

Parvovirus DNA Copy Numbers in the Blood of Cats With and Without Histologically Confirmed Panleukopenia

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Abstract

Use of real time polymerase chain reaction (qPCR) for quantitative detection of FPV and CPV2 DNA in samples from cats (qPCR-PV) has been shown to be both sensitive and specific over a wide range of viral titers in cats with naturally occurring FPV. Parvoviruses induce a viremia and so can be amplified from the blood. The purpose of this study was to determine the range of PV copies/ μ l blood in cats with suspected FPV infection with and without histological evidence of infection.

Blood, jejunum and ileum samples were collected from 25 shelter cats with clinical signs consistent with FPV after humane euthanasia. The majority of cats were vaccinated with a parenteral modified live FPV, FHV-1, and FCV containing vaccine upon intake to the shelter, prior to development of clinical signs. Blood in EDTA was evaluated in a previously validated qPCR-PV. Intestinal tissues were evaluated histopathologically after being stained with hematoxylin and eosin. The tissues were examined by an individual blinded to clinical history and qPCR-PV results. A diagnosis of FPV was given if characteristic lesions were present such as lymphoid depletion, intranuclear inclusions, villus blunting and fusion, or crypt necrosis, loss and abscessation.

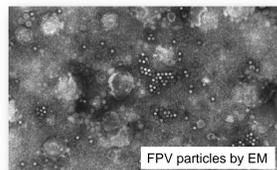
In the 5 cats with histologically confirmed FPV infection, 4 cats were positive in blood by qPCR-PV with the PV copies/ μ l ranging from 0 – 89,333 (mean = 22,929; SD = 33,078). In the 20 cats with no histological evidence of FPV infection, PV copies/ μ l were < 3,000 in all cats with a range of 0 – 2,947 (mean = 319; SD = 790). PV copies/ μ l were < 100 in 17 of 20 cats.

The use of qPCR-PV performed on blood of kittens suspected to have FPV should be evaluated further for clinical utility by comparing to other currently available assays.

Introduction

Feline panleukopenia (FPV) is highly infectious and often fatal in cats. Cats in shelters that show signs consistent with FPV and are positive in a canine parvovirus (CPV) antigen assay performed on feces are often euthanized because of the high mortality and environmental durability of the virus. CPV-antigen assays also detect FPV vaccine strains and so shelter cats vaccinated on intake could be positive and misinterpreted as natural infection leading to needless euthanasia.¹

Use of real time polymerase chain reaction (qPCR) for quantitative detection of FPV and CPV2 DNA in biological samples (qPCR-PV) has been shown to be both sensitive and specific over a wide range of viral titers in dogs.² Parvoviruses induce a viremia and so can be amplified from the blood. In preliminary work in dogs in our laboratory, we determined that qPCR-PV results on blood could be used to discriminate naturally infected from CPV vaccinated dogs.³ However, to our knowledge, this assay has never been evaluated as a discriminating test between vaccinated cats and naturally infected cats with FPV. While transport time is currently required for PCR assays, the initial CPV antigen assay results could be used for screening and suspect cats could be quarantined until qPCR-PV results were available rather than potentially euthanizing the cat needlessly.



Objectives

- Optimize the qPCR-PV for use with feline blood and determine the FPV sensitivity.
- Determine the FPV/CPV copy numbers/ μ l in peripheral blood of shelter cats with clinical signs consistent with FPV by qPCR-PV.
- Evaluate for associations between PV copy numbers/ μ l in peripheral blood and histopathological findings consistent with FPV infection.

Materials and Methods

qPCR-PV

A previously reported qPCR-PV designed to amplify DNA of FPV, CPV2a, 2b, and 2c was optimized to quantify total FPV DNA in feline blood with results expressed as PV copies/ μ l blood.

qPCR-PV sensitivity

Absolute sensitivity of the qPCR-PV was determined by assaying log dilutions of a market leading MLV FVRCP vaccine.

