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Gross pathology and epidemiological features of mule deerpox virus infections in farmed white-tailed deer (*Odocoileus virginianus*) in Florida

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ABSTRACT

Mule deerpox virus (DPV) is a member of the *Poxviridae* family that affects various deer species, causing crustaceous skin lesions and potentially lethal infections. In this study, we aimed to investigate the spatial and temporal distribution of DPV in farmed white-tailed deer (WTD; *Odocoileus virginianus*) in Florida, USA, from 2017 to 2023. Necropsies were performed, and specimens were collected from 776 deer. Focusing on 145 animals with DPV-like lesions, polymerase chain reaction (PCR) testing and whole genome sequencing confirmed the presence of DPV in multiple specimen types. The results indicated that 49 out of 145 deer (33.8 %, 95 % CI: 25.7 %-40.9 %) tested positive for DPV. Mule deerpox virus was identified in 20 of the 37 counties and 8 of the 10 deer management units (DMUs) where we collected specimens. All DPV infection cases occurred in summer and fall. Lesion swabs (4/4, 100 %) and lesion tissues (16/18, 89 %) exhibited the highest PCR-positivity rates among the specimens collected from DPV-infected animals, while no whole blood samples (0/13, 0 %) tested positive. Additionally, DPV nucleic acid was also detected in fecal swabs (4/9, 44 %), suggesting that DPV may be transmitted through the oral-fecal route. Fawns aged 1–3 months (37/70, 52.9 %, 95 % CI: 41.3 %-64.1 %) exhibited statistically higher positivity rates than other age groups. This is the first study investigating the spatial and temporal patterns of DPV in farmed WTD in Florida. The findings emphasize the need for enhanced disease surveillance, non-invasive testing methods, and preventive measures to mitigate the impact of DPV on the deer farming industry and wildlife conservation.

1. Introduction

The family *Poxviridae* is comprised of enveloped double-stranded DNA viruses whose virions are among the largest in terms of physical and genome sizes (Lefkowitz et al., 2006; Haller et al., 2014). Some poxviruses have limited host ranges, while others, such as cowpox virus and monkeypox virus (MPXV), have broad host ranges that might be expanding (Haller et al., 2014). Mule deerpox virus (DPV) is the only recognized species in the genus *Cervidpoxvirus* to date (Carstens and Ball, 2009). In 2010, the deerpox virus was renamed mule deerpox virus in anticipation of the discovery of new related viruses that might infect other deer species. This measure was taken to prevent confusion and accurately reflect its natural host range (Adams and Carstens, 2012). In 2023, the ICTV renamed the scientific name of DPV as *Cervidpoxvirus muledeerpox* to follow the standardized binomial format. Mule deerpox virus has a genome size of 166 kbp (Afonso et al., 2005). The common clinical presentation of DPV infection includes crustaceous scabs, skin lesions, and ulcers on abdomen, chin, coronary bands, ears, eyelids, hard palate, legs, muzzle, neck, rumen, and tongue (Williams et al., 1985; Junge et al., 2000; Moerdyk-Schauwecker et al., 2009; Bildfell et al., 2010; Baughman et al., 2011; Bracht et al., 2013; Sayler et al., 2019; Armien et al., 2020). Some animals also present with abdominal distention (Baughman et al., 2011), arthritis (Sayler et al., 2019), conjunctivitis (Williams et al., 1985; Baughman et al., 2011), depression

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(Bildfell et al., 2010), lameness (Sayler et al., 2019), lymphadenopathy (Sayler et al., 2019), papules and pustules (Bildfell et al., 2010), pyrexia (Bildfell et al., 2010), and secondary bacterial infections (Williams et al., 1985; Bildfell et al., 2010; Baughman et al., 2011; Sayler et al., 2019).

Mule deerpox virus was first identified in free-ranging mule deer (*Odocoileus hemionus*) in Wyoming, USA, in 1983 (Williams et al., 1985). Subsequent reports of cervids with DPV infections included those found in a captive black-tailed deer (*Odocoileus hemionus columbianus*) in California, USA, a wild mule deer in Oregon, USA, and a captive reindeer (*Rangifer tarandus tarandus*) in Ontario, Canada (Moerdyk-Schauwecker et al., 2009). In 2010, DPV was identified in a captive white-tailed deer (WTD; *Odocoileus virginianus*) in Mississippi, USA (Baughman et al., 2011). In the same year, a goitered gazelle (*Gazella subgutturosa*) in a zoo in Minnesota, USA, was diagnosed as DPV-infected; this is the first report of DPV in a host animal other than a cervid species (Bracht et al., 2013). Mule deerpox virus was first found in farmed WTD in Florida, USA, in 2016 (Sayler et al., 2019). These reported cases predominantly involved young animals under five months old, suggesting that the morbidity and mortality rates of DPV in fawns may be higher than in adults. A serosurvey conducted in free-ranging cervid species in Oregon, USA, further suggested the broad species susceptibility and frequency of exposure: DPV antibodies were detected in 52 % of black-tailed deer, 36 % of Columbian WTD (*O. virginianus leucurus*), and 32 % of mule deer (Jin et al., 2013).

