Fecal viral DNA shedding following clinical panleukopenia virus infection in shelter kittens: a prospective, observational study

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Abstract

Objectives The aims of this study were to determine the magnitude and duration of fecal viral DNA shedding after diagnosis of feline panleukopenia (FP) in a group of shelter cats using quantitative real-time PCR (qPCR); to assess the utility of a negative point-of-care test or the resolution of diarrhea and systemic signs as proxy measures for qPCR positivity; and to investigate patterns of additional enteric pathogens in relation to feline panleukopenia viral shedding duration.

Methods Feline panleukopenia virus (FPV) infection in clinically affected shelter cats was confirmed by a commercial qPCR test. Observations were made on days 0, 3, 7, 14 and 21 post-diagnosis. Fecal flotation, FPV qPCR and the canine parvovirus IDEXX SNAP Parvo ELISA (SNAP) test were performed on fecal samples.

Results Forty cats and kittens with confirmed panleukopenia were initially enrolled. Sixteen kittens were sampled until day 14, and 12 were followed to day 21. Median DNA viral copy numbers fell below the diagnostic cut-off by day 7, with 13/16, 6/16, 1/16 and 0/12 testing PCR-positive on days 3, 7, 14 and 21, respectively. The SNAP test was positive in 12/16 kittens on day 0 and only 3/16 on day 3. SNAP test results, diarrhea and systemic signs were inconsistent in relation to qPCR positivity post-diagnosis. Additional enteric pathogens were common. The presence of additional pathogen types was suggestive of a longer PCR shedding duration, but this was not tested statistically owing to the small sample size.

Conclusions and relevance These findings suggest that cats should be isolated for at least 14 days after a diagnosis of FP, but that release from isolation after this point is reasonable, in association with a multifaceted infection control strategy. The study findings did not support using SNAP test results, diarrhea or systemic signs as proxy measures for virus shedding.

Keywords: Panleukopenia; parvovirus; virus-shedding; quantitative real-time PCR; isolation; point-of-care; animal shelter; quarantine

Introduction

Feline panleukopenia virus (FPV), or feline parvovirus, is a highly transmissible pathogen that causes feline panleukopenia (FP), a severe vaccine-preventable illness of domestic cats.[1] Clinical infection is characterized by gastroenteritis, panleukopenia and septic shock.[2] FPV is shed in large quantities in saliva, urine, vomitus and feces, and can persist in an environment for up to a year.[1] It is transmitted primarily by the fecal–oral route, with fomite transmission playing an important role.[1,3,4] Animal shelters are at risk for outbreaks because of shifting populations derived from multiple sources, intake of unvaccinated adults and vulnerable kittens, environmental persistence of the virus and fomite transmission.[5,6] Outbreaks can result in high mortality, euthanasia and shelter closures.[2,6,7]

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The isolation of infected animals is essential to prevent disease transmission within shelters,[1,6,8] but prolonged isolation has implications for shelter capacity, animal welfare, kitten socialization and length of stay before adoption. The required isolation period is dependent on the expected duration and magnitude of pathogen shedding following infection. Reviews and book chapters report that typical FPV shedding duration in cats is 1–2 or 5–7 days,[1,5] but shedding persisted for up to 6 weeks in one cat in an early study.[9]

Previous shedding studies have primarily included experimentally infected cats in controlled environments.[3,9,10] In contrast to these study populations, shelter settings frequently include large numbers of un- or under-vaccinated kittens,[5,6] higher stress levels[11] and a variety of enteric pathogens.[12,13] These factors might influence the magnitude and/or duration of FPV shedding.

Shelters may use a combination of repeat testing, recommended time frames or resolution of clinical signs to determine the appropriate duration of isolation.[14,15] Point-of-care (POC) tests can lack sensitivity,[16,17] and, while FPV PCR is highly sensitive,[18] it is slow and expensive for routine use. It is of interest to shelters to assess whether proxy measures for reduced virus shedding, such as resolution of diarrhea and systemic signs, or a negative POC test, could be used to determine the appropriate timing for release from isolation.

