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

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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 (0.5-1mL) 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.
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.

**TERMS DE INDEXAÇÃO:** Seroepidemiologia, coronavirus felino, FCoV, gatos domiciliados, São Paulo, Brasil, felinos, vírus.

### 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 (Sparks 2006, Frantelli 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 Ponder 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 PIF 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 microtubes 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® (FCov) (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 determine 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.
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 than those that 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 FIP (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+juniors), 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 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       | 04       | 33 (21.8%) |
| Exotic                     | 02       | 02       | 4 (2.6%) |
| Siamese                    | 02       | 02       | 4 (2.6%) |
| Maine coon                 | 01       | 01       | 2 (1.3%) |
| 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%) |

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

| Variables          | p-value a (logistic regression) |
|--------------------|---------------------------------|
| Age range          | 0.0157                          |
| Breed              | 1.0000                          |
| Gender             | 0.0018                          |
| 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.
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 an 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 habbits, wich before used to burry 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 these cats get more contact with these stools, making easier the acute infectious and consecutive cycles of reinfections, with prolonged increasements of seropositivety 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.
Acknowledgments.- The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), for granting the scholarship (Fapesp 2014/02994-6 process) and financing of this project.

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

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