Mule deerpox virus has been confirmed to be transmitted via direct and indirect contact. An experimental study of DPV in black-tailed deer has confirmed that fawns can be infected through intracutaneous and intravenous routes, as well as by commingling an uninoculated animal with experimentally infected fawns (Bildfell et al., 2010). However, whether DPV can be mechanically transmitted through arthropod vectors or vertically transmitted remains unclear. In previous studies on DPV, the primary specimen types tested were skin lesion tissue and serum, with limited information available on the positivity rate and clinical sensitivity of other specimen types. It also remains unclear whether DPV can affect major organs.

According to the USDA 2022 Census of Agriculture report, there are 143 deer farms in Florida, ranking the state fifth in the nation for cervid farming (https://www.nass.usda.gov/AgCensus/). Epizootic hemorrhagic disease virus (EHDV) and blue tongue virus (BTV) infections are among the most extensively studied viral infections in farmed WTD in Florida (Cottingham et al., 2021). However, other infectious pathogens affecting deer remain less well characterized. To better understand infectious diseases in Florida WTD and their preventive measures, the Cervidae Health Research Initiative (CHeRI) at the University of Florida (UF) has been providing on-site necropsy and diagnostic services for deer farms in Florida since 2016. In 2016, the first case of DPV in Florida was identified in a fawn (Sayler et al., 2019). Following this initial case, similar clinical signs were observed annually in deer on multiple farms. To enhance our understanding of DPV infection within Florida's farmed WTD deer populations, we conducted a retrospective analysis, screening past cases for the presence of DPV, with a focus on animals that exhibited clinical signs consistent with DPV infection. This study aimed to better understand the spatial and temporal prevalence and distribution of DPV cases in farmed WTD in Florida, and to determine the types of specimens that contained DPV DNA.

2. Materials and methods

2.1. Necropsy and specimen collection

Necropsies occurred at the behest of deer farmers and were performed at farms. Information such as age, animal ID, initial observations, location, and medical history were documented before initiation of a necropsy. Tissue specimens were cut into approximately 1 cm sizes and then placed into 5 mL sterile Eppendorf snap cap tubes (Thermo Fisher Scientific). Routinely collected tissue specimens included cardiac tissue (CT), hepatic tissue (HT), kidney tissue (KT), lung tissue (LT), grossly normal skin tissue (SK), and spleen tissue (ST). Lesion tissue (LE), including purulent exudates, scabs, and ulcerative lesions on the skin, consistent with DPV infections, was collected from the periphery of the lesions. This area was targeted for sampling as it is where the virus actively replicates and spreads outward, ensuring the highest likelihood of detecting DPV DNA. Gastrointestinal tissue (GI) specimens were collected only when DPV-like lesions, such as ulcerative lesions, were present. Fecal swabs (FS) and nasal swabs (NS) were collected with sterile flocked nylon swabs (Copan Diagnostics). Fecal swabs were kept in empty sterile 15 mL screw cap centrifuge tubes (Thermo Fisher Scientific), and nasal swabs were immersed into 5 mL RNAlater stabilization solution tubes (Invitrogen). Up to 2 mL of whole blood (WB) was collected via a cardiac puncture with a disposable 18 G or 20 G needle (EXELINT International) and 10 mL syringe (EXELINT International), and then transferred into 2 mL BD Vacutainer EDTA tubes (Becton Dickinson) immediately after collection. Photos of the animal and each specimen were taken during necropsy. All swabs, tissues, and whole-blood specimens were refrigerated during transportation and stored in a −80 °C freezer immediately upon arrival of the necropsy team at the UF College of Veterinary Medicine. Lesion swab (LS) specimens were collected with sterile flocked swabs from live animals by the owners and immersed into 5 mL RNAlater stabilization solution tubes (Invitrogen). Lesion swab (LS) and lesion tissue (LE) specimens from live animals were placed in a foam-insulated cooler with ice packs, secured in a secondary container, and shipped overnight to the UF CHeRI laboratory for analysis. The specimens required for bacterial pathogen isolation were delivered directly to the UF Microbiology, Parasitology, and Serology Diagnostic Laboratory of the College of Veterinary Medicine for aerobic or anaerobic bacterial culture, depending on the specimen type and suspicion of bacterial involvement in the lesion. Specimens for bacteriology work-up were cultured on general blood agar, chocolate agar, Columbia Nalidixic Acid (CNA) agar, and MacConkey agar plates for pathogen isolation and incubated as appropriate for aerobic and anaerobic bacteria. Bacterial species identification was performed using MALDI-TOF (MALDI Biotyper Sirius One System, Bruker). Only specimens from animals with skin lesions were selected for DPV testing. The ages, demographics, gross necropsy findings, medical history, sex, and test results of the animals were documented using Microsoft Excel for data management and analysis.

