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Detection of Duck Enteritis Virus by Polymerase Chain Reaction

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Received 28 October 1997

SUMMARY. Duck enteritis virus (DEV), a herpesvirus, is the causative agent of duck viral enteritis in free-flying, feral, and domesticated members of the Anatidae family. HindIII-digested DEV DNA was cloned into the plasmid pBluescript, and a 1.95-kb fragment was sequenced. This fragment codes for the 3’ region of the DEV homologues of varicella-zoster virus (VZV) open reading frame (ORF) UL6 and the 5’ region of VZV UL7. Alignment of the putative peptide fragments for DEV UL6 and UL7 showed a 64% and 37% identity with VZV UL6 and UL7, respectively. Primers located in the highly conserved domain of the UL6 gene were used for a polymerase chain reaction (PCR) assay, which was able to amplify DEV DNA. The PCR assay also amplified DEV DNA from the original outbreak samples and/or after passage in Muscovy duck embryos.

RESUMEN. Detección del virus de la enteritis del pato por medio de la reacción en cadena por la polimerasa.

El virus de la enteritis del pato es un herpesvirus que causa enteritis en patos libres silvestres y domésticos miembros de la familia Anatidae. El DNA del virus de la enteritis del pato fue digerido con la enzima HindIII y clonado dentro del plásmido pBluescript, secuenciando un segmento de 1.95 kb. Este fragmento codifica la región 3’ del virus de la enteritis del pato que es homóloga a la secuencia ininterrumpida que codifica la proteína UL6 del virus de la varicela y a la región 5’ UL7 del mismo virus. La comparación de los polipeptidos de estos fragmentos UL6 y UL7 del virus de la enteritis del pato mostró 64% y 37% de identidad con los segmentos UL6 y UL7 del virus de varicela, respectivamente. Se utilizaron iniciadores localizados en una región altamente conservada del gen UL6 para realizar la prueba de reacción en cadena por la polimerasa que amplificó el DNA del virus de la enteritis del pato. La prueba de reacción en cadena por la polimerasa también amplificó el DNA del virus de la enteritis del pato de muestras del brote original, antes y después de que estas habían sido pasadas por embriones de pato almirizero.

Key words: duck enteritis virus, duck herpesvirus, “viral” enteritis, polymerase chain reaction (PCR)

Abbreviations: CEF = chicken embryo fibroblasts; DEV = duck enteritis virus; DVE = duck “viral” enteritis; EDTA = ethylenediaminetetraacetic acid; MDV = Marek’s disease virus; ORF = open reading frame; PCR = polymerase chain reaction; pBS = pBluescript KS+ TAE = Tris acetate EDTA

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Duck viral enteritis (DVE) is an acute hemorrhagic disease caused by duck enteritis virus (DEV). DEV has been classified as an alphaherpesvirus on the basis of its biological and pathological properties. To our knowledge the molecular structure of the genome has not yet been elucidated, and restriction enzyme maps of DEV DNA have not been published.

Natural infection with DEV is limited to members of the family Anatidae (ducks, geese, and swans) of the order Anseriformes. Outbreaks of DVE have been reported in the United States in commercial duck flocks, feral ducks, and migratory waterfowl (14). Outbreaks of DVE on commercial duck farms can cause high levels of mortality in Muscovy and White Pekin ducks, as well as in other breeds. The first outbreak of DVE in the concentrated duck industry on Long Island (NY) (11) caused economic losses in excess of $1 million due to mortality, condemnation, and decreased egg production (14). Major epizootics have occurred in wild migratory waterfowl in the United States. The first was reported in the Lake Andes National Wildlife Refuge (SD) in 1973, when more than 40,000 of 100,000 wintering mallards died (7). Recently, a major outbreak occurred in the Finger Lakes region of New York State, killing 1150 mallards, Black ducks, and Canada geese and threatening more than 50,000 waterfowl (6). Due to its wide geographic distribution, DVE is now considered enzootic in the United States.

