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Abstract

Feline Panleukopenia Virus (FPV) is a highly contagious and systemic virus that is environmentally stable, targets leukocytes, and affects cats of all ages. Within the United States, particularly in the population-dense downstate region of New York, no molecular surveillance or strain isolation has been conducted for FPV. The main goal of this study is to conduct molecular and serological surveillance of FPV among some shelter cats in this area and to do isolation and genome mining of some of the detected isolates. We used samples (swabs and sera) from 109 shelter cats by qPCR and immunofluorescent assay (IFA) respectively. Our results show that 25.0% (29/108) of the cats tested were FPV positive by qPCR, and 39.4% (43/109) of the cats tested FPV positive by IFA. FPV Viral isolation and identification were conducted using Madin-Darby Canine Kidney (MDCK) cells. Next Generation Sequencing (NGS) confirmed the presence of a novel FPV isolate (Accession: PZ251627) circulating within the tested shelter cat population. Phylogenetic analysis showed that the sequence of the reported FPV isolate had the highest full-length sequence similarity with isolates MH165482 at 99.3%, MN127781 at 99.2%, and MH165481 at 99.2%. Additionally, phylogenetic analysis of the VP2 genome sequence showed the following highest sequence similarities; OQ615264 at 99.2%, KT899746 at 99.1%, and PVMRFD at 99.3%. The following substitutions were noted in comparison of the isolate to the reference sequence (MN45165): Ile101Thr and Glu411Ala. Inverse distance weighting (IDW) interpolation indicated the presence of a higher occurrence of FPV-positive cats in the western region of Downstate New York, encompassing the areas of the 5 boroughs of NYC and Nassau County. Continued FPV surveillance in cats in this region is highly recommended.

Keywords: Feline Panleukopenia Virus (FPV); qPCR; IFA; isolation; molecular epidemiology; Next Generation Sequencing (NGS); shelter cats

1. Introduction

Feline Panleukopenia Virus (FPV) belongs to the family *Parvoviridae*, subfamily *Parvovirinae*, and the genus *Protoparvovirinae* [1]. FPV is non-enveloped, measuring approximately 25nm in diameter

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with icosahedral symmetry and receptor binding sites on the spike surface. FPV's genome is linear, negative-sense, naked single-stranded DNA, at approximately 4.5kb in length [2]. The FPV genome structure contains the following open reading frames formed by alternative splicing and hairpin structures: Non-structural Protein 1 (NSP1), Non-structural Protein 2 (NSP2), Viral Protein 1 (VP1), Viral Protein 2 (VP2), and a Small Alternatively Translated Non-structural Protein (NSP SAT). NSP1, NSP2, and NSP SAT are responsible for the genome section for DNA transcription and replication, while VP1 and VP2 encode nucleocapsid structural proteins [2]. Mutations in the VP2 sequence region are the cause of FPV's ability to infect other animal species, primarily dogs, resulting in the development of multiple forms, such as Canine Parvovirus (CPV), CPV-2, CPV-2a, CPV-2b, and CPV-2c [3]. Decoding the full-length genomes affecting poultry, large and small animals particularly dogs and cats using the NGS and monitoring of any changes in these viral genomes on the genomic levels have many clinical implications [4–18]. Among this application is the design/update of vaccines to match the circulating field strains of these viruses, to fine-tune the currently used diagnostic assays, and to adjust the management and control practices of these viruses. Furthermore, it will also help understanding the molecular pathogenesis, and tissue tropism of the circulating strains of these viruses [19–30].

FPV is transmitted by the oropharyngeal-fecal route but can be up-taken from the environment through fomites [3]. It favors pharyngeal lymphoid tissues (thymus and lymph nodes) and incubates for approximately 2-7 days before causing systemic viremia (targeting bone marrow, spleen, and Peyer's patches [3]. It has tropism towards proliferating cells, particularly those at or near the S-phase during cellular replication due to high concentration of cell membrane transferrin receptors [31]. In adult cats, FPV targets cells of the immune system (lymphocytes, neutrophils, monocytes, and platelets) [31]. Their systemic destruction leads to characteristic clinical signs of profound leukopenia, fluctuating fever, malaise, appetite loss, coat texture change, vomiting, and diarrhea. However, for perinatal and in-utero kittens, FPV targets the cerebellum during critical stages of organogenesis [3]. On the rare occasion they do survive, the infection causes notable ambulatory ataxia, colloquially known as “Wobbly Cat Syndrome” [3].

FPV is documented to be environmentally stable with resistance to heat extremes, pH fluctuations, and normal household disinfection procedures [2,3]. This stability allows it to remain in an environment from hours to days, weeks, or even months at a time. This allows infection to occur at almost any time and place if the environment is not properly cleaned.

