

RESEARCH NOTE**DETECTION OF ANTIBODIES TO FELINE CORONAVIRUS IN STRAY DOMESTIC SHORT-HAIRED CATS (*Felis catus*) AND TIGERS (*Panthera tigris*)**

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ABSTRACT

No study has yet been conducted on the seroprevalence of feline coronavirus antibodies in the Philippines. Sera from 42 stray domestic short-haired cats (*Felis catus*) from Metro Manila and nine captive tigers (*Panthera tigris*) from Manila Zoological and Botanical Gardens (Manila Zoo) were tested for the presence of IgG antibodies against Feline coronavirus (FCoV) using ELISA. Mutant FCoV may result to the development of feline infectious peritonitis (FIP). Results show that 86% (36/42) of cats and 100% (9/9) of captive tigers were seropositive. Chi-square test determined that the acquisition of FCoV antibodies is influenced by both the sex and the age of the animals. Approximate Z-test confirmed that the population of seropositive animals is significant and that a large proportion of the test population was exposed or is currently exposed to FCoV infection. Therefore, it is important that there is strict implementation of sanitation and waste disposal management, continuous stray cat population control measure and constant monitoring of the cats for the possible development of the fatal FIP.

Keywords: cat, feline coronavirus, feline infectious peritonitis, tiger

INTRODUCTION

Feline Coronavirus (FCoV) is ubiquitous and is distributed worldwide in virtually all cat populations including those found in the wild. FCoV is spread through feco-oral route which occurs within a week of exposure (Pedersen, 2008) especially with cats from catteries, multi cat households and shelters where sharing of litter boxes are more common, increasing susceptibility to the infection (Diaz and Poma, 2009). Two biological types of FCoV are known, Feline Infectious Peritonitis Virus (FIPV) and Feline Enteric Coronavirus (FECV) (Pedersen, 1976a, 1987). From the FIPV biotype of FCoV, a fatal, immune-mediated disease of cats, Feline Infectious Peritonitis (FIP) may ensue. Although FCoV is common in cat population worldwide, less than 10% of them may develop FIP, when the FCoV mutates (Kipar *et al.*, 2010; Brown, 2011). Despite the low incidence of FIP among FCoV-infected cats, FIP is a major cause of mortality (Rohrbach *et al.*, 2001). And because it can take weeks to months to develop after the initial infection of FCoV, the disease may only become apparent after a cat has been adopted or sold, resulting in devastating consequences for clients and breeding facilities (Dreschler *et al.*, 2011). The disease is usually fatal in clinically ill cats, but most infections are subclinical (Evermann *et al.*, 1981). The disease has been reported in a variety of species, including mountain lions (*Felis concolor*) (Pedersen, 1988), caracals (*Caracal caracal*) (Leutenegger *et al.*, 1999), lions (*Panthera leo*), tigers (*Panthera tigris*), jaguars (*Panthera onca*), sand cats

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(*Felis margarita*), lynx (*Lynx lynx*) (Kennedy *et al.*, 2003) and cheetahs (*Acinonyx jubatus*) (Benetka *et al.*, 2004).

The seroprevalence of FCoV in both domestic and wild Felidae in the Philippines is still unknown. This is the first study conducted on the detection of FCoV in the domestic cats and captive wild felids in the Philippines. The results of this study can be used to better assess the epidemiology of FCoV in specific areas in the Philippines, especially in Metro Manila. Knowledge of the antibody titers to FCoV present in the test animals in this study may be used as future reference in evaluating the significance of implementing control measures, assessing the need for further in depth study on how the disease can be contained and managed, and in estimating the population of infected animals.

MATERIALS AND METHODS

Blood sera from 42 stray domestic short-haired (DSH) cats (*Felis catus*) captured within the vicinity of a tertiary public hospital in Manila, and from nine captive tigers (*Panthera tigris*) at the Manila Zoological and Botanical Gardens (Manila Zoo) were used in this study. The stray cat sample population consisted of seven adult males, seven juvenile males, 21 adult females and seven juvenile females. Of the nine tigers, four were adult males, one was juvenile male, two were adult females and two were juvenile females. All animals had no history of vaccination against FCoV; however, all captive tigers were vaccinated against rabies, feline panleukopenia, feline viral rhinotracheitis and feline calicivirus, and dewormed semi-annually with ivermectin.