2.2. Polymerase chain reaction

Total DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen) following the spin-column protocol and the recommendation for different specimen types with minor modifications. All tested specimens are listed in Table S1. Negative extraction controls were included in each extraction batch. The concentrations of extracted DNA were measured using a Nanodrop 8000 spectrophotometer (Thermo Fisher Scientific) or a Qubit 3.0 (Life Technologies) with HS DNA reagent. Conventional PCR was conducted in 25 μL reaction mixtures composed of 1 μL of DNA template (up to 100 ng per reaction), 12.5 μL of 2X PCR master mix (Promega), 9 μL of water, and 1.25 μL of each 20 μM low-GC pox virus primers (Eurofins Scientific). The low-GC poxvirus primers were selected due to their proven efficacy in prior studies and the current lack of DPV-specific PCR primers. The forward primer (5'-ACA CCA AAA ACT CAT ATA ACT TCT-3′) targets a binding site within the DPV insulin metalloproteinase-like protein gene, and the reverse primer (5'-CCT ATT TTA CTC CTT AGT AAA TGA T-3′) targets a binding site within the intracellular mature virion membrane protein gene (Li et al., 2010). The expected amplicon size was 220 bp. Thermocycling was performed in a MiniAmp Plus thermal cycler (Applied Biosystems) as follows: 95°C initial denaturation for 2 min, 25 cycles of denaturation at 95°C for 30 s, primer annealing at 50°C for 1 min, and elongation at 72°C for 1 min, and an additional 15 cycles of 95°C initial denaturation for 30 s, 53°C primer annealing for 30 s, and 72°C elongation of 1 min, with a 72°C

final elongation for 5 min, then 4°C for ∞ . Negative extraction and PCR controls, and a positive PCR control, were included during PCR assessments. PCR amplicons were visualized through gel electrophoresis. Specifically, 5 μL of the PCR product was mixed with 1 μL of 6X Tritrack gel dye (Thermo Fisher Scientific) and loaded onto a 1.5 % UltraPure agarose gel (Invitrogen) containing SYBR Safe DNA gel stain (Invitrogen) and 1X TBE buffer (Thermo Fisher Scientific). Electrophoresis was performed at 200 V for 20 min.

2.3. A21 gene amplification and sequencing

To confirm the species of the low-GC poxvirus, the A21 gene from five randomly selected PCR-positive samples was amplified and sequenced. The A21 gene was chosen for analysis because of its diversity in poxviruses and was used for poxvirus phylogenetic analyses (Baughman et al., 2011). The selected samples were OV592 nasal swab (NS), OV1682 skin tissue (SK), OV1846 skin lesion tissue (LE), OV1859 lesion tissue (LE), and OV1863 lesion tissue (LE). The A21 gene PCR primer set was designed by the UF CHeRI team using the Primer3 software version 4.1.0 (Untergasser et al., 2012). Two DPV-A21 primer sets, DPV-A21a (forward primer: 5'-CGG TAG AAA AGA GGA ATC CGC-3′ and reverse primer: 5'-AGT TGC CCA ATC AAT TAA AGA-3′) and DPV-A21b (forward primer: 5'-TGC ACC TCC TGT ATC TTC CA-3′ and reverse primer: 5'-GCG TTT CCA AAG CAA GTT G-3′) were obtained from Eurofins Scientific (Louisville, KY, USA). The expected amplicon size was 726 bp and 621 bp, respectively. PCR was conducted in 30 μL reaction mixtures composed of 3 μL of 10 × buffer solution, 4.5 μL of DNA template (up to 100 ng per reaction), 0.6 μL of 10 mM dNTP mix, 1.2 μL of 50 mM $MgCl_2$, 1.5 μL of each primer from 20 mM stocks, 17.55 μL of water, and 0.15 μL of Platinum Taq DNA polymerase (Invitrogen) with the concentration of 5 U/μL. Thermocycling was performed in a SimpliAmp thermal cycler (Applied Biosystems) as follows: initial denaturation at 95°C for 5 min, 30 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 50°C primer, and elongation at 72°C DNA for 1 min, final elongation at 72°C for 10 min, then 4 °C for ∞ . Negative extraction and negative PCR controls, and a positive PCR control, were included in each PCR run. The results were verified through the gel electrophoresis with 5 μl of the PCR product added to 1 μl of 6X Orange DNA Loading Dye (Thermo Fisher Scientific) in 1.5 % molecular biology grade gel (Genesee Scientific) and 1X TBE buffer (Thermo Fisher Scientific) stained with ethidium bromide (Thermo Fisher Scientific). Electrophoresis was performed at 150 V for 40 min. The PCR product was purified using the QIAquick gel extraction kit (Qiagen). The concentrations of purified amplicons were measured using a Qubit 3.0 (Life Technologies) with HS DNA reagent. Purified amplicons were then submitted to Functional Biosciences (Madison, WI, USA) for Sanger sequencing. The sequences were assembled using CLC Genomics Workbench v2.0 (Qiagen) subjected to BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi) searches against the National Center for Biotechnology Information (NCBI) non-redundant nucleotide database.

