Title: Prevalence of and risk factors for FIV and FeLV infection in two shelters in the United Kingdom (2011-2012)

Jenny Stavisky, School of Veterinary Medicine and Science, The University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD. <u>Jenny.stavisky@nottingham.ac.uk</u>. 01159516660

Rachel Sarah Dean Centre for Evidence-based Veterinary Medicine, University of Nottingham, Nottingham, UK

Michael Henry Molloy, Cockburn Veterinary Group, Coalville, UK

Feline immunodeficiency virus; feline leukaemia virus; cat; feline; retrovirus

Word count (excluding tables, abstract and references) 4076

Prevalence of and risk factors for FIV and FeLV infection in cats in two shelters in the United Kingdom (2011-2012)

Abstract

1

- 2 The aims of this study were to determine the prevalence of FeLV and FIV infection in cats
- 3 presented to two RSPCA animal rehoming centres and to identify risk factors for infection.
- 4 All cats presented at each centre between August 2011-August 2012 were subjected to a
- 5 patient-side test for FeLV/FIV on entry. Kittens under 3 months and cats euthanased within a
- 6 short time of presentation were excluded from the study. Univariable and multivariable logistic
- 7 regression were used to separately determine risk factors for FeLV and FIV infection.
- 8 At shelter A, the prevalence of FIV infection was 11.4% (54/474) and FeLV infection was 3% (14/473),
- 9 with two FIV/FeLV co-infections identified. At shelter B, the prevalence of FIV infection was 3%
- 10 (4/135) and FeLV infection was 0% (0/135). Cats at shelter A were significantly more likely than
- those at shelter B to test positive for FIV (P = 0.0024) and FeLV (P = 0.048). Male cats were more
- likely to be infected with FIV (OR 27.1, p=0.001), and thin body condition and musculoskeletal
- disease were associated with risk of FeLV. Overall, FIV and FeLV positive cats were significantly
- older (median ages 5.1 and 4.75 years respectively) than the uninfected populations (median
- ages 3.4 and 3.5 years respectively).
- 16 This study shows that the prevalence of these diseases varies between shelter populations.
- 17 Local knowledge combined with the risk factors identified may be useful in focussing resources
- 18 for population testing strategies.

Introduction

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

Feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) are diseases of domestic cats and related species. FIV is commonly spread by fighting and biting (blood-to-blood contact), although sexual and vertical transmission are also possible (Hartmann 2011). Once acquired, FIV infection is lifelong, and there is no known recovery or cure (Hartmann 2012). FeLV is considered to be a "friendly cat" disease, with transmission by mutual grooming and sharing of food and water bowls considered to be the most prominent routes of transmission, although transmission by fighting and biting may also occur (Cattori and others 2009; Francis and others 1977; Hartmann 2011, 2012). Exposure may result in a variety of outcomes, including abortive, regressive and progressive infection. If a sufficient humoral and cell-mediate immune response is mounted, the virus will be eliminated, termed "abortive infection". However, it has become clear that apparently "recovered", antigen-negative cats may retain proviral DNA in bone marrow progenitor cells (Lutz and others 2009; Major and others 2010). Such cats, termed "regressively infected" will test negative for circulating antigen and are at low risk of FeLVassociated disease. Some studies have suggested that regressively infected cats may be at increased risk of FeLV-associated disease such as lymphoma and anaemia; however others have not found a link, and the potential role of FeLV in such circumstances is as yet unclear (Gabor and others 2001a; Jackson and others 1993; Stützer and others 2011). It has also been shown that regressive cats can be a source of FeLV infection via blood transfusion (Nesina and others 2015). Progressive FeLV infection occurs when there is an inadequate immune response and a permanent viraemia results, with affected cats typically dying of FeLV-associated disease including lymphoma or aplastic anaemia within three years of infection (Hartmann 2012). Within domestic cat populations, prevalence of these two viruses is variable. Male gender, outdoor access, a history of aggressive behaviour or fight wounds and evidence of ill-health on presentation have been consistently associated with increased risk of both diseases (Bande and others 2012a; Gleich and others 2009b; Hosie and others 1989; Levy and others 2006; Malik and others 1997; Muirden 2002). A variety of clinical presentations have been linked with increased risk of infection, with gingivitis linked most consistently with FIV (Bande and others 2012b; Gleich