The sera were processed with commercial FCoV antibody test kit (Immunocomb® FCoV Antibody Test Kit, BioGal Laboratories, Galed Kibbutz, Isreal) that uses a solid phase Dot ELISA format. A digital imaging scanner (BioGalCombScan®2000, Biogal Laboratories, Kibbutz Galed, Israel) was used to interpret the color change on the test comb kit to eliminate bias and to accurately interpret the quantitative value along with the qualitative classification. The sample spot's color intensifies as the levels of antibodies that are present in the sample increases. Thus, a zero (0) IgG antibody titer is interpreted or classified as 'negative', a 1:10 IgG antibody titer as 'low positive', a 1:20-80 antibody titer as 'positive', and 1:160-320 antibody titer as 'high positive'. This rapid test identifies if an animal has exposure to the disease or was previously vaccinated. The test is 83% specific and 95% sensitive (Sharif *et al.*, 2010).

Statistical analysis using Chi-square ($\alpha = 0.05$) was then performed to determine the correlation between FCoV IgG antibody seropositive animals and sex. Likewise, the same statistical test was used to determine the correlation between FCoV IgG antibody seropositive animals and age. The proportion between seropositive cats and seronegative cats was also analyzed statistically using approximate Z-test ($\alpha = 0.5$).

RESULTS AND DISCUSSION

Table shows the distribution of the results to the FCoV antibody test kit. Eighty-six percent (36/42) stray DSH cats and all nine captive tigers (100%) showed varying levels of antibody titers for FCoV. This is comparable to multi-cat environments found in Italy (82%, Pratelli, 2008), UK (84%, Sparkes *et al.*, 1991), USA (87%, Pedersen, 1976b), and Malaysia (100%, Sharif *et al.*, 2010). However, in Korea the seroprevalence was only 13.7% (Dong-Jun, 2011).

The cats may have acquired the virus from various ways. Interaction with cats on nearby catteries or multiple-cat houses, interaction with infected wild felids at nearby zoological facilities and interaction with dogs and other animals harboring

Table. Detected antibodies to feline coronavirus (FCoV) in stray domestic short haired cats (*Felis catus*, n=42) and captive tigers (*Panthera tigris*, n=9) from Metro Manila using solid phase dot ELISA format, based on sex and age.

IgG titer (classification)	Animal	Male		Female		Total
		Adult	Juvenile	Adult	Juvenile	
0 (Negative)	<i>F. catus</i>	10	20	20	10	6
	<i>P. tigris</i>	0	0	0	0	0
1:10 (Low positive)	<i>F. catus</i>	1	2	3	2	7
	<i>P. tigris</i>	0	1	0	0	1
1:20-80 (Positive)	<i>F. catus</i>	3	2	13	2	20
	<i>P. tigris</i>	4	0	2	2	8
1:160-320 (High Positive)	<i>F. catus</i>	2	1	3	3	9
	<i>P. tigris</i>	0	0	0	0	0

alphacoronaviruses that are infective to felids as well, may be any one of the sources of the FCoV infection in the seropositive cats. Also, because of the unknown origin and history of these stray cats, it is a possibility that they may have been infected before they inhabited the premises of the sample areas.

Leftover food articles attract stray cats to return or to dwell in the sample area. Because of this, a greater number of cats begin to depend and co-exist in the area leading to an increase in density or increase in population of stray cats in one place; thus, creating a multi-cat environment. According to Cave *et al.* (2004), the risk of exposure occurs five times higher in cats living 60 days or longer together. Virus shedding occurs within a week after exposure and remains at high levels for 2 to 10 mo before entering one of three excretion patterns: some cats shed the virus persistently, some have periods of shedding interlaced with periods of non-shedding, and some just cease shedding (Pedersen *et al.*, 2008). Moreover, almost all cats in multicat environments experience some level of stress and exposure to an array of pathogens; thus, higher incidence and outbreaks are expected in stressful environments and cat density is one of the stressors that play a large factor as to whether an FCoV infected cat develops FIP (Addie *et al.*, 2009). This scenario possibly explains why the percentage of FCoV seropositive stray cats in this study is higher compared to the values in similar studies abroad.

Titers lower than 25 may indicate that the cat is shedding low levels of FECV in their feces. A high titer only indicates that the cat has been exposed to FCoV, but it will not be definitive whether the animal will develop FIP or not (Drechsler *et al.*, 2011). Animals with very high positive antibody titers ($\geq 1,600$) are good predictors of the development of FIP; however, none of the tested animals showed this high titer level.

