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## A Comparative Study of the Efficacy of a Canarypox Based Recombinant Leukemia Vaccine against a Natural Contact Felv Challenge in Cats

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### Abstract

#### Objective

Feline leukaemia virus (FeLV) infection is a major infectious disease of cats, but its prevalence has decreased as a result of management of infected animals and vaccination. Efficacy of vaccines is tested in vaccination/challenge experiments which are unfortunately poorly representative of the natural conditions of infection and may in some instances lead to biased conclusions. To overcome this limitation of traditional efficacy studies, we tested the efficacy of a non-adjuvanted recombinant canarypox-FeLV vaccine in a natural contact challenge model, and compared it to an adjuvanted commercially available vaccine.

#### Methods

Vaccinated and unvaccinated control cats were mixed with persistently viraemic cats and kept in close contact for more than 6 months. Cats were regularly tested for p27 antigenaemia and viraemia. Cats that were found positive for either p27 antigenaemia or viraemia for the last three blood samples were considered persistently viraemic.

#### Results

This contact challenge resulted in a high rate of persistent viraemia in control cats (78%). Vaccination efficiently protected cats against persistent viraemia with preventable fractions of 79% for the canarypox vectored vaccine and 68% for the adjuvanted commercial vaccine.

#### Conclusion

The natural contact challenge model proved to be a potent method to reproduce FeLV challenge, confirming that under appropriate conditions FeLV is efficiently transmitted. Despite the severity of the challenge, both vaccines provided a strong and sustained protection against persistent viraemia over a 6-month contact period. This study confirmed the efficacy of a non-adjuvanted canarypox-vectored FeLV vaccine against a contact challenge mimicking the natural conditions of infection.

**Keywords:** FeLV, vaccine; Canarypox virus; Efficacy; Contact challenge

### Introduction

Feline leukaemia virus (FeLV) is a major pathogen of cats causing a wide range of clinical syndromes [1,2]. It has been described as the "most destructive of feline infectious diseases" [3] and an important cause of death. The prognosis for persistently viraemic cats is typically poor with over 80% dying within 3-5 years after diagnosis [4-6].

The prevalence of FeLV depends on the geography and the cat population, typically ranging from 2-3% to more than 10% p27 positive cats in high-risk environment [7-11]. Overall the prevalence

has been reduced in some countries as a result of both vaccination and management of infected cats. Nevertheless, FeLV infection still remains a risk in some cat populations, such as certain free-roaming cats [9,12] or even client-owned cats in some geographical areas [13-15] which display higher prevalence rates. It is well recognized that FeLV p27 antigen negative status can occur following exposure to FeLV, as indicated by the presence of FeLV provirus in tissues or blood [16,17]. Since most studies are based on FeLV p27 antigen detection, it is therefore likely that FeLV exposure and infection are underestimated in such studies.

Although they do not necessarily prevent infection [18,19], FeLV vaccines are potentially able to prevent persistent viraemia and thereby

the diseases caused by FeLV as well as the shedding of the virus [20]. Most vaccines available on the market are classical inactivated or sub-unit adjuvanted vaccines. With the aim to reduce the incidence of injection site reactions, a non-adjuvanted canarypox virus vectored vaccine has been developed where the recombinant canarypox virus vector expresses envelope glycoproteins (p15E and gp70) and capsid proteins of FeLV A, which have been shown to provide protection against FeLV oro-nasal challenge [21-23]. Efficacy data have been published for the different types of vaccines but few comparative studies are available [20]. In addition, most of the published studies rely on artificial challenge models, using the intra-peritoneal route or high doses of challenge virus with simultaneous administration of glucocorticoids [24]. Although less commonly performed, natural challenge models of infection are generally regarded as the most valuable method of assessing FeLV vaccine efficacy, mimicking natural infection as much as possible.

This study was designed to assess the efficacy of a non-adjuvanted canarypox virus vectored vaccine (EURIFEL® FeLV renamed PUREVAX® FeLV, Merial) and a sub-unit vaccine, LEUCOGEN® in a natural challenge model of FeLV infection [25]. LEUCOGEN® is a p45 sub-unit vaccine adjuvanted with aluminium hydroxide and saponin [26].