and others 2009a; Goldkamp and others 2008; Yilmaz and others 2000), and anaemia with FeLV (Hosie and others 1989). Both FIV and FeLV have been linked with an increased risk of neoplasia, particularly lymphomas (Gabor and others 2001a; Gabor and others 2001b). As both diseases can cause immunosuppression and reduced immunosurveillance, recurrent or recalcitrant presentations of common diseases such as feline upper respiratory tract infection ("cat flu") have also been suggested to be potential indicators of infection (Hartmann 2012; Yamamoto and others 1989). Adult cats have been identified as being at greater risk than juveniles for FIV, probably due to greater cumulative opportunities to perform risky behaviours such as fighting and mating (Hosie and others 1989; Levy and others 2006; Malik and others 1997). Adult cats have been identified as at greater risk of FeLV infection than juveniles (Levy and others 2006), however it has been suggested that a degree of age related immunity occurs, meaning that when infection does occur in later life, it may be more likely to be regressive (Hartmann 2012; Hoover and others 1976). Feral cats have been suggested to be at greater risk of FIV infection (Levy and others 2006), whilst cats from multicat households are at increased risk of FeLV infection (de Almeida and others 2012). It has been suggested that the prevalence of FeLV has decreased over recent years, possibly due to an increase in vaccination (Englert and others 2012; Hartmann 2012; Lutz and others 2009). Data for trends over time suggests that FIV prevalence has either remained stable or possibly dropped over the past 30 years at least in specific populations (Courchamp and others 1998; Friend and others 1990; Norris and others 2007); however, definitive local trends are often difficult to identify, as there are relatively few studies repeated within the same population, and improvements in diagnostic tests over time limits comparison between them (Little 2011; Ravi and others 2010; Teixeira and others 2012). Current trends in UK prevalence for both diseases are hard to establish as relatively few data are available, and none recent (Hosie and others 1989; Muirden 2002) Prevalence in owned cats varies widely across the world. Infection prevalence in stray and shelter cats has been shown in different studies to be both higher and lower than the corresponding pet cat populations (Hellard and others 2011a; Little and others 2009; Norris and others 2007). In the UK, a 2002 study at RSPCA Birmingham Animal Hospital (BAH, since re-

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

named to BACH) identified 10.4% positive for FIV infection, and 3.5% for FeLV infection (Muirden 2002). Elsewhere, variable prevalences of 1.7% to 23% for FIV and 1.5% to 6.7% for FeLV have been reported in stray and shelter cats (Courchamp and others 1998; Levy and others 2006; Little 2005). This inconsistency may be partially due to differing strategies for selection of cats for testing in these populations (Levy and others 2006). It may also be possible that pockets of transmission occur within sub-populations, governed by localised risk factors such as neutering or vaccination.

Many cat shelters perform FIV and/or FeLV testing on some or all of their animals, as part of health screening for the individual, population healthcare, and also to provide reassurance to prospective owners. These typically utilise patient-side tests which detect FIV antibody and FeLV antigen respectively. FIV and FeLV infection have been shown to be one of the most common reasons for death or euthanasia in shelters (Murray and others 2008). However, if every one of the estimated 130,000 cats passing through rescue shelters in the UK annually (Stavisky and others 2012) was tested, this would add significantly to the cost of caring for and rehoming these animals. Additionally, when the patient-side test is used as a population screening tool, the relatively low population prevalence and subsequently low positive predictive value leads to difficulties in interpretation of the results. Cats which test positive for FeLV may undergo confirmatory tests such as PCR, and further tests to differentiate progressive from regressive infection, which incurs both a financial cost and potentially an extended stay in a rescue facility. It may therefore be desirable to establish known risk factors within this population of cats, in order to guide selection of cats for testing, to improve cost-effectiveness and accuracy of testing and expedite rehoming of cats.

The aim of this study was to determine the prevalence of FeLV and FIV infection in cats presented to two charities in the UK, as determined by a patient-side test. A secondary aim was to identify risk factors for infection within the populations under study.

Materials and Methods

Shelters

The study was a retrospective case-control study, conducted at two charities. RSPCA Birmingham Animal Centre and Hospital (BACH; Shelter A) is a large charity hospital and rehoming centre in the midlands region of the UK. Block Fen RSPCA (Shelter B) is a rural animal rehoming centre in the east of England. Both organisations accept animals generated by RSPCA animal inspectors, which may be stray, injured or confiscated for welfare reasons. In addition, RSPCA BACH provides charitable veterinary care to owners with low incomes, and members of the public also present sick and injured stray animals.

Study population

The population included any unowned cat presented for admission to the shelters or hospital over the year August 2011-August 2012 that was tested for FIV/FeLV. Publicly-owned animals were excluded (ie those visiting for treatment at BAH). At both shelters, during the study period, the operating policy stated that every cat admitted was tested for FeLV/ FIV as soon after entry as practicable, with the exception of those cats euthanased for welfare reasons on entry, and kittens under 6 months (Shelter A) or under 4 months (Shelter B).

All tests were carried out using either VetLab FASTest FeLV-FIV Combination Test (Shelter A) or IDEXX SNAP FeLV/ FIV Combo (Shelter B) patient-side tests, using anticoagulated whole blood (Shelter A) or separated serum (Shelter B). The SNAP Combo detects p27 antigen for FeLV (sensitivity 98.6%, specificity of 98.2%), and antibodies directed against p24 and gp40 for FIV (sensitivity 93.5%, specificity of 100%) (Hartmann and others 2007).. Similarly, the VetLab FASTest VetLab FASTtest detects p27 antigen for FeLV (sensitivity 95%, specificity 99%) and antibodies directed against gp40 for FIV (sensitivity 96%, specificity of 99%) (Vetlab_Supplies 2012).