Within the last 10 years, FPV outbreaks and epidemics have occurred in areas of the world such as China and Bangladesh, [32–36]. In China, an epidemic strain named “FPLV-CC19-02” (accession OR21195) was found to have two amino acid mutations in the VP2 nucleotide sequence, Ala91Ser and Ile101Thr. These mutations caused increased virulence and indicated further genomic distancing of circulating FPV strains from commercially available vaccines [34]. Without consistent monitoring, positive case isolation, preemptive treatment by vaccination, and strict adherence to cleanliness procedures, FPV will continue to run rampant.

This study aims to fill the informational gap of FPV and its prevalence amongst cat populations present in one of the most populated cities within the USA. We aim to conduct molecular and serological surveillance of FPV in feral and shelter cats in the downstate region of New York, and to perform molecular characterization of any full-length FPV genomes circulating within the feline population.

2. Materials and Methods

2.1. Demographic Information of the Collected Cat Samples

Clinical samples and demographic information were collected from 108 cats, on-site at multiple veterinary clinics. The cats were sourced from cat shelters, rescues, owners, and trappers throughout the downstate New York area, (the 5 boroughs of New York City and Long Island). All demographic data from sample animals was incorporated into a categorized spreadsheet, with each cat receiving a lab-use ID number. Categories within the spreadsheet were: collection date, collection clinic, name, clinic ID number, town, state, zip code, county, gender, approximate age, breed, weight in pounds, body condition score (out of 5), microchip status, surgical history, pregnancy status, endoscopic parasite status, vaccination for Rabies, vaccination for FVRCP, and owned or feral status.

2.2. Clinical Samples Collection, Processing, and Preservation

Clinical samples consisted of approximately 3mL of blood in EDTA-free red-top collection tubes, 1 oropharyngeal swab, and 1 rectal swab. Blood was allowed to coagulate before being centrifuged at 2,500 rpm for 15 minutes at 20ºC. Separated serum was collected in microcentrifuge tubes and stored in a -20 ºC freezer. All swabs were prepared before use in a sterile environment, each containing 4mL of Viral Transport Media (VTM) consisting of the following: 1% penicillin/streptomycin antibiotic (Thermo-Fisher Scientific, REF# 15140-122), 1% antibiotic-antimycotic antifungal 100X (Thermo-Fisher Scientific, REF# 15240-096), 2% heat inactivated Horse Serum (HS) (Thermo-Fisher Scientific, REF# 26050-088), and 96% Dulbecco’s Modified Eagle Medium (DMEM) (Corning, CAT# 10013CV). After swab sample collection, all swabs were vortexed, transferred to 5mL centrifuge tubes, and stored in the -80 ºC freezer.

2.3. Viral DNA Extraction and qPCR

A combined swab sample volume of 500 µL was made from the paired sample sets, consisting of 250 µL of the oropharyngeal swab and 250 µL of the rectal swab fluid. The combined volumes were treated for DNA extraction using the ZYMO Research Quick-DNA/RNA/RNA Viral Kit, (ZYMO Research, Cat# D7020) following the manufacturer’s instructions. qPCR was performed using the resulting dsDNA and the PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, REF# A25742) by following the manufacturer’s instructions. The β-actin was used during the qPCR set up procedure as the housekeeping gene. The oligonucleotides for FPV and β-actin are found on Table 1. The qPCR process was performed using the Quant Studio3 System (Applied Biosystems), Table 1.

2.4. Virus Isolation and Propagation Using the Madin-Darby Canine Kidney (MDCK) Cells

2.4.1. FPV Isolation Protocol

Madin-Darby canine kidney (MDCK) cells (BEI Resources, REF# NR-2628) cultured and maintained in T25 flasks with Eagle’s Minimum Essential Medium (EMEM) (ATCC, CAT# 30-2003), with 1% penicillin/streptomycin antibiotic (Thermo-Fisher Scientific, REF# 15140-122), 1% Non-essential Amino Acids (NEAA) (Thermo-Fisher Scientific, REF# 11140-050), 10% Fetal Bovine Serum (FBS) (Thermo-Fisher Scientific, REF# 2565838RP). The cells were kept in an incubator set to a constant 37℃ with a 5% CO_2 concentration. The MDCK cells were checked daily for growth and confluency and had daily media replacement.