The objectives of this study were to determine the magnitude and duration of fecal viral DNA shedding after diagnosis of FP in a group of shelter cats; assess the utility of a negative POC test or the resolution of diarrhea and systemic signs as proxy measures for viral DNA copy numbers present in fecal samples; and investigate patterns of additional enteric pathogens in relation to the duration of FPV shedding.

Materials and methods

Institutional approval
This observational study was approved by the chief executive officer of the shelter.

Setting
The study was performed at the Toronto Humane Society, a private, limited-admission shelter in Ontario, Canada. Cats were housed singly, in family groups or – in the case of orphan singleton kittens – co-housed with kittens of similar size and weight. The shelter admits animals through owner relinquishment, stray intake and rescue transport, and has a full-service veterinary hospital. FP is seen with some regularity, although case numbers are low (the average in 2019, for locally surrendered cats, was 2.3 cases per month). It is more likely to occur during the spring and summer when large numbers of juveniles are admitted. Shelter-acquired infections are unusual. Based on shelter metrics most cases are diagnosed soon after intake, within the incubation period, and then isolated. Exposed animals are traced and quarantined. Stringent infection control measures are routinely followed in the shelter. Affected cats are regularly transferred from other shelters that lack the resources to treat the disease.

At intake, cats ≥4 weeks of age were vaccinated with a modified live subcutaneous feline viral rhinotracheitis, feline calicivirus and feline panleukopenia virus (FVRCP) vaccine. Kittens were revaccinated every 2–3 weeks based on current recommendations.[19,20] Further standardized intake procedures included examination by a veterinarian or registered veterinary technician; treatment with selamectin, pyrantel (all) and ponazuril (kittens); Wood's lamp and retroviral screening; and rabies vaccination for cats ≥12 weeks of age. Pyrantel was repeated after 2 weeks and continued every 2–3 weeks for kittens. Additional diagnostics and treatment were provided as needed. Blood smears and complete blood counts were not routinely performed because leukopenia is transient and does not occur in all cases.[1]

Case definition and qPCR test
Shelter cats with confirmed FPV infection, and for which follow-up testing was possible, were included in the study. The case definition for FPV infection was compatible clinical signs as described below and a positive FPV quantitative real-time (qPCR) test on a fecal sample or rectal swab.

Clinical criteria for FP were: (1) dehydration, obtundation to coma, hypothermia or hypoglycemia; or, in adult cats (2) anorexia/hyporexia and lethargy in association with vomiting and/or diarrhea; or, in kittens (3) anorexia/hyporexia, lethargy and weight loss, or diarrhea with or without additional signs, or vomiting with anorexia/hyporexia or pyrexia.

Diarrhea was defined as stool score of 5–7 using the Purina Veterinary Diets Fecal Scoring Chart (http://vhc.missouri.edu/wp-content/uploads/2020/07/Nestle-Purina-Fecal-Scoring-System.pdf).

A commercial qPCR test was used (IDEXX Reference Laboratories). The qPCR targeted the FP *VP2* gene EU252145,[12] and was able to detect both FPV and canine parvovirus (CPV). The analytic sensitivity was 10 DNA copies/reaction. The reference laboratory defined a positive diagnosis as cycle threshold (Ct) value of ≤26, which corresponded to ≥1.59×10^6 (1,588,799) viral DNA copies per gram (EA Chan, IDEXX Reference Laboratories, personal communication). Ct values were converted to DNA viral copy numbers by the laboratory, using a proprietary calculation.

Sample collection and monitoring
Following confirmation of FPV infection (day 0), fecal samples were collected on days 3, 7, 14 and 21. Cats were made available for foster or adoption once sufficiently recovered. Information regarding potential shedding and the protection of resident cats was always provided. Fecal samples were collected in the shelter or in the homes of foster volunteers or adopters who opted in. Cats were

Table 1 Duration of fecal viral DNA shedding, and agreement between diagnostic tests, in shelter kittens with clinical feline panleukopenia virus infection

	Day 0 (n = 16)	Day 3 (n = 16)	Day 7 (n = 16)	Day 14 (n = 16)	Day 21 (n = 12)
qPCR positive*	16	13	6	1	0
SNAP positive	12	3	0	1	0
qPCR positive, SNAP negative	4	10	6	1	0

qPCR = feline panleukopenia virus quantitative real-time PCR; SNAP = IDEXX SNAP Parvo point-of-care ELISA
*Cycle threshold value $\leqslant 26$, corresponding to $\geqslant 1.59 \times 10^6$ viral DNA copies per gram

excluded if fecal samples could not be obtained on days 3, 7 and 14. Systemic signs (pyrexia, lethargy, anorexia/hyporexia and/or weight loss) were recorded, when possible, on the days of sample collection.