Data collection

Computerised clinical records for every cat presented at both shelters during the study period were extracted, along with estimated age, sex, neuter status (if known) and test result. Details of clinical presentation were extracted by manually searching the free text records. All information was stored using Microsoft Excel version 10.

Data cleaning

Estimated age was provided at shelter A by calculating the difference between the date of consultation and the estimated date of birth on the record. Age was recorded in Shelter B in categories (0-3 months, >3-6 months, >6-9 months and yearly categories from then on), and therefore age from one year was treated as a categorical variable to ensure consistency within the dataset. For cats of < 1 year of age, interpolation was used to estimate age; for example cats assigned to the 3-6 month age group were nominally assigned an age of 137 days to enable comparison between the two data sets.

Where neuter status was not directly recorded in patient records, description within the records such as "spay scar present" or "no uterus on surgery" were used to assign neuter status. Animals for which a neuter procedure was recorded subsequent to the test were assigned the status of entire, as were pregnant or lactating animals. Pregnancy or admission with a litter was also evaluated separately as a risk factor.

In order to determine the health of each cat at the point of entry, the clinical notes for the first clinical consultation were interrogated by MM, from date of admission to up to three days after admission. Cats were considered healthy if "clinical exam NAD" (no abnormality detected) or similar phrasing was found. Lack of recorded signs was treated as missing data. Each clinical sign recorded was categorised by body system (eg limb fracture would be coded as "musculoskeletal"). In order to investigate a link with feline upper respiratory tract disease ("cat flu"), a separate variable was created where free text mentioned "cat flu/ URTD", or one or more of sneezing, ocular or nasal discharge. Similarly, "fight wounds" and "abscess(es)" were collapsed into a single category, as were "dental disease", "gingivitis" and "stomatitis". Body condition was dichotomised into "thin" (<3/5) or "not-thin" (≥3/5); again where body condition was not recorded this was treated as missing data.

Case definition

Any cat with a positive test result recorded for FeLV, FIV or both was treated as a case. Analysis for each disease was conducted separately; numbers of co-infected animals were too low for

meaningful statistical analysis. Kittens of under 3 months of age were excluded from analysis, due to a possible confounding effect from maternally derived antibodies to FIV (Ueland and Nesse 1992).

Data analysis

Analyses were carried out in Statistical Package for the Social Sciences (SPSS) Version 22.

Continuous data such as age were non-normal and therefore described in terms of medians and interquartile ranges.

Associations between each of the potential risk factors and FeLV and FIV test status was performed separately using univariable binomial regression. Factors with a p<0.2 in univariable analysis were considered for inclusion in a multivariable regression model, which was constructed using backwards elimination. Terms with a plausible biological association were tested for interaction and the most parsimonious model selected, using Pearson's correlation to determine whether to include or reject terms with the appearance of correlation. Rejected terms were rechecked individually against the final model. Age (in days) was non-linear and therefore log-transformed. Goodness-of-fit was assessed using the Hosmer and Lemeshow test. Significance was set at p<0.05 throughout.

Results

Overall, data were obtained from a total of 726 cats, 523 from Shelter A, and 203 from Shelter B. Although the minimum age for testing differed between Shelter A and Shelter B, in practice, the majority of kittens of under 3 months from both shelters were not tested (41/57, 71.9%). Of the 16 kittens aged under 3 months which were tested, all were from Shelter B, and one tested positive for FIV, and none for FeLV. The majority of kittens aged 3-6 months from both shelters were tested (35/44, 79.5%) for both FeLV and FIV. Therefore, 57 kittens aged under 3 months were excluded from the analysis, whilst kittens of 3-6 months were included. The median age for Shelter A was 3 years (Interquartile range [IQR] 1, 5), and for Shelter B was 3 years (IQR 1, 6.5). Test results were missing from a further 60 cats (28 from Shelter A, 32 from Shelter B), giving a total of 609 cats in the final dataset, 474 from Shelter A, and 135 from Shelter B. Of

these, four cats cat had only an FIV result (of which one was positive), and no FeLV result recorded. Therefore the final dataset was 609 cats for FIV and 605 for FeLV (Table 1).