Patient Selection

Shelter cats from north central Colorado <1 year of age with clinical signs (vomiting, diarrhea, depression) consistent with FPV infection.

Sample Collection

- A blood sample was collected from cats showing signs consistent with FPV and placed into an EDTA containing tube.
- Vaccination history and length of time until illness were recorded.
- Cats that died or were euthanized had samples collected from the jejunum and ileum and placed into 10% neutral buffered formalin.
- Total DNA was extracted from blood and assayed in the qPCR-PV.
- Both segments of small intestine was trimmed, paraffin embedded, sectioned at 5 μ m, and stained with hematoxylin and eosin for light microscopy examination.
- Microscopy was evaluated by a pathologist (SM) blinded to clinical history and diagnostic outcomes and characterized as positive or negative for histopathological evidence of FPV infection.

Results

qPCR-PV Sensitivity

- Log dilutions were performed to 2.12×10^{-05} with successful amplification of viral DNA.

Study Population

- A total of 46 cats were identified as having clinical signs consistent with FPV infection.
- Tissues were collected from 25 cats for histopathological assessment.

Histopathology and PCR results

- Of the 25 cats, 5 had histopathological evidence of FPV infection.
- FPV/CPV DNA was amplified from blood of 4 of the 5 cats by qPCR-PV
 - PV copies/ μ l ranged from 0 – 89,333 (mean = 22,929; SD = 33,078).
- FPV/CPV DNA was amplified from blood of 13 of the 20 cats with no histological evidence of FPV infection.
 - PV copies/ μ l were < 3,000 in all cats with a range of 0 – 2,947 (mean = 319; SD = 790).
 - PV copies/ μ l were < 100 in 17 of 20 cats.
- Differences in PV copy numbers were statistically significant in cats with histologic evidence of infection and those without on Mann-Whitney testing (P = .025).

Results (cont.)

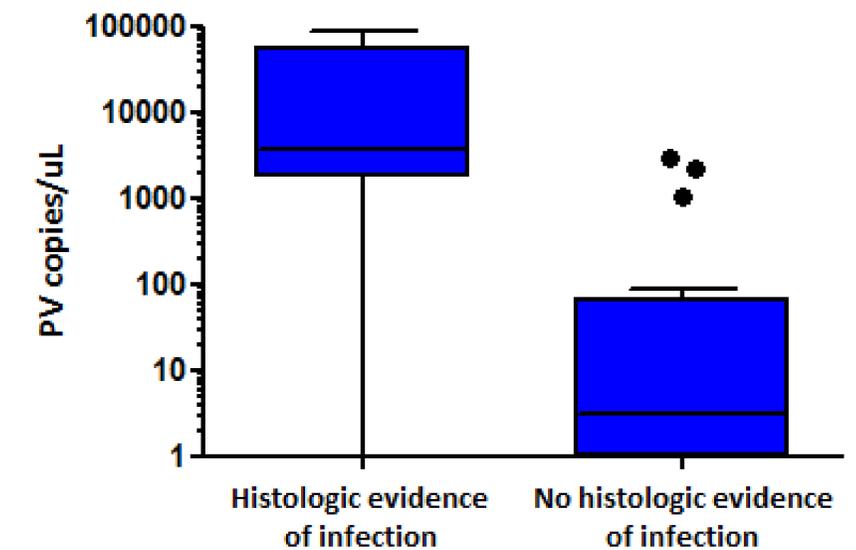


Fig 1. Tukey Box and Whisker plot of FPV/CPV viral copies/ μ l in cats with and without histologic evidence of infection

Summary and Conclusions

- Amplification of FPV/CPV DNA from blood of kittens with no histopathologic evidence of FPV may reflect low level viremia from vaccination
- In puppies, maximal PV copies/ μ l in blood after vaccination was 3,000.
- Studies to determine maximal post-vaccination FPV copy number/ μ l of blood are ongoing.
- Based on the results of these studies, qPCR-PV performed on blood has the potential for discriminating vaccination from natural infection by FPV.

Acknowledgements

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