

Seroepidemiological study of feline coronavirus (FCoV) infection in domiciled cats from Botucatu, São Paulo, Brazil¹

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ABSTRACT.- Almeida A.C.S., Galdino M.V. & Araújo Jr. J.P. 2019. **Seroepidemiological study of feline coronavirus (FCoV) infection in domiciled cats from Botucatu, São Paulo, Brazil.** *Pesquisa Veterinária Brasileira* 39(2):129-133. Laboratório de Virologia, Departamento de Microbiologia e Imunologia, Instituto de Biotecnologia, Universidade Estadual Paulista, Alameda das Tecomarias s/n, Chácara Capão Bonito, Botucatu, SP 18607-440, Brazil. E-mail: arianicristina@yahoo.com.br

Feline coronavirus (FCoV) is responsible for causing one of the most important infectious diseases of domestic and wild felids, the feline infectious peritonitis (FIP), which is an immune-mediated, systemic, progressive and fatal disease. FCoV is highly contagious, and infection is common in domestic feline populations worldwide. The present study aimed to determine the seropositivity of FCoV infection and its associated epidemiological variables (risk factors) in domiciled cats in Botucatu, São Paulo, Brazil. Whole blood samples (0.5-1 mL) were collected from 151 cats, and sera were extracted by centrifugation. These sera were tested by an commercial enzyme-linked immunosorbent assay (ELISA) for the detection of IgG anti-FCoV antibodies. The assessed risk factors were age range, breed, gender, reproductive status, outdoor access and rearing mode (living alone or in a group). The seropositivity was 64.2% (97/151). There was no statistical significance for risk factors related to breed, gender or rearing mode. There were significant differences in seropositivity (p-values ≤ 0.05) for age range (p=0.0157), reproductive status (p=0.0074) and outdoor access (p=0.0001). This study verified a wide dissemination of FCoV in the studied population, with a higher than expected seropositivity for indoor cats. Among the risk factors, age range, reproductive status and outdoor access presented statistically significant differences, thus helping to establish an epidemiological profile of this population.

INDEX TERMS: Seroepidemiology, feline coronavirus, FCoV, domiciled cats, São Paulo, Brazil, cats, viroses.

RESUMO.- [Estudo soropidemiológico da infecção pelo coronavírus felino (FCOV) em gatos domiciliados de Botucatu, São Paulo, Brasil.] O coronavírus felino (FCoV) é responsável por causar uma das mais importantes doenças infecciosas que acometem os felinos domésticos e selvagens, a peritonite infecciosa felina (PIF), que é uma enfermidade imunomediada, sistêmica, progressiva e fatal. O FCoV é altamente contagioso e a infecção é comum nas populações

de felinos domésticos por todo o mundo. O presente estudo objetivou determinar a soropositividade da infecção pelo FCoV e correlacionar variáveis epidemiológicas (fatores de risco) de gatos domiciliados de Botucatu, São Paulo, Brasil. Amostras de sangue total (0,5 a 1 mL) foram colhidas de 151 gatos e os soros foram obtidos após centrifugação. Estes soros foram testados por um teste comercial de ELISA para detecção de anticorpos IgG anti-FCoV. Os fatores de risco avaliados foram faixa etária, raça, gênero, condição reprodutiva, acesso à rua e modo de criação (viver solitário ou em grupo). Observou-se uma soropositividade de 64,2% (97/151). Não houve significância estatística para os fatores de risco relacionados à raça, gênero e modo de criação. Houve significância estatística quanto a soropositividade (p-values $\leq 0,05$) para os fatores de risco faixa etária (p=0,0157), condição reprodutiva (p=0,0074) e acesso à rua (p=0,0001). Através do presente estudo verificou-se que o FCoV está

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amplamente disseminado na população estudada, onde a soropositividade encontrada foi maior do que a esperada para gatos domiciliados. Dentre os fatores de risco, faixa etária, condição reprodutiva e acesso à rua apresentaram diferenças estatisticamente significativas, contribuindo assim, para se estabelecer um perfil epidemiológico desta população.