The natural reservoir for DEV has not yet been identified despite extensive attempts at virus isolation from several species of migratory waterfowl. DEV was reisolated from a few birds associated with the sites of DVE mortality, but not from ducks trapped within a radius of 5–32 miles from the outbreaks (1,2). Like other herpesviruses, DEV can establish latent infections, especially in species that are susceptible to infection but resistant to disease. Differences in susceptibility among members of the Anatidae family have been reported. Pintails (Anas acuta) (8,15) and European teal (Nettion crecca) (8) can be infected with DEV but are relatively resistant to the disease. Mallards (Anas platyrinchoa) can become infected, but the incidence of disease depends on the pathogenicity of the virus strain and the age at exposure (15).

There is a paucity of information on latent or persistent infections in survivors of natural outbreaks. Burgess et al. (4) examined virus shedding using cloacal swabs obtained from several species of ducks that survived natural outbreaks. Virus could be reisolated over a 4-year period, but it is not clear whether these ducks were persistently shedding virus or latent infections were frequently reactivated. The sites

<table>
<thead>
<tr>
<th>Sample</th>
<th>Material</th>
<th>Origin</th>
<th>PCR data</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Spleen</td>
<td>14-day-old Pekin duck vaccinated with modified live virus (16), infected at 15 dpv(^a) with LI(^b) strain and euthanized at 43 days</td>
<td>+ -</td>
</tr>
<tr>
<td>B</td>
<td>liver</td>
<td>see sample A</td>
<td>- -</td>
</tr>
<tr>
<td>C</td>
<td>spleen</td>
<td>42-day-old Pekin duck infected with the LI strain, dead 4 dpc(^a)</td>
<td>+ NT</td>
</tr>
<tr>
<td>D</td>
<td>liver</td>
<td>see sample C</td>
<td>+ +</td>
</tr>
<tr>
<td>E</td>
<td>liver</td>
<td>LI strain used to challenge ducks for samples A–D and J</td>
<td>- +</td>
</tr>
<tr>
<td>F</td>
<td>liver</td>
<td>Muscovy duck with lesions, NY</td>
<td>+ -</td>
</tr>
<tr>
<td>G</td>
<td>ME(^c)</td>
<td>Inoculated with sample F</td>
<td>NT +</td>
</tr>
<tr>
<td>H</td>
<td>ME</td>
<td>DVE outbreak in Muscovy ducks, CA, 1995 (Woolcock, pers. comm.) passage 2 in ME</td>
<td>+ +</td>
</tr>
<tr>
<td>I</td>
<td>liver</td>
<td>Muscovy duckling inoculated with a sample from a NY duck farm outbreak, 1997</td>
<td>NT +</td>
</tr>
<tr>
<td>J</td>
<td>liver</td>
<td>28-day-old Pekin duck infected with the LI strain, dead 3 dpc sample from a Black duck, DVE outbreak, NY, 1994 (6), passage 2 in ME</td>
<td>NT +</td>
</tr>
<tr>
<td>K</td>
<td>ME</td>
<td></td>
<td>NT +</td>
</tr>
</tbody>
</table>

\(^a\)dpv = days postvaccination; dpc = days postchallenge.
\(^b\)LI = Long Island strain of DEV (11).
\(^c\)ME = Muscovy duck embryos.
of truly latent virus infections are unknown, although Burgess (unpubl. data, quoted in Brand and Docherty [2]) mentioned that virus was isolated from trigeminal nerves of apparently healthy carriers.

Virus isolation assays using tracheal or cloacal swabs are currently used to determine if DEV is present in carrier birds; this precludes the detection of truly latent virus. The purpose of this study was to clone and sequence fragments of DEV DNA, and to develop a polymerase chain reaction (PCR) assay for the rapid diagnosis of DEV infection during outbreaks of DVE.

**MATERIALS AND METHODS**

**Virus strain and propagation.** The Lake Andes (LA) strain (7) was propagated in chicken embryo fibroblasts (CEF). The virus was used at passage level 6 to 8 for DNA extraction.

