, AHV2, and CPHV2, form a distinct clade within the *Gammaherpesvirinae* subfamily (Fig. 2).

Consensus primer PCR targeted to the herpesvirus DNA packaging gene amplified a 415-bp product from samples of each of the four cases with lesions characteristic of MCF. Nucleotide sequence analysis demonstrated that a novel gene fragment corresponding to the DNA packaging genes of other herpesviruses had been detected. The sequence of this fragment was determined for amplification products from each of the four deer with MCF and was found to be 100% identical for all samples tested. Exclusion of the primer sequences yielded a 371-bp fragment. Pairwise alignment demonstrated that the nucleotide and deduced amino acid sequences of this fragment were 86.1 and 91.9% identical, respectively, to the corresponding region of the OHV2 genome (Table 2). Slightly lower levels of identity were found for both the nucleotide and deduced amino acid sequences when the putative DHV DNA

<table>
<thead>
<tr>
<th>A)</th>
<th>B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHV</td>
<td>SSDKSTSFLYNLKASERLLNVLNVNYCTDHEFMSMQGLISCPYSLHPSYISIDTQIKNTNLFLDQVFSTELMGS</td>
</tr>
<tr>
<td>OHV2</td>
<td>Q.</td>
</tr>
<tr>
<td>CPHV2</td>
<td>Q.</td>
</tr>
<tr>
<td>AHV1</td>
<td>Q.</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>170</td>
<td>180</td>
</tr>
<tr>
<td>250</td>
<td>260</td>
</tr>
</tbody>
</table>

FIG. 3. Alignment of the nucleotide sequences (A) and deduced amino acid sequences (B) from herpesvirus DNA packaging genes. Only nucleotides or amino acids which differ from the sequence of DHV are shown. Identical bases are shown by dots, and gaps are shown by dashes.
packaging gene fragment was compared to those of CPHV2 and AHV1 (Fig. 3; Table 2). The results of phylogenetic analysis for the deduced amino acid sequence of the DNA packaging gene fragment (Fig. 4) were very similar to those of analysis of the DNA polymerase gene, although sequences for comparison were not available for AHV2 and BLHV.

A DHV-specific PCR assay was designed to allow rapid screening of a larger number of samples without the requirement of a nested amplification strategy. The analytical sensitivity of a single round of amplification with the specific primers was demonstrated to be approximately equivalent to that of both the DNA polymerase and DNA packaging gene nested consensus primer PCR assays (Fig. 5). The use of a cloned, quantitated DNA template indicated that the specific (non-nested) reaction was capable of detecting 0.05 fg of DNA, which corresponds to approximately 10 genomic copies (Fig. 5B). As an initial effort to determine the prevalence of DHV in the deer population of Missouri, DNA extracted from either fresh tissue or formalin-fixed, paraffin-embedded tissues of 39 control white-tailed deer was tested for the presence of this virus by using a DHV-specific PCR assay. None of the control cases had lesions characteristic of MCF, and all were negative by PCR for DHV. However, all four of the previously identified cases which had histologic lesions of MCF were positive by the DHV-specific PCR assay.

**DISCUSSION**

Over a 4-year period, tissues from four captive white-tailed deer were observed to have histologic lesions of severe and systemic lymphocytic vasculitis (arteritis). MCF, epizootic hemorrhagic disease, and bluetongue were included in the differential diagnosis. Initial virological and molecular testing eliminated epizootic hemorrhagic disease virus and bluetongue virus as potential etiologic agents, and other diagnostic testing (including bacteriologic culture) failed to identify plausible causes of disease for these cases. However, consensus primer PCR targeted to conserved regions of the herpesvirus DNA polymer-
sequences for these two gene fragments places DHV within the
(23). Phylogenetic analysis of the deduced amino acid se-
ded amino acid sequences were compared to those of BLHV
were observed when the DNA polymerase gene nucleotide and
ase gene (26) detected viral genomic DNA in each of the four
cases with lesions characteristic of MCF, suggesting, though
possible to detect serial 10-fold dilutions of DNA purified from formalin-fixed,
paraffin-embedded tissue. Amplification products of the second-round
(nested) reaction are shown. For the DNA polymerase gene, primers
TGV and KG1 were used. For the DNA packaging gene, primers A3
and B2 were used. DNA dilutions used were as follows: undiluted
(lane 1 and 7), 1:10 (lane 2 and 8), 1:100 (lanes 3 and 9), 1:1,000
(lanes 4 and 10), and 1:10,000 (lanes 5 and 11). Results of the extraction
(negative) control reaction are shown in lanes 6 and 12. For the DNA
polymerase gene, primers targeting the DHV DNA polymerase region were used to
amplify serial 10-fold dilutions of DNA purified from formalin-fixed,
paraffin-embedded tissue (lanes 1 to 6) or dilutions of a plasmid clone containing the 440-bp fragment of the DHV DNA polymerase gene.
DNA dilutions used were as follows: undiluted (lane 1), 1:10 (lane 2),
1:100 (lane 3), 1:1,000 (lane 4), and 1:10,000 (lane 5). Results of the extraction
(negative) control reaction are shown in lanes 6 and 12. For reactions in which cloned DHV DNA was the template, the quantities of DNA added were as follows: 50 fg (lane 7), 5 fg (lane 8), 0.5 fg (lane 9), 0.05 fg (lane 10), and 0.005 fg (lane 11).