Sample handling and testing

Initial diagnostic samples were processed immediately. Samples from recovering cats were processed immediately or stored at –20°C for up to 60 days. All samples were tested with the IDEXX SNAP Parvo ELISA (SNAP) test and the Diarrhea RealPCR Panel (Comprehensive). Pathogens included in the panel were *Campylobacter* species, *Clostridium perfringens*, *Cryptosporidium* species, feline enteric coronavirus, *Salmonella* species, *Toxoplasma gondii* and *Tritrichomonas foetus*. Additional enteric pathogens were identified by in-house fecal flotation using zinc sulfate centrifugation at intake or as requested by medical staff. The total number of additional pathogens was reported for each kitten, regardless of at which time point(s) they were detected. SNAP tests were performed by a trained research assistant, according to the manufacturer's instructions. qPCR tests were performed by the reference laboratory.

Data analysis

Descriptive statistics were generated using Microsoft Excel. The presence/absence of diarrhea and systemic signs were reported as proportions as data were not available for all cats after day 7, following fostering or adoption. The duration of viral shedding was described in relation to the number and type of additional enteric pathogens identified by fecal flotation and qPCR.

Results

Forty cats and kittens with confirmed FPV infection were enrolled; their median age was 5 weeks (interquartile range [IQR] 4–8; range 2–104); 3/40 cats were >20 weeks of age. Thirty-two of the 40 (80%) cats survived the infection and were subsequently adopted. Eight cats died or were euthanized before 14 days post-diagnosis. One or more samples were missing for 16 cats because of relocation to foster or adoptive homes. Full data sets to day 14 were available for 16 cats, all of which were kittens (median age 5 weeks; range 2–8).

The day of the first positive qPCR test was designated as day 0. Sixteen kittens were followed for 14 days from day 0, and 12 of these were followed for 21 days. On day 0 no kittens were pyrexic, 75.0% had diarrhea, 68.8% had weight loss and 25.0% had vomiting. The qPCR test was positive at the time of onset of clinical signs in 12 cases. For the remaining four kittens, the qPCR was initially negative, but was positive on repeat testing 4 days later (day 0). The median DNA viral copy number (per gram of feces) on initial testing for these four cats was 2.88×10^5 (IQR 2.55×10^4–6.97×10^5). This increased to 7.53×10^9 (IQR 2.30×10^9–8.74×10^9) on repeat testing.

On day 3, 13/16 (81.3%) kittens remained qPCR positive, while only 3/16 (18.8%) had positive SNAP tests (Table 1). Six of 16 (37.5%) kittens tested qPCR positive on day 7, and no SNAP tests were positive at this time point. There was one positive qPCR test on day 14, and one weak positive SNAP test from a different kitten that was qPCR negative. All other SNAP tests for this kitten were negative. On day 21, no samples tested positive on either test. Other than the weak positive SNAP result on day 14, there was no instance in which either type of test was positive following a negative result.

