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Identification of Duck Plague Virus by Polymerase Chain Reaction

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SUMMARY. A polymerase chain reaction (PCR) assay was developed for detecting duck plague virus. A 765-bp *Eco*RI fragment cloned from the genome of the duck plague vaccine (DP-VAC) virus was sequenced for PCR primer development. The fragment sequence was found by GenBank alignment searches to be similar to the 3′ ends of an undefined open reading frame and the gene for DNA polymerase protein in other herpesviruses. Three of four primer sets were found to be specific for the DP-VAC virus and 100% (7/7) of field isolates but did not amplify DNA from inclusion body disease of cranes virus. The specificity of one primer set was tested with genome templates from other avian herpesviruses, including those from a golden eagle, bald eagle, great horned owl, snowy owl, peregrine falcon, prairie falcon, pigeon, psittacine, and chicken (infectious laryngotracheitis), but amplicons were not produced. Hence, this PCR test is highly specific for duck plague virus DNA. Two primer sets were able to detect 1 fg of DNA from the duck plague vaccine strain, equivalent to five genome copies. In addition, the ratio of tissue culture infectious doses to genome copies of duck plague vaccine virus from infected duck embryo cells was determined to be 1:100, making the PCR assay 20 times more sensitive than tissue culture for detecting duck plague virus. The speed, sensitivity, and specificity of this PCR provide a greatly improved diagnostic and research tool for studying the epizootiology of duck plague.

RESUMEN. Identificación del virus de la peste del pato mediante la reacción en cadena por la polimerasa.

Se desarrolló una prueba de reacción en cadena por la polimerasa para detectar el virus de la peste del pato. Un fragmento *Eco*RI de 765 pares de bases clonado del genoma del virus vacunal de la peste del pato fue secuenciado para la obtención de los iniciadores de la prueba de la reacción en cadena por la polimerasa. En investigaciones de alineación en el banco de genes ("GenBank") se encontró que la secuencia del fragmento era similar a los extremos 3′ de un marco de lectura abierto indefinido y al gen para la proteina de la DNA polimerasa en otros virus herpes. Se encontraron tres o cuatro grupos de iniciadores especificos para el virus vacunal y para el 100% (7/7) de los aìslamientos de campo, pero no amplificaron el DNA del virus de hepatitis por cuerpos de inclusión de grullas. Se analizó la especificidad de un primer juego de iniciadores con moldes del genoma de otros virus herpes aviares, incluyendo el águila dorada, águila de cabeza blanca, lechuza de cuernos grandes, lechuza blanca, halcón peregrino, palomas, aves psitácidas y pollos (virus de laringotraqueitis infecciosa), pero no se produjeron los productos finales. Por lo tanto, esta prueba de reacción en cadena por la polimerasa es altamente especifica para el DNA del virus. Dos grupos de iniciadores fueron capaces de detectar un fragmento de DNA de la cepa vacunal equivalente a cinco copias del genoma. Además, se determinó que la proporción de la dosis infecciosa en cultivo celular y copias del genoma del virus vacunal de células de embrión de pato infectadas era de 10 a 100 respectivamente, haciendo la prueba de la reacción en cadena por la polimerasa 20 veces más sensible que el cultivo celular para detectar el virus. La velocidad, sensibilidad y especificidad de la prueba de la reacción en cadena por la polimerasa suministra una herramienta de investigación y de diagnóstico altamente mejorada para el estudio de la epizootiología del virus.

Key words: duck plague, duck virus enteritis, polymerase chain reaction, diagnostic test, field isolates, avian herpesvirus

Abbreviations: APHIS = Animal and Plant Health Inspection Service; BEH = bald eagle herpesvirus; CHV = pigeon herpesvirus; CPE = cytopathic effect; DP-VAC = duck plague vaccine; EDTA = ethylenediaminetetraacetic acid; FHV = prairie falcon herpesvirus; GEH = golden eagle herpesvirus; GHOH = great horned owl herpesvirus; IBDC = inclusion body disease of cranes virus; ILTV = infectious laryngotracheitis virus; MDEF = Muscovy duck embryo fibroblast; MEM = minimum essential medium; NVSL = National Veterinary Services Laboratory; NWHC = National Wildlife Health Center; ORF = open reading frame; PCR = polymerase chain reaction; PFH = peregrine falcon herpesvirus; PHV = psittacine herpesvirus; SOH = snowy owl herpesvirus; TAE = Tris-acetate and EDTA; $TCID_{50}$ = median tissue culture infective dose

Duck plague was first introduced into the United States in 1967 (18). Since then, the disease has spread in the United States, affecting captive-reared waterfowl that are used for commercial sale or maintained in ornamental flocks (19,20,21). Only three disease outbreaks have been documented in migratory waterfowl: one in the Flanders Bay area of Long Island in 1967 (17), a second in 1973 at Lake Andes, South Dakota (11), and the last one in 1994 at Seneca, NY (National Wildlife Health Center [NWHC] files). In addition, other outbreaks in rural nonmigratory flocks of waterfowl of mixed breed species have been reported (25).

