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ORIGINAL RESEARCH ARTICLE

Prevalence of and Exposure Factors for Infectious Diseases in Free-Roaming Cats From Two Florida Counties

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Abstract

***Introduction*:** Ninety-nine deceased free-roaming cats (FRCs) from two nonadjacent counties (Volusia and Alachua) in Florida were used to determine the prevalence of and associated exposure to pathogens postmortem, including Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV), *Dirofilaria immitis*, *Mycoplasma haemofelis, Mycoplasma haemominutum*, and *Cytauxzoon felis*.
***Methods*:** Humanely euthanized FRCs or those FRCs found dead in the community were submitted for postmortem examinations. Blood samples from these FRCs were analyzed using a combination of antibody, antigen, and polymerase chain reaction (PCR) assays.
***Results*:** Male cats were at higher risk of infection for FeLV and FIV, intact cats were less likely to be infected with FeLV, and cats in the Volusia county were more likely to be infected with FIV. *Mycoplasma haemominutum* had the highest prevalence of all surveyed pathogens in this study, and infections were only identified in male cats.
***Conclusion*:** FRCs in this study had similar or higher prevalence rates of infections compared to studies assessing FRCs enrolled in trap-neuter-return programs from Florida.

Keywords: *feral*; *free-roaming cats*; *infectious disease*; *pathogens*

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Free-roaming cats (FRCs) are all around us and include feral, stray, abandoned, and any cat that is not confined to a house or enclosure. While there are many trap-neuter-return (TNR) programs throughout the United States, there remain high levels of breeding among FRCs. The exact population of the FRCs cannot be reported with confidence in the United States.[1] Unfortunately, due to the COVID-19 pandemic, there were restrictions in ability for many agencies to provide TNR services. FRCs pose several problems to society including but not limited to predation of wildlife, public nuisance issues, and the potential to spread infectious and zoonotic diseases.[2,3] Although FRCs present society with a large number of issues, we still must consider the health and welfare of these cats, ultimately maximizing their quality of life.

Multiple studies have assessed FRCs for infectious and zoonotic diseases; however, these have been largely limited to prevalence of infectious diseases from samples collected as part of TNR programs. Locations where these surveillance studies have been performed include Kansas, Florida, Arkansas, Prince Edward Island, and St. Kitts.[4–9] In one study from Florida, it was reported that FRCs had similar or lower prevalence rates for select infectious diseases than pet cats in the United States.[6] A study of feral cats in Arkansas reported increased seroprevalence rates for Feline Leukemia Virus (FeLV) (12.73%) and Feline Immunodeficiency Virus (FIV) (16.36%) compared to other areas within the United States.[7]

Since FRCs do not get routine health assessments, it is important to assess the burden of diseases in this population of cats. It is well known that feline retroviruses (FeLV and FIV) are found throughout the FRC population, and that infection can predispose cats to other infectious diseases. For example, infection with FeLV or FIV has been shown to increase the risk for coinfection with hemotrophic mycoplasmas and *Bartonella henselae* in FRCs.[6] Few studies specifically assess the causes of mortality and comorbidities of FRCs.[10,11] Most studies describe shelter outbreaks of infectious disease or reports of sporadic

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deaths in FRCs.[12–14] There is extremely limited information available pertaining to the prevalence of infectious diseases in FRCs at death.

Given that most FRCs infectious disease studies are performed as part of TNR programs, it is possible that this could lead to underestimates of disease prevalence in FRCs as many of these cats will be presumably younger and healthier. Therefore, the purpose of this study is to investigate the apparent prevalence of select infectious diseases and associated exposure factors at death in FRCs from two nonadjacent counties in Florida.

Methods

Free-roaming cats enrolled in this study came from Alachua county or Volusia county, Florida. Volusia county is located on the east-central coast of Florida and is located between the St. Johns River and the Atlantic Ocean. Alachua County is located in the north central Florida. The distance between these two counties is approximately 160 km.