The median viral DNA viral copy number at day 0 was 5.00×10^8 (IQR 9.59×10^7–5.17×10^9), falling to 5.66×10^6 (IQR 2.00×10^6–3.18×10^8) on day 3 (Figure 1; see also the
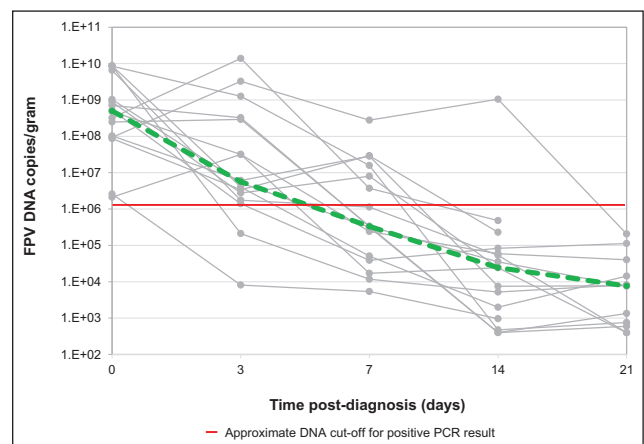
Figure 1 DNA viral copy counts in fecal samples from recovering shelter kittens after a diagnosis of clinical feline panleukopenia virus (FPV) infection by quantitative real-time PCR (n = 16 to day 14; n = 12 at day 21). Dotted line = median DNA copy counts
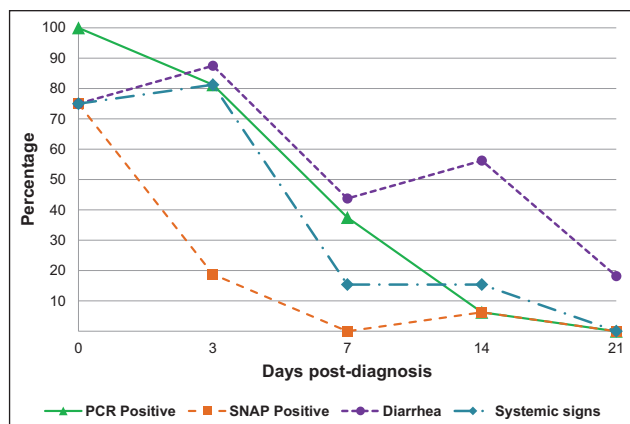
Figure 2 Duration of fecal viral shedding compared with percentage of diarrhea and systemic signs in shelter kittens with clinical feline panleukopenia virus infection (n = 16 to day 14; n = 12 on day 21). Sample sizes for diarrhea and systemic signs varied (see text). PCR = feline panleukopenia virus real-time PCR; SNAP = IDEXX SNAP Parvo point-of-care ELISA

qPCR-positive kitten did not have diarrhea, while 9/15 (60.0%) qPCR-negative cats had diarrhea. The proportion with diarrhea was greater than the proportion with positive qPCR tests at all time points except day 0, and diverged most at day 14, where 6.3% had positive qPCR tests and 56.3% had diarrhea (Figure 2). The presence or absence of systemic signs (pyrexia, lethargy, anorexia/ hyporexia or weight loss) was recorded for 16 kittens on days 0 and 3, 13 kittens on days 7 and 14, and nine kittens on day 21. On day 0, 75.0% of kittens had both systemic signs and a positive qPCR test, compared with 68.8% on day 3 and 0.0% at the subsequent time points (Figure 2, Table 2). On days 7 and 14, systemic signs were absent in all qPCR-positive cats and present in 28.6% (day 7) and 16.7% (day 14) of qPCR-negative cats.

At least one additional enteric pathogen was detected in 14/16 (87.5%) kittens during the study period (Table 2). Eleven had a single additional pathogen (*Isospora felis* n = 4, *C perfringens* n = 4, feline enteric coronavirus n = 3) and three had two additional pathogens – feline enteric coronavirus, and either *C perfringens* or *T cati*. Neither of the kittens with no additional enteric pathogens (n = 2) tested qPCR positive on day 7, while 3/11 kittens with one additional enteric pathogen and 3/3 kittens with two additional pathogens tested qPCR positive on day 7. Those with one additional enteric pathogen all tested qPCR negative by day 14 and all those with two additional enteric pathogens were negative at

table in the supplementary material). Median counts fell below the positive diagnostic cut-off of 1.59×10^6 viral DNA copies by day 7.