2.4. Virus isolation in cultured cells

Lesion tissues from deer OV790 and OV794 were separately suspended in phosphate-buffered saline (PBS) to prepare a 10 % (w/v) tissue homogenate. The tissues were homogenized, thoroughly ground using a tissue grinder (Covidien), and subsequently centrifuged. The resulting supernatant was filtered through a 0.45 μm pore-size polyvinylidene fluoride syringe filter (Thermo Fisher Scientific) to obtain filtrates used to inoculate cells. Three T25 flasks containing nearly confluent monolayers of Vero E6 cells (African green monkey kidney, obtained from the American Type Culture Collection [ATCC] CRL1586) were prepared for the experiment. One flask was mock-inoculated with a complete culture medium, serving as a control, while the remaining two were inoculated with 100 μL of OV790 and OV794 filtrates. The flasks were incubated at 37°C in a humidified 5 % CO_2 atmosphere. Cells were refed with fresh complete media every three days and monitored daily for the appearance of virus-induced cytopathic effects (CPE). Upon observing CPE in over 80 % of the cell monolayers, the cells and spent medium were harvested and stored at −80°C for subsequent analyses.

2.5. Next-generation sequencing and genome analysis

The frozen spent cell growth media from cells inoculated with OV790 and OV794 skin lesion tissue (LE) homogenates were thawed and spun to remove cellular debris prior to extracting viral DNA from virions using a QIAamp DNA Mini kit (Qiagen) according to the manufacturer's protocol. DNA libraries were generated using a NEBNext Ultra DNA Library Prep kit (New England Biolabs), and genomic sequencing was performed using a 2 × 300 cycle V3 kit on an Illumina MiSeq sequencer (Illumina). Cell culture host sequences (Vero E6 cells [African green monkey]) were removed from the sequencing data using Kraken2 (Wood et al., 2019). After removing the host sequences, *de novo* assemblies of the remaining paired-end reads were performed using SPAdes v3.15.3 (Prjibelski et al., 2020).

2.6. Epidemiological analyses

The goal of the epidemiological analyses was to investigate the temporal and spatial patterns of DPV infection and assess associations with factors such as age, geographic region, seasonality, and sex, to better understand the distribution and transmission dynamics of DPV within the population. The total number of retrospective CHeRI necropsy cases, animals with skin lesions, and animals with skin lesions that tested positive for DPV in each Deer Management Unit (DMU) in Florida were compiled and organized using Microsoft Excel.

The Florida Fish and Wildlife Conservation Commission uses DMUs to manage wild WTD populations in the state. These DMUs are designated based on various factors, including deer breeding season, habitat type, hunting regulations, population density, and specific management goals, all of which contribute to the effective management and conservation of WTD in Florida. To determine whether the differences in DPV PCR-positive percentages were statistically significant across age, DMUs, season, and sex, the percentages were analyzed separately. The 95 % confidence intervals (CIs) were calculated using the Wilson interval score via the OpenEpi V3.01 online software (https://www.openepi.com/Menu/OE_Menu.htm) to ensure statistical reliability. This method was chosen due to the small sample size relative to the total farmed white-tailed deer population. In this study, only specimens from animals exhibiting visible skin lesions consistent with potential DPV infection were included for testing. Specimens from the animals without apparent lesions or from asymptomatic individuals were excluded from the analyses to focus on cases with suspected signs of infection.

3. Results

3.1. Clinical presentations

Among the DPV-positive cases, crusty or ulcerative skin lesions were detected on the abdomen, skin around the eyes, ears, hooves, legs, muzzle, and nose (Fig. 1A and B). Some had lesions in their gums, oral cavity, and tongue. Deer OV1355 and OV1392 presented an eroded dental palette and gums to the bone with thick white pus (Fig. 1C), whereas OV585 had the tip of the tongue missing due to severe ulcerative lesions (Fig. 1D). Swollen leg joints were also present in some of the animals, especially in joints with skin lesions (Fig. 1E). An ulcerative lesion was revealed on the rumen mucosa of OV1392 (Fig. 1F). Observations at necropsy described lesions that were circular crusty or ulcerative dermatitis with hair loss and redness, while some were scabs.

