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

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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.

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.[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.[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.[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.[3,4] Despite their relatedness, however, antigenic differences between FPV and CPV-2 exist and are distinguishable using monoclonal antibody panels.[5,6] Although the genetic and amino acid differences among these viruses are small, they

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occur in antigenically important epitopes of VP2, the major capsid protein of the viruses.[6]

Detection of FPV infection is important not only for diagnostic purposes, but also to control infection in populations such as those at rescue facilities. Commercial ELISAs that detect CPV-2 and its variants in feces of infected dogs are available and commonly used in point-of-care settings. In this study, one of these assays—the SNAP Canine Parvovirus Antigen Test Kit (SNAP Parvo, IDEXX Laboratories)—was evaluated for its ability to detect FPV. A previous investigation used SNAP Parvo to detect FPV following vaccination with modified-live or killed FPV vaccines.[7] In this setting, 64 kittens were seronegative at the time of vaccination and feces were tested repeatedly over a 2-week period after vaccination. SNAP Parvo detected vaccinal virus from only one modified-live–vaccinated kitten over the 2-week period. Another investigation examined the ability of SNAP Parvo to detect FPV in cats, but this investigation compared the test with visualization by electron microscopy[8]; thus, characterization of the infecting virus as FPV could not be concluded because CPV-2a, -2b, and -2c variants can infect cats.

In contrast to the two aforementioned studies,[7,8] the current investigation relied on genetic confirmation of viral identity to evaluate the hypothesis that SNAP Parvo is useful for detecting FPV in clinical settings. Using fecal samples collected by veterinarians from cats with signs consistent with feline panleukopenia, we tested for FPV using SNAP Parvo. Samples were then analyzed by polymerase chain reaction (PCR) of viral nucleic acid followed by genetic analysis of the amplified product to confirm viral identity.

MATERIALS AND METHODS

Samples

Collection was biased to obtain sufficient numbers of FPV-infected and -uninfected samples; a total of 97 fecal samples were collected. Fifty-seven of the 97 samples were obtained through a field study program involving animal shelters ($n = 7$) across the United States. An announcement was placed in the monthly Humane Society of the United States newsletter requesting that fecal samples from suspected panleukopenia cases (signs of vomiting and diarrhea, severe dehydration, depression) be sent to IDEXX Laboratories. The shelter received payment on a per-sample basis in return for their sample(s), time, and effort. Participating shelters were located in five states in the Midwest and western United States. Sample collection began in April 2008 and continued through September 2008. Fifty-five of the 57 samples had been tested for parvovirus by SNAP Parvo at the submitting facility.

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.

The other 40 feline fecal samples were submitted to IDEXX for testing by its commercial PCR panel for enteric pathogens (*Tritrichomonas foetus, Toxoplasma gondii, Cryptosporidium* spp, *Salmonella* spp, *Clostridium perfringens* enterotoxin A gene, feline coronavirus, and parvovirus). These samples were submitted by 36 veterinary clinics in 15 states during a 1-week period in June 2009. Of these 40 samples, three had tested positive for par-