Duck plague is difficult to monitor and control because the virus establishes an asymptomatic carrier state in waterfowl that is detectable only during periods of intermittent virus shedding (4). Although avian tissue culture systems are routinely used to isolate duck plague virus, they are not always a reliable indicator of virus presence because cell cultures vary in sensitivity and are less sensitive than animal hosts (5,14,15,27). Molecular diagnostic techniques, such as polymerase chain reaction (PCR), are proving to be exquisitely sensitive and able to detect small quantities of virus (8,26).

We report the establishment of a PCR-based DNA diagnostic test for duck plague virus. The sensitivity and specificity of this test for duck plague virus was determined, and its use to identify duck plague virus from field isolates is reported.

MATERIALS AND METHODS

Viruses and cell culture. The viruses listed in Table 1 were passed in Muscovy duck embryo fibroblasts (MDEFs) prepared by the method of Docherty and Slota (10). Viruses were inoculated into 75-cm^2 flasks in 1.0-ml quantities and allowed to adsorb for 1 hr at 37 C in 4% CO_2 atmosphere. Twelve milliliters of growth medium containing M199-Earles' supplemented with 10% fetal bovine serum, 2 mM L-glutamine, minimum essential medium (MEM) vitamins, MEM nonessential amino acids, and antibiotics (100 IU/ml penicillin G, 100 μg/ml streptomycin sulfate, 100 IU/ml mycostatin, and 50 μg/ml gentamicin) was added to each flask, and the flasks were reincubated until infected cells reached 90%–100% cytopathic effect (CPE). Flasks were then frozen and thawed three times, and the cell debris was pelleted by centrifugation at 800 × *g* for 20 min. The supernatant representing the virus stock was subdivided into vials and stored at −85 C.

Genome preparation. MDEFs infected with 1.0 ml of stock viruses were allowed to reach 90%–100% CPE before cells were mechanically dislodged from the flask surface. Cells and extracellular virus particles were pelleted by centrifugation at 16,000 × *g* for 30 min. Pelleted cells were disrupted in sterile distilled water by freeze-thawing (−85 C to 23 C) three times, then centrifuged at 16,000 × *g* for 5 min, and the pellet was discarded. The supernatant was centrifuged at 16,000 × *g* for 30 min to concentrate virus particles. Pellets were resuspended in 200 μl Tris–ethylenediaminetetraacetic acid (EDTA) buffer (10 mM Tris-HCl, pH 8.0, with 1 mM EDTA), and 10 μg of ribonuclease A (Sigma Chemical Co., St. Louis, MO) was added before incubation at 37 C for 30 min. Proteinase K was added to a final concentration of 0.2 μg/μl, and the mixture was incubated at 56 C for 1 hr. The preparation was made 1% for sodium dodecyl sulfate and incubated at 37 C for 30 min, then NaCl was added to 0.5 M. The mixture was extracted two times with phenol (saturated with 10 mM Tris, pH 8.0) and two times with ether. Samples were made 2.5 M for ammonium acetate, and the DNA was precipitated with two volumes of 95% ethanol held at 21 C for 30 min. The preparation was centrifuged at 16,000 × *g* for 30 min, and the pellet was washed once with 70% ethanol. Viral DNA pellets were resuspended in 40 μl of Tris-EDTA and stored at 4 C. Extracts of normal noninfected MDEF cells were prepared and used as negative controls. One microliter from each extract was run in a 1% agarose gel with 1 × Tris acetate–EDTA (TAE) buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.3) at

Table 1. List of herpesviruses used for genome extraction and template in PCR.

