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SEROLOGICAL PREVALENCE AND HAEMATOLOGICAL PROFILE OF FELINE IMMUNODEFICIENCY VIRUS (FIV) IN SEMI-ROAMER AND OUTDOOR CATS

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SUMMARY

Feline Immunodeficiency virus (FIV) is among the most common infectious diseases diagnosed in cats. In this study, 55 client-owned cats presented to the University Veterinary Hospital, Universiti Putra Malaysia (UVH-UPM) were sampled. Inclusion criteria were semi-roamer and outdoor cats aged more than 6 months old. Blood samples were collected for serological testing using commercial immunochromatographic test kits and haematological analysis. Of the 55 cats tested, 13 cats (23.6%) tested positive for FIV antibodies. There was a significant association ($P < 0.05$) between neuter and health status to FIV seropositivity. FIV infections were more likely occurred in intact cats compared to neutered cats, and in sick cats compared to healthy cats. Erythrocytes, hemoglobin and packed cell volume (PCV) were significantly reduced ($P < 0.05$) in FIV cats compared to FIV-seronegative cats, however these parameters were within the normal ranges.

Key words: Cats, feline immunodeficiency virus, prevalence, risk factors, haematological profile

INTRODUCTION

Feline immunodeficiency virus (FIV) is a retrovirus of the genus Lentivirus. FIV was first isolated in 1986 in Davis, California, by Pedersen *et al.* (1987). There are 6 reported major subtypes of FIV, which includes A, B, C, D, E, and F. FIV has been reported in many regions ranging from the United States, Canada, Africa, South America, Asia and Europe (Greene, 2012). To date, there is no cure for cats diagnosed with FIV; therefore management and supportive therapy of FIV infected cats are vital (Levy *et al.*, 2008). Immunisations through vaccination as prophylaxis may not be protective against the virus, as there are many different antigenic determinants involved (Green, 2012).

In Malaysia, FIV was first reported in 1990 in Selangor (Cheng, 1990). Subsequently, a retrospective study on the prevalence of FIV of hospitalised cats in UVH-UPM (2007-2009) showed that 21 of the 93 cats were positive for FIV, with a prevalence rate of 24.7% (Bande *et al.*, 2009). A cross-sectional prevalence study of FIV which was carried out around Peninsular Malaysia in 2010 reported a prevalence of 31.3% (Bande *et al.*, 2012), where 115 of 368 cats were FIV-seropositive.

Age, sex, health status, lifestyle are described as risk factors associated with FIV. On the other hand, reduced rate of infection is associated with indoor lifestyle and sterilisation, while male gender, adulthood and outdoor access are known risk factors for the infection (Levy *et al.*, 2008). Haematological profiles of FIV positive cats are dependent upon the different stages of FIV infection. In acute phases, neutropaenia and lymphopaenia are normally seen however the abnormalities are no longer evident in the asymptomatic stage. Clinically ill FIV cats may show non-regenerative anemia, neutropaenia,

lymphopaenia, leukopaenia and occasional thrombocytopaenia. However, there have been reports of similar findings in asymptomatic FIV cats in the absence of other identifiable causes (Greene, 2012).

In Malaysia, FIV is usually diagnosed using commercial immunochromatographic test kits. In this preliminary study, the aim was to determine the serological prevalence of FIV infection in semi-roamer and outdoor cats only, as these are the cat populations most susceptible to the disease, using a commercial test kit. Information such as age, sex, neuter status, health status and type of household were obtained for each cat to determine if these were risk factors of FIV infection. Haematological profile of FIV positive cats has not previously been documented in our local setting, therefore this study was conducted to compare the haematological profile of FIV positive cats compared to FIV-seronegative cats.

MATERIALS AND METHODS

Sampling

Client-owned cats presented to the University Veterinary Hospital, Universiti Putra Malaysia (UVH-UPM), Serdang were sourced. The selection criteria were semi-roamer or outdoor cats aged more than 6 months old. Written owner consent was obtained as required by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia, which approved this investigation (FPV/FYP/2013/054).