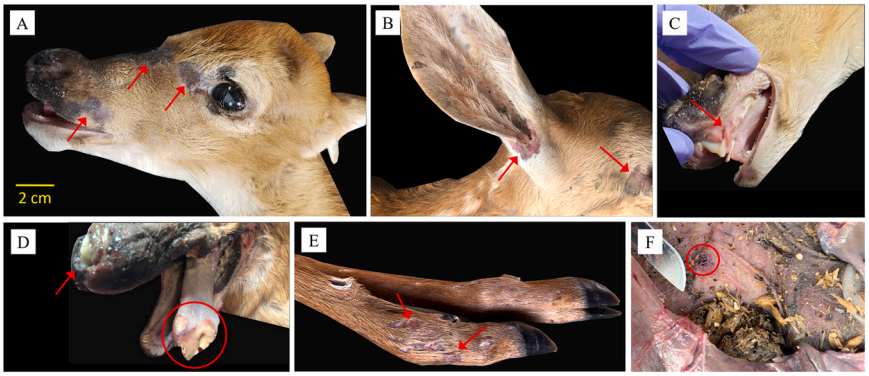

Fig. 1. Gross presentation of DPV lesions in farmed WTD in Florida. (A) Crusty lesions and scabs on the muzzle and around the eyes of OV1355. (B) Ulcerative lesions and scabs on an ear of OV1850. (C) Ulcerative lesions and erosion on the dental pad and hard palate of OV1355. (D) Ulcerative lesions and purulent exudates on the tongue and nose of OV585. (E) Ulcerative lesions on the hooves of OV1846. (F) Ulcerative lesion on the rumen mucosa of OV1392.

3.2. Polymerase chain reaction

From 2017–2023, CHeRI collected specimens from 776 farmed WTD across the state of Florida. These included 752 necropsy cases and 24 shipped-in specimen cases for routine diagnosis and disease surveillance. Of the 776 animals, 145 (18.7 %) animals exhibited gross skin lesions, including 121 deceased animals and 24 live animals. A total of 367 specimens were collected from these 145 animals. Animals were confirmed positive for low-GC poxvirus if at least one of their specimens tested positive. Using conventional PCR, 49 of the 145 animals (33.8 %, 95 % CI: 25.7 %-40.9 %) were confirmed positive for low-GC poxvirus DNA at the time of death or specimen collection. Among the 49 positive animals, eight were alive when lesion swab specimens were collected.

Among the specimens collected from 49 positive animals in this study, lesion swabs (4/4, 100 %) and lesion tissue (16/18, 89 %) were most often positive (Fig. 2). In contrast, none of the whole blood samples (0/13, 0 %) tested positive. Liver tissue (10/12, 83 %), grossly normal skin tissue (8/10, 80 %), spleen tissue (22/33, 67 %), lung tissue (19/32, 59 %), heart tissue (7/12, 58 %), kidney tissue (7/12, 58 %) had more positives than negatives, whereas nasal swabs (6/12, 50 %), and fecal swabs (4/9, 44 %) presented 50 % or lower positive rate. Additionally, one out of three gastrointestinal tissues (1/3, 33 %) tested positive, specifically from the lesion on the rumen mucosa of OV1392.

3.3. Virus isolation

Virus-induced CPE was observed by light microscopy in Vero E6 cell lines 6 days post-inoculation (DPI) with skin lesion homogenates from OV790 and OV794. No CPE was observed in mock-inoculated Vero E6 cells. The CPE of initial cell death was evident in inoculated cells at 6 DPI, and 80–90 % of cell death occurred at 12 DPI.

3.4. A21 gene and DPV genome sequencing

The assembled A21 gene sequences, each 348 base pairs in length, were obtained from all sequenced samples. These samples included lesion tissues from OV1859 and OV1863, nasal swab from deer OV592, skin lesion tissue from OV1846, and skin tissue from OV1682. Sequence analysis revealed 100 % identity with the DPV sequences we previously reported in 2019 (Sayler et al., 2019).

A total of 3910,654 and 4098,664 paired-end reads were obtained from virus propagated in Vero E6 cells that had been inoculated with tissue homogenate filtrates from cases OV790 and OV794. Following the removal of cell culture host sequences, 695,150 reads from OV790 and 584,879 reads from OV794 remained. The genome sequences of DPV from OV790 and OV794 were determined to be 163,341 bp and 163,340 bp in length, respectively, differing by a single base pair from the DPV genome sequence reported by CHeRI in 2019 (NCBI GenBank accessions: NC_006966 and AY689437). Both OV790 and OV794 were found to have a missing thymine (T) in the DPV 29 gene, while OV794 also had an additional thymine at the DPV 166 gene. Both mutations occur in genes encoding hypothetical proteins, which are presumably non-functional. These sequencing results confirmed that the low-GC poxvirus-positive animals were infected with DPV.

3.5. Epidemiological analyses

DPV was detected in many areas in Florida, including 20 out of 26 counties and 8 out of 10 DMUs where CHeRI collected specimens (Fig. 3). Only samples collected in summer (36/85, 42.4 %, 95 % CI:

