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# Levels of feline infectious peritonitis virus in blood, effusions, and various tissues and the role of lymphopenia in disease outcome following experimental infection

Niels C. Pedersen [a,*], Chrissy Eckstrand [c], Hongwei Liu [a], Christian Leutenegger [b], Brian Murphy [c]

[a] Center for Companion Animal Health, School of Veterinary Medicine, University of California, Davis, CA, USA
[b] IDEXX Laboratories, Sacramento, CA, USA
[c] Department Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA, USA

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ABSTRACT

Twenty specific pathogen free cats were experimentally infected with a virulent cat-passaged type I field strain of FIPV. Eighteen cats succumbed within 2–4 weeks to effusive abdominal FIP, one survived for 6 weeks, and one seroconverted without outward signs of disease. A profound drop in the absolute count of blood lymphocytes occurred around 2 weeks post-infection (p.i.) in cats with rapid disease, while the decrease was delayed in the one cat that survived for 6 weeks. The absolute lymphocyte count of the surviving cat remained within normal range. Serum antibodies as measured by indirect immunofluorescence appeared after 2 weeks p.i. and correlated with the onset of disease signs. Viral genomic RNA was either not detectable by reverse transcription quantitative real-time PCR (RT-qPCR) or detectable only at very low levels in terminal tissues not involved directly in the infection, including hepatic and renal parenchyma, cardiac muscle, lung or popliteal lymph node. High tissue virus loads were measured in severely affected tissues such as the omentum, mesenteric lymph nodes and spleen. High levels of viral genomic RNA were also detected in whole ascitic fluid, with the cellular fraction containing 10–1000 times more viral RNA than the supernatant. Replicating virus was strongly associated with macrophages by immunohistochemistry. Virus was usually detected at relatively low levels in feces and there was no evidence of enterocyte infection. Viral genomic RNA was not detected at the level of test sensitivity in whole blood, plasma, or the white cell fraction in terminal samples from the 19 cats that succumbed or in the single survivor. These studies reconfirmed the effect of lymphopenia on disease outcome. FIPV genomic RNA was also found to be highly macrophage associated within diseased tissues and effusions as determined by RT-qPCR and immunohistochemistry but was not present in blood.



## 1. Introduction

In spite of many decades of studies on experimental FIPV infection, there is very little information on how the virus and host interact over an entire disease course. Many experimental FIPV infection studies have also been limited to the final disease outcome, e.g., the testing of vaccine candidates for efficacy or testing of various types and biotypes of feline coronavirus isolates for disease potential (Pedersen, 2009, 2014a). Still other experimental studies have concentrated on specific virus- or host-related inflammatory or immune responses, also measured mainly

* Corresponding author at: University of California, One Shields Avenue, Davis, CA 95616, USA; Tel.: +1 530 752 7402; fax: +1 530 752 7701.
*E-mail address:* ncpedersen@ucdavis.edu (N.C. Pedersen).

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in terminally ill cats (Pedersen, 2014a). There are several longitudinal studies which have followed the entire disease course, but these studies were concerned mainly with disease signs such as fever, antibody responses and cytokine expression and not with viral loads in various tissues (Pedersen and Boyle, 1980; Weiss and Scott, 1981; Gunn-Moore et al., 1998a). Only one other temporal study has been performed on FIPV levels in peripheral blood of experimentally infected cats (de Groot-Mijnes et al., 2005). Although the authors suggested that there was a significant viremia associated with disease, viremia was erratic, observed mainly at week one post-infection and terminally, and the levels of viral genomic RNA, even when detected, were never high.

There has been a long standing belief that FIPV is not shed from the body, at least to the very high levels seen in FECV infection or in an infectious form (Pedersen et al., 2009). FIPV does not appear to replicate in enterocytes (Chang et al., 2010; Pedersen et al., 2012), and was first associated with macrophages by electron microscopy and immunohistochemistry (Ward, 1970; Pedersen and Boyle, 1980). Dean et al. (2003) studied the distribution of FIPV by immunofluorescence at the time of death in experimentally infected cats and also found macrophages to be heavily infected in mediastinal and mesenteric lymph nodes and spleen, with much less evidence of infection in peripheral tissues such as popliteal and cervical lymph nodes and bone marrow.

