

Title: Prevalence of and risk factors for FIV and FeLV infection in two shelters in the United

Kingdom (2011-2012)

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

1 Abstract

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

11 those at shelter B to test positive for FIV (P = 0.0024) and FeLV (P = 0.048). Male cats were more

12 likely to be infected with FIV (OR 27.1, p=0.001), and thin body condition and musculoskeletal

13 disease were associated with risk of FeLV. Overall, FIV and FeLV positive cats were significantly

14 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.

20 Introduction

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).

25 FeLV is considered to be a "friendly cat" disease, with transmission by mutual grooming and 26 sharing of food and water bowls considered to be the most prominent routes of transmission, 27 although transmission by fighting and biting may also occur (Cattori and others 2009; Francis 28 and others 1977; Hartmann 2011, 2012). Exposure may result in a variety of outcomes, including 29 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 30 31 become clear that apparently "recovered", antigen-negative cats may retain proviral DNA in bone 32 marrow progenitor cells (Lutz and others 2009; Major and others 2010). Such cats, termed 33 "regressively infected" will test negative for circulating antigen and are at low risk of FeLV-34 associated disease. Some studies have suggested that regressively infected cats may be at 35 increased risk of FeLV-associated disease such as lymphoma and anaemia; however others 36 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 37 38 shown that regressive cats can be a source of FeLV infection via blood transfusion (Nesina and 39 others 2015). Progressive FeLV infection occurs when there is an inadequate immune response 40 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). 41

42 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

48 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 49 50 neoplasia, particularly lymphomas (Gabor and others 2001a; Gabor and others 2001b). As both 51 diseases can cause immunosuppression and reduced immunosurveillance, recurrent or 52 recalcitrant presentations of common diseases such as feline upper respiratory tract infection 53 ("cat flu") have also been suggested to be potential indicators of infection (Hartmann 2012; 54 Yamamoto and others 1989). Adult cats have been identified as being at greater risk than 55 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 56 1997). Adult cats have been identified as at greater risk of FeLV infection than juveniles (Levy 57 and others 2006), however it has been suggested that a degree of age related immunity occurs, 58 59 meaning that when infection does occur in later life, it may be more likely to be regressive 60 (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 61 62 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 63 64 and others 2012; Hartmann 2012; Lutz and others 2009). Data for trends over time suggests that 65 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 66 67 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 68 69 limits comparison between them (Little 2011; Ravi and others 2010; Teixeira and others 2012). 70 Current trends in UK prevalence for both diseases are hard to establish as relatively few data are 71 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-

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.

83 Many cat shelters perform FIV and/or FeLV testing on some or all of their animals, as part of 84 health screening for the individual, population healthcare, and also to provide reassurance to 85 prospective owners. These typically utilise patient-side tests which detect FIV antibody and FeLV 86 antigen respectively. FIV and FeLV infection have been shown to be one of the most common 87 reasons for death or euthanasia in shelters (Murray and others 2008). However, if every one of 88 the estimated 130,000 cats passing through rescue shelters in the UK annually (Stavisky and 89 others 2012) was tested, this would add significantly to the cost of caring for and rehoming these 90 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 91 92 difficulties in interpretation of the results. Cats which test positive for FeLV may undergo 93 confirmatory tests such as PCR, and further tests to differentiate progressive from regressive 94 infection, which incurs both a financial cost and potentially an extended stay in a rescue facility. It 95 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 96 97 expedite rehoming of cats.

98 The aim of this study was to determine the prevalence of FeLV and FIV infection in cats 99 presented to two charities in the UK, as determined by a patient-side test. A secondary aim was 100 to identify risk factors for infection within the populations under study.

101 Materials and Methods

102 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.

110 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).