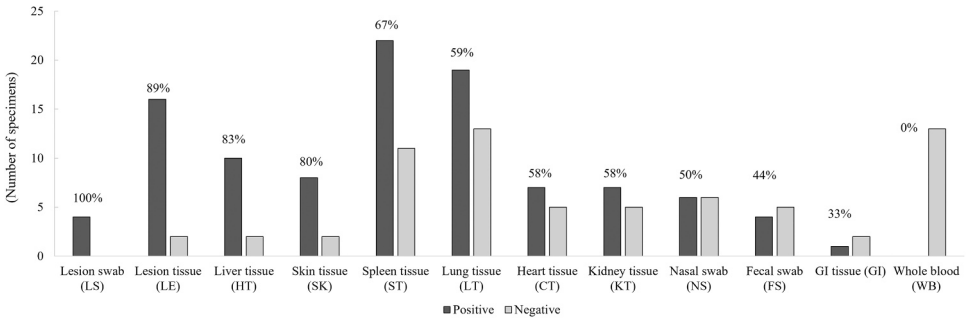

Fig. 2. Testing results of 170 specimens from 49 DPV-positive farmed WTD in Florida from 2017 to 2023 by specimen type. All listed specimens were obtained from animals confirmed to be DPV-positive, with at least one specimen testing positive for DPV. The percentages indicate the positive rates relative to the total number of these specimen types from all positive animals. LS: lesion swab, LE: lesion tissue, HT: hepatic (liver) tissue, SK: grossly normal skin tissue, ST: spleen tissue, LT: lung tissue, CT: cardiac (heart) tissue, KT: kidney tissue, NS: nasal swab, FS: fecal swab, GI: gastrointestinal tissue, WB: whole blood.

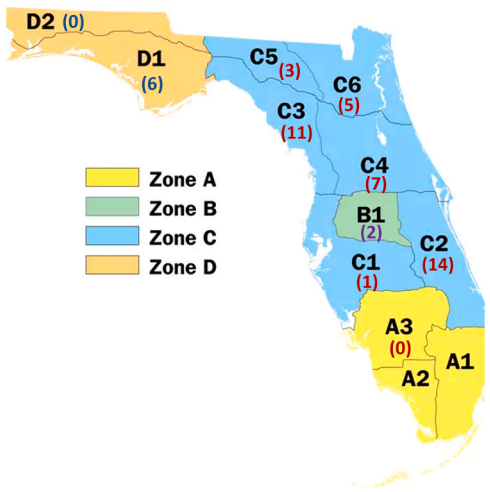

Fig. 3. Case numbers of DPV-positive farmed WTD by Florida DMUs from 2017 to 2023. The numbers in parentheses indicate the DPV infection case numbers in each DMU where CHeRI has collected specimens. DMU map modified from Florida Fish and Wildlife Conservation Commission (FWC, https://geodata.myfwc.com/datasets/myfwc::white-tailed-deer-management-unit-areas-in-florida/about).

32.4 %-53.0 %) and fall (13/51, 25.5 %, 95 % CI: 15.6 %-38.9 %) tested positive (Fig. 4 and Table 1). It is worth noting that DPV was detected every year from 2017 to 2023 (Table S1). Fawns (1–3 months) exhibited a statistically higher DPV-positive rate (37/70, 52.9 %, 95 % CI: 41.3 %-64.1 %) compared to those aged 4–12 months (8/33, 24.2 %, 95 % CI: 12.8 %-41.0 %) and 13 + months (3/36, 8.3 %, 95 % CI: 2.9 %-21.8 %) (Table 1). There was no difference in DPV detections between males (29/66, 43.9 %, 95 % CI: 32.6 %-55.9 %) and females (19/73, 26.0 %, 95 % CI: 17.3 %-37.1 %) (Table 1). The most commonly isolated bacterial pathogens from DPV-positive animals including *Escherichia coli*, *Pseudomonas aeruginosa*, *Trueperella pyogenes*, and *Klebsiella pneumoniae* (Table S1).

Table 1
Percentages and associations of DPV-positive cases with season, age, sex, and geographic area.

Factors	Animals with skin lesions (n = 145)	Animals with skin lesions that tested positive for DPV (n = 49)	% (95 % CI)	Total no. of animals that were necropsied or sampled (n = 776)
Season				
Spring	4	0	0.0 (0.0–49.0)	52
Summer	85	36	42.4 (32.4–53.0)	282
Fall	51	13	25.5 (15.6–38.9)	378
Winter	5	0	0.0 (0.0–43.5)	64
Age				
1–3 months	70	37	52.9 (41.3–64.1)	267
4–12 months	33	8	24.2 (12.8–41.0)	235
13 + months	36	3	8.3 (2.9–21.8)	255
Sex				
Male	66	29	43.9 (32.6–55.9)	376
Female	73	19	26.0 (17.3–37.1)	380
Area				
DMU-A	1	0	0.0 (0.0–79.34)	2
DMU-B	3	2	66.7 (20.8–93.9)	10
DMU-C	107	41	38.3 (29.7–47.8)	430
DMU-D	34	6	17.6 (8.3–33.5)	334

*Spring: March 1st-May 31th, Summer: June 1st-August 31th, Fall: September 1st-November 31th, Winter: December 1st-February 28th.

4. Discussion

In this study, cases of DPV infections were found annually across Florida; however, the emergence of DPV was seasonal, occurring from June to October. Since the identification and reporting of DPV in 1985 (Williams et al., 1985), relatively few studies have been conducted on this pathogen. To date, only a single serosurvey of DPV antibody in deer species in Oregon, USA (Jin et al., 2013), and one DPV challenge study on black-tailed deer (Bildfell et al., 2010) have been conducted. Consequently, a comprehensive understanding of DPV-induced disease characteristics remains elusive, including prevalence estimates of the animals presented with skin lesions across geographical regions, identification of susceptible groups, and inference of transmission mechanisms.