TERMOS DE INDEXAÇÃO: Soroepidemiológico, coronavírus felino, FCoV, gatos domiciliados, São Paulo, Brasil, felinos, viroses.

INTRODUCTION

The feline coronavirus (FCoV) belongs to the order *Nidovirales*, family Coronaviridae, subfamily Coronavirinae, genus *Alphacoronavirus* and species *Alphacoronavirus 1* (ICTV 2017). It is an enveloped virus containing single-stranded RNA and positive polarity (Sparkes 2006, Pratelli 2008).

FCoV infection is widely distributed in domestic cats and sometimes observed in wild cats (Hoskins & Loar 1993, Foley et al. 1997). FCoV remains a habitual pathogen in cat groups because of chronic carriers that make up approximately 20% of the population within heavily populated areas (Hartmann 2005). Antibodies are present in approximately 80-90% of cats living in shelters and 30-50% of domiciled cats (Addie & Jarrett 2006, Brown et al. 2009). Overall, FCoV is a highly contagious virus, transmitted through the fecal-oral route, which usually causes a mild intestinal infection (Addie & Jarrett 2006, Pedersen 2009).

FCoV causes one of the most important infectious diseases affecting domestic and wild cats, feline infectious peritonitis (FIP), which is an immune-mediated, systemic, progressive and fatal disease (Addie & Jarrett 2006). FIP was discovered in the 1960s and has been reported worldwide ever since (Pedersen 2009, Le Poder 2011). There is evidence to suggest that the causative agent of FIP is a FCoV mutation called feline infectious peritonitis virus (FIPV), and its benign counterpart is feline enteric coronavirus (FECV). Both viruses are indistinguishable from one another in terms of their physical and antigenic properties (Addie & Jarrett 2006, Norsworthy 2006, Cornelissen et al. 2007).

The occurrence of FIP is most common in young cats between three months and three years of age (Addie & Jarrett 2006). However, cats older than 10 years may develop FIP as they experience a decline in immune response typical of old age. FIP is more frequent in environments with a high feline concentration, where higher rates of viral infection and dissemination of FIPV variants exposes animals to significant infective doses (Hoskins & Loar 1993, Foley et al. 1997). Approximately 5-10% of seropositive cats may show signs of sickness and, consequently, die from FIP (Addie & Jarrett 2006). Clinical signs of FIP can be variable, because many organs can be involved, as the liver, kidneys, pancreas, eyes and the central nervous system. The FIP can present itself in two forms, the first being the “wet” or effusive form (more common), characterized by with effusions in the abdomen, thorax, and/or pericardium (Hartmann 2005). A second form of the disease is called “dry” or non-effusive (there is no into effusions body cavities), characterized by the presence of granulomas in organs (Pedersen 2009).

Investigations into the seroprevalence of FCoV infection and other viral agents important to feline medicine, such as feline leukemia virus (FeLV) and feline immunodeficiency virus

(FIV), contribute to controlling these agents by identifying risk factors and addressing strategies for infection prevention (Little et al. 2009, Westman et al. 2016). In Brazil, relatively few cases of cats exposed to or infected by FCoV are investigated in labs, except for some cases in certain animal shelters with high sanitary standards. As a general rule, domiciled cats are only investigated in the laboratory if they manifest clinical signs.

Studies describing FCoV seropositivity of domiciled cats are scarce in Brazil. Therefore, regional and national studies of seroepidemiology are necessary to identify the main risk factors of FCoV infection in the household feline population of Brazil. The present study aimed to determine the seropositivity of FCoV infection and the correlated epidemiological risk factors in domiciled cats in Botucatu, São Paulo, Brazil.

MATERIALS AND METHODS

Ethics statement. This work was submitted and approved by the ethics committee (CEUA) of Unesp, Botucatu, with approval protocol 51/2014 (registration number).