Information such as the age, sex, neuter status, type of household and health status was obtained for each cat from the patient file. The risk factors investigated in this study were; 1) age (young [6 months to 1 year old] or adult [>1 year old]); 2) sex (male or female); 3) neuter status (intact or neutered); 4) type of household (single or multiple); and 5) health status (healthy or sick).

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Sample collection

The cats were physically restrained for blood collection via jugular or saphenous venipuncture. Approximately 2 ml of blood was collected into EDTA tubes (Vacutest®, Italy) and were stored at room temperature and used immediately for FIV serological testing and haematological analysis.

Serological testing

The diagnosis of FIV was conducted using a commercial immunochromatographic test kit (SNAP Combo FeLV antigen/ FIV antibody test kit, IDEXX, USA). The kits were stored at 4°C until further use. Briefly, samples and test components were equilibrated at room temperature (18-25°C) for 30 min before use. Three drops of blood followed by 4 drops of conjugate were mixed into a tube by inverting the tube several times. The entire content of the mixture was poured into the well. Within 30-60 sec, the mixture flowed across the result window to reach the activation circle. The activator was pushed firmly when colour appeared in the activation circle and the results were read after 10 min with reference to the instruction from the manufacturer.

Haematological profile

All laboratory analyses were carried out at the Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). A blood smear was made using the blood in EDTA, air-dried and stained with Wright's stain (Sigma-Aldrich, USA). The stained blood smear was covered with a cover slip and mounted with a mounting medium (J.T. Baker®, USA). The glass slide was left to dry overnight and viewed under the microscope (Nikon, Japan) for leukocyte differential count. Total erythrocyte, leukocyte, and thrombocyte counts, as well as the haemoglobin concentration, were determined by the automated cell count machine (Cell-Dyn®3700, Abbott Diagnostics, USA). The packed cell volume was determined using the microhaematocrit method.

Statistical analysis

All statistical analyses were performed using GraphPad Prism® software (Graphpad Software Inc,

USA). The associated risk factors towards FIV seropositivity was analysed using the Chi-square test, with 95% confidence interval (95% C.I). Odds ratio was determined and association was considered statistically significant when $P < 0.05$ (Zar, 1999). Mann Whitney's test, with 95% confidence interval (95% C.I) was used to analyse the blood results of each cat. A significant difference between the blood parameters between FIV positive cats and FIV-seronegative cats was considered when $P < 0.05$.

RESULTS

A total of 55 (n=26 healthy and n=29 sick) cats were included in this study. The 26 healthy cats were presented for a regular check-up (i.e. vaccination and deworming), surgical appointment for ovariohysterectomy, as blood donors or for a minor health-related problem (i.e. mild gingivitis, acute lameness and acute gastroenteritis). The 29 sick cats that were hospitalised were sampled. A total of 23.6% (n=13/55) of the cats were tested positive for FIV antibodies whereas 12.7% (n=7/55) cats were positive for FeLV antigen (Figure 1). Of these 13 FIV-infected cats, only 2 cats were co-infected with FeLV and these cats were from the sick group.

The haematological abnormalities observed in the FIV infected cats are summarised in Table 2. Anaemia, leukopaenia and monocytopenia occurred more frequently in FIV-positive cats compared to seronegative cats. Lymphopaenia were not significant between the two groups of cats. Neutropaenia was not observed in any of the FIV-positive cats (Table 2).

Table 2 shows that both groups of cats were anaemic with higher frequency observed in the FIV-positive cats. However the median values of three erythron parameters (Figure 2) were low normal, which indicate that most of the FIV-positive cats were not anaemic. The values in FIV-positive cats, however, were significantly lower than FIV-negative cats. Masked anaemia due to haemoconcentration as a result of dehydration might contribute to the low normal of these parameters in FIV-positive cats. Overall, the percentages of anaemia observed in FIV-positive cats and FIV-negative cats (Figure 3) (both 7.8% respectively; $P < 0.05$), which indicate that anaemia is not specifically related to the FIV-positive cats, as any diseases will cause anaemia in sick cats.