Herpesvirus identification/ accession no.	Source[A]	Isolation information			Passage[B]
		Year	Location	Species	
DP-VAC (vaccine strain)	APHIS[C]	NA[D]	NA		2
LA-SD-73	NWHC	1973	Lake Andes, SD	Mallard (*Anas platyrhynchos*)	12
SP-MN-73	NWHC	1973	St. Paul, MN	Mute swan (*Cygnus olor*)	6
CO-WI-73	NWHC	1973	Coloma, WI	American black duck (*Anas rubripes*)	4
WA-NY-73	NWHC	1973	Wantagh, Long Is.	Muscovy duck (*Cairina moschata*)	8
SE-NY-94 12628-005	NWHC	1994	Seneca Co., NY	Mallard	8
SA-CA-77 467	NWHC	1977	Sacramento, CA	Muscovy duck	8
SO-CA-92 10700-001	NWHC	1992	Sonoma Co., CA	Muscovy/mallard hybrid	8
IBDC (parental strain)	NWHC	1978	Baraboo, WI	Sandhill crane (*Grus canadensis*)	5
GEH 5386-001	NWHC	1985	Pierre, SD	Golden eagle (*Aquila chrysaetos*)	3
BEH 7475-001	NWHC	1987	Bellevue, WA	Bald eagle (*Haliaeetus leucocephalus*)	4
GHOH 10634-04	NWHC	1992	Grand Island, NE	Great horned owl (*Bubo virginianus*)	5
SOH 2282-25155	NWHC	1991	Duluth, MN	Snowy owl (*Nyctea scandiaca*)	4
PFH 13033-001	NWHC	1994	Frankfurt, KY	Peregrine falcon (*Falco peregrinus*)	4
FHV (ATCC-709)	NVSL	1973		Prairie falcon (*Falco mexicanus*)	5
CHV (ATCC-705)	NVSL	1972		Pigeon (*Columba* sp.)	5
PHV 037-ADV-9301	NVSL	1977		Psittacine (*Psittacidae* sp.)	6
ILTV (ATCC-783)	NVSL	1963		Chicken	4

[A]APHIS = Animal and Plant Health Inspection Service; NVSL = National Veterinary Services Laboratory.
[B]Tissue culture passage level after receipt of virus.
[C]Viruses received from USDA or isolated from diagnostic submissions to the NWHC or part of repository.
[D]NA = not applicable.

Table 2. Sequences of duck plague primers.

Primer set		5′–3′ Sequence
1	Forward	TCATCGACTGCCTTAAATCT
	Reverse	TACTTATCTGTGGTACGCTGTC
3	Forward	GCCAACTCAGAAACCAATAC
	Reverse	CAAATCCCAAGCGTAGAAC
4	Forward	CTTAGAGTCCCCAACAGATG
	Reverse	CAAATCCCAAGCGTAGAAC
5	Forward	GGCTGGTATGCGTGACAT
	Reverse	GTATTGGTTTCTGAGTTGGC

120 V for 1 hr. Gels were stained with ethidium bromide, and results were recorded on Polaroid (Type 655) film (23). The amount of DNA in the duck plague vaccine (DP-VAC) and inclusion body disease of cranes virus (IBDC) extracts was determined by a spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). The DNA concentration in extracts from the duck plague field isolates and other avian herpesviruses was estimated by comparing gel staining intensities of genome extracts with known concentrations of molecular size controls (23).

Sequencing and primer selection. The EcoRI-restricted DP-VAC DNA and pUC18 plasmid were prepared and ligated by the method of Brown *et al.* (3). The recombinant plasmids were transformed into competent *Escherichia coli* TB1 cells. Recombinant clones were screened for DNA inserts by procedures described previously (2). Clone dve_p481 was sequenced by dideoxy sequencing (24) with Sequenase (United States Biochemicals Corp., Cleveland, OH) and ^{35}S-dATP with universal primers and designed primers for primer extension sequencing resulting in sequence data on both strands. The contig sequence assembly and analysis of dve_p481 was done with EuGene/SAM software (16). Plasmid dve_p481 was purified with QIAGEN-tip 20 columns following the manufacturer's protocol (Qiagen, Inc., Valencia, CA), and the recovered DNA served as double-stranded templates in cycle sequencing reactions by standard protocols for ABI Prism 377 DNA sequencer (Perkin-Elmer Cetus Inc., Norwalk, CT). Primers were designed for primer extension sequencing resulting in sequence data for both strands. "Filtered" (see URL http://ncbi.nlm.nih.gov/BLAST/filter.html) and "unfiltered" BLASTIN (1) and BLASTX (13) searches (1,13) of the GenBank or EMBL sequence databases were performed with the reverse complement sequence deposited (AF064639) with GenBank. The sequence information was used to construct PCR primers with Primer 0.5 software (6). Custom primers were purchased from BRL Life Technologies, Grand Island, NY (Table 2).