2.4.2. Propagation of the FPV Using MDCK Cells

MDCK was cultured in 6-well plates until approximately 80% confluency for subsequent infection. Samples with low CT values (<20) were chosen for at least 1 virus to test its ability to propagate. Each sample had a combined 700 µL swab sample volume (350 µL oropharyngeal and 350 µL rectal) that was filtered using a 0.45 µm pore-sized, 30mm sterile syringe filter unit (Whatman, Product # 10462100) attached to a sterile 1 mL syringe. Once filtered, each sample was TPCK-treated by a volume ratio of 100:1, then set on a hotplate at 37℃ for 30 minutes. Once the 6-well plates reached 80% confluency, 50µL of the filtered and TPCK-treated infection samples were added dropwise into an assigned well, except for 1 well left uninfected as the sham. Each well received 400 µL empty EMEM replacement media. The cells were moved into the incubator for infection for 2 hours. The media was replaced with 2 mL of the complete media and then replaced in the incubator for 7 days with daily checks for signs of cytopathic effect (CPE).

2.4.3. Confirmation of the Propagation of the FPV in Cell Culture

The infected plates were moved from the incubator to the -80 ºC freezer, then removed to thaw at room temperature (22ºC). Once thawed, the supernatant from the wells were collected, then treated for DNA extraction as mentioned in Section 2.3. β-actin was as the housekeeping gene. The target oligonucleotides FPV and β-actin can be found in Table 2. The qPCR process was performed using the Quant Studio3 System (Applied Biosystems) as mentioned in Section 2.3.

2.5. Immunofluorescence Assay (IFA)

Serum samples were tested at the Washington Animal Disease Diagnostic Laboratory (WADDL) using indirect immunofluorescent antibody (IFA) assays for FPV antibody. Testing was performed using modified methods adapted from WADDL's standard IFA protocols. Pathogen-specific antigen substrate slides were used for each assay. Before each test, serum and control samples were pre-diluted 1:50 with serum diluting buffer (SDB) containing phosphate-buffered saline (PBS), 1% BSA, preserved with 0.09% sodium azide, pH 7.2. For each assay run, one positive and one negative control serum were included and processed in parallel with test samples.

FPV antigen substrate slides were stored at −20 °C and equilibrated to room temperature for a minimum of 30 minutes before use. Slides were labeled in a predefined layout. Diluted samples and controls were applied to their specified wells. The slides were incubated in a humidified chamber at 37 °C ± 1 °C for 30 minutes serum antibody binding to the antigen substrate. Following primary incubation, slides were rinsed with carbonate-based rinse buffer (pH 9.0) and spent 10 minutes in fresh buffer. After washing, excess buffer at slide margins was removed. Then, a fluorescein isothiocyanate (FITC)–conjugated goat anti-cat IgG (H&L) secondary antibody was set in a conjugate dilution buffer. The conjugate was filtered, equilibrated to room temperature, and applied to each well. Slides were set for secondary incubated in a humidified chamber at 37 °C ± 1 °C for 30 minutes. Afterwards, the slides were washed, mounted with glycerol mounting medium, and covered with glass coverslips. Slides were protected from light and examined using fluorescence microscopy with appropriate FITC filter sets. Fluorescence was evaluated by trained personnel using standardized interpretation criteria. A positive result was defined by specific fluorescence staining corresponding to the viral antigen distribution for each assay, distinguishable from background staining. Negative results were defined by the absence of specific fluorescence-staining pattern. Fluorescence intensity at the screening dilution (1:50) was assessed and recorded quantitatively.

2.6. The Next Generation Sequencing (NGS)

Sample volumes from the DNA extraction and qPCR procedure in Section 2.3 were used for Next Generation Sequencing (NGS). The sample selected was Sample 151. It was selected due to its low CT values in multiple qPCR procedures, averaged to be 13.836. The DNA sample was subsequently submitted to Washington State University's College of Veterinary Medicine: Department of Veterinary Microbiology & Pathology for the full-length genome decoding process. Sequenced reads were processed for adapter removal and quality filtering using Trimmomatic version 0.38 [37], employing a cutoff requiring an average base quality score of at least 20 within a sliding window of three bases, and retaining only sequences trimmed to a minimum length of 20 bases (Parameters: ILLUMINACLIP:adapters.fa:2:20:7 LEADING:20 TRAILING:20 SLIDINGWINDOW:3:20 MINLEN:20). The filtered read pairs were assembled using SPAdes version 3.15.5 [38]. Viral sequences were distinguished from host transcripts by aligning them to the complete genome sequence of FPV reference isolate "BFPV" (accession MN451652.1) using NUCmer version 3.1 from the MUMmer package [39] and visualized using mummerplot version 3.5.

2.7. Bioinformatic Analysis

The resulting sequence generated by the NGS procedure was aligned with the reference isolate (MN451652.1) [31] to organize and annotate the genome sequence using the Geneious Prime 2026.0.2

software. The sequence was finalized and deposited in the NCBI's GenBank database as "FPV/151/LIU/2026" with the given accession number PZ251627.