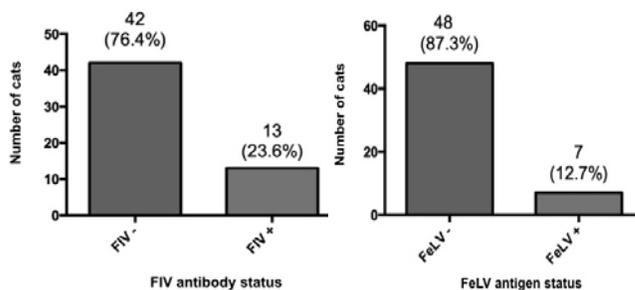


Figure 1. Antigenic prevalence of FIV antibody status and FeLV antigen status of semi-roamer and outdoor cats aged more than 6 months old tested using SNAP Combo FeLV Ag/ FIV Ab test kit (IDEXX, USA)

Table 1. Investigated risk factors, ratio and

percentage of FIV-seropositive cats from a group of semi roamers and outdoor cats (n=55)

Risk factors	Number of Cats (n)	Ratio	Percentage (%) of FIV seropositive cats	Odds ratio (95% CI)	Probability of the outcome occurring
Age					
Young	10	2/55	3.6	1.2 (0.2-7.0)	0.765
Adult	45	11/55	20.0		
Sex					
Male	35	10/55	18.2	2.3 (0.5-9.5)	0.254
Female	20	3/55	5.5		
Neuter status					
Intact	44	13/55	23.6	9.9 (0.5-179.7)	0.039*
Neutered	11	0/55	0		
Health status					
Healthy	26	1/55	1.8	17.7 (2.1-148.7)	0.001*
Sick	29	12/55	21.8		
Type of household					
Single	30	6/55	10.9	1.6(0.5-5.4)	0.487
Multiple	25	7/55	12.7		

Legends: n=number; CI=Confidence Interval, *significant at P<0.05

Table 2. Comparison of the percentage of haematological abnormalities between FIV+ve and FIV-ve cats

Haematological abnormalities	FIV+ve (n=12) Affected cat (%)	FIV-ve (n=39) Affected cat (%)
Anaemia	33.3 (4) ^a	10.3 (4) ^b
Leukopaenia	25.0 (3) ^a	2.6 (1) ^a
Neutropaenia	0.0 (0) ^a	2.6 (1) ^a
Lymphopaenia	25.0 (3) ^a	30.8 (12) ^a
Monocytopenia	8.3 (1) ^a	0.0 (0) ^a

Legends: +ve=positive; -ve=negative; n=number

Values with similar superscript (^a or ^b) were not significantly different at P<0.05.

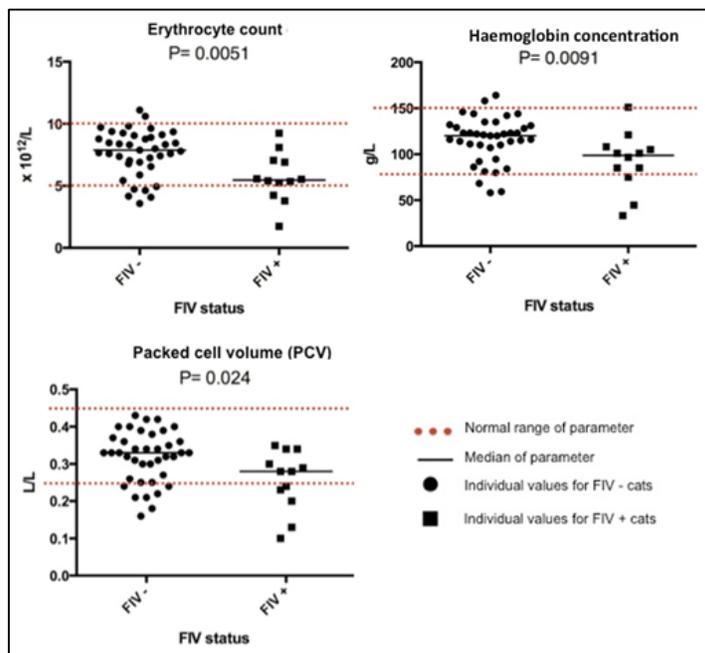


Figure 2. Comparison of the median of erythrocyte count, haemoglobin concentration and packed cell volume (PCV) between FIV-ve and FIV+ve cats

