Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

The protective rate of the feline immunodeficiency virus vaccine: An Australian field study

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ARTICLE INFO

Article history:
Received 28 April 2016
Received in revised form 15 June 2016
Accepted 18 June 2016
Available online xxxx

Keywords:
Feline immunodeficiency virus
Human immunodeficiency virus
Vaccine protective rate
Vaccine effectiveness
FIV vaccine
HIV vaccine
Cats

ABSTRACT

A case-control field study was undertaken to determine the level of protection conferred to client-owned cats in Australia against feline immunodeficiency virus (FIV) using a commercial vaccine. 440 cats with outdoor access from five Australian states/territories underwent testing, comprising 139 potential cases (complete course of primary FIV vaccinations and annual boosters for three or more years), and 301 potential controls (age, sex and postcode matched FIV-unvaccinated cats). FIV status was determined using a combination of antibody testing (using point-of-care test kits) and nucleic acid amplification, as well as virus isolation in cases where results were discordant and in all suspected FIV-vaccinated/FIV-infected cats ('vaccine breakthroughs'). Stringent inclusion criteria were applied to both 'cases' and 'controls'; 89 FIV-vaccinated cats and 212 FIV-unvaccinated cats ultimately satisfied the inclusion criteria. Five vaccine breakthroughs (5/89; 6%), and 25 FIV-infected controls (25/212; 12%) were identified, giving a vaccine protective rate of 56% (95% CI −20 to 84). The difference in FIV prevalence rates between the two groups was not significant ($P = 0.14$). Findings from this study raise doubt concerning the efficacy of Fel-O-Vax FIV® under field conditions. Screening for FIV infection may be prudent before annual FIV re-vaccination and for sick FIV-vaccinated cats. Owners should not rely on vaccination alone to protect cats against the risk of acquiring FIV infection; other measures such as cat curfews, the use of 'modular pet parks' or keeping cats exclusively indoors, are recommended.

1. Introduction

Feline immunodeficiency virus (FIV) was discovered in 1986 in a cat colony in California [1]. FIV is a retrovirus of the genus *Lentivirus*. It has a worldwide distribution and is subdivided into seven clades (subtypes) (A, B, C, D, E, F and U-NZenv) [2–5]. An estimated 14.5 million pet cats are infected with FIV worldwide, and 33.5 million if feral cats are included [2], which is similar to the estimated number (35 million) of individuals infected with human immunodeficiency virus (HIV-1) globally [6]. The FIV-cat model is advocated as a 'test-bed' for HIV infection and HIV-1 vaccine development, and Australia, which has one of the highest FIV prevalence rates in the world (8–15% in client-owned cats with outdoor access; 20–25% in feral cats), is an excellent setting to study FIV transmission and its prevention by vaccination [7–9].

The commercial release of a FIV vaccine[1] for use in domestic cats (USA 2002; Australia 2004) was the first time a vaccine had been registered for preventing infection by a *Lentivirus* in either human or veterinary medicine. More than 5000 laboratory cats were used over 14 years to develop a dual-subtype (A and D), inactivated whole cell (IWC) and inactivated whole virus (IWV) vaccine. 689 client-owned cats were used for safety testing in the field before the vaccine was released commercially. The result was a vaccine registered with a 'preventable fraction' (efficacy) of 68%, based on combined results from two laboratory-based efficacy studies involving 105 cats (52 FIV-vaccinated, 53 FIV-unvaccinated) challenged one year after receiving three FIV vaccinations administered three weeks apart (difference in percentage viraemia between the two groups [25% *vs* 79%] $P < 0.01$) [2].

To date, a total of 262 cats (139 FIV-vaccinated, 123 FIV-unvaccinated) have been tested using the current commercial FIV vaccine in laboratory-based efficacy studies (including the 105 cats from the two pre-registration studies), with reported vaccine efficacy of between 0% and 100%, and an overall preventable fraction of 66% [2,10–16] (Table 1). Extremely high challenge doses, intravenous challenge (which avoids innate immunity barriers), and the use of highly pathogenic strains for challenge (e.g. FIV_{UK8}), have been proffered as possible explanations for the variation in

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[1] Fel-O-Vax® FIV, Boehringer Ingelheim, Fort Dodge, IA, USA.