2.8. Multiple Sequence Alignment and Phylogenetic Analysis

A total of 140 sequences were used for multiple sequence alignment (MSA) and phylogenetic analysis, including our novel FPV-151 isolate and the reference isolate (MN451652.1), other FPV isolates, and representatives from Canine Parvovirus (CPV), Mink Enteritis Virus (MEV), Fox Parvovirus (FoxPV), Panthera leo Parvovirus (PantherPV) [40], and Carnivore protoparvovirus (CPPV). The sequences were chosen accounting for their isolation year, origin country, clade location based on prior sequence analyses [41]. The following commercial vaccine strains included; M38246.1 (Fel-O-Vax® PCT, Zoetis Inc., NY, USA) [41], EU498680 (PUREVAX® Feline 3, Boehringer Ingelheim Inc., Rheinland-Pfalz, Germany) [42], EU498681 (Felocell3®, Pfizer Inc., NY, USA) [42], D88287 (Purevax® RCP, Merial Inc., Paris, France) [43], and OQ615264 (Nobivac® Feline 1-HCP, MERCK Aimal Health USA Inc., Boxmeer, The Netherlands). Chinese isolate "FPLV-CC19-02" (OR21195) was also included during the analyses due its recent epidemic status with the two notable mutations: Ala91Ser and Ile101Thr [34]. All reference sequences and isolates used were retrieved from the NCBI database.

Phylogenetic analysis consisted of two tree constructions; one of full-length genomes consisting of 89 complete genomes and another for 140 VP2 sequences. The phylogenetic trees were generated using the MEGA12.1 software, with maximum likelihood and the Tamaura-Nei model. Bootstrap was set to 1,000 replicates per branch. The resulting Nexius file from MEGA12.1 [44–46] was subsequently uploaded to the online iTOL software for tree structure, coloring, and labeling finalization.

2.9. The Three-Dimensional (3D) Structure of the FPV-VP2 Protein

FPV-151's protein structure prediction of the VP2 protein was made and modeled using the AlphaFold structural prediction and analysis server with the default settings. The protein was modeled using the cartoon structure, while the amino acids of note were labeled and modeled by ball-and-stick.

2.10. Statistical Analysis

2.10.1. Data Management and Preprocessing

All statistical analyses were performed in R (RStudio). FPV PCR results were obtained for individual cats, each tested twice. The cycle threshold (Ct) value from each PCR assay was used as a quantitative measure of viral DNA detection. For subject-level analysis, duplicate PCR measurements from the same cat were summarized the mean Ct value per cat, generating a single representative Ct value. Cats were classified as PCR positive based on Ct-value thresholds used in diagnostic PCR interpretation. Ct values ≥30 were considered negative, while Ct values <30 were considered positive. For categorical interpretation, Ct values were classified as follows: Ct <20 were defined as strong positives, Ct values between 20 and 25 as moderate positives, and Ct values between 25 and 29 as weak positives. These categorizations were applied to the mean Ct value per cat for subject-level inference.

The proportion of PCR-positive cats was calculated as the number of cats with a mean Ct value below the positivity threshold (Ct <30) divided by the total number of cats tested. The 95% confidence interval (CI) for the positivity rate was computed using the exact binomial method (Clopper–Pearson). The overall distribution of Ct values was summarized descriptively by calculating the minimum, maximum, mean, and median Ct values across all PCR measurements. To visualize subject-level viral burden, the distribution of mean Ct values per cat was illustrated using histograms. In addition, cats were grouped according to Ct-value categories (negative, weak positive, moderate

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positive, strong positive), and the frequency and percentage of cats within each category were calculated and displayed graphically.

2.10.2. Comparing the PCR and the IFA Results Concordance

Cats included in the comparative analysis had results available from both qPCR and IFA testing. For PCR, subject-level mean Ct values were calculated, and cats were classified as PCR positive if the mean Ct value was <30, and PCR negative if the mean Ct value was ≥30. IFA testing was performed once for each cat. Cats with detectable antibody titers (reported as positive at 1:50 or greater) were classified as IFA positive, whereas all others were classified as IFA negative. PCR and IFA results were compared at the individual cat level using binary classifications (positive vs negative). A two-by-two contingency table was generated to summarize concordant and discordant test outcomes between PCR and IFA. In addition, Cohen's kappa coefficient (κ) was calculated to assess agreement beyond that expected by chance alone. Kappa values were interpreted according to commonly used criteria, with values near zero indicating agreement no better than chance. All agreement analyses were conducted using unweighted kappa, as the test outcomes were dichotomous.