PCR. All four primer sets (Table 2)were tested for their ability to produce amplicons. Primer sets were prepared according to the manufacturer's recommendation and optimized with 1 ng/10 μl of DP-VAC DNA (7). The PCR used a hot start method with wax (Ampliwax beads; Perkin Elmer Cetus, Norwalk, CT) and reagents from a commercial PCR kit (GeneAmp PCR Reagent Kit; Perkin Elmer Cetus)

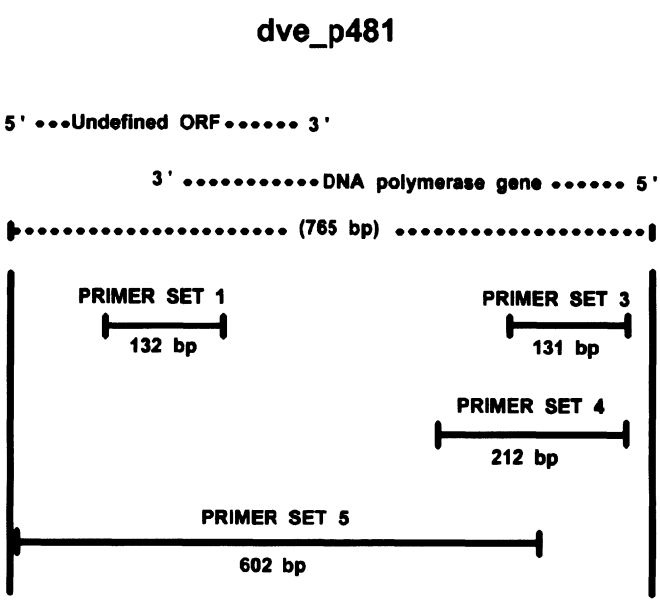

Fig. 1. Schematic of clone dve_p481 submitted to GenBank and the regions amplified by the duck plague PCR primer sets.

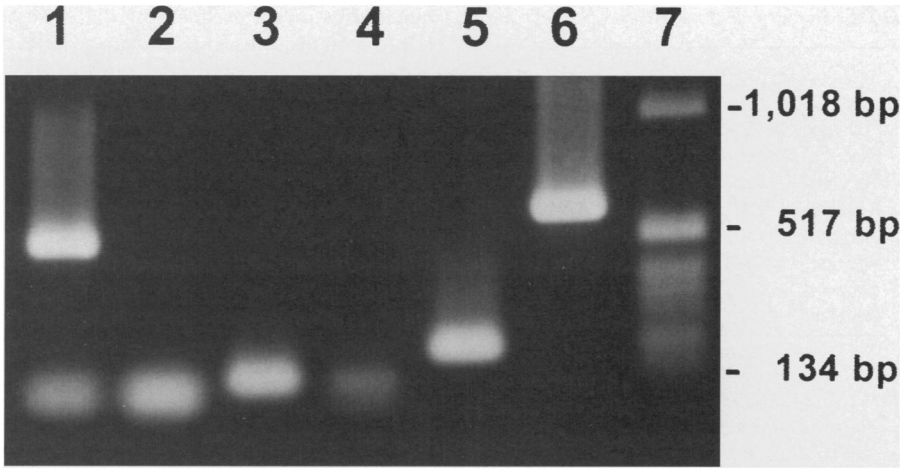

Fig. 2. Identification of 1 ng of DP-VAC genome template by primer sets 1 (lane 3), 3 (lane 4), 4 (lane 5), and 5 (lane 6). A positive lambda template (lane 1) and a negative lambda template (lane 2) were used as controls. A 1-kbp ladder was used as a molecular size marker (lane 7).

according to their setup recommendations. A lambda DNA fragment (500 bp) and primers supplied with the commercial PCR kit were used as a known positive and negative DNA control in each PCR assay.

The specificity of DP-VAC primer sets 1, 4, and 5 was tested with DP-VAC and IBDC genomes diluted to 100 pg/10 μl of reaction mixture. Normal tissue culture extract material diluted to simulate 1.0 ng of template in a reaction mixture was used as a control. A PCR reaction tube without DNA was used to verify that nonspecific amplification did not occur.

Genomes from duck plague field isolates and other avian herpesviruses were diluted to 100 pg/10 μl of DNA material. The duck plague field isolate DNA was tested with primer sets 1, 4, and 5, and DNA from the other avian herpesviruses was tested with primer set 5. PCR products from all field isolate genomes for primer set 5 were sequenced by the University of Wisconsin, Biotechnology Center, Madison, WI, and compared with the dve_p481 clone sequence.