Lymphopenia is a consistent feature of both naturally (Pedersen, 2009, 2014b) and experimentally induced-FIP (Dean et al., 2003; de Groot-Mijnes et al., 2005). The role of lymphopenia in FIP has not been determined, but it has been equated with a decrease in cellular immunity and ultimate disease outcome in experimental infections (de Groot-Mijnes et al., 2005; Vermeulen et al., 2013). Although lymphopenia appears to play a role in FIP, temporal studies on the appearance, magnitude and duration of lymphopenia in cats that succumb or survive experimental infection have not been reported; most cell counts have been taken prior to infection and terminally.

The emphasis of FIP diagnostics has long been on developing a simple blood test that would reliably and specifically detect the causative virus (Pedersen, 2014b). Herrewegh et al. (1995) reported on the detection of viral RNA in blood serum or plasma of cats experimentally and naturally infected with FIPV using a nested RT-PCR that amplified a sequence within the 3′-UTR that was highly conserved among 10 different FIPV and FECV isolates. They were able to detect feline coronavirus RNA in the serum, plasma or ascitic fluid of 14/18 cats with naturally occurring FIP. Unfortunately, they were also able to detect viral RNA in the plasma of 2/7 healthy cats that were concurrently shedding FECV in their feces. An attempt was made to eliminate this problem of specificity by developing an RT-PCR that would only measure forms of coronavirus mRNA that were replication competent (Simons et al., 2005). The rationale was that FECV would not replicate in the blood; therefore, the replicative form of genomic RNA would only be found in the blood of cats with FIP. The authors reported that this test was highly accurate in identifying cats with FIP. Shortly thereafter additional studies using the same assay demonstrated replicating forms of RNA in the blood of healthy cats infected with FECV (Can-Sahna et al., 2007; Kipar et al., 2010), casting doubts on the specificity of such tests. The ability of FECV to replicate in blood monocyte/macrophages was also reported by other groups (Vogel et al., 2010). Chang et al. (2012) subsequently identified two specific mutations within the fusion peptide of the spike protein of FIPVs that were not present in parent FECVs. These would seem to be logical mutations to incorporate into a RT-qPCR. However, a more recent study by Porter et al. (2014) demonstrated the presence of coronavirus with the FIPV-specific fusion peptide mutation in tissues of healthy cats. They concluded that the spike fusion region specific mutation was an adaptation of FECV for growth in blood monocyte/macrophages and not directly related to disease. However, all of these various tests and objections are moot if FIPV genomic RNA is not consistently present in detectable levels by RT-qPCR in blood or blood fractions in cats with FIP.

The goal of this study was to temporally correlate disease signs in experimental FIPV infection with lymphopenia, antibody response, and viremia with disease outcome (death or survival) and specific cell and organ localization of virus in terminal tissue samples by RT-qPCR and immunohistochemistry. We will show that lymphopenia is the strongest predictor of disease outcome, virus is strongly associated with macrophages in lesional tissues and effusions, and viremia is not detectable at any stage of the infection even using a highly sensitive RT-qPCR.

## 2. Materials and methods

### 2.1. Experimental animals

Specific pathogen free cats were bred in the facilities of the Feline Research Laboratory (FRL), Center for Companion Animal Health, UC Davis under IACUC #16989. Cats used in this study were part of a larger experiment concerned with natural immunity (Pedersen et al., 2014) and were 6–9 months of age and equally intact male and female. They were housed in the FRL and cared for by FRL staff under ultimate authority of the Campus Veterinary Services.

### 2.2. Experimental FIPV infection

Cats were infected with a cat-passaged type I field isolate of FIPV (FIPV-m3c-2) (Pedersen et al., 2009), which has a functional mutation in the 3c gene and would not be expected to actively replicate in the intestinal epithelium (Chang et al., 2010; Pedersen et al., 2012). The inoculum was prepared as a cell-free suspension of diseased omentum (starting at 25 g/100 ml) that underwent differential centrifugation to remove particulate matter, bacteria and subcellular debris and then stored at −60 °C. It was diluted 1:80 in Hanks buffered saline prior to use and two ml was injected intraperitoneally to infect each cat. The final inoculate contained $4.32 \times 10^5$ viral genomes as determined by RT-qPCR. Cats were infected under IACUC