**Virus purification.** Supernatant fluids and infected cells were harvested separately when >90% of the cells showed cytopathic effects. Supernatant fluids were clarified by centrifugation at 225 × g for 10 min. Infected cells were harvested with a rubber policeman and resuspended in SPA (218 mM sucrose, 3.8 mM monopotassium phosphate, 7.2 mM dipotassium phosphate, and 1% bovine serum albumin) (5) or phosphate-buffered saline (pH 7.2). Cell pellets were disrupted by two 15-sec ultrasonic treatments or by three freeze/thaw cycles and centrifuged at 225 × g for 10 min. All supernatant fluids were pooled and centrifuged at 65,000 × g for 90 min. Pellets were resuspended in 0.01 M Tris (pH 7.5), layered on 30% sucrose, and centrifuged at 100,000 × g for 60 min. The pelleted virus was resuspended in digestion buffer (100 mM NaCl, 10 mM Tris-Cl [pH 8], 25 mM ethylenediaminetetraacetic acid...
Fig. 2. Nucleotide sequence of the 1.96-kb HindIII clone of DEV (Lake Andes strain). The locations of the EcoRV and SalI sites are indicated and labeled with an E and S, respectively. The locations of primers 1–5 are indicated. The two potential start codons for UL7 are indicated as such. The first 1212 nucleotides are part of UL6, and the termination codon for UL6 is indicated with an asterisk.

[EDTA] [pH 8], and 0.5% sodium dodecyl sulfate (SDS) or 0.01 M Tris (pH 7.5).

**DNA extraction.** Four potential sources of DEV were used for the extraction of DNA: 1) purified virus obtained from infected CEF, 2) spleens and livers from experimentally infected ducks, 3) spleens and livers from outbreaks of DVE, and 4) muscovy duck embryos infected with liver and/or spleen homogenates from domestic or wild waterfowl outbreaks (Table 1).
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HSV-1
VLGEDDETAKGSAAASRLVLRVIMKGMHRGHDINDTVRSYLDEAGGHII

