Epidemiology of Feline Foamy Virus and Feline Immunodeficiency Virus Infections in Domestic and Feral Cats: a Seroepidemiological Study

I. G. Winkler, M. Löchelt and R. L. P. Flower

Epidemiology of Feline Foamy Virus and Feline Immunodeficiency Virus Infections in Domestic and Feral Cats: a Seroepidemiological Study

I. G. WINKLER,1* M. LÖCHELT,2 AND R. L. P. FLOWER1

School of Pharmacy and Medical Science, University of South Australia, City East, North Terrace, Adelaide, South Australia, 5000, Australia,1 and Abteilung Tumorvirologie, Deutsches Krebsforschungszentrum, Heidelberg, Germany2

Received 23 November 1998/Returned for modification 12 April 1999/Accepted 9 June 1999

Although foamy viruses (Spumaviruses) have repeatedly been isolated from both healthy and diseased cats, cattle, and primates, the primary mode of transmission of these common viruses remains undefined. A database of the feline foamy virus (FeFV) and feline immunodeficiency virus (FIV) antibody status, age, and sex of 389 domestic cats presented to veterinarians was assembled. A similar database for 66 feral (wild) cats was also assembled. That FeFV antibody status reflects infection was validated by PCR. Both FeFV and FIV infection rates were found to gradually increase with age, and over 70% of cats older than 9 years were seropositive for FeFV. In domestic cats, the prevalence of FeFV infection was similar in both sexes. In feral cats, FeFV infection was more prevalent in female cats than in male cats. Although both FeFV and FIV have been reported to be transmitted by biting, the patterns of infection observed are more consistent with an interpretation that transmission of these two retroviruses is not the same. The prevalence of FIV infection is highest in nondesexed male cats, the animals most likely to display aggressive behavior. The gradual increase in the proportion of FeFV-infected animals is consistent with transmission of foamy viruses by intimate social contact between animals and less commonly by aggressive behavior.

Cats can be infected with retroviruses from three separate genera. The pathogenesis and epidemiology of infection with feline immunodeficiency virus (FIV) and feline leukemia virus have been well characterized (for reviews, see references 2 and 5). Little is known about naturally occurring foamy virus infection.

FIV is a typical lentivirus that resembles the human immunodeficiency virus in its morphologic features and protein structure (for a review, see reference 2). Infection is persistent, and diagnosis is by the detection of specific anti-FIV antibody. Among domestic cats worldwide, seroprevalences of FIV infection are between 1 and 30% (1, 6, 10, 18). Infection is commonly acquired after 1 year of age, and the prevalence of FIV infection peaks in cats of 10 years of age before it decreases (for a review, see reference 2). Circumstantial evidence suggests that biting is the main means of transmission of FIV (1, 13, 15, 22). Male cats tend to exhibit aggressive behavior such as biting more than female cats, and the highest prevalence of FIV infection is found in mature male cats with unrestricted outdoor activity (1, 13, 15, 23).

Feline foamy virus (FeFV) is a typical spumavirus that resembles the primate foamy viruses and bovine foamy virus in its morphology and molecular structure (4, 19). Infection with FeFV is persistent, and previous studies have indicated that seroprevalences of FeFV infection in domestic cats range between 7 and 100% (1, 3, 9, 11, 12, 16, 20, 23). Several natural modes of transmission have been suggested for FeFV infection. These include vertical transmission from queen to kitten (4) and salivary transfer, either by the respiratory route (3) or through aggressive behavior such as biting (13, 23).

In this report, a detailed study on the epidemiology of FeFV and FIV infections in cats is presented. An enzyme-linked immunosorbent assay (ELISA) was used to investigate the FeFV and FIV antibody status of 389 domestic Australian cats presented to a veterinarian, as well as 66 Australian feral cats. The results were assembled in a database and were subjected to statistical analysis. The prevalences of both FeFV and FIV infections were found to gradually increase with the age of the cat, with over 70% of cats older than 9 years seropositive for FeFV. Statistical analysis suggested that the natural modes of transmission of FeFV and FIV are different, with FeFV transmitted by prolonged intimate contact between cats and FIV transmitted by aggressive behavior.

MATERIALS AND METHODS

Serum samples. Serum from 389 domestic cats submitted for pathological analysis in 1996 and 1997 was generously provided by Veterinary Pathology Services or Vetlab, of Adelaide, South Australia, Australia. Serum from 66 feral cats shot in the Flinders Ranges National Park, South Australia, Australia, as part of an eradication campaign were kindly provided by the Department of Environmental and Natural Resources, South Australia, Australia. The age of the feral cats was estimated by oral examination.

