

## SEROLOGICAL SURVEY OF CATTERIES FOR CATS INFECTED WITH FELINE CORONAVIRUS

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### SUMMARY

A serological survey on antibodies against feline coronavirus (FCoV) was conducted in four catteries in Klang Valley, Selangor, Malaysia. A total number of 24 cats of various breeds, ages and gender were randomly sampled from each cattery. The level of antibody titers were tested using a dot blot ELISA test kit ImmunoComb<sup>R</sup>. All cats showed a previous exposure to FeCoV. All the cats (100%) sampled from the catteries are infected with the virus. Approximately 42% and 58% of the sampled cats exhibited medium and high positive antibodies titers against FeCoV, respectively. This is the first study conducted in Malaysia to gauge the status of FeCoV antibody titer in cats.

Keywords: Feline, feline coronavirus, feline infectious peritonitis, immunoComb kit.

### INTRODUCTION

Feline Infectious Peritonitis (FIP) is a fatal and incurable disease syndrome of domestic and some wild felidae. The disease has been reported since the early 1960s in the United States and late 1960s in Europe (Feldman and Jortner, 1964). In Malaysia, the first case of FIP was reported in 1981 in UVH, Universiti Pertanian Malaysia (Wong *et al.*, 1983).

The disease is caused by feline coronavirus (FCoV). Clinically, FCoV is subdivided into feline enteric coronavirus (FeCV) which causes a disease in cats characterized by self-limiting and mild diarrhoea and feline infectious peritonitis virus (FIPV) which causes fatal disease in cats. Feline infectious peritonitis is characterized by ascites and pleural effusion in wet form and granulomatous inflammation of various organs in dry form. The disease form is associated with the immune status of the infected cats.

The FIPV and FCoV are indistinguishable serologically, morphologically and antigenically (Pederson *et al.*, 1981; Horzinek and Osterhouse, 1979). The FIPV is believed to emerge from an *in situ* mutation of FCoV that enables it to penetrate the intestine and replicate in the macrophages of cats. In addition, mutation

of FCoV may have occurred outside the host, becoming the virulent FIPV before it infects the susceptible cats.

FCoV is ubiquitous among household pets, pedigree and other cats kept in catteries (Sparkes, 2003). Despite exposure to FCoV, incidence of FIP is relatively low. Other risk factors may contribute to the occurrence of the disease such as age, sex, immune status and management of the cats. Currently, there is no report on antibody status of cats to FCoV in Malaysia. The present serological survey was carried out to determine the FCoV antibody status in cats from selected catteries in Klang Valley, Malaysia.

### MATERIALS AND METHODS

#### *Catteries and cats*

A total of 24 cats from four catteries were selected for this study (Table 1). The cats were selected by tracing their client reference number at the small animal clinic office. The criteria was based on the cattery historical background of having had at least one diagnosed or highly suspected FIP positive cases reported to UVH-UPM and private small animals clinics in Selangor. The serum samples were divided into two groups namely the serum

Table 1: List of number of cats and participating catteries

No.	Cattery	Location	Population of cats	Breed
1	A	Petaling Jaya	20-30	Mostly Persian
2	B	K. Lumpur	50-60	Purebred and exotic
3	C	Damansara	20-30	Purebred including Burmese and REX
4	D	Cheras	20-30	Mostly Persian

from cats that have direct contact with cats that died of FIP and serum samples from cats that have no direct contact with cats that died of FIP but were housed together in the respective catteries.

A group of five free-ranging British shorthair cats were included in the study as comparison to cats kept in the cattery.

One each of seropositive and seronegative cat were identified for positive and negative control, respectively.

#### *Sample collection*

About 0.7 to 1.0 ml of blood was drawn from the jugular vein or cephalic vein of the cats using a 2 ml syringe with 23G, 1-inch needle. Blood was transferred into a plain tube and left to stand at room temperature for the blood to clot and form the serum. If the serum was not processed immediately, it was kept in the fridge until it was used (National Panasonic, Japan). The sera were collected and centrifuged at 1000g for 10 min (Hettish, Germany) to clarify the serum. The processed serum was transferred to clean vials and kept in  $-20^{\circ}\text{C}$  (Sanyo, Japan) for further use.