VZV
IHEEDTTTRAGAAASRLVLRVIMKGMHRGHDINDTVRSYLDEAGGHII

BHV-1
LLEGEDDAAASASARLRVILVIMKGMHRGHDINDTVRSYLDEAGGHII

EVH-1
LLEGEDDAAASASARLRVILVIMKGMHRGHDINDTVRSYLDEAGGHII

SHV-1
MLGEDDAASASARLRVILVIMKGMHRGHDINDTVRSYLDEAGGHII

DEV
ALGAQDDAAASASARLRVILVIMKGMHRGHDINDTVRSYLDEAGGHII

HSV-1
APA-VDTGLPPGFKAG--GNSRSAGAGDQGGRAPGLQRAFTRAVNVIN

VZV
GSGSDTSPQDFGKAG--GNSRSAGAGDQGGRAPGLQRAFTRAVNVIN

BHV-1
VSD-VDTSPQDFGKAG--GNSRSAGAGDQGGRAPGLQRAFTRAVNVIN

EVH-1
GAQ-VDTSLPFQGKAG--GNSRSAGAGDQGGRAPGLQRAFTRAVNVIN

SHV-1
S---VDTSPQDFGKAG--GNSRSAGAGDQGGRAPGLQRAFTRAVNVIN

DEV
DT-VDTSPQDFGKAG--GNSRSAGAGDQGGRAPGLQRAFTRAVNVIN

HSV-1
LAAESVTGCGSGRPAAGDDLRA---------D--YDIIVISKSMDDDTTVYA

VZV
HITMA-------------EETRIA---------DLHEVVIDTLLGIDDAVYA

BHV-1
RAGGAGRRPEARVCRPPRA---------ELGHEVVIDSMLGPDYYVA

EVH-1
GSTQPGGSSAILPGSGAKSAG---------CLGHEVIDRNMDGDDAVYA

SHV-1
GTGAAGPGGRAPAGADGVQR---------DLHEVVIDTRAGDDAVYA

DEV
NSLQPIIGGMEPMRLVMSDPTGITTIFRDILGHEVIDLSRMDDAVYA

HSV-1
NSFQHPYIPSQAQDLRLSLWEHELVCHELFCIKLCHRNNQQQETISYSSG

VZV
NSFQSPYIFYYGGDKVRSLSEKLQVLRFCIKLCHRNNQQQETISYSSG

BHV-1
NSFQSPYIFTYADVRSLMSEGGLRCFLRAFNNQQQETISYSSG

EVH-1
NSFQSPYIFAYTADMERSLMSEGGLRCFLRATNNQQQETISYSSG

SHV-1
NSFQSPYIVPPYESDDVRSLMSEGGLRCFLRATNNQQQETISYSSG

DEV
NSFQSPYIFYSPYADELRSLSLWELCFLRATNNQQQETISYSSG

HSV-1
ALAAAFVVPYFESVLARRPVRVGAPITGSQLVEELDEWAVFKKTRLQTYLT

VZV
SISLLLAFYFSLRILLRGLFLVTQSEHRSEELCQLAIFKKTARESTS

BHV-1
SISLLLAFYFSLRILLRGLFLVTQSEHRSEELCQLAIFKKTARESTS

EVH-1
SISLLLAFYFSLRILLRGLFLVTQSEHRSEELCQLAIFKKTARESTS

SHV-1
SISLLLAFYFSLRILLRGLFLVTQSEHRSEELCQLAIFKKTARESTS

DEV
ATILTFVVFYFESVLARRLGFVTVNQCAEYSEELCQLSYKLTARYLT

HSV-1
DIAALFVADVQCA--------MLPPPSVPVAGA---------DLFPG---------

VZV
QIRLEMYMAVRASPTPPTPSWLQDPTDQERPIPKKINQYMY

BHV-1
ELIAIFYATTPRAL---------QLGLASRGARERS---------

EVH-1
ELSTFLVABRNSIAAANSTKRLPVNDEAOSDEEOLKGS---------DERY-

SHV-1
EIAALFADV--------

DEV
DLFAALFVADVQCA--------MLPPPSVPVAGA---------DLFPG---------

HSV-1
------A---------SPRGRSSRSRSPGRARG---------AP

VZV
HVGYKNLH---------FMKGFHPPELRVHKNADSSLLLDRIRANNRSRGRDWRD

BHV-1
------S---------SRSSRSSRSPGRSSRSRSPGRSSRSSRSEEKRKRRR---------R

EVH-1
------YEG---------RDRSSAFQDRDRGRGNYKHKRFSNYYRSGLAR

SHV-1
------RATERLRLDGGRDQA---------DLAR

DEV
GRYRSGSSEYELRDLKPSGGQRNVKRQARQARSEWKAETRVRYKLAG

HSV-1
DQGGIGHRDGRDGR---------R

VZV
VRNKTYQHFRLQDNRQINTSRGVCERRDRRS

BHV-1
EDGRGVRRTYAGRAP

EVH-1
DSSIRDRSQRSGRPTPLL---------DHVG

SHV-1
FPAAQVGARGARADPGHL---------ERLGD

DEV
DTASNVRSSTRGTRHYFAYV--------RRLSHESEGK

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Pelleted virus was incubated in 500 µl digestion buffer at 4°C for 3–4 hr. After addition of RNase (25 µg/ml) and incubation at 38°C with gentle mixing for 1 hr, proteinase K (100 µg/ml) was added, and the mixture was incubated overnight at 37°C with gentle mixing. Viral DNA was purified by standard phenol–chloroform–isoamyl alcohol extractions and ethanol precipitation. The DNA pellets were resuspended in 100 µl glass-distilled water and quantitated by spectrophotometry.

Spleen, livers, and muscovy duck embryo tissues were minced with a scalpel blade and suspended in 1–3 ml digestion buffer. After incubation for 1 hr at 38°C with gentle mixing, proteinase K (100 µg/ml) was added, and the mixture was incubated overnight at 38°C with gentle mixing. Viral DNA was purified as described above and resuspended in 100–500 µl glass-distilled water.

Chicken DNA and Marek's disease virus (MDV) DNA were obtained from chicken embryos and chick kidney cells infected with the RB-1B strain of MDV as described for the duck tissues. Chicken infectious anaemia virus DNA was purified from infected chicken blood using the QIAamp Blood Kit (Qiagen, Santa Clarita, CA).

