J Vet Diagn Invest 2:180-183 (1990)

A serologic survey of Oklahoma cats for antibodies to feline immunodeficiency virus, coronavirus, and *Toxoplasma gondii* and for antigen to feline leukemia virus

Sandra J. Rodgers, Charles A. Baldwin

Abstract. A serologic survey was done on 618 cat sera submitted to the Oklahoma Animal Disease Diagnostic Laboratory between July 1, 1987 and June 30, 1988. The samples were collected from clinically normal and sick cats. The sera were tested for the presence of antibodies to feline immunodeficiency virus by a commercial immunoassay, to a coronavirus by an indirect fluorescent antibody test, and to ***Toxoplasma gondii*** by a commercial latex agglutination test and for the presence of feline leukemia virus antigen with one of 3 different commercial assay kits. Ten percent of the sera had antibodies to feline immunodeficiency virus, 35% had antibodies to a coronavirus, and 22% had antibodies to ***Toxoplasma*** gondii. Feline leukemia virus antigen was detected in 15% of the sera. Thirty-two percent of the sera had evidence of exposure to 2 or more of the agents.

Feline immunodeficiency virus (FIV), feline infectious peritonitis virus (FIPV), ***Toxoplasma gondii,*** and feline leukemia virus (FeLV) are infectious agents that can cause severe health problems or death in cats.

Feline immunodeficiency virus belongs to the family Retroviridae, subfamily Lentivirinae.[9,11,12] The FIV virus causes an immunosuppressive syndrome in cats that may make the cat susceptible to secondary pathogens [2,3,8,12,13] Feline immunodeficiency virus was first identified (and was originally named feline T-lymphotrophic lentivirus) in early 1987 in California during a disease outbreak in a large colony of pet cats.[11] A limited serologic survey in California has shown that FIV is widespread in the California cat population.[7,11] Another study, in Florida, reported a prevalence rate of 8.4%.[6] In addition, other investigators have demonstrated that FIV is widespread in the United States, Canada, Europe, and Japan.[9,14,15] Cats with FIV do not recover from the infection regardless of the severity of the initial stage of the disease? Diagnosis of FIV infection is made by serologic determination of antibodies.[9,11,13-15] A positive correlation between the presence of virus and antibody has been established, although virus has been isolated in seronegative cats.[11,15]

The family Coronaviridae includes feline infectious peritonitis virus, feline enteric coronavirus (FECV), transmissible gastroenteritis virus (TGEV) of swine, and canine coronavirus (CCV). [10] Feline infectious peritonitis virus was first confirmed in the United States in 1963. [10] Once clinical signs develop, it is generally a fatal disease of both domestic and exotic cats. Feline infectious peritonitis virus infection occurs primarily in cats that are between 6 months and 5 years of age, with the highest incidence occurring between 6 months and 2 years. [10] The feline coronaviruses, FIPV and FECV, are morphologically and antigenically similar to TGEV and CCV.

Toxoplasma gondii, a coccidial protozoan parasite, infects many species of mammals and birds. In the United States, the prevalence rates for antibody to ***T. gondii*** have been reported as 38% in domiciled cats and 58% in stray cats.[5] Toxoplasmosis, first recognized in 1942, may occur as an acute, subacute, or chronic disease? Toxoplasmosis is a sporadic disease in cats of all ages, but primary infection usually occurs in young cats.[5] In older cats, clinical disease is usually the result of a reactivation of a latent infection,[5] and twice as many males as females have been hospitalized with ***T. gondii*** infection.[5]

Feline leukemia virus belongs to the family Retroviridae, subfamily Oncovirinae, and was first discovered in Scotland in 1964.[1] Feline leukemia virus infection causes an immunosuppressive syndrome that allows an array of secondary infections to occur. [1,2,4,13] It has been reported that 50% of cats with FIPV, chronic gingivitis, oral ulcers, or chronic stomatitis are concurrently infected with FeLV.[8]

The purpose of this study was to determine the seroprevalence of selected diseases of clinically healthy and sick cats in Oklahoma.

From the Oklahoma Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078.

Presented as a poster at the 31st Annual Meeting of the AAVLD, Little Rock, AK, October 15-18, 1988.

Received for publication October 17, 1988.

Table 1. Number of feline sera tested (6 18) positive for 1 or more antibodies to feline immunodeficiency virus (FIV), feline coronavirus (FIPV), and *Toxoplasma gondii* (Toxo) and for feline leukemia virus (FeLV) antigen.