Samples were subjected to the following in an automated DNA thermal cycler (TempCycler, Coy Laboratory Products Inc., Ann Arbor, MI): the initial cycle was 94 C for 2 min, 37 C for 1 min, and 72 C for 3 min. The reaction mixture was cycled 35 times at 94 C for 1 min, 55 C for 1 min, and 72 C for 2 min. The final cycle was at 72 C for 7 min.

PCR products were examined in 1% agarose gels with 1 × TAE buffer as described above. The sizes of PCR products were compared with a 1-kb pair ladder (BRL Life Technologies) used as molecular size markers.

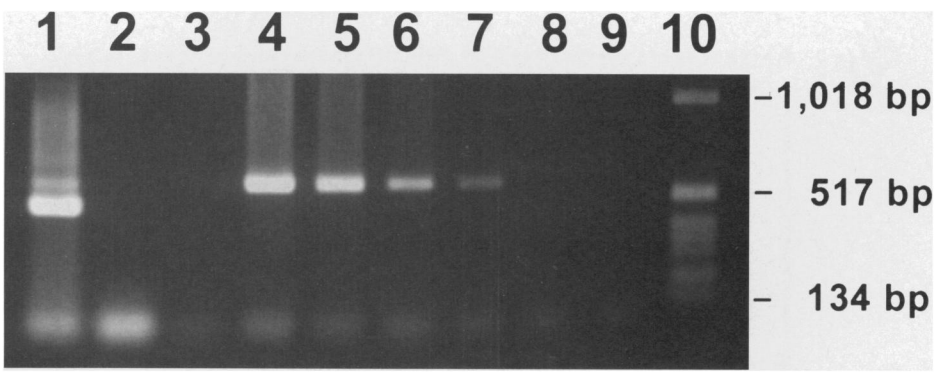

Fig. 3. PCR sensitivity of primer set 5 for detecting DP-VAC template DNA diluted to 1 pg (lane 4), 100 fg (lane 5), 10 fg (lane 6), 1 fg (lane 7), 100 ag (lane 8), and 10 ag (lane 9). A no-template control was used in A lane 3. A negative lambda template (lane 2) and a positive lambda template (lane 1) were used as controls. A 1-kbp ladder was used as a molecular size marker (lane 10).

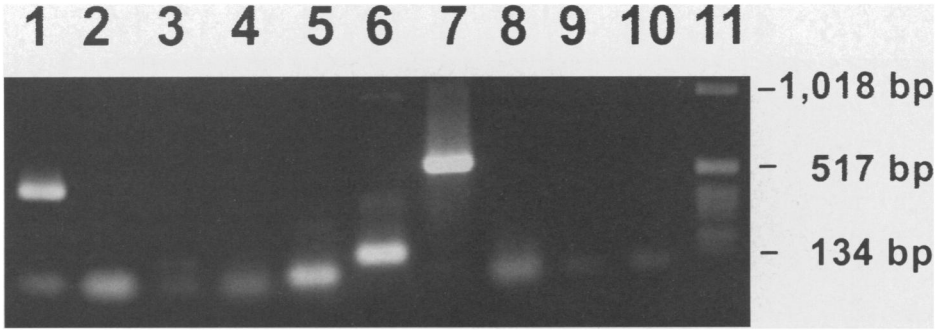

Fig. 4. Specificity of primer sets 1 (lanes 5 and 8), 4 (lanes 6 and 9), and 5 (lanes 7 and 10) for 100 pg of DP-VAC template (lanes 5–7) and IBDC template (lanes 8–10). Normal cell extract control and primer set 5 (lane 3). A no-template control was used in lane 4. A positive lambda template (lane 1) and a negative lambda template (lane 2) were used as controls. A 1-kbp ladder was used as a molecular size marker (lane 11).

Comparison of tissue culture and PCR sensitivity. A 75-cm^2 flask of MDEF cells was infected with DP-VAC virus and incubated for 2 hr at 37 C in 4% CO_2. The inoculum was removed, the cell sheet was washed 2 × with 5 ml of cell culture medium, and 4 ml of growth medium was added before reincubation. The cell sheet was allowed to reach 90%–100% CPE. At harvest, a 100-μl sample was taken, diluted 10-fold, and titrated in a 96-well plate with eight replicates per dilution containing MDEF according to the method of Docherty and Henning (9). After 7 days of incubation at 37 C in 4% CO_2, the virus titer was calculated (22) and expressed as 50% tissue culture doses ($TCID_{50}$). The total flask $TCID_{50}$ was adjusted for the titration sample for comparison with recovered DNA. The remainder of the flask was extracted for DP-VAC DNA. The total DNA in the flask was determined by spectrophotometry and used to calculate the number of DP-VAC genomes recovered based on the genome molecular weight reported by Gardner *et al.* (12).