		Number of cats (%)		
		Shelter A	Shelter B	
Sex	Male	295 (62.2)	65 (48.1)	
	Female	169 (35.7)	70 (51.9)	
	Unknown/ not recorded	10 (2.1)	0	
Neuter status	Neutered	61 (12.9)	5 (3.7)	
	Entire	313 (66)	61 (45.2)	
	Unknown/ not recorded	100 (21.1)	69 (51.1)	
FIV status	Positive	54 (11.4)	4 (3)	
	Negative	420 (88.6)	131 (97)	
FeLV status	Positive	14 (3)	0	
	Negative	459 (96.8)	132 (97.8)	
	Missing	1 (0.2)	3 (2.2)	

Table 1: Overall demographics and test results for cats from Shelter A and Shelter B

Disease prevalence

The overall prevalence of FIV was 9.5 % (95% CI 7.4-12.1%) and of FeLV was 2.3% (95% CI 1.4-3.9). Cats at Shelter A were significantly more likely to be infected with FIV (p=0.0024) or FeLV (p=0.048) than cats at Shelter B. Two cats (both from Shelter A) were co-infected with FIV and FeLV.

193 Age

Cats that tested positive for FIV were significantly older than those that tested negative (p<0.001), with positive cats having a median age of 1864 days (5.1 years; 95% CI 1627-2101

days) as compared to 1244 days (3.4 years; 95% CI 1157-1131 days) for negative cats. Likewise, FeLV positive cats were significantly older than FeLV negative cats, with positive cats having a mean age of 1735 days (4.75 years; 95% CI 1273-2197), as compared to 1289 days (3.5 years; 95% CI 1204 – 1373 days) for FeLV negative cats (p=0.06).

Univariable analysis

Risk factors which were eligible for inclusion in the multivariable logistic regression model for FIV are shown in Table 2.

			95% C.I. for Odds Ratio		
Factor (reference category is	Significance	Odds Ratio	Lower		
disease absence)				Upper	
Sex (Reference category female)	<0.001	43.8	6.03	319.0	
Neurological signs	.035	2.6	1.07	6.2	
Fight wounds/ abscess	<0.001	5.8	3.1	10.7	
Cat flu	.016	2.4	1.2	4.7	
Dental disease/ stomatitis	.172	1.6	.8	3.2	
Fleas	.138	1.8	.8	4.1	
Log age (days)	<0.001	9.5	3.9	23.5	
Body condition (low or not-low)	.177	1.8	.8	4.2	
Shelter	<0.001	4.2	1.5	11.8	

Table 2: Risk factors for FIV infection from univariable analysis which were considered for inclusion in the multivariable analysis

The final multivariable model for FIV suggested that male gender, clinical signs of cat flu and shelter were associated with an increased risk of infection. Increasing age was also associated with increased risk of positive test status (Table 3). Presence of fight wounds or abscesses was

considered for model inclusion but found to be strongly correlated with sex (Pearson correlation 0.251, p< 0.001) and was therefore removed from the final model.

	Significance	Odds ratio	95% CI for odds ratio	
	-		Lower	Upper
Sex (Reference category female)	.001	27.1	3.7	199.1
Shelter (Reference category Shelter B)	0.009	4.3	1.4	12.6
Log age (days)	.000	8.2	2.7	24.6

Table 3: Final multivariable model of risk factors for FIV test positive status. Nagelkerke R

Square value 0.261, Hosmer and Lemeshow p=0.773

Table 4 shows the factors from the univariable analysis which were eligible for the model for FeLV. The final multivariable model retained only thin body condition score and signs of musculoskeletal disease as risk factors for a positive FeLV test result (Table 5).

			95% CI for odds ratio		
Factor (reference category is					
disease absence)	Significance	Odds ratio	Lower	Upper	
Sex (Male = 1)*	.382	1.7	.5	5.4	
Musculoskeletal disease	.028	3.4	1.1	9.9	
Ocular disease	.144	2.4	.7	7.9	
Body Condition	.000	10.3	3.4	31.2	
Log age (days)	.050	5.0	1.0	25.3	
Anaemia	.008	8.8	1.8	44.0	
ĺ					

Table 4: Results of univariable analysis for FeLV test-positive status which were nominated for inclusion in the multivariable model. *Sex was not included in the model, but is shown here for comparison with FIV. Shelter could not be included as a variable, as there were no FeLV positive cats at Shelter B.

Significance	Odds ratio	95% CI for odds ratio

			Lower	Upper
Body condition (thin or not-thin)	<0.001	11.8	3.8	37.1
Musculoskeletal disease	0.016	4.0	1.3	12.5

Table 5: Final multivariable model of risk factors for FeLV test positive status. Nagelkerke R