Unlike many skin diseases of livestock (Foster, 2023), fawns under three months old were prone to DPV-related mortality. Mule deerpox virus-induced skin lesions commonly form on the face and are thought to be associated with nose-to-nose contact between animals. This behavior may increase the likelihood of viral transmission between individuals, facilitating the spread of DPV in close-contact social interactions within deer populations. Additionally, in cases of close contact between animals or exposure to contaminated materials, the virus may be

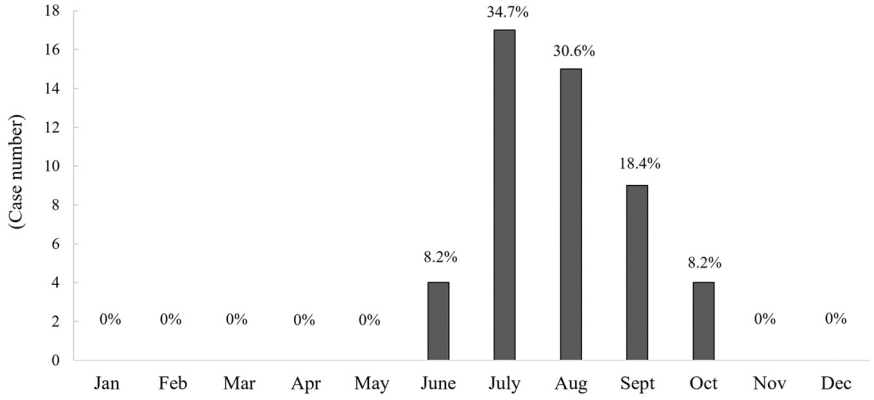

Fig. 4. Case distribution of DPV-positive farmed WTD in Florida from 2017 to 2023, categorized by necropsy/sampling month. The percentages indicate the DPV case numbers in each group relative to the total number of DPV-positive animals.

transmitted via inhalation, similar to other poxviruses that affect the respiratory tract (Milton, 2012; Beeson et al., 2023). Lesions in the oral cavity, including the tongue and gums, might increase difficulties in taking food, which increases the risk of rapid deterioration and probable death. Moreover, even if the skin lesions themselves are not lethal, the viral infection may decrease the immune function (Seet et al., 2003; Saghazadeh and Rezaei, 2022), making the skin lesion vulnerable to a pathogen invasion and secondary infections by bacterial pathogens. Secondary infections are a common complication of skin diseases, and in cases from this study, bacterial coinfection was frequently observed. Most of the bacteria isolated from the cases in this study were opportunistic pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Trueperella pyogenes*. The compromised immunity and skin lesions in DPV animals increase their susceptibility to these opportunistic bacterial infections. These data indicate that DPV can cause mortality either directly through the effects of its lesions or indirectly as a result of secondary bacterial infections.

Maternal antibodies typically persist in fawns for three to six months, with a significant reduction observed by four months of age (Gaydos et al., 2002). As we did not conduct serological analysis, it remains unclear if DPV antibodies were produced in exposed animals, if the fawns in this study failed to acquire maternal DPV antibodies, or if WTD possesses distinct immune mechanisms against DPV compared to other viral pathogens. Given the young age (1–3 months) of many of the positive cases, it is clear that fawns are highly susceptible to infection. To gain a better understanding of the immune mechanisms involved, studies incorporating both antibody and nucleic acid prevalence in whole blood and skin lesion specimens, as well as experimental infections across generations, are needed.

All our DPV-positive cases occurred exclusively in the summer and fall, demonstrating a clear seasonality. The periodic fluctuation of DPV outbreaks was likely due to summer fawning in Florida, which increases the number of naïve animals in the population and may contribute to the high DPV-related mortality observed in fawns due to the seasonality of DPV infection.

Chordopoxviruses have a diversity of transmission modes. Some have been proven to be transmitted through arthropod vectors (Shimizu et al., 2022). Some chordopoxviruses can be vertically transmitted from the mother to the fetus, such as MPXV (Mbala et al., 2017). Mule deerpox virus may have vectors or other reservoir species similar to other poxviruses. To identify the biological vectors of DPV, future work might aim to collect more evidence from candidate vectors and hosts to satisfy the Barnett criteria (Mee et al., 2024), a set of vector incrimination criteria analogous to Koch's postulates. Nowadays, analyzing receptor sequences in animal species can help us better estimate the possible host range of emerging viral diseases (Damas et al., 2020); however, the actual host range and disease prevalence across different regions may vary, reflecting differences in local ecological and epidemiological factors. Understanding the transmission routes in nature can help us better understand the possible outbreak areas and preventive methods.