#### *Serological test using ImmunotestComb<sup>R</sup> antibody test kit*

The serological test was carried out using ImmunotestComb<sup>R</sup> test kit (Biogal Galed Laboratories). The antibody test kit is designed for measuring serum IgG antibody levels to FCoV in cats, to monitor FCoV infections and to assist the diagnosis of FIP. The kit test is based on solid phase "dot"-ELISA technology. The specificity of the test is 100% and the sensitivity is 86%. The test was carried out according to the manufacturer's instructions. Briefly, 5  $\mu\text{l}$  of serum samples were applied to the lowest spot on a comb-shaped plastic card. The Comb is the solid phase and has 12 teeth-sufficient for 12 test samples. The samples were mixed with diluent in the first row of wells of a multi-chamber developing plate. The Comb was then incubated with the samples in the developing plate. Specific IgG antibodies from the samples, if present, will bind to the antigen at the test spots. The middle spot is the positive reference control and the uppermost spot is the internal control. The Comb was then transferred to a well, where unbound antibodies were washed from the antigen spots. In the next step, the Comb was allowed to react with an anti-cat IgG alkaline phosphates conjugate, which will bind to antigen-antibody complexes at the test spots. After two more washes, the Comb was moved to the last well, where enzymatic reaction will result in colour changes. The comb was air-dried and the result was read against its standard scale. The scale consists of internal control and positive reference spot which served to confirm that the test had been properly conducted. The intensity of the colour of the test spots corresponds directly to the antibody level (ie antibody titer) in the test samples. No colour or light

grey indicates no (negative) or low level of antibodies. Higher levels of antibodies are indicated by a darker colour. The scale ranges from 1 to 5. The scale of 0 would indicate seronegative cats, 1-2; low positive; 3-4; medium positive, and 5-6 high positive.

## RESULTS

A total number of 24 cats from four catteries in KlangValley, Selangor were tested for the presence of FCoV antibodies by using ImmunotestComb<sup>R</sup> (Table 2). From the total samples, 54% of the cats have a history of direct contact to FIP cat and 46% cats had no exposure. All the cats were positive to some level of viral exposure. About 42% of the cats showed a Comb scale value of 4-4.5 which is categorized as medium positive and may indicate a previous or current FCoV infection. Most of the cats (58%) showed a Comb value of 5.0-5.5 which is categorized as high positive and may indicate FCoV infection with high rate of virus shedding and the result is consistent with diagnosis of FIP. None of the cats were seronegative.

Of the five free-ranged cats, 2 were seronegative with a Comb value of 0, while 3 cats were low positive with a Comb value of 1-2.

There was no significant difference in age, and gender of the cats in terms of antibody level detected.

## DISCUSSION

This is the first study conducted locally to screen FCoV antibodies in catteries. The study showed that 100% of cats in catteries sampled for the study had been exposed to coronavirus, regardless of their age and gender. The work is consistent with the findings reported by Sparkes (2003).

Cats with typical FIP would have high levels of anti FCoV antibodies. A seronegative result to FCoV in an ill cat usually helps to rule out the diagnosis of FIP. However, serological diagnosis alone is not valuable in the diagnosis of FIP because it shares the same antigenicity with a milder form of feline enteric coronavirus and is indistinguishable by a serological test. Positive results can only show that the cat has been exposed to FCoV and mounted an immune response.

A serological test with a strong positive in conjunction with other biochemistry tests and cytological testing would be useful in the diagnosis of FIP. A positive test alone has limited value in monitoring FCoV infection in healthy cats as the antibodies titre could not be reliably correlated with those obtained with the immunofluorescent antibody test (Addie *et al.*, 2004).

