Detection of Feline Panleukopenia Virus Using a Commercial ELISA for Canine Parvovirus*

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\section*{CLINICAL RELEVANCE}
Feline panleukopenia virus (FPV) is a significant pathogen of cats. Rapid virus detection is critical for treatment and management, especially in populations in which spread may occur. This study investigated the ability of the SNAP Canine Parvovirus Antigen Test Kit (SNAP Parvo, IDEXX Laboratories) to detect FPV with confirmation of viral identity by polymerase chain reaction (PCR) assay and genetic sequencing on fecal samples \((n = 97)\) from cats with suspected FPV infection. Fifty-five samples were positive by SNAP Parvo; 54 of 55 were also positive by conventional PCR assay and were identified as FPV by genetic sequencing. This study demonstrates that SNAP Parvo can detect FPV in clinical samples.

\section*{INTRODUCTION}
Feline panleukopenia virus (FPV) remains an important pathogen of kittens and susceptible adult cats. The virus, a member of the Parvoviridae family, is simple in structure, extremely hardy in the environment, and highly contagious.\textsuperscript{1} It spreads systemically after oronasal infection and targets rapidly dividing cells. In cats older than 4 to 6 weeks, the primary target cells are intestinal crypt epithelia and blood cell precursors in the bone marrow.\textsuperscript{1} The disease manifests as severe depression, vomiting, diarrhea, and profound leukopenia. The virus is of particular concern in shelters in which kittens and immunologically naïve cats are housed, often under stressful conditions. In these situations, mortality may be very high.\textsuperscript{2}

FPV is closely related to canine parvovirus-2 (CPV-2) and its antigenic variants, designated CPV-2a, -2b, and -2c. In fact, some experts speculate that FPV is the ancestral origin of CPV-2.\textsuperscript{3,4} Despite their relatedness, however, antigenic differences between FPV and CPV-2 exist and are distinguishable using monoclonal antibody panels.\textsuperscript{5,6} Although the genetic and amino acid differences among these viruses are small, they

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Genetic analysis of fecal samples identified FPV in 54 of 55 cats testing positive by SNAP Parvo, reflecting the utility of this in-clinic ELISA.
Feline parvovirus by the IDEXX diagnostic PCR assay before our study, 11 were negative by diagnostic PCR for all pathogens tested, and 29 tested positive for at least one enteric pathogen other than FPV, including the three FPV-positive samples, which were also PCR positive for feline coronavirus. The samples were stored at –80°C after PCR analysis and were thawed before testing with SNAP Parvo.

ELISA

Fecal samples were tested by SNAP Parvo according to the manufacturer’s instructions. For fecal swabs, the extraction buffer or conjugate was dispensed into the sample tube via the kit swab. Then, the sample swab was inserted into the tube containing the liquid and vortexed briefly. The extracted fecal sample—conjugate liquid was transferred to the SNAP device by the swab pipette from the test kit according to manufacturer’s instructions.

DNA Extraction and Amplification

Total DNA was extracted from all samples by the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions, with modifications. Fecal material was mixed with an equal volume of phosphate-buffered saline and vortexed, and 200 µl of this mixture was used for extraction. For fecal swabs, 400 µl of phosphate-buffered saline was added to a tube containing the swab; the mixture was vortexed, and 200 µl was used for extraction. The remaining protocol followed the manufacturer’s guidelines.

Five microliters of undiluted extracted nucleic acid was subjected to PCR assay using primers targeting a portion of the VP2 capsid gene.9,10 This PCR product encodes a 583-base pair segment, the nucleotide sequence of which allows differentiation of FPV from CPV-2. Products were analyzed by agar gel electrophoresis.

Sequence Analysis

Amplification products were purified using ExoSAP-IT (USB Corporation, Cleveland, OH) according to the manufacturer’s recommendations. Primers for sequencing were the same as those used for amplification. Purified DNA was sequenced at the Molecular Biology Resources facility at the University of Tennessee with an ABI Prism dye terminator cycle sequencing reaction kit and ABI 373 DNA (Applied Biosystems, Foster City, CA). Phylogenetic tree construction and sequence distances were performed using the MegAlign program with ClustalW align, available in the Lasergene package (DNASTAR, Madison, WI).

Statistical Analysis

The agreement between PCR with genetic sequence confirmation of viral identity and SNAP Parvo was calculated and evaluated using a κ statistic.

RESULTS

Samples were collected from 97 cats with signs compatible with feline panleukopenia. Fifty-seven samples were from regional animal shelters, whereas 40 were submitted by US veterinary clinics for diagnostic purposes. Forty-nine of the 57 shelter animals were 6 months of age or younger, whereas only 9 of the 40 diagnostic samples were from kittens in this age range. The 40 diagnostic samples came from cats between 2 months and 14 years of age, with half of the animals 2 years of age or younger.

Although limited clinical information was available, the shelters occasionally reported a rapid clinical course of depression, anorexia, and loose, watery diarrhea in affected kittens. Leukopenia and death also were reported. Approximately half the shelter animals were vaccinated with a commercial multiway killed or modified-live vaccine upon entry to the facility. Clinical signs of disease and subsequent
Figure 1. Phylogenetic tree depicting sequence variation in the amplified segment of the VP2 gene from positive samples. A high degree of nucleotide homology was found among the samples. The clades tend to segregate by geographic locale and/or shelter (A = Tacoma, WA; B = Modesto, CA; C = Santa Rosa, CA; D = San Francisco, CA; E = Portland, OR [polymerase chain reaction negative and therefore not shown]; F = Burnsville, MN; G = Cleveland, OH). (CPV-2 = canine parvovirus type 2b, accession # FJ222823)
sample collection generally occurred 3 to 7 days after admission to the shelter.