	No. tested	Total positive (%)*	FeLV	FIV	FIPV	Toxo	FeLV, FIV	FeLV, FIPV	FeLV, Toxo	FIV, Toxo	FIPV, Toxo	FIV, FIPV
FIV antibody	618	60 (10	7	15	9	16	17	2	5	16	6	9
FIPV antibody	618	216 (35)	22	9	138	35	2	22	4	6	35	9
Toxoplasma gondii antibody	618	135 (22)	9	16	35	60	5	4	9	16	35	6
FeLV antigen	616	89 (15)	40	7	22	9	7	22	9	5	4	2
All 4 diseases	···	2	2	2	2	2	2	2	2	2	2	2
Negative sera	248	···	—†	—	—	—	—	—	—	—	—	—

* The total number of positive sera does not include those positive for all 4 diseases.
† — = none.

Materials and methods

Clinical specimens. Six hundred eighteen sera from cats were submitted for serologic testing to the Oklahoma Animal Disease Diagnostic Laboratory from veterinarians in 41 of the 77 counties in Oklahoma from July 1987 through June 1988. The samples were collected from clinically healthy and sick cats and from those cats whose health status was unknown.

Serologic determination of antibody to FIV Sera (diluted 1: 100) were tested for the presence of antibodies to FIV by a commercial enzyme immunoassay." Color reactions were analyzed on a reader[b] at a wavelength of 650 nm. Specimens with a sample : positive control ratio ≥ 0.5 were classified as positive.

Serologic determination of antibody to a coronavirus (FIPV-DF_2). Reagent test slides were prepared by inoculating FIPV-DF_2[c] virus on to 8-chambered slides[d] that had previously been seeded with actively growing cultures of Crandell feline kidney cells. Slides were fixed in acetone at 0 C for 10 min 24 hr after viral inoculation. The slides were then dried and stored at -70 C until used. An indirect fluorescent antibody test was used to detect the presence of coronavirus antibody in the cat sera.[1,10] The sera were diluted 1:25 in phosphate buffered saline (PBS), pH 7.4, and 50 µ1 of each diluted serum was added to a well on a reagent test slide. After incubation in a humid chamber at 37 C for 30 min, the slides were washed 3 times for 5 min in PBS. After shaking off any excess buffer, 50 µ1 of optimally diluted fluorescein-labeled rabbit anti-cat IgG (heavy and light chain)[e] containing a rhodamine-labeled bovine albumin[f] counterstain was added to the test well. The slides were incubated again in a humid chamber at 37 C for 30 min and were washed and dried in PBS as above. Coverslips were mounted on the slides with glycerol medium (pH 9.0). The slides were examined for specific immunofluorescence using a microscope equipped with an ultraviolet light source.[g] Samples with a bright-green granular fluorescence in the cytoplasm were considered positive for coronavirus-specific antibody (FIPV-DF_2).

Serologic determination of antibody to Toxoplasma gondii. Sera were tested for the presence of antibodies to *T. gondii* using a commercial latex agglutination test.[h] Sera were screened at the 1:16 dilution and those samples that had a 1 + or greater agglutination pattern were considered positive.

Serologic determination of antigen to FeLV. Sera were tested for the presence of FeLV p27 antigen using one of 3 commercial assay kits.[a,i,j] All commercial assays were performed according to the manufacturers' directions. Sera that produced a color reaction in the test were considered positive.

Results

Six hundred eighteen sera were tested for the presence of antibodies to FIV, coronavirus (FIPV-DF_2), and 7 *gondii.* Only 616 sera were tested for the presence of FeLV antigen. Ten percent of all the serum samples had antibodies to FIV, 35% had antibodies to a coronavirus (FIPV-DF_2), and 22% had antibodies to 7 *gondii.* Fifteen percent of the sera were positive for FeLV antigen. In addition, all of the possible combinations of antibody and antigen were seen (Table I).

One hundred sixty-six sera were submitted from cats that were clinically ill at the time of submission, 194 sera from clinically healthy cats, and 258 sera from cats with an unknown health status. The sick cats had a higher percentage of sera positive for FIV antibodies and for FeLV antigen. The percent positive sera seemed to be equivalent for coronavirus antibody (FIVP-DF_2) and for 7 *gondii* antibody over all 3 health categories (Table 2).

Three hundred sixty-eight sera were tested from cats ≤ 5 years of age, 105 were from cats ≥6 years of age, and 145 were from cats of unknown age. Of those cats ≤5 years old FIV antibody was detected in 7% of the sera, coronavirus antibody (FIPV-DF_2) in 35% of the sera, 7 *gondii* antibodies in 19% of the sera, and FeLV

Table 2. Number and percent of feline sera positive for feline immunodeficiency virus (FIV), coronavirus (FIPV-DF_2), *Toxoplasma gondii,* and feline leukemia virus (FeLV) in relation to their health status.