The proportion of kittens with diarrhea at the five time points was 75.0%, 87.5%, 43.8%, 56.3% and 18.2%, respectively (Figure 2, Table 2). Kittens with diarrhea were frequently qPCR - negative. On day 14, the single

Table 2 Diarrhea, systemic signs (pyrexia, lethargy, anorexia/hyporexia and/or weight loss) and additional enteric pathogens (AEPs)* in relation to quantitative real-time PCR (qPCR) test results in shelter kittens with clinical feline panleukopenia virus infection

		Day 0	Day 3	Day 7	Day 14	Day 21
Sample size		16	16	16	16	11[†]
qPCR positive	Diarrhea	12	11	3	0	0
	No diarrhea	4	2	3	1	0
qPCR negative	Diarrhea	0	3	4	9	2
	No diarrhea	0	0	6	6	9
Sample size		16	16	13	13	9
qPCR positive	Systemic signs	12	11	0	0	0
	No systemic signs	4	2	6	1	0
qPCR negative	Systemic signs	0	2	2	2	1
	No systemic signs	0	1	5	10	8
Sample size		16	16	16	16	12
qPCR positive	No AEPs	2	1	0	0	0
	One AEP	11	9	3	0	0
	Two AEPs	3	3	3	1	0
qPCR negative	No AEPs	0	1	2	2	2
	One AEP	0	2	8	11	9
	Two AEPs	0	0	0	2	1

*Identified by fecal flotation or qPCR panel (*Campylobacter* species, *Cryptosporidium* species, feline enteric coronavirus, *Salmonella* species, *Toxoplasma gondii* and *Tritrichomonas foetus*)
[†]One sample could not be scored due to damage in transit

21 days. Kittens that were positive beyond day 3 had *T cati* (n = 2), feline enteric coronavirus (n = 4) or *C perfringens* infection (n = 3). The kitten that was positive on day 14 was positive for *T cati* and feline enteric coronavirus.

Discussion

In this study, only 1/16 kittens with panleukopenia were qPCR positive at 14 days post-diagnosis, and 0/12 kittens were positive at day 21. The single weak-positive SNAP test at day 14 was most likely a false positive, as this sample was qPCR negative and the kitten had no other positive SNAP test results.

The IDEXX technical cut-off for the FPV qPCR test is based on viral DNA copy numbers considered to be clinically significant (MA Seguin, IDEXX Reference Laboratories, personal communication). However, the precise threshold required for disease transmission is unknown. Infective doses are typically expressed as $TCID_{50}$, which indicates the amount of virus required to produce a cytopathic effect in 50% of inoculated tissue culture cells.[21] It is not possible to relate this directly to viral DNA copy numbers, mainly because PCR amplifies nucleic acid from both viable and non-viable virus,[22] while $TCID_{50}$ measures an effect that is produced only by biologically active virus. Recent studies of SARS-CoV-2[23,24] serve as an important reminder that PCR-detectable virus cannot be extrapolated to be viable virus. Similarly, other human respiratory viruses showed a clear pattern of positive qPCR results persisting beyond the point that virus could be cultured.[25] In the current study, virus DNA remained detectable at low copy numbers in 11/12 kittens at day 21 (Figure 1; see also the table in the supplementary material). Low residual copy numbers are unsurprising when using highly sensitive test modalities such as qPCR.

There is little previously published information about the clinical shedding period for FPV. In an older study using virus isolation, FPV was isolated from the feces of 3/4 cats at days 20–22 post-infection; 0/13 cats at days 27–36; 1/10 cats at days 41–43; and 0/16 cats at days 48–120.[9] In a qPCR study, median DNA viral copy numbers in experimentally infected cats peaked at day 6 post-infection and decreased to low levels (mean count <6.5×10^4 copies/sample) by day 12.[10] Our results showed a similar pattern over time, after taking into account that day 0 of the current study was the day of diagnosis, not of infection (see Figure 1).

More data are available for CPV, which, like FPV, is a carnivore protoparvovirus 1,[1] with very little genetic variation from FPV.[18] In puppies with parvovirus infection, CPV was detectable by PCR for a median of 46 days post-infection.[22] For the reasons provided above, it is important to distinguish between detectable and clinically significant viral loads. Median counts in these puppies were consistently low after day 15 post-infection,[22] with a similar pattern to our study. In contrast to prolonged low viral DNA counts using qPCR, studies using viral

isolation found that viable CPV was shed only briefly, for a maximum of 8–14 days post-infection.[22,26–30] This time period corresponded with peak viral DNA copy numbers in PCR studies of FPV and CPV,[10,22] and is consistent with findings in the current study. It is unknown if the single cat that shed viable virus (positive on culture) on days 41–43 in an older study[9] represents an anomaly or a more frequent event, but available data suggest that the former may be the case.