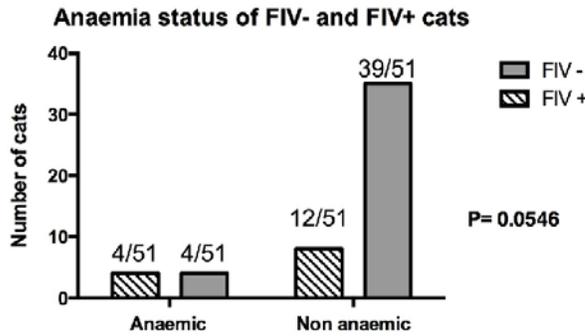


Figure 3. Comparison of anaemia status between FIV- and FIV+ cats

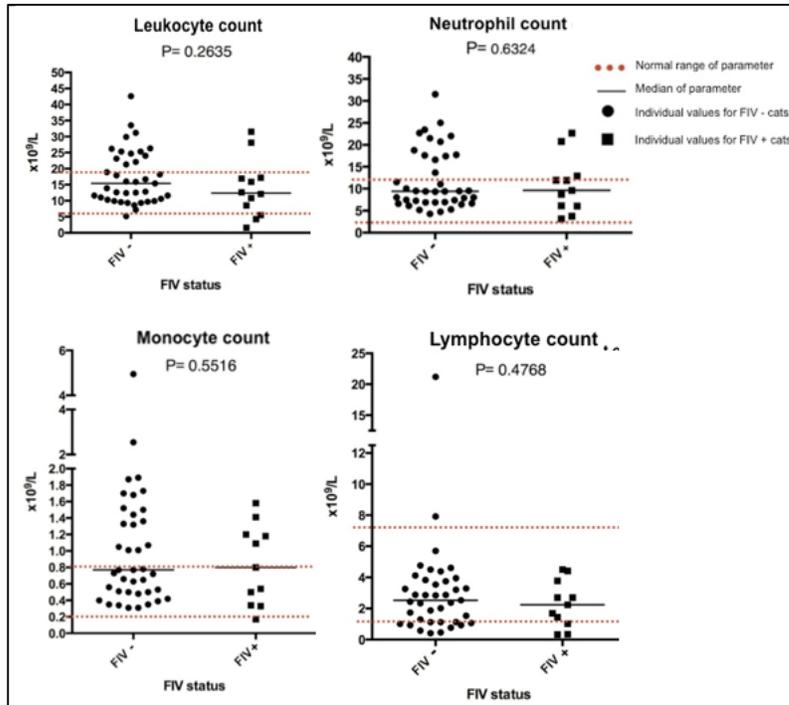


Figure 4. Comparison of the median leukocyte, neutrophil, monocyte and lymphocyte counts between FIV-ve and FIV+ve cats

There were no significant differences ($P > 0.005$, respectively) in the leukocyte, neutrophil, monocyte and lymphocyte counts between the FIV-seropositive and FIV-seronegative cats. Neutropaenia were not observed in this group of FIV-seropositive cats. Leucocytopenia, lymphopaenia and monocytopenia were not a significant findings in this group of FIV-seropositive cats (Figure 4).