Health status	No. tested	No. of positive sera (%)			
		FIV*	FIPV-DF_2*	*T. gondii**	FeLV†
Sick	166	21 (13)	55 (33)	40 (24)	24 (21)
Well	194	15 (8)	69 (36)	55 (28)	23 (12)
Unknown	258	26 (10)	94 (36)	42 (16)	34 (13)

* Antibodies detected for FIV at 1:100, FIPV-DF_2 at 1:25, and *T. gondii* at 1:16.
† Antigen detected in undiluted sera.

Table 3. Age distribution of positive feline sera in Oklahoma for feline immunodeficiency virus (FIV), coronavirus (FIPV-DF_2), *Toxoplasma gondii,* and feline leukemia virus (FeLV).

Age (yr)	No. tested	No. of positive sera (%) FIV*	FIPV-DF_2*	*T. gondii**	FeLV†
<1	119	2 (2)	40 (34)	15 (13)	17 (14)
1	87	6 (7)	36 (41)	18 (21)	17 (20)
2	62	3 (5)	25 (40)	15 (24)	16 (26)
3	44	6 (14)	12 (27)	11 (25)	4 (9)
4	29	2 (7)	12 (41)	5 (17)	6 (21)
5	27	5 (19)	5 (19)	7 (26)	4 (15)
≥6	105	24 (23)	29 (28)	36 (34)	10 (10)
Unknown	145	14 (10)	59 (41)	30 (21)	17 (12)

* Antibodies detected for FIV at 1:100, FIPV-DF_2 at 1:25, and *T. gondii* at 1:16.
† Antigen detected in undiluted sera.

Table 4. Distribution by sex of positive feline sera for feline immunodeficiency virus (FIV), coronavirus (FIPV-DF_2), *Toxoplasma gondii,* and feline leukemia virus (FeLV).

Sex	No. tested	No. of positive sera (%) FIV*	FIPV-DF_2*	*T. gondii**	FeLV†
Male	257	32 (13)	93 (36)	63 (25)	46 (18)
Female	256	18 (7)	85 (33)	52 (20)	30 (12)
Unknown	111	12 (11)	40 (36)	22 (20)	15 (14)

* Antibodies detected for FIV at 1:100, FIPV-DF_2 at 1:25, and *T. gondii* at 1:16.
† Antigen detected in undiluted sera.

antigen was detected in 17% of the sera. In cats ≥6 years old, 23% had FIV antibodies, 28% had coronavirus (FIP-DF_2) antibodies, 34% had *T. gondii* antibodies, and 10% had FeLV antigen. Cats with an unknown age had 10%, 41%, 21%, and 12% sera positive for FIV, coronavirus (FIPV-DF_2), *T. gondii,* and FeLV, respectively (Table 3).

Even though sera were submitted from the same number of males (257) and females (256), approximately twice as many male cats (13%) were positive for FIV as female cats (7%). Otherwise, exposure and seroconversion to the other 3 agents appeared to be similar. The same trend was seen for those cats where the sex was not known (Table 4).

Submissions were from 41 of 77 Oklahoma counties evenly distributed across the state. Cats in 59% of the counties had antibody for FIV and antigen for FeLV, 61% had antibody for a coronavirus (FIPV-DF_2), and 66% had antibody for *T. gondii.*

Discussion

The data obtained in this study demonstrate that FIV, FIPV, *T. gondii,* and FeLV occur in the Oklahoma cat population and that 32% of the cats have been exposed to multiple agents.

The number of sera from cats with a known health status was evenly distributed with 27% from sick cats and 31% from well cats. The health status of 42% of the cats was unknown. A larger number of the FIV- and FeLV-positive sera were from sick cats. Approximately the same percent of FIPV- and *T. gondii*-positive sera were from sick cats or healthy cats.

Four times the percentage of cats ≥4 years of age were seropositive for FIV. There was no apparent age-related incidence of positive sera for FIPV, *T. gondii,* or FeLV. The broad range of ages of cats positive for FIPV and *T. gondii* may be more of a reflection of previous exposure rather than current or active infection, particularly in light of the nonspecific nature of the coronavirus antibody test.

There was a remarkable difference between the number of FIV-positive males and females, which also has been reported by others.[2,13,15] Twice as many males (13%) were positive as compared with females (7%). This may be a reflection of an increased potential of exposure to FIV because males are often free-roamers. This may also account for the slightly higher percentage of FeLV-positive males compared with females.