http://dx.doi.org/10.1016/j.vaccine.2016.06.060
0264-410X/

Table 1
Summary of laboratory-based efficacy studies in which Fel-O-Vax FIV® was given according to the manufacturer's guidelines (three subcutaneous injections 2–4 weeks apart, followed by a single annual booster in the long-term studies). Experimental vaccine efficacy (preventable fraction) = ([percentage viraemia in controls − percentage viraemia in vaccinates]/percentage viraemia in controls) [2]. Fel-O-Vax FIV® used in the first trial for USDA (United States Department of Agriculture) approval was a slightly different version to what was eventually registered and released commercially[a] [14,37]. Otherwise, studies where Fel-O-Vax FIV® was modified before administration, where Fel-O-Vax FIV® was administered via non-registered routes (e.g. intranasally) and where non-commercial vaccines (e.g. single- subtype FIV vaccines) were trialed are excluded. FDAH = Fort Dodge Animal Health, the parent company that developed and registered Fel-O-Vax FIV® (the FDAH vaccine range has since been acquired in Australia by Boehringer Ingelheim). CID_{50} = cat infectious dose 50, which is equivalent to the amount of virus required to cause infection in half of susceptible subjects. Conflicting CID_{50} doses are both presented[b] [12,15]. IM = intramuscular, IV = intravenous. Origins of homologous challenges: FIV_{Pet} (A) = California, USA; FIV_{Shi} (D) = Shizuoka, Japan, FIV_{UK8} (A) = Glasgow, UK. Origins of heterologous challenges: $FIV_{FD/US}$ (A) = California, USA; FIV_{FC1} (B) = Florida, USA; FIV_{Ao2} (B) (Aomori) = Aomori, Japan; FIV_{NZ1} (F′/C) = Auckland, New Zealand (prime sign represents that a full sequence of subtype F has yet to be identified) [15]; $FIV_{FD/DutA}$ (A) = Netherlands; FIV_{Bang} (A/B) = Massachusetts, USA. NA = not available.

Author	Challenge virus, clade, % difference from vaccine *env* sequence (FIV_{Pet} and FIV_{Shi})	Source	Dose ($\times CID_{50}$), route	Time after final vaccination	Viraemia in FIV-vaccinated cats	Viraemia in placebo controls	Vaccine efficacy (Preventable fraction, %)
FDAH (Study 1 for USDA license approval)[a] [10,15]	$FIV_{FD/US}$, A, 9% and 20%	*In vitro*	×1.47, IM	1 year	9/27 (PCR)	25/34 (PCR)	55
Huang (Study 2 for USDA license approval) [12,15]	$FIV_{FD/US}$, A, 9% and 20% (overall 11% difference in sequence)	*In vitro*	×1.79/11[b], IM	375 days	4/25 (PCR)	17/19 (PCR)	82
Pu [10]	FIV_{FC1}, B, 19% and 19.2%	*In vivo*	×15, IV	21 days	0/8 (VI)	9/9 (VI)	100
Kusuhara [14]	FIV_{Ao2}, B, 18.5% and 19.6%	*In vitro*	Natural, biting	21 days-19 months	0/6 (nested PCR)	4/8 (nested PCR)	100
Dunham [11]	FIV_{UK8}, A, NA	NA	×10, IM	28 days	5/5 (VI, RT-PCR)	6/6 (VI, RT-PCR)	0
Yamamoto [2,10]	FIV_{FC1}, B, 19% and 19.2%	NA	×100, IV	3–4 weeks	3/4	4/4	25
Yamamoto [15]	$FIV_{FD/DutA}$, A, NA	NA	×1.73, IM	NA	3/24	13/15	86
Huang [10,13]	FIV_{FC1}, B, 19% and 19.2%	*In vivo*	×1000 PMBC, IV	54 weeks	4/14 (PCR, RT-PCR)	5/5 (PCR, RT-PCR)	71
Coleman [10,16]	(i) FIV_{Bang}, A/B, NA	*In vivo*	NA, IV	3–4 weeks	3/4 (VI, PCR)	4/4 (VI, PCR)	25
	(ii) FIV_{FC1}, B, 19% and 19.2%	*In vivo*	NA, IV	3–4 weeks	0/8 (VI, PCR)	4/4 (VI, PCR)	100
	(iii) FIV_{FC1}, B, 19% and 19.2%	*In vivo*	NA (higher than [iii]), IV	3 weeks	7/9 (VI, PCR)	5/5 (VI, PCR)	22
	(iv) FIV_{NZ1}, F′/C, NA	*In vivo*	NA, IV	3–4 weeks	3/5 (VI, PCR)	10/10 (VI, PCR)	40
Total					41/139	106/123	66%

reported protection rates [2,17]. It has therefore been suggested that Fel-O-Vax FIV® efficacy may have been underestimated and there has been speculation that field trials involving natural challenge might report a preventable fraction higher than 66–68% [15,17]. Despite uncertain efficacy, millions of FIV vaccine doses have been sold worldwide, with no unequivocal 'vaccine breakthroughs' reported following in-field use in Australia (personal communication, Dr. Phillip McDonagh [Head of Regulatory Affairs for Animal Health, Boehringer Ingelheim Australia] and Dr. Elvira Currie [Australian Pesticides and Veterinary Medicines Authority]) or elsewhere [2,15].

The aim of this study was to determine the 'protective rate' (effectiveness) for the Fel-O-Vax FIV® vaccine in the field in Australia.