Animals

Humanely euthanized FRCs or those FRCs found dead in the community were submitted to the 'A Cat Has No Name' program by animal service agencies, law enforcement agencies, veterinarians, and the public. Cats were euthanized at the discretion of the submitting agency and were sick, debilitated, and/or injured. No cats were euthanized by the 'A Cat Has No Name' program, and all euthanasia were performed for reasons unrelated to this study. Biological samples were collected from FRCs that were submitted to the 'A Cat Has No Name' at the University of Florida College of Veterinary Medicine, from June 2020 to July 2021. Briefly, the 'A Cat Has No Name' program is a free service dedicated to the postmortem examination of FRCs. All postmortem examinations were performed by a board-certified veterinary pathologist. Postmortem examinations were performed according to standard operating procedure in the laboratory, including complete external and internal examinations.[15] For each cat, approximate age, spay/neuter status, and sex were recorded. Cats were classified juvenile or adult based on overall appearance and the appearance of the teeth. Cats that had deciduous teeth were classified as juveniles; all others were classified as adults. Cats enrolled in this study were from Alachua county or Volusia county, Florida. In total, 99 cats were enrolled in this study between June 2020 and July 2021. This study was approved by the Institutional Animal Care and Use Committee Animal Care Committee of the University of Florida.

Sample Collection

Heart blood was collected from each cat during the autopsy. Blood was put in potassium ethylenediaminetetraacetic acid (EDTA) tube and serum activator tube (Becton Dickinson, East Rutherford, New Jersey). In some instances, there was insufficient blood recovered to fill both tubes, and blood was only put in a potassium EDTA tube. Blood samples were refrigerated at 4°C prior to testing for FeLV, FIV, and *Dirofilaria immitis* (heartworm) and then subsequently placed in -80°C storage until polymerase chain reaction (PCR) testing for the other infectious agents was performed.

Testing Methodology

FeLV, FIV, and Heartworm

The SNAP Feline Triple Test was used for the detection of FIV antibodies, FeLV p27 antigen, and *D. immitis* antigen (IDEXX Laboratories, Westbrook, Maine, USA). According to the manufacturer's instructions, a small amount of blood from the potassium EDTA tube was analyzed using a point of care enzyme-linked immunoabsorbent assay (ELISA).[16] The manufacturer reports sensitivity for FeLV antigen, FIV antibody, and heartworm detection as 100, 100, and 89.3%, respectively, and specificities as 98.6, 99.2, and 99.5%, respectively.[17]

DNA Extraction

DNA was extracted from the serum activator tubes when available. In case blood was not collected in the serum activator tube, EDTA blood was used. DNA was extracted using the DNeasy Blood and Tissue Extraction Kit (QIAGEN, Hilden, Germany). The manufacturer's instructions were followed for extraction of DNA from 50 µL of whole blood.[18] The extracted DNA was quantified using a Qubit™ 3 Fluorometer and the Qubit™ dsDNA HS Assay Kit (Invitrogen, Waltham, MA, USA). Extracted DNA was stored at -20°C.

Mycoplasma PCR

The detection of *Mycoplasma haemofelis* and *Mycoplasma haemominutum* was done using a conventional PCR assay. Primer sequences were selected to specifically amplify the 16S rRNA gene as previously.[19] Since *M. haemofelis* and *M. haemominutum* contained a non-conserved region that was bordered by areas of high homology, the selected forward primer for sequence used was 5′-ACGAAAGTCTGATGGAGCAATA-3′, and the reverse primer sequence was 5′-ACGCCCAA TAAATCCGAATAAT-3′. PCR reactions were set up with 10 µL of DreamTaq Green Master Mix (2X) (Thermo Fisher Scientific, Waltham, MA), 0.6 µL of each forward and reverse primer (10µM final concentration), and 10–50 ng of template DNA and water to make the reaction volume to 20µL. An initial incubation was performed at 20°C for 10 min, followed by PCR under the following conditions: 95°C for 2 min for an initial

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denaturation, 40 cycles of amplification (95°C for 60 sec, 61°C for 60 sec, and 72°C for 30 sec), and a final extension at 72°C for 5 min. Products were analyzed on 3% agarose gels, and bands were noted. Positive and negative controls were assessed with both PCR assays, and only cases confirmed by sequencing were considered positive.