DISCUSSION

The serological prevalence of FIV in semi-roamer and outdoor cats presented to UVH-UPM was 23.6%. This present study reflects the serological prevalence of FIV in client-owned cats presented to UVH. Previous similar studies conducted in UVH-UPM revealed a variable seroprevalance between 21.0-31.3% (Cheng, 1990; Bande *et al.*, 2009), but these studies included a larger number of stray cats. In addition, healthy cats were included in this study with a specific inclusion criteria of semi roamer and outdoor cats of more than 6

months of age which were not studied in the study carried out by Bande *et al.* (2009). Early diagnosis of FIV is important due to the fact that FIV causes persistent infection that leads to high titers of circulating FIV-specific antibodies, a definitive diagnosis is made based on the detection of the antibodies in blood. In addition, vaccination against FIV is not a common practice in Malaysia; therefore, FIV-positive results would be most likely due to infection and not immunisation (Sapian, 2011). Development of detectable antibodies in some cats may be delayed, but most cats produce antibodies 60 days post-exposure to the virus (Green, 2012).

Many serological studies have demonstrated that FIV is endemic in domestic cat populations worldwide. The seroprevalence of FIV is highly variable between regions and estimated to be between 1-14% in asymptomatic cats and up to 44% in sick cats (Hartmann, 1998). In this study, one of the 26 healthy cats that were tested positive for FIV infection had mild

gingivitis without any FIV-associated clinical signs whereas the other 12 FIV-infected sick cats were hospitalised due to other diseases such as Feline Upper Respiratory Tract Disease (FURD), Feline Infectious Peritonitis (FIP), wounds, fractures, and dermatophytosis. A clinical staging was not carried out in our study due to the limited number of cats. It has been further described that FIV can be subdivided to two stage; known as an acute stage or asymptomatic stage or phase of nonspecific clinical signs (which include Aids Related Complex (ARC), lymphadenopathy, and others) and the last stage, a terminal AIDS-like stage (Hartmann, 1998). The first stage may occur weeks to months whereas the asymptomatic stage may occur for several years. An experimental study documented an infected cat, which had been kept in isolation with persistent viraemia for more than 8 years without apparent clinical signs (Greene, 2012). The third stage which involves observation of lymphadenopathy may last for several months to 1 year, and the terminal stage for several months.

There are several risk factors associated with FIV infection. A study done in North America revealed that FIV prevalence is reported to be higher in males compared to females, castrated males compared to intact males, intact females compared to spayed females, adult cats compared to young cats, outdoor cats compared to indoor cats, and sick cats compared to healthy cats (Levy *et al.*, 2006). However, some of these risk factors were not consistently found in this study. Among the risk factors investigated, health and neuter status were found to be significant risk factors towards FIV seropositivity, while age, sex, and type of household were not. Age was not a risk factor for FIV seropositivity in this study, but others have shown that the risk is higher in adult cats compared to young cats (Liem *et al.*, 2013; Bande *et al.*, 2012; Levy *et al.*, 2006). This could be due to the heterogenous/variable number of cats of different age groups sampled in this study, whereby there were only 10 young cats sampled compared to 45 adult cats. Type of household was not a noteworthy risk factor and a similar finding was observed by Fromont *et al.* (1997). FIV infection is spread more by unfriendly exchanges, mainly biting, and therefore cats in households with a stable social structure are at lower risk for acquiring FIV infection (Levy *et al.*, 2006).

In this study, the prevalence of FIV was higher in intact males compared to castrated males, and spayed females compared to intact females (Bande *et al.*, 2012). A study that recruited owned cats showed that there was no association observed between sex and FIV seropositivity (Bandecci *et al.*, 2006). It is speculated that this risk factor would depend on the cat population sampled. However, neuter status was a significant risk factor and similar to the study by Bande *et al.* (2012), FIV antibodies were more prevalent among intact male and female cats compared to their neutered counterparts.