PCR sensitivity was determined for the DNA extract by diluting whole DP-VAC template to 1 ng/10 μl of reaction material after the DNA concentration was determined by spectrophotometry. A serial 10-fold dilution set was prepared, and 10 μl of each dilution was added to reaction tubes containing one of the three primer sets. PCR product was evaluated in ethidium bromide-stained 1% agarose gels run with 1 × TAE buffer as described above.

RESULTS

One 765-bp cloned fragment designated dve_p481 was selected for primer development. In filtered BLASTN and BLASTX searches, dve_p481 contained high sequence similarity to an undefined open reading frame (ORF) and a DNA polymerase gene in other herpesviruses. The dve_p481 clone showed sequence similarity for a number of herpesvirus DNA polymerase genes, ranging from 59% to 75% similarity depending on the virus. Hence, dve_p481 appears to contain a 3′ end of an undefined ORF and the 3′ end of the DNA polymerase gene located on opposite strands of the DNA clone (Fig. 1). Primer sets were selected for PCR testing by their ability to detect different regions of the cloned fragment (Fig. 1). The sequences for primer sets 1, 4, and 5 were compared with other herpesviruses. None of the primer sets had complementary base matches for both primers to the same virus. For example, primer 4f had the highest base match (18) to Gallid herpesvirus 2, but the 4r primer did not match any herpesvirus in the sequence databases.

Three of four primer sets prepared from dve_p481 produced amplicon molecular weights of expected size in an optimized PCR test (Fig. 2). Primer set 1 targets a unique region of an undefined gene producing a 132-bp product. Primer set 4 locates a conserved genome region in the DNA polymerase gene producing a 212-bp product, and primer set 5 produces a product that spans both ORFs producing a 602-bp product. Primer set 3 was not used for additional work because of poor PCR performance.

The time required to obtain results was rapid compared with traditional virologic methods. The entire procedure, starting with the DNA extraction method until visualization of the results, required only 36–48 hr.

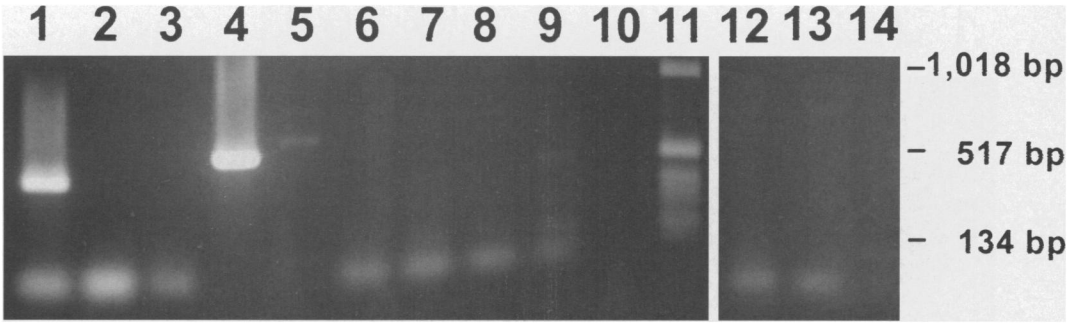

Fig. 5. PCR cross-reactivity of DP-VAC primer set 5 with other avian herpesvirus isolates. Avian herpesvirus templates were used at 100 pg as follows: no DNA (lane 3), DP-VAC (lane 4), golden eagle herpesvirus (GEH) (lane 5), bald eagle herpesvirus (BEH) (lane 6), great horned owl herpesvirus (GHOH) (lane 7), snowy owl herpesvirus (SOH) (lane 8), peregrine falcon herpesvirus (PFH) (lane 9), prairie falcon herpesvirus (FHV) (lane 10), pigeon herpesvirus (CHV) (lane 12), psitticine herpesvirus (PHV) (lane 13), and infectious laryngotracheitis virus (ILTV) (lane 14). A positive lambda template (lane 1) and a negative lambda template (lane 2) were used as controls. A 1-kbp ladder was used as a molecular size marker (lane 11).