Cloning and subcloning of DEV DNA. DEV DNA was digested with HindIII (New England BioLabs, Beverly, MA), cloned into pBluescript KS+ (pBS) (Stratagene, Inc., La Jolla, CA), and transformed into DH5α Escherichia coli cells following standard procedures (13). Plasmid DNA was extracted from randomly selected white colonies, digested with HindIII, and analyzed for inserts by gel electrophoresis on 1.0% TAE (40 mM Tris-acetate and 1 mM EDTA) agarose gels. The presence of DEV DNA fragments was confirmed by Southern blotting, followed by hybridization to 32P-labeled DEV DNA (Rediprime DNA Labeling Kit, Amersham, Arlington Heights, IL). Subclones of HindIII DEV fragments were prepared as needed in pBS following standard procedures.

DNA sequencing. A 1.95-kb HindIII fragment of DEV DNA was sequenced completely from both directions by the Biotechnology Resource Center (Cornell University) using a Perkin-Elmer/Applied Biosystems Division 377 automated DNA sequencer and universal primers T and T,. Additional primers (primers 2, 3, 4, and 5) (see Fig. 2) were generated based on the obtained sequence data and used to confirm specific sequences. The sequence data were analyzed using the BLAST P function of Genbank and Megalign of DNASTar (Lasergene, Dynastare. Inc., http://www.dnastar.com).

Primers. Two primers were designed to amplify a 421-bp product of the conserved region of the proposed DEV UL6 homologue. Primer no. 1 (5'-'GAGC GTATTAGATGAACTGC-3') and primer no. 2 (5'-'TGTTGTGATGTGTTCC-3') correspond to nucleotide numbers 114–135 and 521–535 of the 1.96-kb HindIII fragment, respectively. Primer sequences were checked for primer–dimer and hairpin formation using the DNAStar Primer Design function. All primers were synthesized by DNA Services (Cornell University).

PCR amplification. Samples containing 100–200 ng DNA were used in 100 µl reaction volumes containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 µM MgCl2, dATP, dCTP, dGTP, and dTTP (each at 20 µM), primer nos. 1 and 2 (each at 100 pmol), and 2.5 U Taq polymerase (Gibco BRL Life Technologies, Gaithersburg, MD). Amplification was performed in a thermocycler (Perkin-Elmer GeneAmp System 2400) using the following protocol: 94°C for 5 min; 25 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec; followed by a 5-min 72°C extension step. Samples were held at 4°C until analysis. PCR products were analyzed by electrophoresis of 10–20 µl of the samples in a 2% TAE agarose gel at 100 V, stained with ethidium bromide, and visualized under UV light. The specificity of the some fragments was confirmed by Southern blotting and hybridization to the 32P-labeled, 1.96-kb HindIII DEV DNA fragment. A positive control (DEV genomic DNA) and a negative control (glass-distilled water) were included with each PCR assay. In addition, DNA from MDV, chicken infectious anemia virus, CEF, and plasmids were used in some assays. DNA fragments in ethidium bromide-stained gels were visualized using an Eagle Eye II still video system with Eagle Sight v3.1 image capture and analysis software to acquire positive images (Stratagene, La Jolla, CA).

RESULTS

DEV clones and sequence. Seven white pBS clones were selected for analysis, and two colonies of each clone were screened for DEV DNA inserts. Three clones containing inserts of approximately 4 (Fig. 1a, lanes 4 and 5), 2 (Fig. 1a, lanes 12 and 13), and 7 kb (Fig. 1a, lanes 14 and 15), respectively, hybridized to a DEV

Fig. 3. Alignment of the aa sequences of the translated ORF UL6 for herpessimplex virus 1 (HSV-1) (Genbank protein accession no. 136794), varicella-zoster virus (VZV) (no. 136796), bovine herpesvirus 1 (BHV-1) (no. 971328), equine herpesvirus 1 (EHV-1) (no. 73996), suid herpesvirus 1 (SHV-1) (no. 1478623), and duck enteritis virus (DEV). The two regions with strong homology are shaded.
probe prepared from full-length DEV DNA (Fig. 1). This probe did not hybridize to HindIII-digested chicken DNA (Fig. 1b, lane 1), but did hybridize to HindIII-digested DEV DNA (Fig. 1b, lanes 2 and 3). The other four clones (Fig. 1b, lanes 6–11, 16, and 17) did not hybridize with the probe and were discarded. The 2-kb fragment (Fig. 1b, lane 12) was selected for complete sequencing from both directions. Based on preliminary sequence data, three fragments (5′HindIII–3′EcoRV [945 bp], 5′EcoRV–3′SalI [231 bp], and 5′SalI–3′HindIII [778 bp]) were subcloned for further sequencing. In addition, four primers were constructed (Fig. 2) to confirm the sequence for specific regions. The complete sequence (Fig. 2) was compiled using the Seqman function of DNAStar and has been submitted to GenBank (http://www.ncbi.nlm.nih.gov) (accession no. AF043730).