2.10.3. Spatial Analysis and Mapping

Geographic locations were obtained based on recorded place information associated with each individual cat. Locations were geocoded to latitudinal and longitudinal coordinates and imported into ArcGIS Pro (Version 3.6.2) for spatial analyses, with each location representing at least one cat tested for FPV. To visualize the spatial distribution, a point-based choropleth map was created where each location symbolized the number of PCR-positive cats or the proportion of positive tests identified at that location. Spatial patterns of FPV detection was further analyzed using inverse distance weighting (IDW) interpolation. IDW was applied to estimate the spatial distribution of PCR positivity based on the assumption that locations closer in space are more similar than those farther apart. Interpolated surfaces were generated using the number of PCR-positive cats or the proportion of positive tests per location as the input variable, producing continuous spatial gradients of predicted positivity across the study area. Interpolated surfaces were visualized as continuous raster maps to highlight potential spatial hotspots and gradients in positive detections.

3. Results

3.1. Molecular and Epidemiological Analysis of FPV in Downstate New York

A total of 108 cats were tested for feline parvovirus using duplicate qPCR assays, yielding two cycle threshold (Ct) values per individual. For subject-level analyses, duplicate measurements were found to be the mean Ct value. Using a Ct threshold of <30 to define overall qPCR positivity, 27 of 108 cats were classified as PCR positive with a positivity rate of 25.0%, and 95% confidence interval (CI) from 17.16% to 34.25%, found by the exact binomial method.

3.1.1. Distribution and Categorization of the Mean Ct Values of the Tested Samples by qPCR

The minimum Ct value was 13.50, the maximum 39.23, the mean 31.95, and the median 31.4. The distribution of Ct values per cat exhibited a right-shifted pattern, with most cats clustering near the diagnostic threshold (Ct 30-33) and high Ct value (>39.0), while a smaller number of cats displayed lower Ct values (Figure 1A). The distribution showed a concentration immediately above the negativity cutoff, with few cats exhibiting Ct values below 25 and fewer with Ct values below 20, consistent with high viral loads. Categorization based on mean Ct values revealed that 75.0% (81/108) were classified as PCR negative (Ct ≥30) (Figure 1B). Among PCR-positive cats, 17.59% (19/108) were classified as weak positives, 3.7% as moderate positives, and 3.7% as strong positives.

Figure 1. Resulting charts from the statistical analysis run on the accumulated qPCR data. **(A)** Distribution of mean PCR cycle threshold (Ct) values per cat with a notable right-shifted skew. **(B)** Distribution of cats by Ct-

value category with percentages shown above bars. Ct ≥30 was considered negative; Ct <20 was classified as strong positives; Ct >= 20 & Ct < 25 as moderate positives; and Ct >= 25 & < 30 as weak positives. **(C)** Concordance between duplicate qPCR Ct measurements per cat. Scatter plot showing the relationship between paired Ct values obtained from duplicate PCR tests for each cat. The dashed line represents the line of identity (y = x), indicating perfect agreement.

3.1.2. Concordance Analysis Between the Duplicate PCR Measurements

Comparison of paired Ct values from duplicate PCR assays demonstrated strong concordance between replicates across the full range of observed Ct values (Figure 1C). Most paired measurements clustered tightly along the line of identity, indicating high analytical reproducibility. Minor dispersion between replicates was observed, particularly among Ct values near the diagnostic cutoff, but no systematic bias between the two measurements was evident.

3.1.3. Spatial Distribution of FPV Positivity

The spatial distribution of feline parvovirus (FPV) PCR results demonstrated geographic heterogeneity across the study area (Figure 2). When visualizing the number of PCR-positive cats at each geocoded location (Figure 2A), positive detections were concentrated in several localized areas, particularly in the western and central portions of the study region. Multiple locations exhibited more than one PCR-positive cat, suggesting spatial aggregation of positive detections rather than a random distribution. Spatial interpolation of the number of PCR-positive cats per location using inverse distance weighting (IDW) further highlighted these patterns (Figure 2C). The interpolated surface revealed distinct areas of elevated predicted positivity surrounded by regions with lower predicted values, forming spatial gradients consistent with localized clusters of positive cats.

When accounting for differences in sampling intensity by examining the proportion of PCR-positive cats at each location (Figure 2B), similar spatial patterns were observed. Several locations demonstrated a high proportion of positive cats, including locations where all or most tested cats were PCR positive. IDW interpolation of the proportion of PCR-positive cats (Figure 2D) produced a continuous surface that reinforced the presence of spatially structured patterns in FPV positivity. Areas of elevated interpolated positivity proportion overlapped with, but were not identical to, regions identified using absolute positive counts, suggesting that both localized clustering and variable sampling density contributed to the observed spatial patterns. Together, these analyses indicate a non-uniform spatial distribution of FPV PCR positivity across the study area, with evidence of localized areas of increased positivity.