Release of infected animals from isolation requires reasonable assurance that the released animal will not be contagious, and is therefore based on knowledge of typical shedding times. The main limitation of PCR as a means to determine shedding duration is its detection of non-viable virus. However, these more conservative results may act as a safety net, to avoid early release of cats with longer shedding durations that have not been captured in the relatively small body of literature. Our study showed consistently negative qPCR results following the first negative result, demonstrating that intermittent, clinically significant, shedding did not occur in this study.

The study findings did not support using a negative SNAP test or resolution of diarrhea or systemic signs (pyrexia, lethargy, inappetance and/or weight loss) as a means to determine when to release a recuperating animal from isolation. These measures were inconsistent in relation to qPCR, the reference standard, and were therefore unreliable proxy measures. Determining the duration of isolation based on these measures would have resulted in premature release from isolation in some cases and delayed release in others. As has been mentioned,[5] diarrhea was not reliably present at the time of FPV diagnosis in our study. The later presence of diarrhea in a substantial proportion of cats that tested qPCR negative was most likely from a combination of intestinal damage and intestinal microbiota disruption from FPV, additional enteric pathogens, shelter stress and diet changes, including weaning.

Previous shelter recommendations were to hold recovered cats for 3 weeks after *recovery*,[14] while more recent recommendations were to isolate for 4 weeks after *diagnosis*;[15] this can take place in an adoptive or foster home. The results of this and previous studies suggest that clinically significant shedding is unlikely to occur beyond 14 days after diagnosis in the majority of animals, and that release from isolation after this point is reasonable. Biological variation means that some risk upon release at this time point is unavoidable. A multifaceted approach of vaccination upon intake, cohorting, robust cleaning and disinfection protocols, and strategic placement of recovered cats (directly to foster or adoptive homes, or in adoption rooms containing fully vaccinated adults) is required to concurrently avoid prolonged isolation time and minimize risks upon release from isolation. The use of PCR testing could accelerate release from isolation in

some cases but has the disadvantages of cost, time delay and the need for more than one pre-release test in animals that are initially positive on retesting.

The majority of kittens in our study had additional enteric pathogens, which could have prolonged the duration of diarrhea and PCR positivity. Additional enteric pathogens are common in shelter cats.[12,13] Coinfections may have synergistic or antagonistic interactions with viruses, facilitating or competing with their establishment.[31] In a study of *Giardia* species and CPV coinfections in three dogs, giardiasis was associated with more severe clinical signs but did not appear to impact CPV shedding duration.[27] Although the sample size in our study was small and statistical analysis was not performed, the maximum FPV shedding duration of cats with additional enteric pathogens increased with the number of enteric pathogens present. Further studies with larger sample sizes would be necessary to clarify this relationship.

While not the focus of the study, the survival rate of 80% for the 40 cats that met the FP case definition is noteworthy, and contrasts with previously reported mortality of 50–90%.[1,2,6,7] This may reflect the medical care and resources available to the shelter, in particular the ability to provide intravenous fluids and medications to very small kittens.

The study had several limitations, the most important of which were the small sample size, the lack of sampling between 14 and 21 days, and the unknown virus viability at the time points following diagnosis. The study was performed in field conditions, in which many variables cannot be controlled. However, these conditions more closely reflect the realities faced by shelters. Larger sample sizes and a study design that includes virus isolation would provide valuable additional information.

Conclusions

The study findings and previous literature suggest that kittens recovering from FPV infection should be isolated for at least 14 days after diagnosis. Release from isolation after this point appears to be reasonable, in association with a multifaceted infection control strategy. The study findings did not support using diarrhea, systemic signs or SNAP test results as proxy measures for virus shedding.

Supplementary material The following file is available online:
Supplementary table: SNAP and PCR test results for 16 kittens with clinical feline panleukopenia virus infection.

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