Cytauxzoon Felis PCR

The detection of *C. felis* was performed using a conventional PCR assay. Primer sequences were selected to specifically amplify a 284 bp fragment of the 18S rRNA gene as previously described by Haber et al.[20] The selected forward primer sequence was 5′-GCGAATCGCATTGCTTTATGCT-3′, and the reverse primer sequence was 5′-CCAAATGATACTCCGG AAAGAG-3′. PCR reactions were set up with 10 µL of DreamTaq Green Master Mix (2X) (Thermo Fisher Scientific, Waltham, MA), 0.6 µL of each forward and reverse primer (insert concentration), and 10–50 ng of template DNA and water to make the reaction volume to 20 µL. PCR conditions were set at 95°C for 5 min for an initial denaturation, 40 cycles of amplification (95°C for 45 sec, 60°C for 45 sec, and 72°C for 60 sec), and a final extension at 72°C for 5 min. Products were analyzed on 1.2% agarose gels, and bands were noted. Positive and negative controls were assessed with this PCR assays, and only cases confirmed by sequencing were considered positive.

Data Analysis

The presence of FeLV antigen, FIV antibody, *D. immitis* antigen, *M. haemofelis* DNA, *M. haemominutum* DNA, and *C. felis* DNA in the blood indicates a current infection. Prevalence estimates of infectious diseases (FeLV, FIV, Heartworm, *M. Haemofelis*, *M. haemominutum*, and *C. felis*) were calculated and reported as the percentage cats with a positive test result divided by the total number of samples tested. Furthermore, the prevalence of infectious diseases was examined for sex and neuter status of cats. Odds ratio and 95% confidence intervals were calculated using univariable logistic regression to assess exposure factors location, sex, and spay/neuter status for diseases. A univariable level of significance of $P \leq 0.2$ was required for a potential risk factor to be entered into the initial model. Variables that passed the univariable screening were used to develop the final multivariable model. Stepwise forward logistic regression was used, and any variable significant $P \leq 0.1$ remained in the model. Finally, multivariable logistic regression was used to calculate overall effect of exposure factors on FIV and FeLV. The interaction between sex and neuter status for FeLV, and location and sex for FIV were examined. The goodness-of-fit of the multivariable models was explored by use of the Hosmer-Lemeshow goodness-of-fit chi-squared statistic. Results were considered significant when P value ≤ 0.05. Statistical analysis was performed using Statistix10® (Analytical Software, Tallahassee, FL).

Results

Blood samples of 99 FRCs from Alachua county and Volusia county were analyzed for infectious diseases (Tables 1 and 2). Due to specimen consumption and occasional small amount of blood being recovered, not all tests could be performed on all cats. Out of 98 cats, 14.3% of cats were positive for FeLV, 14.3% of cats were positive for FIV, and none of the cats were positive for *D. immitis*. Sixty-six cats were tested for *M. haemofelis* and *M. haemominutum*, and these pathogens were detected in 4.5 and 15.1% of cats, respectively. Fifty-six cats were tested for *C. felis* and were detected in 7.1% of cases.

