Prevalence and risk factors of feline leukaemia virus and feline immunodeficiency virus in peninsular Malaysia

Faruku Bande1,2, Siti Suri Arshad1*, Latiffah Hassan1, Zunita Zakaria1, Nurul Asyikin Sapian1, Noor Almah Rahman3 and Amer Alazawy1

Abstract

Background: Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are major causes of morbidity and mortality in domestic and wild felids. Despite the clinical importance of feline retroviruses and the growing interest in cats as pets, information about FeLV and FIV in Malaysia is presently insufficient to properly advise veterinarians and pet owners. A cross-sectional study was carried out from January 2010 to December 2010 to determine the prevalence and risk factors associated with FeLV and FIV among domestic cats in peninsular Malaysia. Plasma samples were harvested from the blood of 368 domestic cats and screened for evidence of FeLV p27 antigen and FIV antibodies, using an immunochromatographic kit. Additionally, data on cat demographics and health were collected using a structured questionnaire, and were evaluated as potential risk factors for FeLV or FIV status.

Results: Of the 368 cats that were evaluated in this study, 12.2% (45/368; 95% CI = 8.88 - 15.58) were positive for FeLV p27 antigen, 31.3% (115/368; 95% CI = 26.51 - 35.99) were seropositive to FIV antibodies, and 4.3% (16/368; 95% CI = 2.27 - 6.43) had evidence of both viruses. Factors found to significantly increase the risk for FeLV seropositivity include sex, age, behaviour, sickness, and living in a multi-cat household. Seropositive response to FIV was significantly associated with sex, neuter status, age, behaviour, and health status.

Conclusions: The present study indicates that FeLV and FIV are common among domestic cats in peninsular Malaysia, and that factors related to cat demographics and health such as age, sex, behaviour, health status and type of household are important predictors for seropositive status to FeLV or FIV in peninsular Malaysia. High prevalence of FeLV or FIV observed in our study is of concern, in view of the immunosuppressive potentials of the two pathogens. Specific measures for control and prevention such as screening and routine vaccination are needed to ensure that FeLV and FIV are controlled in the cat population of peninsular Malaysia.

Keywords: Feline leukaemia virus, Feline immunodeficiency virus, Prevalence, Risk factors, Cats, Peninsular Malaysia
and health status [6]. Although several studies have been carried out on important viruses of cats in Malaysia [9-11], little is known about seroprevalence of FeLV or FIV. Information on the prevalence of FeLV and FIV from different part of the world will be crucial in understanding the distributions and epidemiological parameters related to feline retroviruses worldwide. The aims of this study were to determine the seroprevalence of FeLV and FIV among domestic cats in peninsular Malaysia, and to evaluate the risk factors associated with positive serological responses.

Results
Prevalence
Out of the 368 cats tested in the present study, 12.2% (45/368; 95% CI = 8.88 - 15.58) were positive for FeLV p27 antigen, 31.3% cats (115/368; 95% CI = 26.51-35.99) were positive for FIV antibody, and 4.3% (16/368; 95% CI = 2.27-6.43) had evidence of both viruses. Prevalence of FeLV or FIV was greater in clinically sick (FeLV, 23.2%; FIV, 38.4%) compared to healthy cats (FeLV, 5.1%; FIV, 23.6%), and in client-owned (13.1%) compared to shelter cats (10.3%). The seroprevalence of FeLV or FIV in each of the putative risk categories is presented in Tables 1 & 2.

Risk factors
In this study, several factors were significantly associated with FeLV or FIV seropositive status (Tables 1). In particular, risk of FeLV or FIV seropositive status was significantly higher in male cats, in aggressive cats, and in cats showing evidence of concurrent illness. Young cats were more likely to test positive for FeLV antigen while adult cats were more at risk for FIV seropositive status. Seropositivity to FeLV was more frequent in cats living in multi-cat households, compared to those living in shelters or single cat households. FIV antibodies were more prevalent among intact male and female cats, while positive response to FeLV testing was more frequent among intact males and neutered females. Factors such as breed, ownership, and sampling location, did