2. Material and methods

2.1. Sample population

Criteria for recruitment have been described previously [18]. Briefly, client-owned cats were recruited through veterinary clinics in Australia during 2013–15, most commonly at the same time as an annual health check or routine procedure (e.g. dental procedures). Two groups of cats were recruited: a FIV-vaccinated group ('cases') and a FIV-unvaccinated group matched to cases for age, sex and postcode ('controls'). Cats in the FIV-vaccinated group had been FIV antibody-tested before FIV vaccination was commenced (unless younger than six months-of-age when first vaccinated, due to the low risk of FIV infection and the possibility of false-positive antibody results from maternal antibodies) [19], given a primary course of three FIV vaccinations 2–4 weeks apart, and vaccinated annually against FIV for at least three years. Cats were excluded from the FIV-vaccinated group if FIV nucleic acid amplification (PCR) testing had been performed instead of FIV antibody-testing before FIV vaccination was commenced (due to the PCR assay's lower sensitivity) [18,20,21], if any primary FIV vaccinations were more than two weeks overdue (i.e. greater than 6 weeks interval between vaccinations), and if any of the annual FIV vaccinations were more than three months overdue (i.e. greater than 15 months interval between vaccinations). Cats included in the FIV-unvaccinated group had never been given the FIV vaccine. Outdoor access was a requirement for cats in both groups. Information pertaining to outdoor access, as well as number of suspected cat fights based on medical records and owner recollection, was collected at the time of sampling via a questionnaire. Owners of cats meeting the criteria of either group were offered free FIV testing in return for enrolling their cat in the study, and participating clinics were given free vaccines (FIV and/or non-FIV core vaccines) as an inducement, in return for their assistance recruiting cats.

Animal ethics approval was granted by the University of Sydney (Approval number N00/1-2013/3/5920).

2.2. Blood collection and determining FIV infection status

Procedures for venipuncture, FIV antibody testing of EDTA blood using point-of-care test kits (SNAP FIV/FeLV Combo[2], Witness FeLV/FIV[3] and Anigen Rapid FIV/FeLV[4] concurrently), nucleic acid amplification of blood using a commercial PCR assay that detects proviral DNA and viral RNA by targeting a conserved region

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[3] Zoetis Animal Health, Lyon, France.
[4] BioNote, Gyeonggi-do, Korea.

Please cite this article in press as: Westman ME et al. The protective rate of the feline immunodeficiency virus vaccine: An Australian field study. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.06.060

of the *gag* gene (FIV RealPCR)[5], collection of blood for virus isolation (VI)[6,7] and final assignment of FIV status have been described previously [18]. All FIV-vaccinated/FIV-infected cats ('vaccine breakthroughs') were confirmed by VI, reverse transcription (RT) assay and proviral PCR testing using primers targeting the *env* gene. For cats where FIV was isolated in cell culture, sequencing of the *env* product was performed and compared to sequences in GenBank to determine the clade of breakthrough FIV isolate.[8] For all FIV-infected cats, FIV subtype was determined by FIV RealPCR testing using subtype-specific primer pairs for clades-A, -B, -D and -F [22].

2.3. Statistical analysis

Sample size calculations were made using statistical software (Minitab 16th Edition)[9] based on projected FIV prevalence rates of 3% and 16% in the FIV-vaccinated and FIV-unvaccinated groups, respectively, and statistical power of 80%. A study design aiming for a 1:3 vaccinate (case) to control ratio was chosen to improve the power [23]. Numerical analyses were performed at the conclusion of the study using commercial software (Genstat 16th Edition).[10] Significance was considered at $P < 0.05$ and 95% confidence intervals (CIs) were calculated using Microsoft Excel.[11] Shapiro-Wilk tests were used to assess data for normality. When data was normally distributed, means were reported and a two-sample *t*-test (two-sided) used (days between last FIV vaccination and sampling, breakthroughs *vs* FIV-uninfected cases; C_T value from FIV RealPCR testing, breakthroughs *vs* controls). When data was not normally distributed, medians were reported and Mann-Whitney *U*-tests used (age, cases *vs* controls). Fisher's exact tests (two-tailed) were used to investigate whether there was a significant difference in recruitment criteria (sex, breed, outdoor access and number of suspected cat fights) or FIV prevalence rate between the FIV-vaccinated and FIV-unvaccinated groups. Protective rate (effectiveness) of the FIV vaccine was calculated using the formula:

$$\mathrm{PR} = (1 - \mathrm{OR}) \times 100$$

where PR = protective rate and OR = odds ratio (an approximation of relative risk) [24–26].