Animals and samples. The samples (n=151) were randomly collected. The cats lived in several neighborhoods around Botucatu’s urban zone (22°53’09”S, 48°26’42”O), located in the South-Central region, in the State of São Paulo. The source of the samples was through personal contacts, veterinary clinics the city and municipal kennel (from cats that were taken for free neutering). The samples were collected from 52 houses, each one having from one to eleven cats, all cats being part of the research. The State of São Paulo houses a population of around 947.539 domestic cats, and the city of Botucatu houses 3684 animals, claiming 0.4% total (Pasteur Institute 2016). The number of samples was calculated having in mind the number of cats in Botucatu in 2016, based on estimated prevalence of 90% (literature worldwide data about FCoV seropositivity, ever since there isn’t national results available) with a margin of error allowable error of 5% and confidence level of 95%. The sample calculation resulted in 134 samples, however were collected 151.

Blood samples (0.5 to 1mL) were collected aseptically by cephalic or jugular vein puncture and stored in a siliconized glass tube containing clot activator gel (Vacutainer®, Becton Dickinson) to obtain serum. Then, samples were centrifuged at 4000g for 10 minutes, and the sera were stored in 1.5mL microtubules free of nucleases (Axygen®) and frozen at -20°C until they were used. Individual data for each animal, such as age range (kitten, junior, prime/mature, senior/geriatric), breed, gender, reproductive status (whole/castrated), environment (outdoor access or confined), and rearing mode (in group/solitary) were recorded on an epidemiological card.

Serological test. Sera were tested using the ImmunoComb FCoV kit® (FIP) (Biogal Galed Labs, Acs. Ltd.) following manufacturer recommendations. The ImmunoComb test is a modification of ELISA test, based on immunoassay tenet on solid phase (DOT-ELISA). The test is able to determinate a semi-quantitative measure of the FCoV antibody titer present in whole blood, plasma, serum, effusion or cerebrospinal fluid (Bell et al. 2006b). The antibodies levels are determined according to the intensity of the test color result. Thus, the absence of color or a light gray color indicates negative or low level of antibodies. Higher levels of antibodies are indicated by darker color results. The results were scanned by Combo Scan software to classify specimens as seropositive or seronegative. These analyses were performed by Laboratory of Virology at Unesp, IBTEC, Botucatu, São Paulo, Brazil.

Statistical analysis of data. The data were analyzed with Statistical Analysis System software (SAS 9.3) and Microsoft Office Excel 2007.

All variables were described using descriptive statistical methods and expressed in frequency and percentage. Logistic regression models were used to verify the existence of significant differences in seropositivity (0 = seronegative, 1 = seropositive) between groups of each variable, with a statistical significance level of $p \leq 0.05$. The difference was considered statistically significant when 1 was not included within the 95% confidence interval.

RESULTS

The study revealed a seropositivity of 64.2% (97/151). The descriptive statistics of all variables are shown in Table 1. In total were sampled 151 animals from 52 different houses, where 40.3% (21/52) had only one cat; 28.8% (15/52) had 2 cats; 5.7% (3/52) had 3 cats; 7.7% (4/52) had 4 cats; 1.9% (1/52) had 5 cats; 5.7% (3/52) had 7 cats; 3.8% (2/52) had 9 cats; 3.8% (2/52) had 10 cats and 1.9% (1/52) had 11 cats. The risk factor analysed, were found meaningful statistics differences for the age range variable ($p=0.0157$), reproductive status ($p=0.0074$) and outdoor access ($p=0.0001$) (Table 2), where three variable combined helps to explain the seropositive phenomenon on the researched population (p -values ≤ 0.05) (Table 3).