Alachua County

Sixty-seven FRCs from Alachua County were analyzed (Tables 3 and 4). There were more female cats than male cats submitted to this study from this county. Sixty-seven cats were tested for FeLV, FIV, and *D. immitis*. Ten were positive for FeLV (14.9%), 6 were positive for FIV (9%), and none was positive for *D. immitis* (0%). Out of the 10 cats that tested positive for FeLV, 8 were males (3 intact and 5 castrated) and 2 were female (1 intact and 1 spayed). Out of the 6 cats that were positive for FIV, 4 were intact adult males, 1 was a castrated male, and one was a neonatal female. Due to the potential for transfer of maternal antibody to nursing kittens, it is unknown if the neonatal female was a true infection or if this was a response to maternal antibodies. None of the 41 cats tested positive for *Mycoplasma haemofelis* (0%) and 7 of 36 tested positive for *M. haemominutum* (19.4%). All *M. haemominutum* positive cats were male (4 intact and 3 castrated). *Cytauxzoon felis* was detected in 2 of 35 (5.7%) cats, and both were intact females (1 adult and 1 juvenile). Multiple coinfections were observed, but there were no coinfections of *M. haemofelis* and *M. haemominutum* (Table 3).

Volusia County

Thirty-two FRCs from Volusia county were analyzed (Tables 3 and 4). There were more male cats than female

Table 1. Number and percentages of feral cats sampled in Alachua and Volusia counties by sex and neuter status

Variable	Alachua (%)	Volusia (%)	Total (%)
Male	36 (63.2)	21 (36.8)	57 (100)
Intact	22 (59.5)	15 (40.5)	37 (100)
Neutered	14 (66.7)	7 (33.3)	21 (100)
Female	31 (75.6)	10 (24.4)	41 (100)
Intact	19 (79.2)	5 (20.8)	24 (100)
Neutered	12 (70.6)	5 (29.4)	17 (100)

Table 2. Apparent prevalence and 95% confidence interval of infectious disease by spay/neuter status

Sex/neuter status	FeLV (*n* = 98)	FIV (*n* = 98)	*M. haemofelis* (*n* = 66)	*M. haemominutum* (*n* = 66)	*C. felis* (*n* = 56)
Male-intact	11.1 (4–25)	30.6 (18–46)	8.3 (2–25)	29.2 (15–49)	0
Male-castrated	38.1 (20–59)	4.8 (0.8–22)	5.6 (0.9–26)	16.7 (6–39)	6.3 (1–28)
Female-intact	4.2 (0.7–20)	4.2 (0.7–20)	0	0	22.2 (6–54)
Female-spayed	5.9 (1–26)	5.9 (1–26)	0	0	11.1 (2–43)
Total	14.3 (8–22)	14.3 (8–22)	4.5 (15–12)	15.1 (8–25)	7.1 (3–17)

Table 3. Coinfections from cats sampled in Alachua and Volusia counties

Outcome	Exposure	Total	Alachua	Volusia
FeLV	*M. haemominutum*	1	1	0
	C. felis	1	0	1
	M. haemofelis	1	0	1
FIV	*M. haemominutum*	5	3	2
FeLV	*FIV and M. haemominutum*	2	1	1

cats submitted to this study from this county. Thirty-one cats were tested for FeLV, FIV, and *D. immitis*. Four were positive for FeLV (12.9%), 8 were positive for FIV (25.8%), and none was positive for *D. immitis*. Three of the four cats that tested positive for FeLV were male (1 intact and 2 castrated), and the sex of the fourth cat was unknown as the reproductive tract was not identified as this animal died of predation. One FIV positive cat was a juvenile female, and all others positives were intact males. Three out of 25 cats tested positive for *M. haemofelis* (12%) and *M. haemominutum* (12%). Multiple coinfections were observed, but none of the coinfections were for *M. haemofelis* and *M. haemominutum* (Table 3). All *M. haemofelis* positive cats were male (2 intact and 1 castrated), and all *M. haemominutum* positives were intact males. *Cytauxzoon felis* was detected in 2 of 22 (9.1%) cats, and both were adults (1 castrated male and 1 spayed female).