3. Results

3.1. Sample population

Blood samples were obtained from 440 client-owned cats recruited from 13 clinics distributed over five jurisdictions within Australia (New South Wales [NSW], Victoria [VIC], Queensland [QLD], South Australia [SA] and Australian Capital Territory [ACT]) (online Supplement 1). There were 139 FIV-vaccinated cats (cases) and 301 FIV-unvaccinated cats (controls). 139 cats were excluded from further analysis for various reasons (online Supplement 2). All cats recruited from VIC and QLD ($n = 92$) were excluded because FIV infection was not detected in any cats, removing the presumption of meaningful FIV exposure. 301 cats remained for final analysis (89 FIV-vaccinated, 212 FIV-vaccinated; case: control ratio of 1:2.4).

3.1.1. Cases (n = 89)

The 89 FIV-vaccinated cats recruited ranged from 3 to 18 years (median 8 years; interquartile range [IQR] 5–11 years). These cats comprised 46 castrated males and 43 spayed females. Most had been antibody-tested prior to vaccination (60/89; 67%), and a summary of the number of annual FIV vaccines administered to cases is provided in Table 2. All cats had been vaccinated against FIV within the previous 15 months (range 2–443 days; mean 224 days; IQR 141–307 days). Most cats were described by their owner as having mainly day-time outdoor access (70/89; 79%), with fewer described as having unlimited outdoor access (17/89; 19%) or mainly night-time outdoor access (2/89; 2%). The majority of cases were suspected of having been in at least one cat fight (64/89; 72%), with 36/89 (40%) involved in more than three fights (Table 3).

3.1.2. Controls (n = 212)

The 212 FIV-unvaccinated cats ranged from 3 to 20 years (median 7 years; IQR 6–11 years). The cats comprised 102 castrated males and 110 spayed females. 120 cats had mainly day-time outdoor access (120/212; 57%), 90 cats had unlimited outdoor access (90/212; 42%) and one cat had mainly night-time outdoor access (1/212; 0.5%). The majority of controls were suspected of having been in at least one fight (144/212; 68%), with 78/212 (37%) involved in more than three fights.

Controls matched cases when age ($P = 0.83$), sex ($P = 0.61$), breed ($P = 1.00$) and number of fights ($P = 0.58$ for at least one fight) were compared between groups (Table 3). The only statistical difference between groups was in relation to outdoor access; cases were more likely than controls to have day-time only outdoor access, while controls were more likely to have unlimited outdoor access ($P < 0.001$).

3.2. FIV testing

3.2.1. Cases (n = 89)

The prevalence of FIV infection in the FIV-vaccinated cohort was 6% (5/89). The five FIV-vaccinated/FIV-infected cats were 6–8 years-of-age, comprising four castrated males and one spayed female. Of these vaccine breakthroughs, 4/5 had received their first vaccination when they were older than six months-of-age, and thus had been FIV antibody-tested before vaccination commenced; the fifth cat (# 404) was 16 weeks-of-age when first vaccinated and antibody-testing had therefore not been performed (Table 4). A summary of cat fight incidents requiring veterinary intervention, in relation to timing of FIV vaccination, is provided in Table 4.

Information regarding two additional cats that were possibly vaccine breakthroughs, but were excluded from further analysis because they did not meet the strict inclusion criteria, is provided in online Supplement 3.

Subtyping results from both VI and FIV RealPCR testing for the five vaccine breakthroughs are presented in Table 5. FIV subtype A infection was identified in all cases. None of the five cats were co-infected with other clades of FIV. The mean C_T value from FIV RealPCR testing for vaccine breakthroughs was 31.1.

3.2.2. Controls (n = 212)

The FIV prevalence rate in the FIV-unvaccinated cohort was 12% (25/212). The 25 FIV-unvaccinated/FIV-infected cats ranged from 3 to 16 years-of-age (median 7 years; IQR 5–10 years), comprising 18 castrated males and 7 spayed females. FIV RealPCR testing identified two subtypes (i.e. co-infection) in over half of FIV-infected controls (13/25; 52%). FIV subtype A infection was most common (20/25 cats; 80%), followed by subtype F (5/20 cats; 25%) and subtype D (4/25 cats; 16%) (Table 6). The mean C_T value from FIV RealPCR testing for FIV-infected controls was 31.0 (using the lower

[5] IDEXX Laboratories, East Brisbane, Queensland, Australia.
[6] Yamamoto Laboratory, The University of Florida, Gainesville, FL, USA.
[7] Veterinary Diagnostic Services, The University of Glasgow, Scotland, UK.
[8] https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch.
[9] Minitab 16th Edition for Windows, State College, PA, USA.
[10] GenStat 16th Edition for Windows, VSN International, Hemel Hempstead, United Kingdom.
[11] Microsoft Excel 2010 for Windows, Microsoft, Redmond, WA, USA.