Nevertheless, the serological test is useful in the screening of cats before introducing to a cattery or household with FCoV-free colony. Seropositive cats may shed infectious virus particles through the feces and this can be a source of contamination to feed, water, bedding

**Table 2: Serological analyses of cats from four catteries for FeCoV antibodies titre using ImmunoComb<sup>R</sup>.**

Cattery	Sample no.	Age	Sex	Result*	Direct contact with FIP cats	
					Yes	No
A	01	2-year-old	F	5.5		/
	02	2-year-old	F	5.5		/
	03	1-year-old	F	5.0		/
	04	2-year-old	F	5.5	/	
	05	2-year-old	F	5.5	/	
	06	1-year-old	M	4.0	/	
	07	3-year-old	F	5.5	/	
B	08	1-year-old	M	4.5	/	
	09	4-year-old	F	5.0	/	
	10	3-year-old	M	5.0		/
	11	4-yearsold	F	5.5	/	
	12	7-month-old	F	4.0		/
	13	1-year-old	F	5.0	/	
	14	8-month-old	M	4.5		/
	15	2-year-old	F	5.0		/
C	16	4-year-old	F	5.0	/	
	17	3-year-old	F	5.0	/	
	18	4-year-old	F	4.5		/
	19	1-year-old	CM	4.5	/	
D	20	2-year-old	M	4.0		/
	21	6-month-old	F	4.0		/
	22	2-year-old	F	5.0		/
	23	3-year-old	M	4.0	/	
	24	1-year-old	M	4.0	/	
Free-ranged cats	25	2-year-old	F	1.0		/
	26	7-month-old	F	0		/
	27	3-year-old	M	0		/
	28	4-year-old	M	2.0		/
	29	7-month-old	M	0		/
Control positive	30	2-year-old	M	5.5	Unknown	
Control negative	31	1-year-old	M	0		/

Legends:\*Immunocomb scale: 0 = negative; 1-2 = low positive; 3-4= medium positive; 5-6 high positive.  
F= female, M= male, CM = castrated male.

and other utensils. Although, FCoV of the milder strain may only cause mild diarrhoea and a limited form of the disease, but infection to kittens may predispose them to secondary infection. A negative result with the test is likely to be reliable for screening cats prior to entry into a FCoV-free cattery or stud.

Most of the cats worldwide are exposed to FCoV and the infection is asymptomatic in the majority of cats. The viability of the virus in dried secretions for as long as seven weeks enhances the fecal-oral route infection. The risk of infection is higher in catteries and multiple-cat households. Close contact plays a crucial role in promoting persistent infection amongst the cats in the catteries. Keeping these cats close together by mixing the cats, using the same feed and water bowls and litter tray, and the same worker to handle the cats in the cattery may contribute to widespread prevalence of the virus amongst the cats. These factors may contribute to persistent infection of FCoV amongst the cats, which may lead to a

high antibody titre against FCoV. In contrast, free ranging cats have no or low positive antibodies titre to FCoV. These cats may have an exposure to FCoV during their life time but since they are free-ranging cats, their feces, which constitute the greatest source of virus infection in cats is buried outside. The persistent infection does not develop and therefore the free-ranging cats are more likely to eliminate the infection.

All cats in the catteries showed positive antibodies, regardless of the direct or lack of direct contact to FIP cats. Cats having a high titre may pose a risk of shedding to other member of the cats.

Catteries having high seropositive cats may want to control the virus transmission amongst the cats. In order to breach the cycle of virus infection, the high seropositive cat should be segregated from seronegative cats and its antibody titre should be monitored regularly. Once the cycle of reinfection is interrupted, the antibody titre will be reduced and some cats have been able to eliminate the

virus from the body and become seronegative (Addie, pers. comm.).

## CONCLUSION

Feline coronavirus is highly prevalent in catteries where 100% of the cats sampled were positive for FCoV, regardless of previous exposure to FIP diseased cats. The study shows that FCoV is highly prevalent in the environment and most cats exposed to the virus remained asymptomatic.

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