117 All tests were carried out using either VetLab FASTest FeLV-FIV Combination Test (Shelter A) or 118 IDEXX SNAP FeLV/ FIV Combo (Shelter B) patient-side tests, using anticoagulated whole blood 119 (Shelter A) or separated serum (Shelter B). The SNAP Combo detects p27 antigen for FeLV 120 (sensitivity 98.6%, specificity of 98.2%), and antibodies directed against p24 and gp40 for FIV 121 (sensitivity 93.5%, specificity of 100%) (Hartmann and others 2007).. Similarly, the VetLab 122 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 123 2012). 124

125 Data collection

126 Computerised clinical records for every cat presented at both shelters during the study period 127 were extracted, along with estimated age, sex, neuter status (if known) and test result. Details of 128 clinical presentation were extracted by manually searching the free text records. All information 129 was stored using Microsoft Excel version 10.

130 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.

143 In order to determine the health of each cat at the point of entry, the clinical notes for the first 144 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 145 146 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 147 148 "musculoskeletal"). In order to investigate a link with feline upper respiratory tract disease ("cat 149 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 150 collapsed into a single category, as were "dental disease", "gingivitis" and "stomatitis". Body 151 condition was dichotomised into "thin" (<3/5) or "not-thin" (≥3/5); again where body condition was 152 153 not recorded this was treated as missing data.

154 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).

160 Data analysis

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

162 Continuous data such as age were non-normal and therefore described in terms of medians and163 interquartile ranges.

164 Associations between each of the potential risk factors and FeLV and FIV test status was 165 performed separately using univariable binomial regression. Factors with a p<0.2 in univariable 166 analysis were considered for inclusion in a multivariable regression model, which was 167 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 168 169 determine whether to include or reject terms with the appearance of correlation. Rejected terms 170 were rechecked individually against the final model. Age (in days) was non-linear and therefore 171 log-transformed. Goodness-of-fit was assessed using the Hosmer and Lemeshow test. 172 Significance was set at p<0.05 throughout.

173 Results

174 Overall, data were obtained from a total of 726 cats, 523 from Shelter A, and 203 from Shelter B. 175 Although the minimum age for testing differed between Shelter A and Shelter B, in practice, the 176 majority of kittens of under 3 months from both shelters were not tested (41/57, 71.9%). Of the 177 16 kittens aged under 3 months which were tested, all were from Shelter B, and one tested 178 positive for FIV, and none for FeLV. The majority of kittens aged 3-6 months from both shelters 179 were tested (35/44, 79.5%) for both FeLV and FIV. Therefore, 57 kittens aged under 3 months 180 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, 181 6.5). Test results were missing from a further 60 cats (28 from Shelter A, 32 from Shelter B), 182 giving a total of 609 cats in the final dataset, 474 from Shelter A, and 135 from Shelter B. Of 183

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)

- 187 Table 1: Overall demographics and test results for cats from Shelter A and Shelter B
- 188 Disease prevalence
- 189 The overall prevalence of FIV was 9.5 % (95% CI 7.4-12.1%) and of FeLV was 2.3% (95% CI
- 190 1.4-3.9). Cats at Shelter A were significantly more likely to be infected with FIV (p=0.0024) or
- 191 FeLV (p=0.048) than cats at Shelter B. Two cats (both from Shelter A) were co-infected with FIV
- and FeLV.
- 193 Age
- 194 Cats that tested positive for FIV were significantly older than those that tested negative
- 195 (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.
- 197 Likewise, FeLV positive cats were significantly older than FeLV negative cats, with positive cats
- 198 having a mean age of 1735 days (4.75 years; 95% CI 1273-2197), as compared to 1289 days
- 199 (3.5 years; 95% CI 1204 1373 days) for FeLV negative cats (p=0.06).
- 200 Univariable analysis
- 201 Risk factors which were eligible for inclusion in the multivariable logistic regression model for FIV202 are shown in Table 2.
- 203

			95% C.I. for Odds Ratio		
Factor (reference category is					
disease absence)	Significance	Odds Ratio	Lower	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

205 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

208 with increased risk of positive test status (Table 3). Presence of fight wounds or abscesses was

- 209 considered for model inclusion but found to be strongly correlated with sex (Pearson correlation
- 210 0.251, p< 0.001) and was therefore removed from the final model.