Please cite this article in press as: Westman ME et al. The protective rate of the feline immunodeficiency virus vaccine: An Australian field study. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.06.060

Table 2
Summary of number of annual FIV vaccinations received by FIV-vaccinated cats recruited for the study and included in the final analysis (cases). The FIV-vaccinated/FIV-infected cats (vaccine breakthroughs) are identified in brackets.

Years vaccinated/ potentially exposed to FIV	No. FIV-vaccinated cats (n = 89)	No. FIV-infected cats (vaccine breakthroughs; n = 5)
3	15	1 (# 415)
4	28	1 (# 106)
5	24	0
6	9	2 (# 1, # 404)
7	12	1 (# 152)
8	1	0

Table 3
Summary of criteria used to match controls to cases. IQR = interquartile range. All cats recruited had been neutered. All control cats sampled lived in the same or an adjacent postcode to the matching cases. Only level of outdoor access was statistically significant between groups; cases were more likely than controls to have day-time only outdoor access, while controls were more likely to have unlimited outdoor access ($P < 0.001$)[a]. Number of suspected cat fights was estimated using a combination of medical records and owner recollection.

Category	FIV-vaccinated (cases) (n = 89)	FIV-unvaccinated (controls) (n = 212)
Total age range (years)	3–18	3–20
Age IQR (years)	5–11	6–11
Male:female ratio	52:48	48:52
Proportion of domestic crossbred cats (%)	88	88
Outdoor access 'mainly day-time' (%)[a]	79	57
Outdoor access 'mainly night-time' (%)	2	0.5
Outdoor access 'unlimited' (%)[a]	19	42
'0' cat fights (%)	28	32
'1' cat fight (%)	16	16
'2' cat fights (%)	11	9
'3' cat fights (%)	4	6
'More than 3' cat fights (%)	40	37

C_T value when two subtypes were identified simultaneously in the same cat).

There was no significant difference in age or C_T value from FIV RealPCR testing when FIV-infected cases and controls were compared (P = 0.54 and 0.89, respectively).

3.3. Vaccine effectiveness (protective rate)

A summary of results by clinic and group (FIV-vaccinated *vs* FIV-unvaccinated) is provided in Table 7. The overall protective rate for Fel-O-Vax FIV® was 56% (95% CI −20 to 84). The difference in FIV prevalence rates between the two groups (i.e. 5/89; 6% *vs* 25/212; 12%) failed to reach significance (P = 0.14).

A *post hoc* power analysis identified that the higher than predicted rate of FIV infection in cases and lower rate of FIV infection in controls reduced the power to detect a significant difference between groups to 40%. Had the intended 1:3 vaccinate (case) to control ratio been achieved, the power to detect a significant difference between groups would have only increased to 43%. Given the prevalence rates reported in the current study, to have achieved a statistically significant effect of the vaccine (assuming one exists) with power of 80% and 1:3 case to control ratio would have required 207 FIV-vaccinated cats and 621 matching FIV-unvaccinated controls.

Table 5
FIV *env* sequencing results following virus isolation and subtyping results from FIV RealPCR testing for the five vaccine breakthroughs. *Env* sequences were compared to stored sequences in GenBank to determine subtype. The FIV RealPCR assay included primer pairs for FIV subtypes-A, -B, -D and -F. C_T = cycle threshold value for FIV RealPCR testing.

Cat (case) no.	Subtyping results (virus isolation)	Subtyping results (FIV RealPCR), C_T
# 1 (FIV-vaccinated)	FIV-Dixon (A)	FIV-A, 31.7
# 106 (FIV-vaccinated)	FIV-Sendai 1 (A)	FIV-A, 32.0
# 152 (FIV-vaccinated)	FIV-Dixon (A)	FIV-A 29.7
# 404 (FIV-vaccinated)	FIV-UK8 (A)	FIV-A, 31.5
# 415 (FIV-vaccinated)	FIV-Dixon (A)	FIV-A, 30.5

Table 6
Subtyping results from FIV RealPCR testing for 25 FIV-unvaccinated cats (controls). Primers pairs for FIV subtypes-A, -B, -D and -F were included in the PCR reaction. Virus isolation was not performed for FIV-infected controls.

FIV subtype	Frequency
FIV A only	11/25 = 44%
FIV B only	0
FIV D only	0
FIV F only	1/25 = 4%
FIV A/F	9/25 = 36%
FIV D/F	4/25 = 16%

4. Discussion

The nominal protective rate for Fel-O-Vax FIV® in this study, the first field trial conducted for this vaccine anywhere in the world, was 56%. Five confirmed vaccine breakthroughs were detected, as well as two additional cases which became FIV-infected but where there were lapses in timing of vaccine administration. The FIV vac-

Table 4
FIV vaccination and suspected cat fight history (based on retrieved medical records only) of the five vaccine breakthroughs. Ab = FIV antibody test, Y = yes, N = no, P = primary vaccine, A = annual vaccine, d = days since last vaccination, m = months since last vaccination. P1 (first primary FIV vaccine) is taken as time = 0. Fight history (requiring veterinary intervention and verified using clinic medical records) is displayed as the time elapsed since the previous vaccination when the fight occurred. A negative symbol (cat # 415) indicates the fights occurred before FIV vaccinations had commenced. [a]Cat # 106 did not have any fight history. As per the manufacturer's guidelines, antibody-testing was not performed prior to commencing FIV vaccination in kittens less than six months-of-age at the time of the first vaccination (cat # 404).