Sequence analysis. The 1954 bases were analyzed for potential open reading frames (ORFs). Two overlapping large ORFs in the same direction were found. A putative incomplete ORF coding for 428 amino acids (aa) and a stop codon was found between nucleotides 1 and 1212 (Fig. 2). A gapped BLASTP search of the Genbank database for proteins showed homology of this ORF to the UL6 protein of herpes simplex virus 1 (HSV-1) and varicella-zoster virus (VZV), with 50% and 54% identity based on the examination of 298 and 300 aa, respectively. There was also considerable similarity to the UL6 homologues in bovine herpesvirus 1 (BHV-1) (49% identity based on 368 aa), suid herpesvirus 1 (SHV-1) (56% identity based on 298 aa), and equine herpesvirus 1 (EHV-1) (46% identity based on 409 aa). In contrast, there was less homology with Ul104 of human cytomegalovirus (HCMV), which is a betaherpesvirus, and BBRF1 of Epstein-Barr virus (EBV), with 23% and 22% identity based on 274 and 286 aa, respectively.

A clustal alignment using weight matrix PAM250 of five alphaerpesviruses (HSV-1, VZV, BHV-1, EHV-1, and SHV-1) with the translated proximal DEV ORF showed a significant homology between the UL6 peptides of these viruses and DEV. Two regions with strong homology were found (Fig. 3). The first region was approximately 130 aa in length, starting at nucleotide (nt) 5 (Fig. 2), and codon for a potential leucine zipper motif. A second region of 130 aa was found downstream from this motif, starting at nt 542 (Fig. 2). The primers for the PCR assay were selected to amplify the former region of the proposed UL6 of DEV.

The distal ORF has two potential start codons with Kozaks consensus sequences at nt 1026 and nt 1047. ORFs originating from these start codons are in frame with each other.
Fig. 5. PCR amplification of DEV DNA using serial dilutions. PCR products were separated by electrophoresis through 2.0% agarose and stained with ethidium bromide (a). DNA was transferred to a nylon filter and hybridized to the 1.96-kb HindIII fragment of DEV DNA (b). Lane 1, HaeIII-digested PhiX174 RF DNA; lane 2, 10 ng DNA; lane 3, 4 ng DNA, lane 4, 800 pg DNA; lane 5, 160 pg DNA; lane 6, 32 pg DNA; lane 7, 6.4 pg DNA.

A gapped BLASTP search showed significant homology to the UL7 protein homologues of HSV-1 (34% identity, 284 aa), BHV-1, SHV-1, VZV ORF53 (37% identity, 290 aa) (data not shown). The arrangement of the two ORFs in the DEV fragment resembles the UL6/UL7 ORF arrangement found in HSV-1 and other herpesviruses in which the UL6 ORF overlaps with UL7 ORF.

**PCR assay.** Three independently prepared batches of viral DNA obtained from the cell culture-propagated LA strain of DEV were tested in the PCR assay. A discrete band of the expected size (421 bp) was produced from the DEV DNA templates, but no bands were detected with CEF DNA or MDV DNA (Fig. 4).

One of the DEV DNA preparations was used to determine the minimal amount of virus that could be detected in the PCR assay. DEV DNA concentrations from 10 ng to 0.08 pg were amplified and examined by hybridization to the 32P-labeled 1.96-kb HindIII fragment. A 32-pg concentration of viral DNA was detected in this assay by hybridization (Fig. 5). This amount of DNA represents approximately 100–200 mean tissue culture infective doses (TCID50) based on the titer of the virus preparation used for the extraction of DNA.

