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Development and application of a quantitative real-time PCR assay to detect feline leukemia virus RNA

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Abstract

We previously defined four categories of feline leukemia virus (FeLV) infection, designated as abortive, regressive, latent, and progressive. To determine if detectable viral DNA is transcriptionally active in the absence of antigenemia, we developed and validated a real-time viral RNA qPCR assay. This assay proved to be highly sensitive, specific, reproducible, and allowed reliable quantitation. We then applied this methodology, together with real-time DNA qPCR and p27 capsid antigen capture ELISA, to examine cats challenged with FeLV. We found that circulating viral RNA and DNA levels were highly correlated and the assays were almost in perfect agreement. This indicates that the vast majority of viral DNA is transcriptionally active, even in the absence of antigenemia. The real-time qPCR assays are more sensitive than the most commonly used FeLV diagnostic assay, the p27 capsid antigen capture ELISA. Application of qPCR assays may add greater depth in understanding of FeLV–host relationships.

Keywords: FeLV; Real-time PCR; Immune response; Latent

1. Introduction

Feline leukemia virus (FeLV) was first identified as a naturally occurring viral infection of cats more than 40 years ago by electron microscopy (EM) (Jarrett et al., 1964a; Jarrett et al., 1964b). Since that time, advances in sensitivity, specificity, and speed of FeLV diagnosis have prompted greater insight into the complexity in the FeLV–host relationship. The clone 81 cell line used to detect infectious virus (VI) by focus formation (Fischinger et al., 1974; de Noronha et al., 1977) and the direct immunofluorescent antibody (IFA) assay used to detect intracellular *gag* proteins (Hardy et al., 1973; Hardy and Zuckerman, 1991a) provided the first major insights into FeLV detection and led to recognition of progressive and regressive host–virus relationships (Hoover et al., 1981). Development of an antigen capture ELISA using monoclonal antibodies directed against different epitopes of the p27 capsid protein (Lutz et al., 1983a) provided a rapid and sensitive diagnostic assay applicable to testing animals on site at low cost.

Use of the VI, IFA, and ELISA assays in combination led to recognition of cats with 'discordant' results (Lutz et al., 1980; Jarrett et al., 1982; Lutz et al., 1983b; Hardy and Zuckerman, 1991b), which in turn provided the first indication of more complex host–virus relationships among FeLV infec-

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tions (Hoover and Mullins, 1991). With the advent of molecular diagnostics, conventional PCR to detect FeLV DNA was employed in attempt to better understand these discrepant results (Jackson et al., 1996; Miyazawa and Jarrett, 1997; Herring et al., 2001), but the assay did not appear to have increased sensitivity or specificity.

We, and other investigators, developed a quantitative real-time FeLV DNA PCR assay (qPCR) as means to both diagnose FeLV infection and seek further insight into the determinants of the virus–host relationship (Hofmann-Lehmann et al., 2001; Flynn et al., 2002; Tandon et al., 2005; Torres et al., 2005). When we applied DNA qPCR, together with the antigen capture ELISA, in vaccinated and unvaccinated cats challenged with FeLV-A/61E, we identified a spectrum of host–virus relationships in FeLV-exposed cats which did not develop persistent antigenemia (Torres et al., 2005). Some animals experienced *abortive* infections—i.e. infections marked not only by undetectable antigenemia at all timepoints, but also the absence of infected cells in both circulation and tissues. Because these animals appeared not to maintain a tissue reservoir, it would be impossible to distinguish these animals from those never exposed to FeLV. By contrast, two additional groups of FeLV-exposed cats which did not develop persistent antigenemia were found to maintain either low or moderate levels of infected cells in circulation and tissues—and were designated as having experienced *regressive* or *latent* infections, respectively, depending on whether transient antigenemia was recognized. Finally, cats which developed overt persistent antigenemia with persistent high circulating and tissue viral DNA burdens represented those with *progressive* infection.

The finding of previously covert viral DNA in some cats which ostensibly totally resisted FeLV infection was not restricted to FeLV-A/61E, as other investigators found a similar phenomenon in cats challenged with the FeLV-A/Glasgow-1 (Hofmann-Lehmann et al., 2001; Flynn et al., 2002; Tandon et al., 2005; Cattori et al., 2006; Gomes-Keller et al., 2006a; Gomes-Keller et al., 2006b; Hofmann-Lehmann et al., 2006). It remained unknown, however, whether the viral DNA we detected by our qPCR assay represented intact, replication-competent provirus or replication-defective viral DNA sequences. To determine whether this viral DNA is transcriptionally active in the absence of antigenemia we developed a qPCR assay to quantitate FeLV RNA in feline plasma. The validation and application of this assay is described here.

2. Materials and methods

2.1. Experimental animals and challenge virus

Forty specific-pathogen-free (SPF) cats were obtained from Harlan Sprague Dawley, Inc. (Mt. Horeb, WI). The cats were randomly housed up to 5 cats per enclosure at Charmany Instructional Facility at the University of Wisconsin-Madison School of Veterinary Medicine (Madison, WI). The animals were housed in accordance with the university animal care and use committee regulations. At 34–35 weeks of age, all cats were challenged intraperitoneally with 200 μL of 5×10^4 $TCID_{50}$/mL FeLV-A/61E. Cats were observed twice daily for signs of illness after virus inoculation. Sample collections were performed on cats anesthetized with an intramuscular administration of ketamine hydrochloride (11 mg/kg).

2.2. Sample collection and processing

Blood samples were collected at challenge and every week thereafter through 8 weeks post-challenge (PC). Whole blood was shipped overnight on ice to Colorado State University (Ft. Collins, CO) where they were immediately processed upon arrival. Buffy coat cell pellets were stored at −70 °C until analysis for FeLV DNA by qPCR. Plasma samples were separated into 1 mL aliquots and stored at −70 °C until analysis for FeLV RNA by qPCR and FeLV p27 capsid antigen by capture ELISA.

DNA was extracted from buffy coat cells using a QIAamp DNA blood mini kit (QIAGEN, Inc., Valencia, CA), eluted in 100 μL of elution buffer, and DNA concentrations determined spectrophotometrically. RNA was extracted from 140 μL of plasma using a QIAamp viral RNA mini kit (QIAGEN). On-column digestion of DNA during RNA purification was performed using the RNase-free DNase set (QIAGEN). The RNA was eluted in 80 μL of elution buffer.

2.3. Primers and probe for RNA and DNA qPCR assays

We designed a primer/probe set to amplify exogenous and not endogenous FeLV sequences within the U3 region of FeLV-A/61E (Casey et al., 1981; Berry et al., 1988) as previously described (Torres et al., 2005). These primers and probe were used to detect both FeLV RNA and FeLV DNA.