Cat (case) no.	Ab	P1	P2	P3	A1	A2	A3	A4	A5	A6	A7
# 1	Y	0d	21d	21d	12m	11m	11m	14m	12m	13m	–
FIGHT HISTORY					9m		8m		5m		
# 106	Y	0d	14d	14d	12m	8m	14m	12m	–	–	–
FIGHT HISTORY[a]											
# 152	Y	0d	12d	18d	11m	11m	11m	11m	12m	12m	12m
FIGHT HISTORY							7m	11m			
# 404	N	0d	14d	21d	11m	12m	12m	13m	12m	12m	–
FIGHT HISTORY				3m							
# 415	Y	0d	27d	29d	15m	12m	12m	–	–	–	–
FIGHT HISTORY	−48d, −156d				9m	7m					

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Table 7
FIV prevalence by veterinary clinic (n = 301). In total, 89 FIV-vaccinated cats and 212 FIV-unvaccinated cats were recruited. GWAH = Great Western Animal Hospital, EDAH = Elizabeth Drive Animal Hospital, CAH = Campbelltown Animal Hospital, MAVH = Mt Annan Veterinary Hospital, ISVH = Inner South Veterinary Hospital, BVH = Bankstown Veterinary Hospital, FGVS = Fulham Gardens Veterinary Surgery, CVH = Casula Veterinary Hospital. NSW = New South Wales, ACT = Australian Capital Territory, SA = South Australia. CAH and MAVH were pooled together as they are located in adjacent suburbs and cats recruited were from the same area. Cats excluded from final analysis (n = 139) are not shown. The difference in FIV prevalence rates between groups did not reach statistical significance using a Fisher's exact test (P = 0.14).

Veterinary clinic	FIV prevalence (FIV-vaccinated cats)	FIV prevalence (FIV-unvaccinated cats)
GWAH (NSW)	1/19 = 5%	8/75 = 11%
EDAH (NSW)	1/19 = 5%	2/23 = 9%
CAH/MAVH (NSW)	0/13 = 0%	4/34 = 12%
ISVH (ACT)	1/12 = 8%	1/15 = 7%
BVH (NSW)	2/11 = 18%	5/27 = 19%
FGVS (SA)	0/10 = 0%	3/26 = 12%
CVH (NSW)	0/5 = 0%	2/12 = 17%
Total	5/89 = 6%	25/212 = 12%

cine was shown not to significantly reduce the risk of client-owned cats becoming infected with FIV, although there was a trend for some protection. A study with greater numbers is required to resolve this issue, although based on our experiences, it is very difficult to recruit cases using the present approach due to vaccine protocol compliance issues. We also found it difficult to recruit matching control (FIV-unvaccinated) cats, since most cats from the 13 participating clinics with outdoor access were vaccinated against FIV. A prospective study would likely be easier to manage, but would take longer to generate meaningful data. Owners wanting to prevent their cat acquiring FIV infection should consider measures in addition to vaccination, such as cat curfews, 'modular pet parks' or keeping their cat(s) exclusively indoors. Cats vaccinated against FIV should undergo annual testing prior to booster FIV vaccination, e.g. using a Witness FeLV/FIV or Anigen Rapid FIV/FeLV antibody test kit [18], to check infection has not occurred in the preceding year.

The benefit of field studies is that they involve natural challenge in terms of dose, route, and type (i.e. a selection of genetically different viruses, with a range of pathogenicities and *env* sequences). There are, however, some disadvantages to field studies compared to experimental studies for evaluating vaccine effectiveness. The frequency and extent of viral challenge cannot be predicted, since challenge relies on bite(s) from FIV-infected cat(s) (hence use of the terms 'vaccine protective rate' and 'effectiveness' for the current study, instead of 'preventable fraction' and 'efficacy'). It is possible (based on the FIV prevalence rate in controls) that many of the FIV-uninfected cats in the study were never exposed to FIV, although the retrospective quantification of cat fight incidents documents at least possible exposure for many cats. The need to exclude cats recruited from VIC and QLD due to an absence of FIV infection in controls was surprising considering previous studies, including a large recent serosurvey from Australia documenting FIV prevalence rates of 10–16% in healthy client-owned cats in these states [8,27]. Differences in housing conditions and lifestyles between groups was unavoidable (e.g. amount of time spent outdoors). Thus despite our best efforts to match controls to cases on the basis of age, sex and postcode it is possible there was some mismatch in relation to level of exposure to FIV between groups. Since this was a retrospective field study, there were no housing restrictions to eliminate the risk of exposure to FIV during the primary course of FIV vaccination. Furthermore, it was impossible to determine when cats became FIV-infected (cases or controls), and it was also impossible to ensure controls were FIV-negative at the start of the study period with antibody-testing (as was done for vaccinates). This may have introduced a slight bias towards the FIV vaccine showing a protective effect, although this seems unlikely, given the comparable FIV seroprevalence in the control group to previous Australian studies [7–9]. In future, a prospective study design, with FIV-testing of both vaccinates and controls on day 0, would circumvent this possible bias. This type of study design would require a considerably larger sample population to account for the inevitable losses that occur in a longitudinal study of this required duration (8–10 years).