The sensitivity of the PCR assay was determined by testing serial dilutions of DP-VAC genome DNA. Primer set 1 amplified down to a genome concentration of 1 pg of DNA (data not shown). The other two primer sets (4 and 5) produced detectable amplicons at 1 fg of DNA, as shown in Fig. 3 for primer set 5.

To determine the virus particle to $TCID_{50}$ ratio for DP-VAC virus, we compared the total $TCID_{50}$ for an infected flask with the total DNA extracted and found a ratio of one infectious unit per 100 genome copies. The total virus titer of the infected flask was estimated at $10^{8.1}$ $TCID_{50}$, and the amount of DNA extracted was calculated to be $10^{10.1}$ genomes.

Primer sets 1, 4, and 5 were tested for their specificity to duck plague DNA (Fig. 4). The three primer sets produced amplicons from DP-VAC DNA but not from IBDC DNA. Primer sets 1, 4, and 5 did not amplify a product from the normal cell extract material (see Fig. 4 for primer set 5, data for others not shown). The genomes of nine avian herpesviruses were used as templates in the PCR test with primer set 5 (Fig. 5). No specific amplification products were detected from genomes for these herpesviruses.

The three primer sets were able to identify templates from field isolates of duck plague virus (Fig. 6). The three primer sets produced amplicons from all seven field isolate genomes tested. The amplicons from the field isolate genomes for primer set 5 were verified as duck plague by comparing their DNA sequences with the dve_p481 clone.

DISCUSSION

The PCR assay developed for duck plague offers a rapid, specific, and sensitive method for detecting and identifying duck plague virus in field isolates without detecting other avian herpesviruses (Figs. 4–6). On the basis of the comparisons made, the oligonucleotide primers selected for this PCR were specific for duck plague DNA (Figs. 4, 5). Despite the sequence similarity of the dve_p481 clone to the DNA polymerase gene of the Marek's disease virus, a conserved region in herpesvirus genomes, none of the primer set sequences showed 100% sequence identity with other herpesviruses. These results suggest that the duck plague primer sets may not be good for detecting other herpesviruses. However, additional herpesviruses need to be tested to determine how specific the duck plague primer sets are.

The PCR was found to be 20 times more sensitive than tissue culture for detecting duck plague virus when the number of infectious units per sample was compared with the number of genome copies. These PCR characteristics, and a quick sample processing and run time of 36–48 hr, provide an assay that is more reliable and accurate than traditional virus isolation and serologic identification methods used for detecting duck plague virus.