From subclinical stage of disease, titers of cats were observed to progressively rise as it approaches the clinical stage. The problem, however, is that continuous and progressive monitoring of titers is done very rarely and titers are just usually measured whenever clinical signs begin to appear and titers by then have already plateaued. In addition, a dramatic fall in titers may also occur at the end stage of the disease, which occurs in cats with fulminating effusive FIP (Pedersen, 2009). It is suggested that large amounts of virus present in the cat's body bind to antibodies and render them unavailable

to the antigen in the test, or the antibodies maybe lost in the effusions (Dreschler *et al.*, 2011). A positive titer only indicates that a given cat has been exposed to FCoV but it cannot be determined if it is an active FIP infection nor can it be predicted whether or not FIP will develop in the animal. On the contrary, a negative FCoV titer is a good predictor of the absence of FIP infection (Hartmann *et al.*, 2003).

No statistical analysis was done for the results of the tigers due to the small sample size of this species. For the results with stray cats, an approximate Z-test ($\alpha = 0.05$) was used to test if there is a significant number of seropositive cats among the population ($n=42$). Statistical analysis showed that with 36 cats positive to FCoV IgG, a large proportion of the population was exposed or is currently exposed to FCoV infection.

Statistical analysis using Chi-square test ($\alpha = 0.5$) showed that FCoV IgG and sex are not independent from each other. This means that the presence of FCoV antibodies is influenced by the sex of the cat, and that male and female cats are not equally susceptible to acquiring FCoV antibodies. Unfortunately, the statistical test was limited only to the determination of the relationship between the two parameters and the values that will specifically describe how much of a difference there is to male and females were not obtained. Among the stray cats, 89% (25/28) of the female cats and 79% (11/14) of the male cats turned out to have FCoV antibodies. This is contradictory with the study of Oguzoglu *et al.* (2013) in Ankara, Turkey wherein male cats at 48% (42/88) had a greater population of seropositives for FCoV antibodies than the female cats at 45% (45/100). The disparity in results may perhaps be due to the non-uniform sample distribution based on the sex of the 42 tested animals because the population of female samples ($n=28$) is twice as much as that of the male samples ($n=14$).

Statistical analysis using Chi-square test ($\alpha = 0.5$) showed that FCoV IgG and age are not independent from each other. This means that the presence of FCoV antibodies is influenced by the age of the cat, and that adult and juvenile cats are not equally susceptible to acquiring FCoV antibodies. The level of immunocompetence of an animal may be affected by age. Among the stray cats, 79% (11/14) of the juvenile cats and 89% (25/28) of the adult cats turned out to have FCoV antibodies. This is in agreement with the study of Oguzoglu *et al.* (2013) wherein juvenile cats at 45% (32/71) had a lesser population of seropositives for FCoV antibodies than the adult cats 47% (56/119).

The adult female cats comprise the majority of the seropositive population in this study. Most of these female animals transmit maternal antibodies to their kittens that protect the latter from various infections including FCoV infection. The problem, however, is that maternal antibodies against FCoV wane at around four to six weeks of age (Hickman *et al.*, 1995) and the kittens will still be infected if they are not separated or isolated from their FCoV-infected mother and environment. The results suggest that seropositive juvenile cats in this study were exposed to the virus at an early age. The ELISA test kit used in this study, however, cannot determine the exact time of exposure of the juvenile seropositive cats or if the antibodies that were detected were maternal antibodies. The results also confirm that there is an active source of infection in the area.

Based on the results of this study, 21% or nine out of the 42 cats sampled were classified as high positive for FCoV antibody titer. This finding reflects that a little more than 20% of the stray domestic short-haired cat population are high-level shedders of FCoV. Cats with persistently high antibody titers are consistently shedding. Placing the high level shedders of FCoV in individual quarantine areas may result to cessation of shedding and consequent decrease in antibody titer level (Mullin, 2009) as result of decreased exposure to infection from other shedders.

Commercially available assay has a sensitivity of 95% and specificity of 83%, although sensitivity is relatively high; the specificity gives a possible 17 % non-specificity. However, no assay can make a distinction between FECV, FIP and other coronaviruses (Goodson *et al.*, 2009). Exposed cats to other non-virulent coronaviruses such as Canine

Coronavirus and Transmissible Gastroenteritis virus will turn seropositive (Goodson *et al.*, 2009).

The results of this study suggest that the employment of a cat population control program is necessary because the cat population in the sampled area will only grow if not acted upon. FCoV shedders will gradually increase over time. According to Dreschler *et al.* (2011) the removal of these consistent shedders from multi-cat environments should be taken into consideration if isolation alongside stress reduction failed to promote a decrease in shedding.

For the captive tigers, it is recommended that, since eradication of the captive tigers is out of the question, the zoo management should constantly monitor the health status of these animals for the development of the fatal FIP. Newly acquired captive felines should not be placed in the same enclosure with the resident tigers, and stray cats should be eliminated from the area.

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