Table 4 shows a summary of the risk of infectious diseases by location, sex, and spay/neuter status. Multivariable logistic regression identified sex and spay/neuter status as risk factors for infection with FeLV and location and sex as risk factors for FIV (Table 5). Cats in Alachua County were at a 70% lower risk for infection with FIV compared to Volusia county (Table 5). Male cats were at significantly higher risk for infection with FIV (4.5 times) and FeLV (5.4 times) than female cats. Intact cats were less likely (~72%) to be infected with FeLV. There was a significant combined effect of sex and spayed/castrated on cats classified as seropositive to FeLV but not FIV (Tables 6 and 7). The sex of the cat appears to be an important factor for infection with *M. haemofelis* and *M. haemominutum*; however, odds ratios could not be calculated since all infected cats were male. There was no identifiable risk of infection with *C. felis* by location, sex, or spay/neuter status.

Discussion

This study investigated the prevalence of select infectious diseases in FRCs from two nonadjacent counties in Florida. All of the pathogens except *D. immitis* were identified in both populations of FRCs. Overall, *M. haemominutum* was the most prevalent pathogen detected in these cats.

The prevalence rates of both FeLV and FIV were similar as both had a prevalence of 14.3%. The rates for FeLV and FIV are in contrast to three previous feral cat studies from Florida, which had prevalence rates for FeLV and FIV at 3.6 and 3.3%, 3.3 and 3.7%, and 5.2 and 4.3%, respectively.[5,6,21] In another study assessing sick and high-risk pet cats for FIV in Florida, the FIV prevalence rate was 8.4%.[22] The rates for FeLV and FIV were similar to a study performed in Arkansas with reported FIV and FeLV prevalence rates of 12.7 and 16.7%, respectively.[7] FRCs found in Alachua county were found to have a 70% lower risk for infection with FIV compared to cats from Volusia county. Male cats were at an increased risk for infection with FIV, and this is consistent with a study looking at FRCs in Raleigh, NC and Gainesville, FL.[21] There was no significant difference for infection with FeLV by location. Neutered male cats were at significantly higher risk for infection with FeLV, and intact cats were less likely to be infected with FeLV. While it is not unexpected that cats in the current study had higher FeLV and FIV rates, it does support the theory that these sick cats may serve as a possible source for infection of other FRCs, particularly male cats, that they come in contact with.

Location and spay/neuter status did not play a significant role in infection with hemotrophic mycoplasmas. A previous study assessing disease prevalence in feral cats from north Florida identified *M. haemominutum* as the most commonly encountered hemotrophic mycoplasma in feral cats.[6] In another study, all hemotrophic mycoplasma positive feral cats were male.[8] Similar to that study, all cats infected with hemotrophic mycoplasmas in this study were male. The exact mode of transmission

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Table 4. Risk of infectious diseases by county, sex, and spay/neuter status in feral cats

Infectious organism	Prevalence		Odds ratio	95% CI	*P*
	Alachua	Volusia			
FeLV	14.9 (10/67)	12.9 (4/31)	1.18	0.34–4.11	0.79
FIV	9 (6/67)	25.8 (8/31)	0.28	0.09–0.90	0.03
D. immitis	0 (0/67)	0 (0/31)	NA	NA	NA
M. haemofelis	0 (0/41)	12 (3/25)	NA	NA	NA
M. haemominutum	17.1 (7/41)	12 (3/25)	1.51	0.35–6.45	0.57
C. felis	5.9 (2/34)	9.1 (2/22)	0.63	0.08–4.78	0.65
	Male	Female			
FeLV	19.6 (11/56)	4.9 (2/41)	4.77	1.01–22.60	0.05
FIV	21.4 (12/56)	4.9 (2/41)	5.32	1.13–24.99	0.03
D. immitis	0 (0/56)	0 (0/41)	NA	NA	NA
M. haemofelis	7.3 (3/41)	0 (0/24)	NA	NA	NA
M. haemominutum	24.4 (10/41)	0 (0/24)	NA	NA	NA
C. felis	2.7 (1/37)	16.7 (3/18)	0.14	0.01–1.44	0.09
	Intact	Neutered			
FeLV	8.3 (5/60)	23.7 (9/38)	0.29	0.09–0.96	0.04
FIV	20 (12/60)	5.3 (2/38)	4.5	0.95–21.21	0.05
D. immitis	0 (0/60)	0 (0/38)	NA	NA	NA
M. haemofelis	5.4 (2/37)	3.4 (1/29)	1.60	0.14–17.7	0.7
M. haemominutum	18.9 (7/37)	10.3 (3/29)	2.02	0.48–8.56	0.34
C. felis	6.5 (2/31)	8 (2/25)	0.79	0.10–6.05	0.82

FeLV: Feline Leukemia Virus.
FIV: Feline Immunodeficiency Virus.