In this second study to describe detection of the newly rec-
ognized gammaherpesvirus of white-tailed deer, both similar-
ities to and differences from the first report were noted. First,
in the initial report of this virus causing disease in white-tailed
deer (14), the deer that were positive had concurrent contact with a broad range of species, including exotic ruminants. In
contrast, contact species for the deer studied in the present
report were generally limited to other domestic ruminants and
most often included cattle, sheep, elk, and/or goats. Only one of the four deer had contact with exotic species, and this was not concurrent with the onset of clinical disease. Similar to the
report of Li et al. (14), the cases described here occurred during the late fall or early winter. Both the extent of contact with other species (especially ruminants) and the potential
seasonal nature of clinical MCF associated with DHV may
offering a starting point for epizootiological investigations. For
MCF caused by OHV2, an association of contact by susceptible
species with lambing ewes suggests that perinatal lambs play an
important role in transmission. Similarly, the peak incidence of
AHV1 causing MCF in cattle occurs when young wildebeests
are in maximum numbers (21). Infected wildebeests up to the
age of 3 months have a continuous viremia, and AHV1 can be
detected in a cell-free form in both nasal and ocular secretions.
Thus, for investigation into the epizootiology of the disease
cased by DHV, efforts directed toward increased awareness and/or surveillance would logically be focused during the late
fall and early winter months and possibly on contact animals that have recently given birth.

A second major distinction between the present report and that of Li et al. (14) is the level of morbidity and subsequent
mortality. From the herd histories gathered for this report, it
was noted that while some deer were fatally afflicted, other
white-tailed deer on the same premises remained clinically
normal. In the epizootic investigated by Li et al. (14), five of six
deer in the zoological collection died of MCF and the sixth was
euthanized and found to be PCR positive for DHV. The sporadic
nature of the cases described here is similar to the typical
epizootiologic patterns for MCF of cattle in which the mor-
bidity and mortality is generally low, presumably due to the
fact that transmission of AHV1 and OHV2 does not readily
occur following contact between infected and uninfected cat-
tle. This similarity suggests that deer-to-deer transmission of
DHV may not readily occur. Furthermore, if the biology of the
two other MCF viruses, AHV1 and OHV2, can be used as a
guide, DHV probably does not endemically infect deer but
rather is carried by a different ruminant species and transmitted
to the deer by direct contact.

To facilitate screening of additional samples from tissue
archives and future epizootiologic investigations, a specific
PCR assay was developed to amplify a region of the DHV
DNA polymerase gene. A single (nonnested) round of ampli-
fication with the DHV-specific primers was shown to be
equally sensitive to the nested, consensus primer reactions that
were initially used to detect both the DNA polymerase and
DNA packaging genes of DHV. This DHV-specific assay failed
to detect virus in any tissues from deer without lesions char-
acteristic of MCF, suggesting that DHV was not widespread in
the white-tailed deer population of Missouri. However, it is
important to recognize that the presence of this virus may be
difficult to detect if the host is latently infected. It is anticipated
that this assay will be useful in future efforts to identify the
reservoir host for DHV, which will likely require screening of

FIG. 5. Detection of the DHV DNA polymerase and packaging
genes by PCR. (A) Consensus primer PCR for the DNA polymerase
gene (lanes 1 to 6) and DNA packaging gene (lanes 7 to 12) was used
to detect serial 10-fold dilutions of DNA purified from formalin-fixed,
paraffin-embedded tissue. Amplification products of the second-round
(nested) reaction are shown. For the DNA polymerase gene, primers
TGV and KG1 were used. For the DNA packaging gene, primers A3
and B2 were used. DNA dilutions used were as follows: undiluted
(lanes 1 and 7), 1:10 (lanes 2 and 8), 1:100 (lanes 3 and 9), 1:1,000
(lanes 4 and 10), and 1:10,000 (lanes 5 and 11). Results of the extraction
(negative) control reaction are shown in lanes 6 and 12. For the DNA
polymerase gene, primers targeting the DHV DNA polymerase region were used to
amplify serial 10-fold dilutions of DNA purified from formalin-fixed,
paraffin-embedded tissue (lanes 1 to 6) or dilutions of a plasmid clone containing the 440-bp fragment of the DHV DNA polymerase gene.
DNA dilutions used were as follows: undiluted (lane 1), 1:10 (lane 2),
1:100 (lane 3), 1:1,000 (lane 4), and 1:10,000 (lane 5). Results of the extraction
(negative) control reaction are shown in lanes 6 and 12. For reactions in which cloned DHV DNA was the template, the quantities of DNA added were as follows: 50 fg (lane 7), 5 fg (lane 8), 0.5 fg (lane 9), 0.05 fg (lane 10), and 0.005 fg (lane 11).
a large number of antemortem samples such as nasal and/or ocular swabs.

In summary, additional cases of fatal MCF have been associated with a newly recognized herpesvirus of white-tailed deer (14). These cases were sporadic, spanned a period of 4 years, and involved three separate and unrelated captive herds of white-tailed deer located within the state of Missouri. Phylogenetic analysis based on the deduced amino acid sequences of both the DNA polymerase and DNA packaging gene fragments clearly demonstrated that this herpesvirus detected in deer was similar to AHV1 and OHV2, two gammaherpesviruses that are known etiologic agents of MCF in cattle and other ruminants.

ACKNOWLEDGMENTS

We thank John M. Kreeger and Sunny J. Troxell for invaluable discussions during the preparation of the manuscript.

REFERENCES