Exposure to FIV, FIPV, *T. gondii,* and FeLV appeared to be widespread throughout the state of Oklahoma; approximately 60% of the counties tested had positive sera. Data were not available to compare results between urban and city cats and between indoor and outdoor cats. No breed predilection was observed for FIV, *T. gondii,* or FeLV. However, 87% of the sera tested was from mixed or unknown breeds.

Acknowledgements

We thank D. Dirato for computer analysis of the data and M. Simer and G. Schemenaur for assistance in preparation of the manuscript. We thank Iddex Corp. for providing the PetChek® Anti-FTLV assays. We also thank our director, Dr. D. Goodwin, for his continued support and encouragement.

Sources and manufacturers

a. PetChek Anti-FTLV Antibody Test Kit, CITE Feline Leukemia Virus Test Kit, AgriTech Systems, Inc., Portland, ME.
b. Dynatech MR-580 reader, Dynatech Laboratories, Inc., Alexandria, VA.
c. Feline infectious peritonitis virus patent strain DF, (ATCC VR-2004), Daryl Laboratory, Santa Clara, CA.
d. Lab-Tek slides, Nunc, Inc., Naperville, IL,
e. CooperBiomedical, Inc., Malvem, PA.
f. Becton, Dickinson and Co., Cockeysville, MD.
g. Leitz Orthoplan Microscope, Xenon arc lamp (400 HBO) and exciter (KP500) and barrier (KP515) filters, E. Leitz, Inc., Rockleigh, NJ.
h. Toxotest-MT "Eiken," Tanabe U.S.A., Inc., San Diego, CA.
i. FeLV-Flex II, TechAmerica Diagnostics, Fort Collins, CO.
j. Leukassay F, Pitman-Moore, Inc., Washington Crossing, NJ.

References

1. Barlough JE: 1984, Serodiagnostic aids and management practice for feline retrovirus and coronavirus infections. Vet Clin North Am [Small Anim Pract] 14:955-969.
2. Chalmers S, Schick RO, Jeffers J: 1989, Demodicosis in two cats seropositive for feline immunodeficiency virus. J Am Vet Med Assoc 194:256-257.
3. Connaughton D: 1989, What you need to know about feline immunodeficiency virus. J Am Vet Med Assoc 194:169-173.
4. Cotter SM, Hardy WD, Essex M: 1975, Association of feline leukemia virus with lymphosarcoma and other disorders in the cat. J Am Vet Med Assoc 166:449-454.
5. Frenkel JK, Holzworth J: 1987, The coccidia. *In:* Disease of the cat, ed. Holzworth J, pp. 359-390. W. B. Saunders Co., Philadelphia, PA.
6. Fisch H, Altman NH: 1989, Feline immunodeficiency virus infection in a population of pet cats from southeastern Florida. J Vet Diagn Invest 1:339-342.
7. Harbour DA, Williams PD, Gruffydd-Jones TJ, et al.: 1988, Isolation of a T-lymphotropic lentivirus from a persistently leucopenic domestic cat. Vet Rec 122:84-86.
8. Hardy WD: 1988, Feline T-lymphotropic lentivirus: retrovirus-induced immunosuppression in cats. J Am Anim Hosp Assoc 24:241-243.
9. Ishida T, Washizu T, Toriyabe K, Motoyoshi S: 1988, Detection of feline T-lymphotropic lentivirus (FTLV) infection in Japanese domestic cats. Jpn J Vet Sci 50:39-44.
10. Pedersen NC: 1984, Feline coronavirus infections. ***In:*** Clinical microbiology and infectious diseases of the dog and cat, ed. Greene CE, pp. 514-526. W. B. Saunders Co., Philadelphia, PA.
11. Pedersen NC, Ho EW, Brown ML, Yamamoto JK: 1987, Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. Science 235:790-793.
12. Pedersen NC, Yamamoto JK, Ishida T, Hansen H: 1989, Feline immunodeficiency virus infections. Vet Immunol Immunopatho1 21:111-129.
13. Shelton GH, McKim KD, Colley PL, et al.: 1989, Feline leukemia virus and feline immunodeficiency virus infections in a cat with lymphoma. J Am Vet Med Assoc 194:249-252.
14. Witt CJ, Moench TR, Gittelsohn AM, et al.: 1989, Epidemiologic observations on feline immunodeficiency virus and ***Toxo***plasma gondii coinfection in cats in Baltimore, MD. J Am Vet Med Assoc 1941229-233.
15. Yamamoto JK, Hansen H, Ho EW, et al.: 1989, Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. J Am Vet Med Assoc 194:213-220.