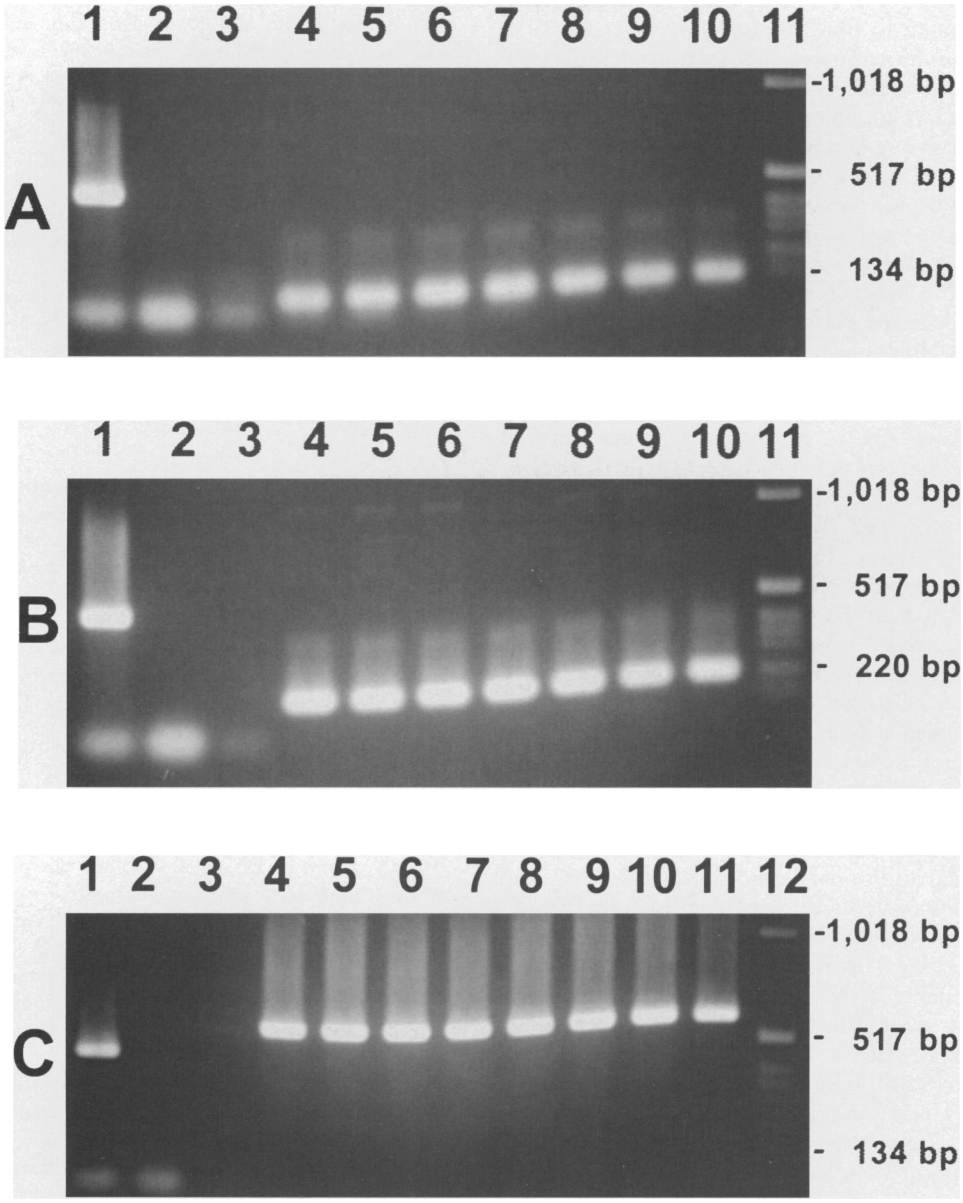

Fig. 6. PCR specificity of primer sets 1 (A lanes 3–10), 4 (B lanes 3–10), and 5 (C lanes 3–11) for 100 pg of DNA from the DP-VAC (C lane 4), and duck plague field isolates LA-SD-73 (A and B lane 4; C lane 5), SP-MN-73 (A and B lane 5; C lane 6), CO-WI-73 (A and B lane 6; C lane 7), WA-NY-73 (A and B lane 7; C lane 8), SE-NY-94 (A and B lane 8; C lane 9), SA-CA-77 (A and B lane 9; C lane 10), and SO-CA-92 (A and B lane 10; C lane 11). Lambda positive and negative controls (A–C lanes 1 and 2) and a 1-kbp molecular size ladder (A and B lane 11; C lane 12) were used for comparison. A no-template control (A–C lane 3) was used for each primer set.

This PCR assay is very sensitive for detecting small numbers of duck plague viral genome material. The estimated number of viral genome copies required to produce a PCR product was calculated to be five, based on the 1 fg of DNA detected (Fig. 3) and the molecular weight estimates of the duck plague genome provided by Gardner *et al.* (12). These results

are similar to the PCR for varicella-zoster virus that has an estimated DNA detection sensitivity of six genome copies (8). The PCR for infectious laryngotracheitis virus (ILTV) was able to detect five infectious virus particles per 100 μl, but no estimate of the actual number of genome copies present was made (26). These results also suggest that very little contaminating cellular DNA was in the viral extract material affecting spectrophotometer readings used to dilute the templates.

The duck plague PCR was able to uniformly identify historic as well as more recent duck plague field isolates from the United States. The PCR was able to detect the genomes from all seven duck plague field isolates used as templates (Fig. 6). These isolates represent disease outbreaks from three geographic regions of the United States, including east, midwest, and west coast, and span 21 yr (Table 1).

PCR technology has proven useful for detecting acute infections and latent carriers of herpesviruses (8,26). The three primer sets used for this PCR also provide the ability to diagnose duck plague in tissues from suspect cases. Because of their high specific sensitivity to duck plague DNA and the high molecular weight amplicons, both primer sets 4 and 5 can be used to identify DNA by a standard PCR assay without additional verification. Primer set 1 can be used to verify duck plague by nested amplification of the standard PCR product from primer set 5 if initial amplicon concentrations are small (7). Nested primer sets have proven useful for detecting small concentrations of DNA in latent infections of the trigeminal nerve (8,26). Thus, the increased sensitivity of nested primers can be used to detect low-level duck plague virus shedding in waterfowl. Work is in progress to apply these PCR assay methods to diagnose suspect cases of duck plague submitted to the NWHC. Field studies are under way to evaluate the ability of the duck plague PCR to identify asymptomatic virus-shedding waterfowl. The PCR assay provides a powerful new tool for studying the epizootiology of duck plague in waterfowl populations.

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