Figure 2. Spatial distribution and interpolation of feline parvovirus PCR positivity. **(A)** Point map showing the number of PCR-positive cats at each geocoded location, with symbols scaled to reflect the absolute count of positive cats per location. **(B)** Point map showing the proportion of PCR-positive cats at each location, calculated as the number of PCR-positive cats divided by the total number of cats tested at that location. **(C)** Inverse distance weighting (IDW) interpolation of the number of PCR-positive cats, illustrating spatial gradients and areas of elevated predicted positivity across the study area. **(D)** IDW interpolation of the proportion of PCR-positive cats, representing the spatial distribution of relative PCR positivity while accounting for variation in sampling intensity across locations.

3.1.4. Indirect Fluorescent Antibody (IFA) Testing for FPV

Indirect fluorescent antibody (IFA) testing identified serologic evidence of feline parvovirus exposure in a substantial proportion of cats. Based on detectable antibody titers (positive at 1:50), the estimated proportion of IFA-positive cats was 39.4% (95% CI: 30.22-49.27) (Figure 3A). These findings demonstrate widespread exposure to feline parvovirus within the study population and complement the molecular PCR results by reflecting cumulative infection history rather than active viral detection.

Figure 3. Resulting charts from the statistical analysis run on the accumulated IFA data. **(A)** Estimated positive proportion of IFA-positive cats with the 95% CI range (30.22-49.27) denoted as error bar. **(B)** Agreement between

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PCR and IFA test results. Heatmap illustrating concordant and discordant classifications between PCR and indirect fluorescent antibody (IFA) testing at the individual cat level. Numbers within cells indicate the number of cats in each category.

3.1.5. Agreement Between the PCR and the IFA Testing

Agreement between PCR and indirect fluorescent antibody (IFA) test results was evaluated at the individual cat level for the 78 cats (Figure 3B). Overall percent agreement between PCR and IFA results was 62.82%, indicating that the two tests yielded concordant classifications for slightly more than half of the cats. However, Cohen's kappa analysis demonstrated only slight agreement beyond chance ($\kappa = 0.057$, $p = 0.60$), indicating that the observed agreement was not significantly greater than would be expected by chance alone. These findings reflect limited concordance between molecular detection of FPV DNA and antibodies serological detection.

3.2. Molecular Characterization

3.2.1. Establishment of the Genome Structure and Organization of the Reported FPV Isolate

The resulting sequence received from the NGS procedure was compared and analyzed against the reference strain (MN45165) to produce the full genome structure and organization of the isolate FPV-151. Our isolate's genome contains palindromic sequences at the 5′ and 3′ terminals that form complex hairpin structures; NSP1 and NSP2 (encoded within the same region); VP1, VP2, and a SAT protein (encoded within the same region) (Figure 4A). The novel isolate was found to be 4,271 nucleotides (nt) in length and the complete protein sequences as follows: NSP1 ranging from nucleotides 1-2007 (669 aa in size); NSP2 ranging from nucleotides 1-260 and 1733-1970 (166 aa in size); VP1 ranging from nucleotides 2014-2044 and 2117-4269 (728 aa in size); VP2 ranging from nucleotides 2515-4269 (585 aa in size); and NSP SAT ranging from nucleotides 2522-2728 (69 aa in size) (Table 2). The full-length genome sequence alignment found that isolates FPV-151 and "BFPV" (MN45165) were 98.7% similar (Table 2).

Figure 4. Schematic representation of the FPV-151 genome organization and the phylogenetic trees generated by the MEGA12.1 software and finalized using iTOL. **(A)** The representative FPV genome structure contains hairpin structural 5′ and 3′ palindromic terminal sequences; NS1 and NS2 (shown in blue); VP1 and VP2 (shown in green); and a small alternatively translated (SAT) protein is located within the structural protein region (shown in red). **(B)** Phylogenetic tree of 89 full-length FPV and related genomes with FPV-151 noted in fuchsia font color, bolded, and increased in font size, which is found in Clade B (green). **(C)** Phylogenetic tree of 140, FPV and related genomes' VP2 sequences with FPV-151 noted in fuchsia font color, bolded, and increased in font size, which is found in Clade B (green).