vovirus 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

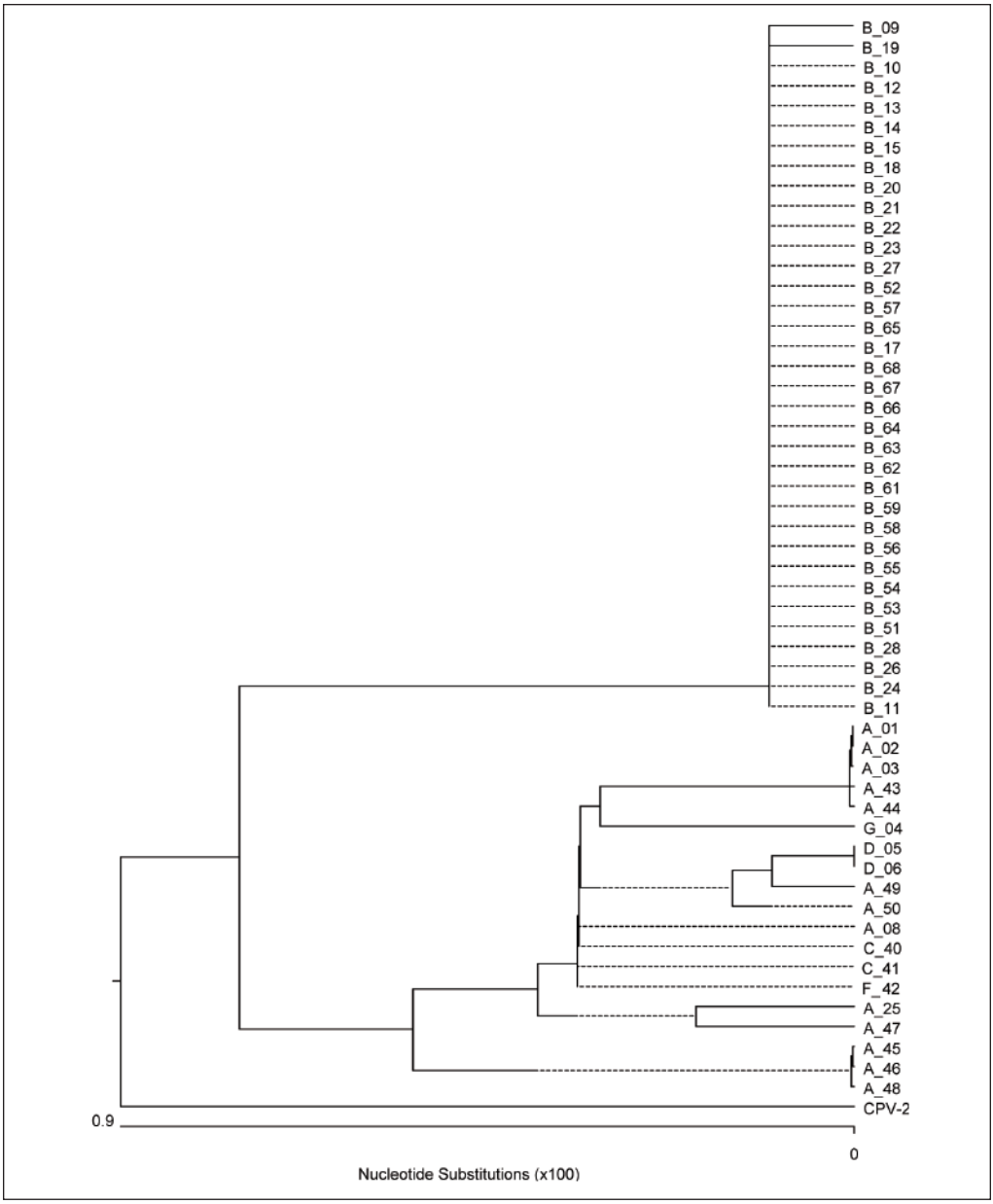

Figure 1. *Phylogenetic tree depicting sequence variation in the amplified segment of the VP2 gene from positive samples. A high degree of nucleotide homology was found among the samples. The clades tend to segregate by geographic locale and/or shelter (*A = *Tacoma, WA;* B = *Modesto, CA;* C = *Santa 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 κ of 0.92.

TABLE 1. PCR with Genetic Sequence Confirmation of Viral Identity versus SNAP Parvo*

		Polymerase Chain Reaction (No.)	
		+	–
SNAP 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[7] and that positive ELISA results in dogs and cats have a high positive predictive value for CPV-2 and FPV infections, respectively.[8,9] Thus, the positive results from SNAP Parvo were considered useful diagnostic information for the clinically ill animals in this

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.[11] 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.[3]

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.

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