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Shedding of feline leukemia virus RNA in saliva is a consistent feature in viremic cats

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Abstract

The purpose of this investigation was to characterize the shedding pattern of feline leukemia virus (FeLV) RNA in saliva, and to correlate it with the proviral load in whole blood, viral load in plasma, levels of p27 in saliva and plasma, the isolation of infectious FeLV from saliva, and the titers of FeLV-specific antibodies of the IgG and IgA isotypes. We evaluated 24 experimentally FeLV-infected cats for these parameters using real-time RT-PCR and PCR, cell culture assay and sandwich ELISA. We observed that shedding of viral RNA in saliva was a consistent feature in viremic cats. Latently FeLV-infected cats, displaying a very low proviral load, did not shed infectious virus in saliva, but occasionally shed viral RNA. Consequently, salivary shedding of FeLV RNA may not necessarily indicate a transmission potential for susceptible cats. This study also confirmed previous results from our laboratory, showing that a negative result for p27 in plasma, or for viral RNA in plasma or saliva does not exclude FeLV infection, considering that blood cells from those cats contained provirus. We also showed that FeLV RNA and DNA were stable for more than 64 days in saliva samples stored at room temperature. We conclude that the detection of FeLV RNA in saliva may be a useful indicator of viremia, and that the detection of salivary viral RNA by RT-PCR could become a reliable tool for the diagnosis of FeLV infection, which is facilitated by the low invasive method of collection of the samples.

Keywords: FeLV; Saliva; RNA; Viral shedding

1. Introduction

Feline leukemia virus (FeLV) is a horizontally transmitted retrovirus that infects cats worldwide, and causes disease and eventually death in persistently infected animals. FeLV infection is a disease of high veterinary importance and it serves as an animal model for certain aspects of human cancerous diseases, anemia, and immunodeficiencies (Rohn et al., 1996).

Once FeLV infection has taken place, four outcomes have been observed (Hoover and Mullins, 1991). A portion of cats become persistently viremic over many months (outcome 1), shedding virus and

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posing a risk to susceptible cats. In a large proportion of the cats, an effective immune response is elicited immediately after infection, and these cats do not become detectably positive in the p27 assay (outcome 2). A number of cats, however, first develop a transient viremia, which is overcome after a few weeks, and can shed virus transitorily (outcome 3). A small number of cats harbor a localized infection, which is characterized by virus replication in certain tissues, such as mammary, salivary and urinary epithelium, resulting in low levels of p27 in plasma (outcome 4). Cats of outcomes 2 and 3 may remain latently infected. Infection can eventually be reactivated under stressful conditions or by administration of corticosteroids (Rojko et al., 1982) due to the presence of provirus in a latent form.

It is assumed that mutual grooming and sharing of eating and drinking bowls represent the major means of FeLV transmission, due to the fact that saliva is the primary source of virus (Francis et al., 1977), leading up to 10^6 infectious FeLV particles per milliliter of saliva. However, once released in the environment, the infectiousness of salivary FeLV decreases relatively rapidly on dry surfaces (Francis et al., 1979). In addition, saliva contains a variety of non-specific anti-FeLV factors, such as mucins and cystatins, which have potent antiviral activity in a variety of viral infections, and could theoretically play a role in inactivating free FeLV in saliva (Amerongen and Veerman, 2002).

Saliva has been used as a substrate for the detection of various systemic diseases. The collection of saliva specimens for diagnostic purposes in the cat is well accepted by the owner based on its low invasiveness. In addition, no trained personnel are required for collection of samples, and saliva can be obtained easily from very young kittens and difficult patients with a minimum stress. Saliva collection is also suitable for protocols requiring repetitive sampling, and for screening and monitoring of specific populations. As saliva is not a reliable substrate for p27 detection by enzyme-linked immunosorbent assay (ELISA), it is not recommended for testing of individual sick cats (Lutz and Jarrett, 1987). With the advent of the polymerase chain reaction (PCR) technique, the detection of FeLV-specific sequences in different specimens became much more reliable, allowing it to be used as a tool to study the viral pathogenesis and to diagnose the infection.

The present study was undertaken to characterize the pathogenesis of FeLV infection by studying the shedding of FeLV RNA in saliva specimens collected from experimentally infected cats, and to correlate the results with the presence of infectious FeLV in saliva, proviral load in blood, viral load in plasma, and the presence of p27 in saliva and in plasma. In addition, we investigated and compared the presence of antibodies to FeLV in saliva and serum by ELISA.

2. Materials and methods

2.1. Animals

Twenty-four specific-pathogen-free (SPF) cats (16 males and 8 females) were acquired from Charles River Laboratories (L'Arbresle, France), and kept under barrier conditions in groups of eight animals in large rooms furnished with running boards, climbing trees, ladders, hammocks and elevated sleeping places, under optimal ethological conditions. Cats were infected intraperitoneally with FeLV-A/Glasgow strain at 17–19 weeks of age. Forty SPF cats acquired from Liberty Research, Inc. (Waverly, NY) were kept under the same conditions and used as negative controls.

2.2. Blood collection and processing

Blood specimens were obtained by jugular venipuncture using evacuated tubes (Becton Dickinson, Plymouth, UK) containing K_3EDTA one year after experimental infection. Two hundred microliters of whole blood were aliquoted for DNA extraction. Plasma was obtained by centrifuging blood at $2300 \times g$, for 10 min.

2.3. Saliva collection and processing

Saliva specimens were collected without stimulation with the aid of commercially available cotton wool swabs (Primella, Migros Genossenschafts-Bund, Switzerland), similar to Q-tips. Swabs were inserted into and rubbed gently along the cheek pouches and under the tongue of the cats. Swabs were immediately