Medical records of the five FIV-vaccinated/FIV-infected cats suspected of representing vaccine breakthroughs were scrutinized to investigate the prospect that some vaccine breakthroughs occurred before the primary course of FIV vaccination had been completed. No cats had received veterinary treatment for possible cat fight wounds during the primary course of FIV vaccination (Table 4). Cat # 415, when one year-of-age, was in a suspected cat fight 48 days prior to FIV antibody testing (SNAP FIV/FeLV Combo) and commencement of the primary course of FIV vaccination. Most cats produce detectable antibodies to FIV within four weeks of experimental inoculation [28,29], although in rare cases this response may be delayed [30]. Consequently, current recommendations are to retest cats with possible recent retrovirus exposure after 56 or 60 days [31,32]. It is therefore possible this cat was already infected before FIV vaccination commenced. In retrospect, it would have been helpful to have retested this cat (antibody and/or PCR testing) [18] at the end of the primary course of FIV vaccination to investigate this possibility. Given the short duration of time in relation to the overall study period (12 weeks *vs* minimum 156 weeks) and the ages of these five cats when first vaccinated (all one year-of-age or younger) we think it unlikely that vaccine breakthroughs occurred during the primary course of FIV vaccination. This is because young cats lack confidence and are thus less inclined to fight than older cats; they are therefore much less likely to be FIV-infected than a mature, territorial cat older than three years [8]. In addition, we contend that owners are less likely to allow prolonged periods of outdoor access to kittens (<6 months-of-age), reducing further the possibility of FIV exposure in the one breakthrough cat that was vaccinated as a kitten (# 404). Future research should consider a prospective field trial to address these concerns by ensuring cats have not been in a cat fight in the 12 weeks prior to recruitment and commencement of FIV vaccination, enforcing strict housing restrictions during the initial course of primary FIV vaccination (indoors only) and sequential testing to establish if and when cats become FIV-infected.

Despite 5/25 FIV-infected controls in the current study being infected with subtypes D and F, only subtype A was identified in the five vaccine breakthroughs (and both additional possible breakthroughs), suggesting that the vaccine may provide superior immunity against these other subtypes. To further investigate this prospect, a larger Australian field study to increase the number of vaccinates potentially exposed to other FIV subtypes, as well as field studies in countries where non-A subtypes are more common (e.g. Taiwan and Japan where subtypes C and D, respectively, are more prevalent [2,33,34]), is required. The diverse subtyping results in the FIV-infected controls (with presence of clades A, D and F) compared to previous Australian studies (which found a marked preponderance of subtype A, with rare subtype B isolates) was surprising [35,36]. For these results to be considered valid, sequencing of the *env* gene of FIV isolates from all FIV-infected cats (not just vaccinates) needs to be performed in the future to confirm the accuracy of the PCR subtyping results.

The exact mechanism(s) by which the FIV vaccine provides sterilizing immunity against certain subtypes is still unclear. In experimental studies, protection appeared to rely on both cell-mediated and antibody-mediated immunity [16,37]. Humoral immunity, specifically the production of antibodies directed against the

Please cite this article in press as: Westman ME et al. The protective rate of the feline immunodeficiency virus vaccine: An Australian field study. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.06.060

hypervariable V5 region of the FIV envelope, is important for homologous challenge [2,37,38]. Passive-transfer studies (using pooled serum from FIV-vaccinated cats) have consistently conferred good protection against homologous (FIV_{Pet}) challenge, but not heterologous (FIV_{FC1}) challenge [15,16]. In contrast, cell-mediated immunity (CMI) is important for both homologous and heterologous challenge [2,37,38]. T-cell responses likely important in CMI include T-helper 1 activity mediated by specific cytokines (IL-2 and IFNγ), as well as cytotoxic lymphocyte activity, in particular the increased production of the cytotoxic-effector molecule perforin [39]. Adoptive-transfer studies (using B-cell depleted, T-cell enriched preparations from MHC-matched FIV-vaccinated donor cats) have demonstrated good protection against both homologous (FIV_{Pet}) and heterologous (FIV_{FC1} and FIV_{NZ1}) challenge. Accordingly, it is believed that CMI is more critical for protection against FIV than humoral immunity [2,15,16]. The failure of the FIV vaccine to protect against multiple strains of subtype A in the current study (as well as in other studies involving homologous challenge with FIV_{UK8} [11,40]), but possible protection against subtypes D and F, supports the notion that any sterilizing protection induced by the FIV vaccine is reliant predominantly on CMI rather than antibody-based immunity.