**PCR assay for detection of DEV in field samples.** DNA samples (100 ng in 10 μl) obtained from different sources (Table 1) were provided in a blinded fashion to one of us for
Fig. 6. PCR amplification of DNA samples for the detection of DEV DNA using primers 1 and 2. All samples were blinded by one operator prior to amplification by another operator. Refer to Table 1 for the description of samples A–F. The PCR products were separated by electrophoresis through 2.0% agarose, stained with ethidium bromide, and visualized using the Eagle Eye II still video system. Lane 1, genomic DEV DNA (positive control); lane 3, CEF DNA (negative control); lanes 2 and 4–9, DVE DNA samples A–F, as indicated below lane numbers (see Table 1 for details). Size in bp is based on electrophoresis of HaeIII-digested PhiX174 RF DNA.

use in the PCR assay. The results of two independent assays are presented in Figs. 6 and 7 and Table 1. All negative controls were negative, whereas samples from ducks with lesions typical for DVE were positive. Two samples from Muscovy ducks were passed twice in Muscovy duck embryos, resulting in 100% mortality in the second passage. Lesions in the dead embryos varied from slight to severe cutaneous hemorrhage, liver congestion or necrosis and atrophy, and in some embryos thickening of the chorioallantoic membrane. In both cases DNA samples prepared from the second embryo passage were positive in the PCR assay. A spleen sample obtained from a 43-day-old duck vaccinated with modified live virus vaccine at 14 days of age and challenged at 28 days of age was positive in the PCR assay. However, DNA obtained from the liver of the same duck was negative. Some samples were positive in one assay, but not in a second assay (Table 1).

DISCUSSION

To our knowledge this report provides the first sequence information for DEV and confirms that DEV belongs in the group of alphaherpesviruses. The 1954-bp DEV fragment codes for two large ORFs. The translated distal ORF is homologous to the amino terminus of HSV-1 UL7 peptide. The proximal ORF is ho-
mologous to the carboxy terminus of HSV-1 UL6 protein. UL6 codes for an essential protein needed for the cleavage and packaging of viral DNA to form viable virions (10). Comparison of the DEV UL6 ORF to other members of the herpesvirus family suggests that it is more homologous to alphaherpesvirinae than to beta- or gammaherpesvirinae. A 130-aa region with a leucine zipper motif is highly conserved among members of the alphaherpesvirus group. This region is also present in the HindIII fragment of DEV. Because this region is shared among the different viruses and codes for an important domain of an essential protein, it is expected that all DEV viruses have this gene with very little genetic variation. The primers for the PCR assay (nos. 1 and 2) are located in this region. It is expected that the use of these primers in a PCR assay will allow the detection of most if not all DEV strains.

Virus isolation assays followed by virus neutralization are used to confirm the presence of DEV in suspected outbreaks (14). Muscovy duck embryo fibroblasts are the preferred cells for the isolation of DEV (9). However, some less virulent strains only cause plaques in these cells when the cultures are incubated at 40.5–41.5 C (3). Three samples (G, H, and K) were obtained after two passages in Muscovy duck embryos incubated at 37 C. These isolates caused late occasional mortality in inoculated embryos, with inconsistent lesions, but all three samples were positive in the PCR assay, thus demonstrating the potential usefulness of this assay for diagnostic purposes.

Discrepancies were noted between the two PCR assays using blinded samples. Samples A and F were positive in the first assay and negative in the second assay. The most likely explanation for this change is that the DNA was not fully purified and degraded during storage. Unfortunately, these samples were not longer available for DNA extraction. Primers to amplify chicken actin DNA did not amplify duck DNA (Schat and O'Connell, unpubl. data), making it impossible to determine whether degradation had occurred. Sample E was still available, and positive results were obtained from the second preparation.

All herpesviruses are able to establish latency in their natural host. The alphaherpesvirinae establish latency often but not exclusively in the neural ganglia (12). The location of latency for DEV has not been established, although the ganglia have been suspected (Burgess, unpubl. data, quoted in Brand and Docherty [2]). It may be possible that blood lymphocytes carry latent virus, based on positive results obtained with the spleen sample from the duck vaccinated with modified live virus followed by challenge. Experiments to further establish the locations for latency are planned. If blood samples can be used for the detection of latent DEV infections it will become possible to examine the prevalence of virus in wild duck populations.

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