3.2.2. Phylogenetic Analysis Based on the Full-Length Genome and the VP2 Sequences

Phylogenetic analysis against 89 full-length FPV and related genomes found that FPV-151 clustered with other FPV isolates in Clade B (Figure 4B). MSA and phylogenic analysis resulted in isolate FPV-151 being clustered closest to the Chinese FPV isolate MH165482 at 99.3%, FPV Thailand isolate MN127781 at 99.2%, and Chinese FPV isolate MH165481 at 99.2%. Notably, OR921195 and FPV-151 had a 98.7% similarity. Clade A contained FPV strains solely. Clade B contained strains of mostly FPV, with singular strains of CPPV1, MEV, and PantherPV. Clade C contained MEV strains solely. Clade D contained isolates from CPPV, CPPV1, CPV, and CPV2 [35]. The calculated maximum log likelihood for this tree was -29,906.38.

The phylogenetic analysis against 140 VP2 nucleotide sequences found that FPV-151 clustered closest with other FPV isolates in Clade B (Figure 4C). MSA and phylogenic analysis resulted in isolate FPV-151's VP2 clustering closest to Brazilian strain OQ615264 at 99.2%, Chinese strain KT899746 at 99.1%, and Japanese strain PVMRFD at 99.3%. Notably, the VP2 regions of OR921195 and FPV-151 had 98.7% similarity. Clade A contained all FPV, except for one MEV isolate. Clade B contained strains of almost entirely FPV, with singular strains of PantherPV and CPV, 2 MEV strains, and 2 CPPV1 strains. Clade C contained FPV strains solely. Clade D contained isolates largely from CPV, CPPV1, and CPV2, with singular strains from FPV and MEV. The calculated maximum log likelihood for this tree was found to be -10,368.86.

3.2.3. FPV Genome Mining and the 3D Structural Representations of the FPV-151 VP2 Protein

MSA was done to find the precise locations of any mutations within the FPV-151 isolate's VP2 nucleotide sequence when compared and analyzed against the reference strain (MN451652.1), the Chinese isolate "FPLV-CC19-02" (OR21195), commercially available FPV vaccine strains (accession numbers PVFPVC, EU498680, OQ615264, EU498681, D88287), and representative strains from each clade [34,47] (Figure 5A). In our isolate, 3 nts are missing at points 86 to 88 and 14 nts at points 228 to 241. These missing nucleotides are attributed to the NGS process itself, as the mechanisms did recognize the presence of nucleotides, but could not identify the exact nucleotides. The following synonymous amino acid VP2 sequence mutations were found: T300C, 11232C, G1521A, and T1572C. The following non-synonymous mutations were found in the VP2 nucleotide sequence: Ile101Thr and Glu411Ala (Figure 5B). The VP2 regions of the vaccine strains analyzed during this study had the following similarity to the FPV-151 isolate: PVFPVC at 98.7%, EU498680 at 98.3%, OQ615264 at 98.6%, EU498681 at 98.5%, and D88287 at 98.5%.

Figure 5. Mutation identification and structural modeling of FPV-151's VP2 sequence. **(A)** MSA of the amino acid variations of FPV-151's amino acid sequence (boxed in red) against vaccine strains and other isolates representing each phylogenic clade. MSA conducted using Geneious Prime 2026.0.2. **(B)** 3D protein structure modeling of FPV-151's VP2 resulting protein using the AlphaFold Server online program. It indicates the locations of the two non-synonymous mutations (Ile101Thr and Glu411Ala), referring to them in ball-and-stick models against the protein's cartoon model.

3.3. FPV Isolation and Identification

FPV-151 was cultured using the MDCK cell line simultaneously with an MDCK culture sham well on two separate occasions. The sham showed no signs of morphological changes in the form of the cytopathic effect (CPE), with normal shape and growth patterns of an MDCK culture. Conversely, the FPV 151-infected well yielded significant CPE (Figure 6). This was seen in morphological changes such as cell rounding and swelling, cell rupture, and monolayer cellular detachment photographed at 5 days post infection (dpi) (Figure 6). These results remained constant both times that the FPV-151 field isolate was cultured.

Figure 6. Cellular morphology changes in MDCK cells in sham and FPV 151-infected cells at 5dpi. (**A-C**) The sham is shown at 3 different magnifications as a point of comparison where in which the MDCK cellular morphology remains unchanged. (**D-F**) Changes in cellular morphology of FPV 151-infected cells with notable cell rounding, rupturing, and detachment.

3.4. Immunofluorescence Assay (IFA) Results

Our results from the IFA procedure found that there was notable detection of FPV antibodies from the sample serum on pathogen-specific antigen substrate slides. This was seen as visually distinguishable fluorescent green staining against a black background when comparing the resulting fluorescence with control slides (Figure 7).

Figure 7. Results of the IFA proceedure testing serum samples in the form of images taken of the pathogen-specific antigen substrate slides. (**A, B**) The sham and negative results were identified by the lack of distinguishable staining against the black background. (**C, D**) Positive fluorescence results detected as the presence of notably distinct fluorescence seen in green against the black background.