Square value 0.157, Hosmer and Lemeshow p=0.916

Discussion

220

221

222

223

224

225

226

227

228

229

230

231

232

233

234

235

236

237

238

239

240

241

242

243

This study has shown a variable prevalence of FeLV and FIV in two populations of animals entering charity hospital and rehoming services. Despite the similar criteria for admission to these two sites, the difference in the disease prevalence in these two populations suggests there must be some critical distinctions between them. Both populations of cats consisted of those presented by RSPCA inspectors as stray, injured or confiscated for reasons of animal welfare, as well as some directly relinquished by owners (Shelter B only). It is possible that the proportions of these subcategories differed between the two shelters, or that factors such as inferred greater population density in the urban environment may have contributed to this variation in risk; cats from inner city environments have previously been found to be at increased risk of FIV infection (Malik and others 1997). Additionally, the proportion of male cats was higher in Shelter A than Shelter B (62.2% as compared with 48.1%), which could have also contributed to the higher prevalence of FIV. Although these specific results cannot be generalised across the whole feline population, the disparity between the prevalences found does reinforce the importance of knowledge of local patterns of infection when designing and administering a testing policy. Interestingly, the prevalence of both FeLV and FIV at RSPCA BACH seems to be remarkably similar to the prevalence revealed in 2002, in the same hospital, also using patient-side testing (Muirden 2002). It is uncertain why this may be the case, especially given that no FeLV positives were detected in Shelter B, but it is possible that factors such as vaccine coverage (for FeLV) and neutering rates could be implicated. Sub-populations of cats with limited engagement with veterinary care have been identified (Aegerter J. and others 2017), and it may be hypothesised that the BACH cats could be largely drawn from one such sub-population.

In terms of seropositivity to FIV, entire males are clearly at a much increased risk of infection, which agrees with previous studies (Courchamp and others 1998; Levy and others 2006). The additional link between FIV seropositivity and presence of fight wounds and abscesses supports the previously demonstrated relationship with behaviours associated with increased likelihood of transmission. FIV positive cats were significantly older than FIV-negative cats, and this is consistent with other studies suggesting that middle-aged cats are more commonly infected (Levy and others 2006; Spada and others 2012), due to cumulative risk of infection over time, or to the time taken to develop FIV-associated illness. In studies where the test is used for diagnosis rather than screening, the cats may be presented at an older age due to a delay between infection and development of clinical signs of sufficient severity to be presented for veterinary treatment. On univariable analysis, clinical signs consistent with cat flu were also associated with increased risk of FIV seropositivity, presumably due to immunosuppression in the presence of near-ubiquitous respiratory pathogens. This factor was eventually excluded from the multivariable model; however this may be worthy of further investigation, as an association with cat flu would agree with previous findings (Hosie and others 1989), and may be useful in guiding choices about which cats to test. For cats testing positive for FeLV, being thin on presentation (BCS<3/5) was significantly associated with infection (OR 11.8, 95% CI 3.8-37.1). Presence of musculoskeletal disease, such as lameness or fractures, was also identified as a risk factor (OR 4.0, 95% CI 1.3, 12.5) and again may reflect a link with risky behaviours such as roaming and fighting. Again, FeLV-positive cats were typically older than the remainder of the study population, with a mean age of 4.75 years. Whilst a little older than FeLV-positive populations in previous studies, this finding is broadly consistent with previous data showing the highest FeLV prevalence to be in young to middle aged adults (de Almeida and others 2012; Gleich and others 2009a; Hellard and others 2011b). However, the complex interplay of the risk of infection, a cat's immune response and the curtailed lifespan of progressively infected cats make it difficult to generalise about the interaction

between risk of FeLV infection and age on a population basis.

244

245

246

247

248

249

250

251

252

253

254

255

256

257

258

259

260

261

262

263

264

265

266

267

268

269

Limitations must be considered when interpreting these data. The results were collected over a year, from two shelters recruited on a convenience basis. Therefore, they may not be representative of all such organisations. Data were collected retrospectively, meaning that some information was missing. This may have caused an underestimation of prevalence, as animals sick enough to be euthanased without testing, soon after presentation, may have been at increased risk of infection. Similarly, missing data, either due to incomplete recording of test results or regarding specific clinical signs could have potentially affected study power, although the effect of this is difficult to quantify. Animal origin (stray, relinquished etc) was too inconsistently recorded to be included in the analysis. This incompleteness is reflected in the relatively low R² values for both models, which suggests the importance of other, unmeasured factors is significant.

The prevalence of FeLV in this study was low, limiting the statistical power to examine risk factors; a larger study would be required to more fully investigate the impact of risk factors. Finally, the combination of a relatively low population prevalence and imperfect test specificity could have led to some false positives, inflating the apparent FeLV prevalence. However, without the use of PCR tests, regressive infections would have been missed, increasing uncertainty around the FeLV prevalence estimates.