ELISA for detection of antibodies to FeFV. An ELISA for detection of antibodies to FeLV was performed as described previously (20). Briefly, a recombinant biotinylated protein from the FeFV nucleocapsid domain was produced in Escherichia coli and was linked to streptavidin-coated 96-well ELISA plates. Cat sera were tested at 1/100 dilutions, followed by incubation with protein A and G-horseradish peroxidase conjugate and a chromogenic substrate. A positive reaction was defined as one in which the A450 in the test well was greater than three times the absorbance for the same serum in a negative control well. Validation of the assay and correlation of results with viral isolation has been reported elsewhere (20).

Confirmation of ELISA results by PCR analysis. PCR was performed with the DNA extracted from 105 cat peripheral blood mononuclear cells by using sense primer 2610S (5′-AACAGCAACACTCTGATGTTCCCG-3′) and antisense primer 3085A (5′-TGCTGCTAACCAGGTCTTTCCTCCC-3′) as described previously (21).

ELISA for detection of antibodies to FIV. Crandell feline kidney cells were infected with FIVpetaluma or were mock infected in 96-well ELISA plates, and the plates were incubated until the cells were confluent and numerous multinucle-
ated syncytia were observed (approximately 5 days). The wells were washed and blocked in phosphate-buffered saline with 0.5% dried skim milk for 30 min and were then fixed with ethanol-acetone (95:5) for 20 min at -20°C. The plates were then rinsed three times in phosphate-buffered saline-Tween, and 100 µL of each standard and direct ELISA was performed as described above. The sera were diluted 1/200 and were incubated 2 h. For 50 different serum samples, the sensitivity and specificity of this ELISA were compared with those of a commercial kit (PetChek; IDEXX, Portland, Maine).

**Construction of database and statistical analysis.** A database detailing the age, sex, and FeFV and FIV antibody status was compiled in Microsoft Excel, version 5.0. Statistical analysis of the data was performed by a two-way Student’s t test for comparison of age, a two way χ² test for detection of variation between the FeFV and FIV antibody status, age, and sex, or two-tailed Fisher analysis when the expected number of cats in each category was less than 5. In statistical tests, P values of less than 0.05 were considered to represent a significant association.

**RESULTS**

**Validation of ELISAs.** For 30 randomly selected cats, detection of antibodies to FeFV by ELISA was compared to detection of proviral nucleic acid in 10⁵ peripheral blood mononuclear cells by PCR. All cats that were PCR positive for FeFV DNA (14 of 30) were positive for FeFV antibody. One PCR-negative cat was also FeFV antibody positive by ELISA. This negative PCR result most likely reflects the low proviral load in the peripheral blood mononuclear cells of FeFV-infected cats (approximately 1 copy per 10³ to 10⁴ peripheral blood mononuclear cells [18a]). All cats negative for FeFV antibody by ELISA were also negative for FeFV proviral nucleic acid sequences by PCR.

The FIV antibody statuses of 50 different cat serum samples were compared to the results from a commercial assay (PetChek; IDEXX). With PetChek, eight cats were found to be seronegative for FIV. The same eight cats were positive by ELISA; however, one ELISA-positive but PetChek-negative serum sample was identified. This cat may not have had antibodies to the recombinant FIV p24 target antigen in the PetChek kit.

**Prevalence of FeFV infection in domestic cats.** By the FeFV ELISA described above, 57% of domestic cats and 36% of feral cats were seropositive for FeFV. A strong association between prevalence of FeFV infection and age of the cat was observed (Table 1). Less than 5% (1 of 20) of domestic cats under the age of 1 year had detectable antibody to FeFV. The prevalence of antibodies to FeFV increased steadily with the age of the cat until 73% (143 of 195) of cats 9 years of age and over were positive for FeFV (P = 0.003).

Over half (58%; 227 of 389) of the domestic cats were recorded as having been desexed. The prevalence of FeFV infection in young desexed domestic cats (<5 years of age) was 44% (16 of 36), whereas it was 15% (7 of 48) for cats of the same age which were not recorded as being desexed (P = 0.003) (Table 1). FeFV did not appear to be associated with desexing in older cats.

In domestic cats, no significant association was found between FeFV infection and sex.

**Prevalence of FIV infection in domestic cats.** The seroprevalence of FIV in both domestic cats (10%) and feral cats (9%) was similar. The prevalence of infection with FIV was also found to increase with the age of the cat. Only 2% (2 of 84) of domestic cats under 5 years were seropositive for FIV. This rose to 15% (16 of 110) of domestic cats between 5 and 9 years of age, while in old cats, a lower prevalence (8%; 8 of 96) was observed (Table 1).

FIV infection was more common in male domestic cats (16%) than female domestic cats (4%) (P = 0.0002). The prevalence of FIV infection was not found to be altered by desexing (Table 1).