Were found meaningful statistics differences when different categories of age range, reproductive status and street access were compared (Table 4). Prime/mature animals are 4.5 times more likely to be seropositive when compared to kittens, and prime/mature+senior/geriatric (analyzed in group) are 6.7 times more likely than kittens+junior animals. The chance of seropositivity for whole animals is greater (2.76 times) than for castrated animals. Animals without outdoor access

Table 1. Frequencies of seroprevalence classifications for age range, breed, gender, reproductive status, outdoor access and rearing mode (solitary or group)

Variables	Positive	Negative	Total
Age range			
Kitten: 1 to 12 months	36	34	70 (46.3%)
Junior: >1 to 3 years	20	09	29 (19.2%)
Prime/mature: >3 to 8 years	25	07	32 (21.1%)
Senior/geriatric: >8 years	16	04	20 (13.2%)
Breed			
Mongrel cat	63	48	111 (73.5%)
Persian	29	4	33 (21.8%)
Exotic	02	0	2 (1.3%)
Siamese	02	02	4 (2.6%)
Maine coon	01	0	1 (0.6%)
Gender			
Male	45	33	78 (51.6%)
Female	52	21	73 (48.3%)
Reproductive status			
Whole	31	07	38 (25.1%)
Castrated	66	47	113 (74.8%)
Outdoor access			
Yes	22	32	54 (35.7%)
No	75	22	97 (64.2%)
Rearing mode			
Solitary	8	5	13 (8.6%)
Group	89	49	138 (91.4%)

are 4 times more likely to be seropositive than those that have outdoor access (Table 5).

DISCUSSION

This study revealed that FCoV infection is widely disseminated in the assessed feline population, with a seropositivity of 64.2%. The presence of antibodies, which normally varies from 30-50%, is higher than expected for domiciled cats, according to global data in the literature (Addie & Jarrett 2006, Brown et al. 2009, Pedersen 2009). There are no data available from studies conducted in Brazil.

Regarding the age groups, the study sample had a large number of kittens (1 to 12 months), representing 46.3% of the total animals sampled. Age is considered an important risk factor for the development of PIF (Hartmann 2005, Horzinek et al. 2008). Cats may become infected by FCoV in all age ranges, but the highest risk of developing FIP is for cats from three months to three years old (kitten and junior). Senior/geriatric cats older than 10 years are also considered high-risk animals due to the decline of their immune system (Rohrbach et al. 2001, Addie & Jarrett 2006). Statistical analysis demonstrated that prime/mature animals are more likely to be seropositive than kittens. When analyzed in groups (prime/mature+senior/geriatric and kitten+junior), the prime/mature+senior/geriatric group is more likely to have anti-FCoV antibodies. In another seroprevalence study, Akkan & Karaca (2009) also found greater seropositivity in adult and elderly individuals. These animals, possibly due to their age, have a greater chance of coming into contact with the virus and producing antibodies, though this may occur in any age range.

Moreover, 73.5% of the specimens were "mixed breed" cats (MBC). No significant differences were found related to the breeds we analyzed, but this could be due to the low number of animals sampled from certain types. All cat breeds can become infected with FCoV and develop FIP. However, some purebred cats seem to have a genetic predisposition to systemically manifest the disease (Horzinek et al. 2008).

Table 2. Statistical significance (p-value) for each variable

Variables	p-value ^a (logistic regression)
Age range	0.0157
Breed	1.0000
Gender	0.0818
Reproductive status	0.0074
Outdoor access	0.0001
Rearing mode	0.8325

^a Significant p-values ≤ 0.05 .

Table 3. Logistic regression of the combined statistically significant variables

Variables	Degrees of freedom	Chi-square statistic	p-value ^a
Age range	3	10.42	0.0153
Reproductive status	1	4.39	0.0361
Outdoor access	1	12.49	0.0004

^a Significant p-values ≤ 0.05

Table 4. Statistical significance of the differences between several categories, including age, range, reproductive status and outdoor access

Comparison	Degrees of freedom	Chi-square statistic	p-value ^a
Kitten x Junior	1	2.35	0.1256
Junior x Prime/mature	1	1.46	0.2275
Prime/mature x Senior/geriatric	1	0.23	0.6318
Prime/mature x Kitten	1	8.89	0.0029*
Senior/geriatric x Kitten	1	3.44	0.0635
Senior/geriatric x Junior	1	0.29	0.5902
(Kit. + Sr./geriat.) x (Jr. + Prime/mat.)	1	1.50	0.2201
(Prime/mat. + Sr./geriat.) x (Kit. + Jr.)	1	4.62	0.0317*
Reproductive status (castrated or whole)	1	4.39	0.0361*
Outdoor access (yes or no)	1	12.49	0.0004*