Conclusions

These findings suggest that within the cats at BACH (Shelter A), neither FIV nor FeLV prevalence markedly changed over the ten years prior to data collection. This implies that factors such as FeLV vaccination and neutering coverage could be improved upon in this population of cats, although it is also important to note that in both populations, FeLV infection was relatively uncommon. Initiatives to improve feline welfare and control over-population have been instigated over recent years, including a move to prepubertal neutering and multi-agency approaches to engage hard-to-reach owners (Joyce and Yates 2011; Roberts and Clements 2015), and outcomes from such projects should be closely monitored to determine where resources can be used for maximum impact. In the study population, being male, presence of fight wounds and abscesses and clinical signs of cat flu were associated with seropositivity for FIV infection. Thin

299 body condition and signs of musculoskeletal disease were associated with increased risk of 300 testing FeLV positive. These factors could be used to prioritise at-risk cats for testing, informing the use of FIV and FeLV tests within a charity context, to maximise test predictive value and 301 302 improve efficient uses of resources to promote feline health and wellbeing. 303 **Acknowledgements** 304 The authors would like to acknowledge all the staff at the RSPCA BACH and Block Fen Animal 305 Centre especially Rebecca Willby, Rebecca Ailsby and Laurie Curtis for allowing us access to 306 their records and help with data extraction. **Ethics** 307 308 This project has been ethically reviewed and approved by a panel at the University of Nottingham 309 School of Veterinary Medicine and Science 310 **Funding** The Centre for Evidence-based Veterinary Medicine is supported by an unrestrictive grant from 311 312 Elanco Animal Health and the University of Nottingham Conflict of Interest 313 The authors declared no potential conflicts of interest with respect to the research, authorship, 314 315 and/or publication of this article. References AEGERTER J., FOURACRE D. & G.C., S. (2017) A first estimate of the structure and density of the 316 317 populations of pet cats and dogs across Great Britain. PLoS One BANDE, F., ARSHAD, S. S., HASSAN, L., ZAKARIA, Z., SAPIAN, N. A., RAHMAN, N. A. & ALAZAWY, A. 318 319 (2012a) Prevalence and risk factors of feline leukaemia virus and feline immunodeficiency virus in 320 peninsular Malaysia. BMC Veterinary Research [Electronic Resource] 8, 33 321 BANDE, F., ARSHAD, S. S., HASSAN, L., ZAKARIA, Z., SAPIAN, N. A., RAHMAN, N. A. & ALAZAWY, A. 322 (2012b) Prevalence and risk factors of feline leukaemia virus and feline immunodeficiency virus in 323 peninsular Malaysia. BMC Veterinary Research 8, 33 CATTORI, V., TANDON, R., RIOND, B., PEPIN, A. C., LUTZ, H. & HOFMANN-LEHMANN, R. (2009) The 324 325 kinetics of feline leukaemia virus shedding in experimentally infected cats are associated with