Table 5. Final multivariable logistic regression analysis model for the risk of FeLV and FIV in feral cats

Infectious organism	Exposure	Adjusted OR	95% CI	*P*
FeLV	Sex (male)	5.47	1.12–26.61	0.035
	Spay/neuter status (intact)	0.28	0.08–0.98	0.047
FIV	Sex (male)	4.84	1.01–23.13	0.048
	Location (Alachua)	0.30	0.09–0.99	0.048

Table 6. Observed and expected combined effects of sex and spayed/castrated on cats classified as seropositive to Feline Leukemia Virus

Sex	Spayed/castrated	*N*	Positive	Negative	OR	95% CI	*P*
Male	Intact	36	4	32	1	Reference	NA
Male	Castrated	20	7	13	4.3	1.07–17.3	0.04
Female	Intact	24	1	23	0.34	0.03–3.32	0.6
Female	Spayed	17	1	16	0.5	0.05–4.85	1

of hemotrophic mycoplasma to cats has not been definitively determined. Possible vectors for transmission that have been considered include fleas and ticks.[23,24] It has also been hypothesized that fighting behavior of male cats increases their chance of infection.[8] Given the fact that only male infections were identified in this study, biological relevance of this appears likely and provides support of male–male interactions being involved in transmission.

Cytauxzoon felis had a lower prevalence in this population of FRCs compared to hemotrophic mycoplasmas and retroviral infections. Sex, spay/neuter status, and location did not play a significant role in infection with *C. felis*. In an FRC study from Kansas, 25.8% of TNR cats were positive for *C. felis*.[9] Compared to a previous study looking at cats in Florida which found 2/494 FRCs with *C. felis*,[20] we identified 4/56 (7.1%) cats to be positive for *C. felis*. This discrepancy in findings may be due to the different types of study populations as the previous study assessed 'apparently healthy' FRCs enrolled in TNR programs, and in this study, we assessed deceased FRCs. One of the cats in this study was spayed 1 day prior to its death and died as a result of its infection. Another *C. felis* positive cat in this study was estimated to be 3–4 weeks old, and given the young age of this cat, it is possible that this was a case of vertical transmission of *C. felis*. To date, there has been limited research on perinatal transmission of *C. felis*, and in one study looking at two litters of cats, perinatal transmission was not identified.[25] It is unlikely

Table 7. Observed and expected combined effects of location and sex on cats classified as seropositive to Feline Immunodeficiency Virus

Location	Sex	*N*	Positive	Negative	OR	95% CI	*P*
Alachua	Male	36	5	31	1	Reference	NA
Alachua	Female	31	1	30	4.8	0.5–43.8	0.2
Volusia	Male	20	7	13	0.3	0.08–1.12	0.09
Volusia	Female	10	1	9	1.4	0.14–14.0	1

that the neonatal cat had a false positive for *C. felis* as we minimized potential sources of contamination during our analysis and simultaneously ran a no-template control to identify sources of contamination.

There were no *D. immitis* cases identified in this study; however, this is not unexpected as we utilized an antigen test kit. Heartworm antigen tests detect proteins from mature female worms, and in cases of low heartworm burden infections or male-only heartworm infections, there is a higher frequency of false-negative antigen test results.[26] A positive result on a heartworm antibody test would have indicated that a cat has or has had a previous heartworm infection. While it is possible that a cat had a low heartworm burden that could have had a false-negative antigen test, in all cats, the heart and lungs were thoroughly examined, including all chambers of the heart, the pulmonary arteries were opened, and the lungs were sectioned and inspected for the presence of adult worms. None of the cats in this series had any adult heartworms identified.