All 97 samples were tested by conventional PCR assay using primers that were previously used to amplify a portion of the VP2 gene in both canine and feline parvoviruses. Analysis of the genomic region encompassed by these primers and its predicted amino acid sequence allowed discrimination of FPV from CPV-2 and its variants. Fifty-four of the 57 shelter samples and 3 of the 40 diagnostic specimens tested positive by conventional PCR for the VP2 gene of canine and feline parvoviruses. The amplification products from all positive samples were confirmed as FPV by nucleotide sequence analysis. Sequence analysis of all products showed a high degree of nucleotide homology. In fact, only 10 nucleotides showed variability in the entire sequence among all the isolates, none of which led to a change in amino acid sequence. Sequence variation correlated with the population, with clades segregated primarily by geographic locale and/or shelter (Figure 1). The exception was viruses from one queen and her four kittens at a single shelter. The strains from one kitten (A_47), the queen (A_49), and the remaining three kittens (A_45, A_46, A_48) segregated into different clades, although the genetic differences were minor.

Fecal material from 53 of 57 clinically ill shelter animals and two of 40 samples submitted by veterinary clinics tested positive by SNAP Parvo. Compared with the conventional PCR assay results, two shelter samples and one diagnostic sample that were confirmed positive by PCR and genetic sequencing tested negative by the CPV-2 ELISA. Additionally, one shelter sample tested strongly positive by the CPV-2 ELISA but was not found to have molecular evidence of FPV by conventional PCR (Table 1). The remaining samples (54 of 57 shelter samples and 39 of 40 diagnostic samples) gave equivalent results, thus yielding 96% agreement and a $\kappa$ of 0.92.

| TABLE 1. PCR with Genetic Sequence Confirmation of Viral Identity versus SNAP Parvo* |
|---------------------------------|-----|-----|
| **Polymerase Chain Reaction (No.)** |     |     |
| SNP Parvo (No.) | +   | 54  | 1   |
|                  | –   | 3   | 39  |
| *Results obtained from 97 feline fecal samples. |

**DISCUSSION**

Genetic analysis of fecal samples identified FPV in 54 of 55 cats testing positive by SNAP Parvo, reflecting the utility of this in-clinic ELISA. Although these animals were symptomatic, indicating they likely were naturally infected, we cannot rule out that in some cases vaccinal virus was detected, particularly in cats in rescue facilities where modified-live vaccines were given upon the cats’ arrival. Twenty-seven animals at the shelter facilities were known to have been vaccinated 3 to 12 days before sample collection, but only 13 received modified-live vaccines. Twenty-one animals at these facilities were not vaccinated, and the vaccination status of seven animals was unknown. Despite these differences in vaccination, the frequency of positive SNAP Parvo results was similar across the groups. Because the cats in this study were ill with signs compatible with FPV and their fecal samples were strongly positive on ELISA, it is more likely that the viruses detected were field strains. Previous studies showed that SNAP Parvo infrequently detects virus resulting from modified-live vaccines and that positive ELISA results in dogs and cats have a high positive predictive value for CPV-2 and FPV infections, respectively. Thus, the positive results from SNAP Parvo were considered useful diagnostic information for the clinically ill animals in this.
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study. Nevertheless, recent vaccination with modified-live vaccines should be taken into consideration when interpreting either antigen or PCR test results, even in sick patients.

Although this study did not specifically evaluate the sensitivity and specificity of SNAP Parvo for FPV, agreement between the test methods was high. Only three shelter samples and one diagnostic sample gave discrepant results when comparing ELISA with conventional PCR. Of these, FPV was detected by conventional PCR in three samples that tested negative on SNAP Parvo. This is not surprising given the increased sensitivity of PCR compared with other virus detection methods. This methodology can detect the presence of low levels of virus, but because conventional PCR was done in this study, the amount of virus in the samples could not be assessed. The other discrepant sample tested positive by ELISA but was not confirmed by PCR (ELISA positive, conventional PCR negative). This sample, which tested strongly positive on SNAP Parvo, was from a 2-month-old kitten that had been vaccinated with a killed vaccine 6 days before sample collection. Feces from this kitten had tested positive on ELISA both at the shelter facility and at IDEXX Laboratories. Possible explanations for the negative PCR result include the presence of PCR-inhibitory substances in the sample, nucleic acid degradation in the sample, or a false-positive ELISA result.

Sequence analysis of amplification products showed very little nucleotide variation in the region analyzed among all the isolates, and no amino acid changes were predicted. These findings are consistent with those of previous studies, indicating that FPV appears to be in evolutionary stasis. Nucleotide mutations noted among some samples likely were the result of random genetic changes. Parvoviruses in general are known to have a higher mutation rate than most DNA viruses.

CONCLUSION

Despite known genomic and antigenic differences between FPV and CPV-2 and its variants, this study conclusively shows that SNAP Parvo can detect FPV in clinically affected cats. Point-of-care testing, such as with commercial antigen detection ELISA kits, is vital for rapid and cost-effective diagnosis of parvovirus infection. It is especially critical in rescue facilities and other housed populations, in which infections may spread rapidly, leading to high mortality. Rapid detection will allow appropriate control measures to be implemented to prevent viral spread.

REFERENCES