| Factors          | Categories    | Prevalence | P value | OR   | 95% CI     |
|------------------|---------------|------------|---------|------|------------|
| Sex              | Female        | 12/163 (7.4%) | Ref     | NA   | NA         |
|                  | Male          | 33/205 (16.1%) | 0.011* | 2.414 | 1.204 - 4.842 |
| Neuter status    | Intact female | 6/116 (5.2%) | Ref     | NA   | NA         |
|                  | Spayed female | 6/46 (12.8%) | 0.093   | 2.683 | 0.819-8.793 |
|                  | Intact male   | 12/163 (17.4%) | 0.469   | 1.356 | 0.592-3.105 |
| Age              | Adult         | 24/251 (9.6%) | Ref     | NA   | NA         |
|                  | Young         | 21/117 (17.9%) | 0.022* | 2.069 | 1.099-3.894 |
| Breed            | Domestic      | 36/305 (11.8%) | Ref     | NA   | NA         |
|                  | Pedigree      | 9/63 (14.3%) | 0.584   | 1.245 | 0.567 - 2.735 |
| Household type   | Single        | 3/68 (4.4%) | Ref     | NA   | NA         |
|                  | Multi-cat     | 30/184 (16.3%) | 0.013* | 4.219 | 1.243-14.285 |
|                  | Shelter       | 12/116 (10.3%) | 0.156   | 2.500 | 0.6798 - 9.174 |
| Lifestyle        | Indoor        | 13/134 (9.7%) | Ref     | NA   | NA         |
|                  | Outdoor       | 20/184 (16.9%) | 0.890   | 1.900 | 0.900-4.011 |
|                  | Shelter       | 12/116 (10.3%) | 0.866   | 1.074 | 0.470-2.456 |
| Behaviour        | Non-aggressive | 27/281 (9.6%) | Ref     | NA   | NA         |
|                  | Aggressive    | 18/87 (20.7%) | 0.006* | 2.500 | 1.300 - 4.808 |
| Ownership        | Shelter       | 12/116 (10.3%) | Ref     | NA   | NA         |
|                  | Owned         | 33/252 (13.1%) | 0.454   | 1.305 | 0.648 - 2.631 |
| Sampling location| Other states  | 6/65 (9.2%) | Ref     | NA   | NA         |
|                  | Selangor State | 39/303 (12.9%) | 0.416   | 1.452 | 0.588-3.590 |
| FIV status       | Negative      | 27/253 (10.7%) | Ref     | NA   | NA         |
|                  | Positive      | 18/115 (15.7%) | 0.176   | 1.553 | 0.817-2.952 |
| Health status    | Healthy       | 9/178 (5.1%) | Ref     | NA   | NA         |
|                  | Sick          | 36/190 (18.9%) | < 0.001* | 4.390 | 2.048-9.408 |

OR = Odds ratio; CI = confidence intervals; NA = not applicable; * = statistically significant difference; Ref = Reference category
not significantly influence FeLV or FIV seropositive status ($p > 0.05$).

**Discussion**

**Prevalence**

The prevalence and risk factors associated with FeLV or FIV were determined among domestic cats in peninsular Malaysia. Prevalence of FeLV or FIV in this study was greater than in the United Kingdom, North America, and Taiwan [12-14]. The observed differences might be attributed to variation among geographic regions, cat population densities, lifestyles and control policies and practices among different countries. Lack of routine vaccination against FeLV or FIV in Malaysia might be associated with high prevalence of feline retroviruses observed in our study. The impact of retrovirus infection has been drastically reduced in countries where vaccination, test-and-removal programs, and education campaigns are practiced [15,16].

**Risk factors**

Seropositive response to FeLV or FIV was significantly higher in sick compared to healthy cats. Sick cats were 5 times more likely to test positive for FeLV p27 antigen, and 2 times more likely to be positive for FIV antibody. These observations are similar to studies carried out in the United Kingdom and Canada [12,17]. Since both FeLV and FIV are immunosuppressive in nature, it is likely that FeLV- or FIV-infected cats become predisposed to opportunistic or secondary infections [18].