Health status was a significant risk factor where seropositivity was higher among sick cats than among healthy cats (Levy *et al.*, 2006). Clinical signs for FIV infection are nonspecific and commonly go unnoticed, except for FIV-induced neurological disease. The acute

stage may last several days to few weeks and these FIV infected cats appear clinically healthy. Clinical signs during the later stages are a reflection of opportunistic infections, neoplasia, neurological diseases and myelosuppression. Investigation of cats with chronic stomatitis in several studies showed that FIV infection is associated with persistent feline calicivirus infection (Knowles *et al.*, 1989; Tenorio *et al.*, 1991; Waters *et al.*, 1993; Reubel *et al.*, 1994). Other diseases that are reported to be associated with FIV include feline calicivirus infection (Knowles *et al.*, 1989), toxoplasmosis (Witt *et al.*, 1989), trichomoniasis (Gothe *et al.*, 1992), *Chylamydophila felis* infection (O'Dair *et al.*, 1994), and several fungal infections (Walker *et al.*, 1995). Presence of feline coronavirus antibodies, which if mutated causes feline infectious peritonitis (FIP), is not associated with FIV (Cohen *et al.*, 1990). Among cases of FIV-associated neoplasia, lymphoid malignancies were slightly more common in the FIV-infected cats (n=16/75, 21.3%) than the uninfected cats (n=30/230, 13%) (Liem *et al.*, 2013). Furthermore, skin disease of chronic nature specifically seen following biting, pustular dermatitis, facial dermatitis, chronic military dermatitis and others were also observed (Chalmers *et al.*, 1989).

There are several haematological abnormalities associated with retroviral infections. According to many studies in naturally and experimentally infected cats, neutropaenia, lymphopaenia, anaemia, and thrombocytopenia are among the common findings of FIV infection (Shelton *et al.*, 1990; Sparkes *et al.*, 1993; Dua *et al.*, 1994; Gleich *et al.*, 2009; Liem *et al.*, 2013). Although red blood cells, haemoglobin and packed cell volume were reduced in FIV cats compared to seronegative cats, the values were all within the normal ranges although it was low normal. Anaemia status was not a significant finding and rarely reported in FIV cats (Gleich *et al.*, 2009). However, anemia is a complex, multifactorial problem and its cause or causes may not always be identified in a sick cat with multiple problems (Liem *et al.*, 2013). Since FIV infection usually involves co-infection with other diseases caused by known opportunistic pathogens; therefore, abnormalities observed in FIV-positive cats cannot be definitively ascribed to the virus (Dua *et al.*, 1994). This observation explains the lack of association between the white blood cell differential count and FIV seropositivity. Neutropaenia was the only significant haematological change observed in a group of FIV infected cats compared with non-FIV infected cats (Gleich *et al.*, 2009). Another study has shown that the FIV infected cats had 7.13 times odds of decreased monocyte counts compared to the uninfected cats. However, leukopaenia, neutropaenia, and lymphopaenia were not significantly associated with FIV in this group of cats (Liem *et al.*, 2013). Variations of haematological profiles of FIV cats in different studies are dependent on the different stages of the infection (Greene, 2012).

Results of this study must be interpreted cautiously due to the limitations of the study. Neuter and health status were found to be significant risk factors associated with FIV seropositivity, while age, sex, and type of

household were not significant. Therefore, it is important for cat owners to provide proper healthcare for their cats with regular check-up, as well as to neuter their pets to reduce outdoor access, since it is known to be a high risk factor (Levy *et al.*, 2006). The sample population was obtained from only one hospital, therefore a wider sampling area and an increased sample size of cats from both healthy and diseased cats could reflect a true serological prevalence and distribution for the evaluation of the risk factors. Clinical staging of FIV cats would allow further correlation with the clinical signs, the associated diseases, as well as the haematological profile. Serum biochemistry analysis is recommended, as it would give a meaningful correlation with the stage of FIV infection as there have been reports of several abnormalities in FIV positive cats which include hypergammaglobulinaemia, lower serum activities of aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH) levels (Gleich *et al.*, 2009). Molecular detection of FIV would be a good comparative study to serological testing and to determine the FIV clades that are present in Malaysia, which has yet to be characterized.

CONCLUSION

Routine screening for FIV antibodies in cats is highly recommended especially in diseased and un-neutered cats. Anaemia was significantly observed, however these parameters (i.e. erythrocytes, haemoglobin and packed cell volume) were within the normal ranges. Lymphopaenia and monocytopenia were frequently observed in FIV seropositive cats.

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