**Feral cats.** Feral cats represent a distinct feline population with social behavior different from that of domestic cats, so data for feral cats were analyzed separately. The 66 feral cats showed no signs of disease, were not desexed, and were all under 5 years of age. In contrast to the equal distribution of FeFV infection found in female and male domestic cats, the prevalence of FeFV infection was over twofold higher in fe-
male feral cats (52%; 16 of 31) than in male feral cats (23%; 8 of 35) \((P = 0.006)\) (Table 1).

The prevalence of FIV infection in the feral cat population was higher in male cats (14%) than female cats (3%). This was similar to the sex distribution of FIV infection found in domestic cats.

**Association between FeFV and FIV infection.** Analysis of data for domestic cats revealed no statistical association between FIV infection and FeFV infection (Table 1).

In the feral cat population examined (all under 5 years of age), FeFV infection was, however, found to be significantly associated with FIV infection (Table 1). Male feral cats with FIV infection (5 of 5 cats) were more likely than male feral cats with no FIV infection (3 of 30 cats) to becoinfected with FeFV \((P = 0.01)\). Differences in social behavior in the feral cat population compared to the social behavior of the predominantly desexed domestic cat population may be the basis of this association.

**DISCUSSION**

Domestic cats were introduced into Australia by European settlers in the 18th and 19th centuries, and feral (wild) cat populations established soon after initial settlement (8). Infection with FeFV and FIV is persistent, and the detection of specific antibodies by ELISA can be considered diagnostic of infection (14, 16). In this study, the seroprevalence of FeFV infection was found to increase steadily with the age of the cat, suggesting cumulative spread from infected to noninfected cats. Although vertical transmission of FeFV from queen to kitten has been reported (4), this mechanism did not appear to be the predominant mode of transmission in the two populations of cats studied. The strong association of FeFV infection with the age of the cat found in this study is consistent with the results of a previous study with 286 healthy male domestic cats by Pedersen et al. (12) and may explain the diversity of earlier reports on the prevalence of FeFV infection in cat populations of unspecified age (range 7 to 100%).

Infectious FIV and FeFV are present in the saliva of many infected cats, and transmission of both FIV and FeFV through experimental bites has been reported (13, 22). In previous reports on the prevalence of FIV infection in domestic cats (1, 15, 23), a greater proportion of male cats than female cats was infected with FIV. The results of this study are consistent with those reports and consistent with the hypothesis that the major mode of transmission of FIV is by aggressive behavior such as biting between male cats (1, 13, 15, 23). In contrast to the results for FIV, the prevalence of naturally occurring FeFV infection was found to be similar in both male and male domestic cats, suggesting that the predominant mode of transmission of FeFV is not by biting, as for FIV, but that transmission occurs slowly with intimate, social contact between individuals. This hypothesis also applies to the population of feral cats, in which a significantly greater proportion of female feral cats (52%) than male feral cats (23%) was infected with FeFV. The major mode of FeFV transmission proposed in this study is similar to that proposed previously for bovine foamy virus, for which infection is believed to be spread through saliva, mainly by social licking between infected and noninfected individuals (7).

Social interactions between individual animals in domestic cat populations are different from the interactions observed in feral cat populations and are greatly influenced by the desexing of domestic cats. Over half of the domestic cats in this survey were recorded by their veterinarian as having been desexed. The prevalence of FeFV infection was significantly higher in young domestic cats which were recorded as having been desexed (44%) than in domestic cats of the same age group which were not recorded as having been desexed (15%). This observation suggests that either the visit to the veterinary surgery for desexing (and association with other cats) or behavioral modifications following desexing are associated with an increased risk of FeFV infection in cats.

It is interesting that although saliva appears to be a medium for transmission of both FeFV and FIV, the major mode of transmission in cats appears to be different for these two retroviral groups. One possible explanation for this difference is that FIV requires contact with peripheral blood mononuclear cells for initial infection, while FeFV can directly infect and replicate in the cells of the oropharyngeal mucosa. This hypothesis is supported by two previous studies that have reported that transmission of simian foamy virus type 1 into rabbits and bovine foamy virus into cows is more effective when virus is administered by the oropharyngeal route than by systemic inoculation (7, 17).

This is the first report of the prevalence of foamy virus infection in a large number of naturally infected animals. The prevalence of FeFV infection was found to steadily increase with age, a pattern commonly found with endemic infections with pathogens of low virulence. Although speculative, the data suggest that for FeFV, social contact and not aggressive behavior may be the most important factor in the spread of the infection.

**ACKNOWLEDGMENTS**

We thank Jeanine Baker and Chris Holden for devotion in collecting samples from feral cats. We are also grateful to Mary Barton, Margaret Allanson, and Sue Fitzsimmons for the provision of blood samples from domestic cats.

**REFERENCES**


Winkler, I. Unpublished data.