Some of the risk factor results identified in this study are in agreement with those of previous studies including sex and/or spay/neuter status in regard to retroviral infectious. In contrast to previous studies from Florida assessing the health status of FRCs, we found higher apparent prevalence rates for FeLV, FIV, and *C. felis* in the current study. In Kreisler et al.,[5] it was reported that the FIV prevalence decreased by 0.16% per year and the FeLV prevalence decreased by 0.18% per year over the study period. This was likely due to a combination of sterilization, removal of positive cats, and vaccination against FeLV, and the fact that this was a geographically isolated (3 sides surrounded by water), which could have decreased the chance of cats migrating from adjacent areas. Unlike the Kreisler et al.'s[5] study, in the current study, locations were not geographically isolated, and it is possible that numerous cats in the locations were not sterilized (and likely not vaccinated for FeLV), thus increasing the potential for exposure to pathogens. It is also possible that some of the infected cats were older and therefore had a greater potential for exposure to these pathogens. Interestingly, we did identify location as a risk factor for infection with FIV. As reason for this is not known, however, since fighting/biting is the primary mode of transmission of FIV, one possible explanation is that there are an increased number of FIV positive roaming male cats in the Volusia county. We also saw a high prevalence of FeLV, it is possible that this is due to prolonged direct contact with FeLV positive cats from mutual grooming in colonies or possibly from clustering due vertical transmission by exposure via the placenta, grooming by the infected mother, or milk during feeding. Future studies assessing additional locations including a comparison between rural and urban settings within individual counties and investigating larger numbers of FRCs are warranted to future explore locations as a risk factor for infectious disease in FRCs. Additionally, tracking FRCs over time possibly at both the TNR phase and at death may provide additional information as to the long-term health status of these cats. Analysis of *D. immitis* antibodies is recommended, given the shortfall of antigen testing alone as infections in cats may be of low burden and male-only infections. Since we identified a neonatal cat with a *C. felis* infection, further exploration of possible vertical transmission of this pathogen in cats should be considered.

There are several limitations of this study. This study provided a convenience sample of FRCs as these cats were submitted to our program by investigators and the public. Many of the FRCs did not come from a known colony, and these cats were most often found by people concerned with the welfare of FRCs and in locations where the cat could be located such as the street or backyard. Since the FRCs examined were convenience samples, we cannot generalize the results of the entire population of FRCs since we do not know how cats that die in a more secluded location (such as a wooded area) might differ from cats that are injured or die in locations that are more public. While we saw an interaction between sex and neuter status for FeLV and not FIV, there is the potential of lack of power to detect differences between the comparison groups. Additionally, the results of this study only provide us with a single snap-shot in time for each of these cats. Although we were able to determine the apparent prevalence of these pathogens at death, it is not possible to determine when these cats were infected with each

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pathogen; therefore, we do not know how long each cat was infected for.

Conclusion

The results from this study do show that at death, there appears to be a greater prevalence of infections with FIV, FeLV, *C. felis*, and *M. haemominutum* than previously reported in apparently healthy FRCs; therefore, these cats may serve as a source of infection for other FRCs, or if the cats live longer, they have a greater chance of infection from other cats. Future studies investigating larger number of FRCs, assessment of FRCs from more secluded locations, evaluating diseases when cat–cat interactions (such as mating and fighting) are known, and further characterization of how infections correlate with the cause of death are needed in order to improve our ability to improve the health and welfare of FRCs.

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Conflict of Interest and Funding

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Credit Author Statement

Stern: Conceptualization, Methodology, Investigation, Writing, Project administration, Funding acquisition. **Muralidhar**: Investigation, Writing – Original draft preparation. **Demagamage**: Formal analysis, Writing – Review & Editing.

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