Mule deerpox virus was identified in many areas in Florida, including 20 out of 26 counties and 8 out of 10 deer management units (DMUs), suggesting that the pathogen is widely distributed across deer farms. Because no surveillance on wild deer in Florida has been conducted, it is unclear what role wild deer play as a reservoir for the pathogen. Nonetheless, the legal movement of deer within Florida farms likely contributes to the distribution of DPV across farms. Testing for DPV should be considered before transporting their animals, such as a gross examination for external skin lesions or a more thorough protocol that includes molecular testing for DPV.

Disease surveillance and farm management practices will benefit if non-invasive specimens such as lesion swabs can be utilized for DPV testing. Skin lesion tissues were tested in most of the previous DPV studies, and little is known about the positivity rate and clinical sensitivity of other specimen types, such as those collected using swabs, and main organ tissues. For example, spleen tissue is a common specimen type collected for many viral pathogen detections since the spleen functions as a blood filter in mammals. Examining the detection of the virus in various specimen types will not only enhance the accuracy and efficiency of testing protocols but also provide critical insights into potential transmission pathways. According to our findings, lesion tissue and lesion material collected using swabs are the best specimen types, and main organ tissues are the best alternative if lesions are not available. Also, internal organs have less chance for postmortem contamination than skin lesion tissue, especially for farmed deer field necropsies, which might be conducted in an outdoor area and a time after the animal was found dead. We also detected DPV nucleic acid in fecal swab specimens, suggesting another non-invasive testing method for DPV on live animals and, more importantly, indicting the possible indirect transmission of DPV through fecal contaminants. We did not obtain positive results from any of the 13 whole blood samples from DPV-positive animals. However, it is worth noting that all whole blood samples were collected from necropsy animals using cardiac puncture, and many of them were already coagulated when collected. The whole blood samples used in this study might not be of optimal quality, and it is possible that the viremic phase had already concluded by the time the animals exhibited skin lesions or were diseased. Fresh whole blood from live animals could potentially yield different results. Also, DPV testing results are not consistent throughout the infection course (Bildfell et al., 2010), so it is better to test more than once and also test for anti-DPV antibodies for diagnosis and survey.

This study focused on farmed WTD in Florida exhibiting lesions like those expected to be caused by DPV, with most specimens collected postmortem. The overall case number might be different if asymptomatic and mildly symptomatic animals were included in the analysis. The gross presentations of the lesions in DPV-infected animals in this study align with those of previous reports: the animals exhibited crustaceous or ulcerative skin lesions and scabs. However, it is noteworthy that papules and pustules, which are typical early clinical signs of poxvirus infection, were rarely observed in this or in previous reports. This rarity may be attributed to the nature of papules, which are small, raised bumps on the skin that can form beneath the fur or hair in animals, making them less visible in the early stages of DPV infection. Papular and pustular lesions have been described primarily in a challenge study where deer were shaved at the lesion sites; in that study, pustules formed within 24 h after papules and progressed quickly to crusts and ulcers (Bildfell et al., 2010). Despite their rarity, papules and pustules can still be detected by palpation and may become noticeable if the fur is parted or shaved.

The A21 gene sequence and whole genome sequence data support that our cases are due to DPV infections, and the DPV strains in this study are very conserved. The observed genetic stability may be attributed to either a low evolutionary rate or limited sequence data. However, given that the genome sequence has shown minimal nucleotide variation across samples collected over several years, we believe this stability likely reflects a relatively low evolutionary rate for the genome. The result corresponds to the fact that DNA viruses have lower mutation rates than RNA viruses and do not have many serotypes or strains (Holmes, 2010; Stern and Andino, 2016).

5. Conclusions

In summary, DPV infections in farmed WTD were identified across Florida between 2017 and 2023. Additionally, the virus did not mutate rapidly, suggesting a long-term relationship and adaptation between the virus and the deer population. The results will enhance our understanding of potential transmission routes and improve testing methods and preventive measures for DPV in farmed WTD in Florida. Future studies, such as examining the possible vectors and challenge studies, are needed to confirm the potential transmission routes of DPV, including animal transportation, fecal matter, and insect vectors.

According to previous studies on EHDV and BTV in farmed and wild deer in Florida, farmed WTD in Florida may serve as sentinels for infectious disease circulation in adjacent free-ranging animal populations (Cauvin et al., 2020; Cottingham et al., 2021). Future works might aim to test wild deer for DPV, particularly at the wildlife-livestock interface, where close contact between wild and farmed animals can occur. Additionally, DPV might have hosts other than what we know; though the lower evolutionary rate of DNA viruses compared to RNA viruses suggests a lower risk of spillover events. Mule deerpox testing is recommended for deer farmers when relocating their animals, particularly those with skin lesions. Based on our findings, lesion tissue and lesion swabs are the most reliable specimen types for both live and deceased animals. If lesions are unavailable in live animals, fecal swabs serve as a viable alternative. For deceased animals, primary organ tissues such as liver and spleen are the best alternative specimens. Moreover, the development of a vaccine and a DPV-specific molecular assay would be valuable in preventing the movement of infected animals, thereby reducing the risk of further viral transmission.

Ethics statement

Ethical approval was not required for the diagnostic research work performed in this study.

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetmic.2025.110608.

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