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Markers of feline leukaemia virus infection or exposure in cats from a region of low seroprevalence

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Molecular techniques have demonstrated that cats may harbour feline leukaemia virus (FeLV) provirus in the absence of antigenaemia. Using quantitative real-time polymerase chain reaction (qPCR), p27 enzyme-linked immunosorbent assay (ELISA), anti-feline oncornavirus-associated cell-membrane-antigen (FOCMA) antibody testing and virus isolation (VI) we investigated three groups of cats. Among cats with cytopenias or lymphoma, 2/75 were transiently positive for provirus and anti-FOCMA antibodies were the only evidence of exposure in another. In 169 young, healthy cats, all tests were negative. In contrast, 3/4 cats from a closed household where FeLV was confirmed by isolation, had evidence of infection. Our results support a role for factors other than FeLV in the pathogenesis of cytopenias and lymphoma. There was no evidence of exposure in young cats. In regions of low prevalence, where the positive predictive value of antigen testing is low, qPCR may assist with diagnosis.

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Following the isolation of feline leukaemia virus (FeLV) from pet cats over 45 years ago, this gammaretrovirus became recognised as one of the most important pathogens of domestic cats and other Felidae worldwide.[1–3] Using diagnostic tests established to detect viral antigen (by enzyme-linked immunosorbent assay [ELISA] or immunofluorescence assay [IFA]) it became clear that up to 30% of exposed cats become persistently antigenaemic following exposure and have a significantly reduced life-expectancy with 83% mortality within 3.5 years.[4,5] The clinical consequences of FeLV are predominantly non-specific resulting from bone marrow disorders and immunosuppression, while around 25% of antigenaemic cats develop lymphomas or leukaemias.[6,7] Persistently antigenaemic cats were identified as the source of infection for susceptible cats and detection of antigen or virus formed the basis of test-and-removal programmes which, together with vaccination, have been successful in reducing the worldwide prevalence of FeLV.[8,9]

During the last decade the availability of molecular techniques to detect provirus (DNA) and free plasma virus (RNA) has enhanced our understanding of the pathogenesis of FeLV infection. It has become apparent that antigen detection is a relatively insensitive indicator of infection and that cats previously considered to have recovered using conventional techniques can harbour low levels of virus. Following experimental exposure most cats show evidence of persistent, transcriptionally active virus.[10–15] In the field, up to 10% of antigen-negative cats test positive for provirus in peripheral blood by polymerase chain reaction (PCR).[10,16–18] The absence of viral RNA in the saliva of 96% of provirus-positive/antigen-negative cats, the success of test-and-removal programs that relied on antigen detection to identify 'infected' cats and the results of attempted experimental

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transmission from latently infected cats,[4,17,19] suggest that regressive infection, characterised by absent antigenaemia and low, transient proviral load,[15] does not play a major role in natural transmission. The potential of FeLV to contribute to disease in antigen-negative cats requires clarification. While some epidemiological data demonstrate an increased risk of lymphoma and reduced survival in FeLV-exposed, antigen-negative cats compared with cats never exposed to the virus,[5,20,21] the results of immunohistochemical and molecular studies to date have yielded conflicting results.[22–28]

Cats in Eastern Australia are commonly diagnosed with problems that are potentially related to FeLV, such as anaemia and lymphoma, but FeLV antigenaemia is an uncommon finding.[29] ELISA-based, in-house antigen detection kits, despite their high specificity, have a low positive predictive value in this population where the prevalence of antigenaemia is similar to the incidence of false-positive results.[30] The aim of this study was to use a battery of serological and molecular techniques to look for evidence of infection with FeLV in cats from an area of low antigen prevalence. Cats at risk of infection by virtue of their clinical signs, cats with known exposure to persistently antigenaemic cats and a population of young, healthy cats undergoing routine procedures were tested. The derived information would more accurately describe the potential threat to Australian cats from FeLV, and inform testing and vaccination recommendations for cats in low-risk areas. This is of global importance as the prevalence of FeLV falls worldwide.

Materials and methods

Peripheral blood samples, obtained prospectively from three groups of cats, were tested for FeLV provirus, p27 antigen, and anti-feline oncornavirus-associated cell-membrane-antigen (FOCMA) antibodies. Informed consent was obtained and the study was approved by the University of Sydney's Animal Ethics Committee, N00/1-2009/1/4939.

Group A (sick cats)

This group comprised 75 cats presented to the Valentine Charlton Cat Centre (VCCC), University of Sydney over a 7-month period (July 2007–January 2008). Cases were included if anaemia or another cytopenia was identified or if intermediate or high-grade lymphoma was diagnosed and there was residual sample after running a complete blood count. Data on signalment, environment (indoor only or outdoor access, single cat or multicat household) and feline immunodeficiency virus (FIV) vaccination status were recorded.

Group B (in-contact cats)

These four cats were free-ranging from a multicat household and had been in-contact with a persistently antigenaemic cat during the previous 12 months. FeLV was isolated in culture from the antigenaemic cat, confirming its infection status and providing an isolate of Australian origin, FeLV Syd-1, for further study (unpublished data). The cats in group B had been in the household for 5 years or longer.

Group C (young, healthy cats)

This group consisted of 169 healthy cats up to 1 year of age that were presented for routine veterinary procedures to one of three inner city veterinary clinics over a 12-month period (January–December 2009). Data on age, breed, sex, source and FIV vaccination status were recorded.

Samples

Blood was collected into ethylenediamine tetra-acetic acid (EDTA) and was stored at 4°C for up to 7 days, centrifuged at 12,000 *g* for 2 min and the plasma was decanted. The cell pellet and plasma were stored at −20°C for up to 3 months or at −80°C for up to 3 years.

FeLV quantitative real-time polymerase chain reaction (qPCR)

Cell pellets were thawed and mixed with an equal volume of sterile phosphate buffered saline (PBS). Polypropylene tubes (Matrix Storage Tubes, Thermo Fisher Scientific, New Hampshire, USA) were loaded with 200–400 µl of each sample and placed at −80°C. The plates were shipped on dry ice to the University of Bristol, UK. DNA extraction and qPCR were carried out as described previously using primers targeting the U3 region of the exogenous retroviral long terminal repeat (LTR).[16] Primers amplifying feline 28S rDNA were included to verify adequate DNA extraction. FeLV positive and negative controls were included as described previously.[16]

Serology

FeLV p27 antigen and antibodies to FIV p15 and p24 were detected using a commercial in-clinic ELISA (Snap Combo, Idexx Laboratories, NSW, Australia) according to the manufacturer's instructions. Whole blood in EDTA was tested immediately or after storage at 4°C for up to 7 days. EDTA plasma samples were shipped on dry ice to the University of Glasgow for detection of antibodies to FOCMA, as described previously.[21]

Virus isolation (VI)

Plasma from cats in group A that tested provirus positive ($n = 2$) and from all cats in group B ($n = 4$) was submitted for VI (Jarrett O et al, unpublished) using QN10 cells.

Statistical analysis

Chi squared tests were used to compare group A with the entire VCCC hospital population to test for its