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4. Discussion

In this study, 25% of cats tested positive by qPCR, consisting with previous studies. However, comparing studies is difficult due to differences in case definitions, study locations and designs, vaccination status, housing structures, and outbreak settings. In Germany, Parvoviral DNA shedding was detected in 28-49% of cats across 4 shelters [48]. In Canada, PCR-confirmed FPV infections were detected in 12% of clinically suspected shelter cats [49]. Despite the collection of demographic information, such as weight and gender, we found no statistically significant metrics for FPV positivity.

Differences in the Ct value distribution were observed, where most positive cats were classified as weak, and few exhibited moderate or strong positivity. This finding agrees with a previous study [49], suggesting that high viral load is usually seen in cats with acute disease, but in the same shelter, other cats recovering from infections exhibited low-level shedding. However, low levels of viral shedding are important for infection prevention [50], as it poses a transmission risk in high-density shelter environments [51].

In our study, IFA testing identified FPV-specific antibodies in approximately 39% of tested cats, indicating a prior exposure or vaccination. Seroprevalence exceeded qPCR positivity, indicating antibodies persisted long after viral shedding stopped. High seroprevalence was also reported in Australia and regions with active outbreaks, suggesting cumulative exposure or vaccination rates affecting the feline populations' seropositivity [52]. The presence of antibodies should be interpreted as evidence of prior exposure or existing immune response, and not as a protective measure against infections. The presence of antibodies does not limit viral shedding, particularly in vaccinated cats or those with non-protective or waning immunity [53,54].

No significant agreement was observed between PCR and IFA testing, which could be explained by differences in diagnostic targets. PCR detects viral DNA, indicative of active infection or viral shedding [55]. IFA indicates past exposure or vaccination and cannot distinguish between recent and distant immune events [55]. Additionally, cats with high fecal viral loads are more likely to test positive on antigen assays, while cats with lower levels or convalescent infections frequently test negative despite serologic evidence [56]. Our findings emphasize the different roles of antigen and antibody-focused tests in FPV surveillance. PCR is ideal for identifying cats actively shedding to contain outbreaks, while IFA provides information on population-level exposure, immunity gaps, and vaccination effectiveness [48,51].

We observed spatial heterogeneity in FPV qPCR positivity rates, suggesting a non-uniform geographic pattern with localized clustering. Spatial clustering of FPV outbreaks in cats has been previously described, explained by heterogeneous vaccination coverage, animal movement networks, differences in viral strains, and shared environmental reservoirs [48,52,57]. Further investigation found a higher concentration of positives towards the western region The eastern region had very few positive FPV cases. The higher concentration also aligns with the trend of population concentration in both humans and cats for this area [58]. Population-dense areas increase the likelihood of direct contact with other cats or fomites due to increased proximity. These areas would benefit from continued monitoring for feline viral infections and implementing more effective cleaning methods to limit disease spread.

The overall genome structure of FPV-151 in comparison to the reference strain (MN45165) resulting in a 98.7% similarity, reflecting the similarities between FPV-151 and the commercial vaccine strains at approximately 98.6%. Full-length genome and VP2 phylogenetic analysis found FPV-151 to be predominantly closer to Chinese, Japanese, and Thai FPV strains at approximately 99.0%. It is possible that a parent or earlier version of this strain initially traveled by fomites or a pet's latent infection from one of these countries due to FPV's notorious environmental stability. Despite sharing the Ile101Thr mutation with the "FPLV-CC19-02" (OR21195) isolate, FPV-151 was found to have a unique non-synonymous mutation among FPV isolates: Glu411Ala. This is a notable difference due to the mutation only occurring in MEV and FoxPV isolates with the indication of recombination or a shift in host virulence.

Our findings indicate frequent, on-going mutation in the currently locally circulating FPV strains. These mutations and they effect on the shape and function of structural proteins, specifically VP2, could lead to conformational changes to the secondary, tertiary and quintenary structure. Further effects could be seen on the virus-host binding site, causing higher virulence, increasing ability to evade the immune system, or potentially decreasing vaccine and drug effectiveness [34].

The use of IFA, qPCR and in-vitro viral propagation using the MDCK cell line continue to be useful tools in diagnostic procedures for feline viral identification in research and diagnostics [15]. These tools could be applied in combination with in-silico testing, such as docking models, to further test drug and vaccine efficacy against ongoing viral mutation as preventative measures [59]. Continued molecular and serological surveillance of FPV and other highly infectious feline viruses is recommended for the maintenance and safeguarding of the environment, local wildlife, and the health of our animal companions.

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