infection outcome. Veterinary Microbiology 133, 292-296

- 327 COURCHAMP, F., YOCCOZ, N. G., ARTOIS, M. & PONTIER, D. (1998) At-risk individuals in Feline
- 328 Immunodeficiency Virus epidemiology: evidence from a multivariate approach in a natural
- 329 population of domestic cats (Felis catus). Epidemiol Infect 121, 227-236
- 330 DE ALMEIDA, N. R., DANELLI, M. G. M., DA SILVA, L. H. P., HAGIWARA, M. K. & MAZUR, C. (2012)
- 331 Prevalence of feline leukemia virus infection in domestic cats in Rio de Janeiro. Journal of Feline
- 332 Medicine & Surgery 14, 583-586
- 333 ENGLERT, T., LUTZ, H., SAUTER-LOUIS, C. & HARTMANN, K. (2012) Survey of the feline leukemia
- virus infection status of cats in Southern Germany. Journal of Feline Medicine & Surgery 14, 392-398
- 335 FRANCIS, D. P., ESSEX, M. & HARDY, W. D. (1977) Excretion of feline leukaemia virus by naturally
- 336 infected pet cats. Nature 269, 252-254
- 337 FRIEND, S. C. E., BIRCH, C. J., LORDING, P. M., MARSHALL, J. A. & STUDDERT, M. J. (1990) Feline
- 338 immunodeficiency virus: prevalence, disease associations and isolation. Australian Veterinary
- 339 Journal 67, 237-243
- 340 GABOR, L. J., JACKSON, M. L., TRASK, B., MALIK, R. & CANFIELD, P. J. (2001a) Feline leukaemia virus
- 341 status of Australian cats with lymphosarcoma. Australian Veterinary Journal 79, 476-481
- 342 GABOR, L. J., LOVE, D. N., MALIK, R. & CANFIELD, P. J. (2001b) Feline immunodeficiency virus status
- of Australian cats with lymphosarcoma. Aust Vet J 79, 540-545
- 344 GLEICH, S. E., KRIEGER, S. & HARTMANN, K. (2009a) Prevalence of feline immunodeficiency virus
- 345 and feline leukaemia virus among client-owned cats and risk factors for infection in Germany. J
- 346 Feline Med Surg 11, 985-992
- 347 GLEICH, S. E., KRIEGER, S. & HARTMANN, K. (2009b) Prevalence of feline immunodeficiency virus
- and feline leukaemia virus among client-owned cats and risk factors for infection in Germany.
- 349 Journal of Feline Medicine and Surgery 11, 985-992
- 350 GOLDKAMP, C. E., LEVY, J. K., EDINBORO, C. H. & LACHTARA, J. L. (2008) Seroprevalences of feline
- 351 leukemia virus and feline immunodeficiency virus in cats with abscesses or bite wounds and rate of
- 352 veterinarian compliance with current guidelines for retrovirus testing. J Am Vet Med Assoc 232
- 353 GOMES-KELLER, M. A., GONCZI, E., TANDON, R., RIONDATO, F., HOFMANN-LEHMANN, R., MELI, M.
- L. & LUTZ, H. (2006) Detection of feline leukemia virus RNA in saliva from naturally infected cats and
- 355 correlation of PCR results with those of current diagnostic methods. J Clin Microbiol 44, 916-922
- 356 HARTMANN, K. (2011) Clinical aspects of feline immunodeficiency and feline leukemia virus
- infection. Veterinary Immunology & Immunopathology 143, 190-201
- 358 HARTMANN, K. (2012) Clinical aspects of feline retroviruses: a review. Viruses 4, 2684-2710
- 359 HARTMANN, K., GRIESSMAYR, P., SCHULZ, B., GREENE, C. E., VIDYASHANKAR, A. N., JARRETT, O. &
- 360 EGBERINK, H. F. (2007) Quality of different in-clinic test systems for feline immunodeficiency virus
- and feline leukaemia virus infection. Journal of Feline Medicine & Surgery 9, 439-445
- 362 HELLARD, E., FOUCHET, D., SANTIN-JANIN, H., TARIN, B., BADOL, V., COUPIER, C., LEBLANC, G.,
- POULET, H. & PONTIER, D. (2011a) When cats' ways of life interact with their viruses: a study in 15
- 364 natural populations of owned and unowned cats (Felis silvestris catus). Preventive Veterinary
- 365 Medicine 101, 250-264
- 366 HELLARD, E., FOUCHET, D., SANTIN-JANIN, H., TARIN, B., BADOL, V., COUPIER, C., LEBLANC, G.,
- POULET, H. & PONTIER, D. (2011b) When cats' ways of life interact with their viruses: a study in 15
- 368 natural populations of owned and unowned cats (Felis silvestris catus). Prev Vet Med 101
- 369 HOOVER, E. A., OLSEN, R. G., HARDY, W. D., JR., SCHALLER, J. P. & MATHES, L. E. (1976) Feline
- leukemia virus infection: age-related variation in response of cats to experimental infection. J Natl
- 371 Cancer Inst 57, 365-369
- 372 HOSIE, M., ROBERTSON, C. & JARRETT, O. (1989) Prevalence of feline leukaemia virus and antibodies
- to feline immunodeficiency virus in cats in the United Kingdom. Veterinary Record 125, 293-297
- 374 JACKSON, M. L., HAINES, D. M., MERIC, S. M. & MISRA, V. (1993) Feline leukemia virus detection by
- immunohistochemistry and polymerase chain reaction in formalin-fixed, paraffin-embedded tumor
- tissue from cats with lymphosarcoma. Canadian Journal of Veterinary Research 57, 269-276