The probability of seropositive response for FeLV or FIV was significantly higher among male cats. This is consistent with some epidemiological findings previously reported for retrovirus screening [13,19]. Danner et al. [20] reported that the sex of a cat significantly influences risk for FIV but not FeLV. On the other hand, Bandecchi et al. [21], observed no significant association between sex and seropositivity to FeLV or FIV. These variations might be related to difference in the type of cat populations being studied. For example, Danner et al. [20] exclusively sampled feral cats, while Bandecchi et al. [21] sampled owned cats. In contrast, our study included owned and shelter cats that probably had varying medical and behavioural characteristics [22].

In our study, cats with aggressive behaviour were 2 times more likely to test positive for FeLV or FIV, as

### Table 2 Results of chi-square and univariate logistic regression analyses for risk of FIV in peninsular Malaysia

| Factors          | Categories | Prevalence | $P$ value | OR      | 95% CI   |
|------------------|------------|------------|-----------|---------|----------|
| Sex              | Female     | 41/163 (25.2%) | Ref       | NA      | NA       |
|                  | Male       | 74/205 (36.1%) | 0.024*    | 1.681   | 1.067 - 2.648 |
| Neuter status    | Intact female | 35/116 (30.2%) | 0.020*    | 2.951   | 1.149-7.576 |
|                  | Spayed female | 6/47 (12.8%) | Ref       | NA      | NA       |
|                  | Intact male | 53/138 (38.4%) | 0.323    | 1.366   | 0.735-2.538 |
|                  | Castrated male | 21/67 (31.3%) | Ref | NA      | NA       |
| Age              | Young      | 28/117 (23.9%) | Referent | NA      | NA       |
|                  | Adult      | 87/251 (34.7%) | 0.039*    | 1.686   | 1.025 - 2.777 |
| Breed            | Domestic   | 93/305 (30.5%) | Ref       | NA      | NA       |
|                  | Pedigree   | 22/63 (34.9%) | 0.490    | 1.223   | 0.690 - 2.168 |
| Household type   | Single     | 22/68 (32.4%) | Ref       | NA      | NA       |
|                  | Multi-cat  | 55/184 (29.9%) | 0.707    | 0.8912  | 0.490 - 1.621 |
|                  | Shelter    | 38/116 (32.8%) | 0.955    | 1.018   | 0.538 - 1.931 |
| Lifestyle        | Indoor     | 35/134 (26.1%) | Ref       | NA      | NA       |
|                  | Outdoor    | 42/118 (35.6%) | 0.103    | 1.563   | 0.912 - 2.680 |
|                  | Shelter    | 38/116 (32.8%) | 0.250    | 1.378   | 0.798 - 2.381 |
| Behaviour        | Non-aggressive | 75/281 (26.7%) | Ref       | NA      | NA       |
|                  | Aggressive | 40/87 (46.0%) | 0.001*   | 2.336   | 1.420-3.846 |
| Ownership        | Owned      | 77/252 (30.6%) | Ref       | NA      | NA       |
|                  | Shelter    | 38/116 (32.8%) | 0.672    | 1.107   | 0.691 - 1.774 |
| Sampling location| Other states | 15/65 (23.1%) | Ref | NA      | NA       |
|                  | Selangor state | 100/303 (33.0%) | 0.117 | 1.642   | 0.879 - 3.067 |
| FeLV status      | Negative   | 97/323 (30.0%) | Ref | NA      | NA       |
|                  | Positive   | 18/45 (40.0%) | 0.176    | 1.553   | 0.817 - 2.952 |
| Health status    | Healthy    | 42/178 (23.6%) | Ref | NA      | NA       |
|                  | Sick       | 73/190 (38.4%) | 0.002*   | 2.020   | 1.284 - 3.178 |

OR = Odds ratio; CI = confidence intervals; NA = not applicable; * = statistically significant difference; Ref = Reference category
compared with non-aggressive cats. A relationship between aggressive behaviours and seropositivity to FIV has been reported by several authors [19,23,24]. However, the high prevalence of FeLV observed in aggressive cats in our study contradicts the earlier notion that FeLV is primarily a disease of friendly or socialized cats [25]. Thus, there is the need to re-consider social behaviour in assessing FeLV transmission patterns [19,22]. It is possible that demographic factors that influence serological status to FeLV or FIV may change over time or with geography, climate and other factors.