^a Significant p-values ≤ 0.05

Table 5. Estimates and confidence intervals (95%) for the odds ratio

Variable	Variable	Estimate	L. L. ^a	S. L. ^b
Whole	Castrated	2.7624	1.0276	7.4254 [#]
No outdoor access	Outdoor access	4.0044	1.8293	8.7653 [#]
Junior	Kitten	2.1357	0.7951	3.1482
Prime/mature	Kitten	4.5367	1.5854	12.9817 [#]
Senior/geriatric	Kitten	3.1562	0.8785	11.3395
Prime/mature	Junior	2.1242	0.6196	7.2766
Senior/geriatric	Junior	1.4780	0.3518	6.2090
Prime/mature	Senior/geriatric	1.4372	0.3294	6.2703
Prime/mat. + Sr./geriat.	Kitten + Junior	6.7050	1.1300	39.7866 [#]

^a L.L = Lower limit, ^b S.L. = superior limit; the interval confidences (95%) without value 1.

Abyssinus, Bengal, Burmese, Himalayan, Ragdoll, Rexes, Burmese, Exotic Shorthair, Manx, Persian, Russian Blue and Siamese are some of the breeds especially prone to developing the disease (Bell et al. 2006a, Pesteanu-Somogyi et al. 2006, Horzinek et al. 2008). The increased prevalence in these purebred cats may be due to a concentration of hereditary risk factors caused by inbreeding (Foley & Pedersen 1996).

Related the gender of the sampled animals, 51.6% were males and 48.3% females. There was no statistically significant difference in seropositivity between the gender groups. These results corroborate the findings of Bell et al. (2006a). Some studies point out a greater predisposition for FIP in male cats (Robison et al. 1971, Rohrbach et al. 2001, Pesteanu-Somogyi et al. 2006). For the reproductive condition variable, 74.8% of the individuals were castrated and 58.4% of the castrated individuals were seropositive. In whole animals, seropositivity was 81.5%. Statistical analyses showed that whole animals were 2.7 times more likely to be seropositive than castrated animals. Other authors describe a greater risk of developing the sickness in whole cats (Robison et al. 1971, Rohrbach et al. 2001, Pesteanu-Somogyi et al. 2006, Worthing et al. 2012). Male and whole indoor cats easily go out, being subjected to a higher stress from fights disputing territory or females. This may become them more vulnerable to PIF, also increasing the contact with a innumerous variety of FCoV strains.

Concerning rearing mode, 91.4% of the animals lived in groups of 2 to 10 cats. Environments with multiple cats appear to be at greater risk for the development of the disease, because the infection prevalence is higher in houses with more than

one cohabitant (Addie & Jarrett 2006). However, no significant differences were found for this risk factor despite most cats in the studied population cohabitating with others. There were statistically significant differences linked to outdoor access. Animals kept inside were 4 times more likely to be seropositive compared to those with outdoor access. The cat's creation in closed environments has contributed to increase the exposure to a great quantity of infectious agents, specially when created in groups. The confinement has brought changes for the specie's hygiene habits, wich before used to bury it's stools and nowadays use shared sandboxes. The cat's main way to eliminate FCoV is through the stools, and the sandboxes has made theses cats get more contact with theses stools, making easier the acute infectious and consecutive cycles of reinfections, with prolonged increasements of seropositivity and the risk of developing PIF. Suitable waste management (cleaning and disinfection, not overcrowding single spaces) by their owners is fundamental for PIF prevention.

CONCLUSIONS

This seroepidemiological study demonstrated that FCoV is widely disseminated in the studied cat population. Seropositivity was higher than expected for domiciled cats relative to data from other parts of the world.

The statistically significant differences found in risk factors, such as age range, reproductive condition and outdoor access, help to create an epidemiological profile of this population.

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Conflict of interest statement.- The authors have no competing interests.

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