- 377 JOYCE, A. & YATES, D. (2011) Help Stop Teenage Pregnancy!: Early-Age Neutering in Cats. Journal of
- 378 Feline Medicine and Surgery 13, 3-10
- 379 LEVY, J. K., SCOTT, H. M., LACHTARA, J. L. & CRAWFORD, P. C. (2006) Seroprevalence of feline
- 380 leukemia virus and feline immunodeficiency virus infection among cats in North America and risk
- factors for seropositivity. J Am Vet Med Assoc 228, 371-376
- 382 LITTLE, S. (2011) A review of feline leukemia virus and feline immunodeficiency virus seroprevalence
- in cats in Canada. Veterinary Immunology and Immunopathology 143, 243-245
- 384 LITTLE, S., SEARS, W., LACHTARA, J. & BIENZLE, D. (2009) Seroprevalence of feline leukemia virus
- and feline immunodeficiency virus infection among cats in Canada. Can Vet J 50, 644-648
- LITTLE, S. E. (2005) Feline immunodeficiency virus testing in stray, feral, and client-owned cats of
- 387 Ottawa. Can Vet J 46, 898-901
- 388 LUTZ, H., ADDIE, D., BELÁK, S., BOUCRAUT-BARALON, C., EGBERINK, H., FRYMUS, T., GRUFFYDD-
- JONES, T., HARTMANN, K., HOSIE, M. J., LLORET, A., MARSILIO, F., PENNISI, M. G., RADFORD, A. D.,
- 390 THIRY, E., TRUYEN, U. & HORZINEK, M. C. (2009) Feline Leukaemia: ABCD Guidelines on Prevention
- and Management. Journal of Feline Medicine and Surgery 11, 565-574
- 392 MAJOR, A., CATTORI, V., BOENZLI, E., RIOND, B., OSSENT, P., MELI, M., LUISA, HOFMANN-LEHMANN,
- 393 R. & LUTZ, H. (2010) Exposure of cats to low doses of FeLV: seroconversion as the sole parameter of
- 394 infection. Vet. Res. 41, 17
- 395 MALIK, R., KENDALL, K., CRIDLAND, J., COULSTON, S., STUART, A. J., SNOW, D. & LOVE, D. N. (1997)
- 396 Prevalences of feline leukaemia virus and feline immunodeficiency virus infections in cats in Sydney.
- 397 Aust Vet J 75, 323-327
- 398 MUIRDEN, A. (2002) Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency
- 399 virus and feline coronavirus in stray cats sent to an RSPCA hospital. Veterinary Record 150, 621-625
- 400 MURRAY, J. K., SKILLINGS, E. & GRUFFYDD-JONES, T. J. (2008) A study of risk factors for cat mortality
- in adoption centres of a UK cat charity. Journal of Feline Medicine and Surgery 10, 338-345
- 402 NESINA, S., KATRIN HELFER-HUNGERBUEHLER, A., RIOND, B., BORETTI, F. S., WILLI, B., MELI, M. L.,
- 403 GREST, P. & HOFMANN-LEHMANN, R. (2015) Retroviral DNA—the silent winner: blood transfusion
- 404 containing latent feline leukemia provirus causes infection and disease in naïve recipient cats.
- 405 Retrovirology 12, 105
- 406 NORRIS, J. M., BELL, E. T., HALES, L., TORIBIO, J. A., WHITE, J. D., WIGNEY, D. I., BARAL, R. M. &
- 407 MALIK, R. (2007) Prevalence of feline immunodeficiency virus infection in domesticated and feral
- 408 cats in eastern Australia. J Feline Med Surg 9, 300-308
- 409 RAVI, M., WOBESER, G. A., TAYLOR, S. M. & JACKSON, M. L. (2010) Naturally acquired feline
- 410 immunodeficiency virus (FIV) infection in cats from western Canada: Prevalence, disease
- associations, and survival analysis. The Canadian Veterinary Journal 51, 271-276
- 412 ROBERTS, M. & CLEMENTS, J. (2015) Using early neutering to control unwanted litters. Veterinary
- 413 Record 176, 570-571
- 414 SPADA, E., PROVERBIO, D., DELLA PEPA, A., PEREGO, R., BAGGIANI, L., DEGIORGI, G. B.,
- 415 DOMENICHINI, G., FERRO, E. & CREMONESI, F. (2012) Seroprevalence of feline immunodeficiency
- 416 virus, feline leukaemia virus and Toxoplasma gondii in stray cat colonies in northern Italy and
- 417 correlation with clinical and laboratory data. Journal of Feline Medicine & Surgery 14, 369-377
- 418 STAVISKY, J., BRENNAN, M. L., DOWNES, M. & DEAN, R. (2012) Demographics and economic burden
- of un-owned cats and dogs in the UK: results of a 2010 census. BMC Veterinary Research [Electronic
- 420 Resource] 8, 163
- 421 STÜTZER, B., SIMON, K., LUTZ, H., MAJZOUB, M., HERMANNS, W., HIRSCHBERGER, J., SAUTER-LOUIS,
- 422 C. & HARTMANN, K. (2011) Incidence of persistent viraemia and latent feline leukaemia virus
- 423 infection in cats with lymphoma. Journal of Feline Medicine and Surgery 13, 81-87
- 424 TEIXEIRA, B. M., HAGIWARA, M. K., CRUZ, J. C. M. & HOSIE, M. J. (2012) Feline immunodeficiency
- virus in South America. Viruses 4, 383-396
- 426 UELAND, K. & NESSE, L. L. (1992) No evidence of vertical transmission of naturally acquired feline
- 427 immunodeficiency virus infection. Veterinary Immunology & Immunopathology 33, 301-308

- 428 VETLAB_SUPPLIES (2012) FASTest FeLV-FIV test kit. West Sussex
- 429 YAMAMOTO, J. K., HANSEN, H., HO, E. W., MORISHITA, T. Y., OKUDA, T., SAWA, T. R., NAKAMURA, R.
- 430 M. & PEDERSEN, N. C. (1989) Epidemiologic and clinical aspects of feline immunodeficiency virus
- infection in cats from the continental United States and Canada and possible mode of transmission.
- 432 Journal of the American Veterinary Medical Association 194, 213-220
- 433 YILMAZ, H., ILGAZ, A. & HARBOUR, D. A. (2000) Prevalence of FIV and FeLV infections in cats in
- 434 Istanbul. J Feline Med Surg 2, 69-70