The release of the FIV vaccine in 2002 was the culmination of ten years of collaborative work and heralded as a triumph of veterinary vaccinology. Many approaches to FIV vaccine design were tried, including IWC, IWV, recombinant (e.g. p24), gene-deletion, vector-based and DNA-based vaccines, formulated with a range of adjuvants and administered in different prime and boost protocols [2,15,37,41]. Vaccinating cats against FIV using an IWC vaccine was found to induce higher VNA levels than a IWV vaccine, although the duration of protection following IWC vaccination may be shorter [37]. The prototype FIV vaccine, which contained only IWV (no IWC), outperformed the commercial FIV vaccine in several studies [15,16,42]. Ultimately, the lower production cost of IWC over IWV led to the compromise of the combined IWC/IWV formulation in Fel-O-Vax FIV® [15].

The identification of five vaccine breakthroughs, and a further two equivocal cases, casts doubt over the ability to induce solid protection against an immunodeficiency virus through vaccination and is a setback in the quest to develop a uniformly effective HIV-1 vaccine. Research is already underway towards the development of a FIV epitope vaccine targeting T-cell immunity [15]. It is possible that for both FIV and HIV-1, sterilizing immunity is unattainable. A more realistic aim might be a vaccine that reduces viral load to a level that delays or prevents the onset of clinical signs [17]. If the aim of FIV vaccine development shifts from sterilizing to protective immunity, like the core vaccines against feline calicivirus and feline rhinotracheitis, then future research will need to focus on reduction of FIV-associated disease in vaccinated individuals rather than the prevention of infection.

5. Conclusion

A field study into the effectiveness of a commercial FIV vaccine determined a protective rate of 56% in client-owned Australian cats and documented the first convincing in-field vaccine breakthroughs. FIV infection rate was not significantly different between FIV-vaccinated and FIV-unvaccinated cats, although there was a trend for some protection. The result is disappointing for veterinarians wanting to use the vaccine in high risk situations, as well as for researchers working on developing a HIV-1 vaccine, but is a reminder of the difficulties associated with vaccinating against any *Lentivirus*. Recently, the World Small Animal Veterinary Association upgraded the FIV vaccine classification from 'Not Recommended' to 'Non-Core', a change which may encourage more veterinarians to administer this vaccine [43]. We recommend FIV-vaccinated cats should undergo annual testing to ascertain whether they are still FIV-uninfected before administering the booster FIV vaccine, with testing commencing at the end of the primary course of FIV vaccination, to check infection has not already occurred. Complete protection from FIV infection is only possible by eliminating FIV exposure through the use of 'modular pet parks' or keeping cat exclusively indoors. Further research needs to be conducted where the FIV vaccine is available, FIV prevalence is high and other FIV subtypes are present (e.g. Taiwan, Japan) to establish the protective rate of Fel-O-Vax FIV® against the full range of FIV subtypes.

Conflict of interest

The authors have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Acknowledgements

This study was supported financially by the Australian Companion Animal Health Foundation (ACAHF), Feline Health Research Fund (FHRF), and Boehringer Ingelheim, Australia. We are grateful to the significant in-kind contribution of diagnostic kits by IDEXX Laboratories, Zoetis Animal Health and BioNote, without which this study would not have been possible. Derek Brown, Senior Veterinary Laboratory Scientist for IDEXX Australia was generous with his time and expertise processing samples for FIV RealPCR testing. Yamamoto Laboratory (Janet Yamamoto, The University of Florida, USA) and Veterinary Diagnostic Services (The University of Glasgow, Scotland) kindly performed virus isolation and detection by RT assay and proviral PCR, with special thanks to Yasmin Parr for her assistance with virus isolation. Mark Westman was supported by an Australian Postgraduate Award (APA), the Neil and Allie Lesue Scholarship and the Herbert Johnson Travel Grant Scholarship. Richard Malik was supported by the Valentine Charlton Bequest. Alan Marcus provided helpful comments to the manuscript. Thanks to the staff at the 13 veterinary clinics for assistance with recruiting and sampling. Finally, and most importantly, thanks to all of the cats and owners who supported this study, in particular the vaccine breakthroughs who had several health checks - Zeb, Ridgi, Kiwi, Mitch, Meggs, George and Gollum.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.06.060.

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