FIV seropositive status was more frequent in intact male and female cats, compared to their neutered counterparts. On the other hand, positive response to FeLV testing was higher in intact males and neutered females. Reports of the relationship between sex and risk for feline retroviruses vary considerably. For example, in a study involving all parts of North America, FeLV or FIV seropositive outcomes were more frequent in intact females compared to spayed females, and in castrated males compared to intact males [13]. However, when only cats from the United State were considered, positive response to FeLV or FIV was observed more frequently among neutered male and female cats [26]. Greater seropositive status with respect to retrovirus infections in intact cats might be explained by their frequent involvement in territorial aggression and free-roaming behaviours that could increase risk for contact with infected cats. Although, in our study, seropositive test and odds of FeLV or FIV were greater among cats with outdoor access, this relationship was not significant statistically as reported recently in Canadian and German studies [17,19]. It is possible that lack of history on the original lifestyle of cats (indoor or outdoor) before their arrival at the shelter may affect our conclusion on the relationship between retrovirus seropositive response and lifestyle of cat population sampled. High prevalence of FeLV or FIV among free-roaming sexually intact cats might explain why the American Association of Feline Practitioners (AAFP) and The European Advisory Board for Cat Diseases (ABCD) recommended neutering as a means of reducing the frequency of feline retrovirus infections [27,28].

In our study, cats living in multi-cat households were most likely to be seropositive for FeLV, followed by cats living in shelters. FeLV seropositive status was least common among cats from single cat households. Conversely, seropositivity to FIV was not significantly influenced by type of household. This finding agrees with Fromont et al. [23], who also observed that prevalence of FeLV is more likely to be affected by population density compared to FIV. Overcrowding associated with multi-cat households often results in stress, poor hygiene, and increased direct contact among cats. FeLV transmission may likely be facilitated in these circumstances, since the virus is transmitted predominately by sharing of food and water containers [5,29].

The age of the tested cat was significantly associated with FeLV and FIV. Seropositive response to FeLV was 2.5 times higher in young cats, whereas seropositive response to FIV was 1.8 times higher in adult cats. This finding is in agreement with Levy et al. [13], and consisted with previous observations that increased susceptibility to FeLV was higher among young cats while susceptibility to FIV increases as cats grow older [30].

Although 4.3% of the sampled cat population was positive for both FeLV and FIV, we could not demonstrate any association between the two viruses statistically. There are conflicting opinions about the epidemiological relationship between FeLV and FIV. Some authors argued that FeLV and FIV occur independently [12,31], and others reported significant associations [19,32]. The debate notwithstanding, co-infection of cats with FeLV and FIV could lead to more negative health outcomes, compared to single infection with either virus [33].

It has been recommended that asymptomatic cats tested positive for FeLV p27 antigen should be re-tested within 6-12 weeks. This is because of low positive predictive value of single ELISA-based assays particularly in population with low seropositive rate [28,34]. No follow-up test was performed in the population we sampled, due to logistical problems and lack of willingness on the part of cat owners. In addition, cats in animal shelters usually remain for brief time periods as a result of adoptions and limited shelter space. Caution is therefore needed to avoid overgeneralizing the present findings.

**Conclusion**

From the result of the present study, it was concluded that FeLV and FIV seropositive responses are high among cats in peninsular Malaysia and that seropositive status to FeLV and FIV is significantly influenced by several risk factors related to cat’s demography and health. High prevalence of FeLV and FIV suggest the need for increased use of specific control measures such as screening and vaccination against feline retroviruses in peninsular Malaysia. As most of the identified risk factors are similar with those reported in other parts of the world, it is possible to adopt and implement current guidelines by the international associations for feline practices such as AAFP and ABCD to achieve better control and prevention strategies against feline retrovirus infections in peninsular Malaysia. Overall, this study has provided valuable insight on the occurrence of feline retroviruses in peninsular Malaysia, and has identified important risk factors.
Methods
Study area
The study was carried out in peninsular Malaysia which is located to the southern part of the Malay peninsula, extending from latitude 1°20’N to 6°40’N and from longitude 99°35’E to 104°20’E. Peninsular Malaysia is the most densely populated part of Malaysia and covers an area of 124,450 Km². The region can be divided into South, East, North, and Central regions. The climate is relatively humid (94-100%); with averagely high and uniform temperature (25-28°C) and rainfall (1,750 mm to 5,000 mm) throughout the year [35,36]. The people of this region have decades of history in keeping cats as their pets.