	Significance	Odds ratio	95% CI for odds r 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

- 211 Table 3: Final multivariable model of risk factors for FIV test positive status. Nagelkerke R
- 212 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
- 214 FeLV. The final multivariable model retained only thin body condition score and signs of
- 215 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	

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

|--|

			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

222 Discussion

223 This study has shown a variable prevalence of FeLV and FIV in two populations of animals 224 entering charity hospital and rehoming services. Despite the similar criteria for admission to 225 these two sites, the difference in the disease prevalence in these two populations suggests there 226 must be some critical distinctions between them. Both populations of cats consisted of those 227 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 228 229 these subcategories differed between the two shelters, or that factors such as inferred greater 230 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 231 232 (Malik and others 1997). Additionally, the proportion of male cats was higher in Shelter A than 233 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 234 235 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. 236

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.

244 In terms of seropositivity to FIV, entire males are clearly at a much increased risk of infection, 245 which agrees with previous studies (Courchamp and others 1998; Levy and others 2006). The 246 additional link between FIV seropositivity and presence of fight wounds and abscesses supports 247 the previously demonstrated relationship with behaviours associated with increased likelihood of 248 transmission. FIV positive cats were significantly older than FIV-negative cats, and this is 249 consistent with other studies suggesting that middle-aged cats are more commonly infected 250 (Levy and others 2006; Spada and others 2012), due to cumulative risk of infection over time, or 251 to the time taken to develop FIV-associated illness. In studies where the test is used for 252 diagnosis rather than screening, the cats may be presented at an older age due to a delay 253 between infection and development of clinical signs of sufficient severity to be presented for 254 veterinary treatment. On univariable analysis, clinical signs consistent with cat flu were also 255 associated with increased risk of FIV seropositivity, presumably due to immunosuppression in 256 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 257 258 with cat flu would agree with previous findings (Hosie and others 1989), and may be useful in 259 guiding choices about which cats to test.

260 For cats testing positive for FeLV, being thin on presentation (BCS<3/5) was significantly 261 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 262 263 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 4.75 264 265 years. Whilst a little older than FeLV-positive populations in previous studies, this finding is 266 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 267 2011b). However, the complex interplay of the risk of infection, a cat's immune response and the 268 curtailed lifespan of progressively infected cats make it difficult to generalise about the interaction 269 270 between risk of FeLV infection and age on a population basis.

271 Limitations must be considered when interpreting these data. The results were collected over a 272 year, from two shelters recruited on a convenience basis. Therefore, they may not be 273 representative of all such organisations. Data were collected retrospectively, meaning that some 274 information was missing. This may have caused an underestimation of prevalence, as animals 275 sick enough to be euthanased without testing, soon after presentation, may have been at 276 increased risk of infection. Similarly, missing data, either due to incomplete recording of test 277 results or regarding specific clinical signs could have potentially affected study power, although 278 the effect of this is difficult to quantify. Animal origin (stray, relinquished etc) was too 279 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 280 281 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.

288 Conclusions

289 These findings suggest that within the cats at BACH (Shelter A), neither FIV nor FeLV 290 prevalence markedly changed over the ten years prior to data collection. This implies that factors 291 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 292 293 uncommon. Initiatives to improve feline welfare and control over-population have been instigated 294 over recent years, including a move to prepubertal neutering and multi-agency approaches to 295 engage hard-to-reach owners (Joyce and Yates 2011; Roberts and Clements 2015), and 296 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 297 298 abscesses and clinical signs of cat flu were associated with seropositivity for FIV infection. Thin

- 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
- 301 the use of FIV and FeLV tests within a charity context, to maximise test predictive value and
- 302 improve efficient uses of resources to promote feline health and wellbeing.
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- 314 The authors declared no potential conflicts of interest with respect to the research, authorship,
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