Study design, participants and sampling
A cross sectional study was carried out to sample a total of 368 domestic cats from 12 veterinary hospitals or clinics (n = 252) and two animal protection shelters (n = 116) in peninsular Malaysia. Inclusion to this study was based on the willingness of veterinary hospitals and shelter management to participate in the study. In addition, consent was obtained from cat owners prior to the sampling. Sampling was carried out by convenience from January 2010 to December 2010. Blood samples of 1-3 mL were collected into EDTA tubes via jugular vein puncture. All samples used in this study were collected by attending clinicians as part of routine practice.

Testing protocol
Blood samples were centrifuged to collect plasma that was tested for the presence of FeLV p27 antigen and FIV antibodies, using an immunochromatographic kit (SensPERT FeLV Ag/FIV Ab kit). Testing protocols were as recommended by the manufacturer. The manufacturer reported test sensitivities for FeLV antigen and FIV antibody assays as 98.5% and 99.7%, respectively, while specificities for the assays were 97% and 99%, respectively (VetAll™, Korea).

Risk factors
To evaluate risk factors associated with FeLV and/or FIV seropositivity, a structured questionnaire was completed for each sampled cat. Data recorded included sex, age (young [≤ 1 year] vs. adult [> 1 year]), breed (domestic [domestic short hair, domestic long hair, cross-breed] vs. pedigree), neuter status, household type (multi-cat household vs. single cat household or shelter), behaviour (aggressive vs. non-aggressive), health status (healthy vs. sick), lifestyle (indoor, outdoor, or shelter), ownership (owned vs. shelter), and sampling location (Selangor State vs. other States). Health status was evaluated from clinical records and general physical examination, while aggression was assessed based on cat demeanour as determined by the owners or shelter veterinarian.

Data analysis
Information recorded on the questionnaires was arranged into Microsoft Excel 2007 spreadsheets (Microsoft corporation Berkshire UK). Data was inspected and imported into an SPSS version 19.0 for statistical analysis (SPSS Inc. Chicago USA).

In the first step, descriptive statistics and frequency distributions were calculated and prevalence was determined as number of cats with positive serological test divided by the total number of cats evaluated. In addition, confidence intervals for each prevalence rates (FeLV, FIV and FeLV and FIV) were calculated at 95% level. Chi-square and univariate logistic regression analyses were used to determine association between the putative risk factors and seropositive response to FeLV or FIV. All statistical associations were considered significant at p < 0.05.

Abbreviations
FeLV: Feline Leukaemia Virus; FIV: Feline Immunodeficiency Virus; CI: Confidence Interval; SPSS: Statistical Package for Social Sciences; UVH-UPM: University Veterinary Hospital Universiti Putra Malaysia, Malaysia; AAFP: American Association of Feline Practitioners; ABCD: Advisory Board for Cat Diseases.

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Author details
1Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia. 2Department of Veterinary Services, Ministry of Animal Health and Fisheries Development, PMB 2109 Usman Faruk Secretariat Sokoto, Sokoto State, Nigeria. 3University Veterinary Hospital, Universiti Putra Malaysia 43400 UPM Serdang, Selangor, Malaysia.

Authors’ contributions
FB carried out the study, analysed the data, and drafted the manuscript. SSA conceived and coordinated the study, edited the manuscript, and approved the final submission. LH participated in the study design, data analysis, and proof-reading of the manuscript. ZZ participated in the study design and proof-reading of the manuscript. NAS, NAR, and AA participated in sample collection, study design, and proof-reading of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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