## Material and Methods

### Animals and challenge

Specific pathogen free (SPF) kittens were used for the study and housed in a confined environment. All kittens were FeLV negative by p27 antigen screening (IDEXX, Westbrook, USA) prior to inclusion in the study. The kittens were divided into four groups:

- Group A comprised 18 kittens which served as the source of natural challenge. They were challenged at 8-9 weeks of age with 1 × 106 focus forming units of Glasgow-1 strain of FeLV-A with 0.25 ml administered into each nostril and 0.5 ml orally to induce persistent viraemia.
- Groups B and C comprised 24 kittens in each and received one of the two vaccines being tested
- Group D comprised 23 kittens given placebo to serve as controls.
- Cats from groups B, C and D were also shown to be free of antibodies against gp70 at the time of inclusion. Allocation to these groups B, C and D was performed using a computerized randomization program based on age, sex, parentage and weight in descending order of priority.

### Vaccination

Kittens in Groups B and C were vaccinated according to the manufacturer's recommendations. Kittens in group B were vaccinated at 8-9 weeks of age and four weeks later with EURIFEL® FeLV (renamed PUREVAX® FeLV), a non-adjuvanted recombinant canarypox virus expressing the *env* and *gag/pro* genes of FeLV A (vCP97). Kittens in group C were vaccinated at 9-10 weeks of age and again three weeks later at 12-13 weeks of age with LEUCOGEN® purchased from a veterinary drug wholesaler. Group D kittens received subcutaneous injections of sterile phosphate buffered saline at 8-9 weeks of age and at 12-13 weeks of age as a placebo.

The four groups were initially kept in separate rooms. Two weeks after cats in groups B and C received their second vaccination; the kittens from all four groups were mixed and housed in the same room.

They shared litter pans, food bowls and water dishes for the remainder of the trial. Kittens in Group A were approximately four weeks older than kittens from Groups B, C and D.

### Post-challenge follow-up

Blood samples were collected by jugular venipuncture from all kittens every two weeks for the first 14 weeks and then every three weeks until the end of the trial, 27 weeks after mixing. The blood samples were tested using both a standard commercial ELISA for FeLV p27 antigen (Inochem, Carnforth, UK or IDEXX, Westbrook, USA) and virus isolation on QN10S cells based on the method of Jarrett et al. (1982) [27]. Persistent viraemia was defined as positive viraemia and/or antigenemia for the final three blood samples of the study or, for the cats which were euthanized before the end of the trial, for the three samples prior to death. Vaccine efficacy was calculated as the preventable fraction (Table 2).

### Statistical Analysis

The proportion of persistently viraemic cats in each group was compared by 2 test or Fisher's exact test (comparison between vaccinated groups). The Kaplan-Meier Product-Limit method was used to plot the "survival" curves defining the time to viraemia or antigenaemia, and to compare the median time to viraemia or antigenaemia (Log-rank test). The analyses were two-tailed and conducted with SAS 9.2 (SAS Institute Inc, Cary, NC) or Statgraphics Plus 5.1 (StatPoints Technologies Inc., Warrenton, VA). The type I error was set to  $\alpha=5\%$ .

### Results

Eleven out of 18 kittens in Group A became antigenaemic/viraemic within four weeks of challenge and 14 were antigenaemic/viraemic by the time of mixing (six weeks after challenge). Three of the remaining kittens became antigenaemic/viraemic before the end of the trial and the final kitten remained negative throughout. Four of the viraemic kittens from Group A were euthanized during the trial-two due to severe anaemia at 28 and 32 weeks after challenge, one due to an abdominal mass at 23 weeks after challenge, and one due to paraparesis one week before the trial terminated.

Results of p27 ELISA and virus isolation were highly consistent overall, although viral isolation was slightly more sensitive than p27 ELISA (Table 1). Eighteen of 23 (78%) kittens from Group D became persistently viraemic - one died of unrelated causes during the trial but was persistently viraemic.

Four of the remaining 5 cats in this group became antigen and/or virus isolation positive during the last three weeks of the trial but did not fulfil the criteria for persistent viraemia. Four kittens from Group B became persistently viraemic (time to onset of viraemia was respectively 6, 10, 12 and 18 weeks, Figure 1). Only one other kitten in this group tested positive (on virus isolation), at the end of the trial. Six kittens from Group C became persistently viraemic (time to onset of viraemia was 6 weeks for one cat and 18 weeks for the remainder) and one other kitten became antigenaemic/viraemic on the two final two tests of the trial.

The proportions of persistently viraemic kittens for each group and the preventable fractions achieved by the two vaccines are shown in Table 2.

Week	Criteria	Canarypox-FeLV #/24	LEUCOGEN #/24	Control #/23
1	Neg	24	24	23
	p27	0	0	0
	VI	0	0	0
4	Neg	24	24	20
	p27	0	0	2
	VI	0	0	3
6	Neg	23	23	19
	p27	0	1	3
	VI	1	1	4
8	Neg	23	23	18
	p27	1	1	5
	VI	1	1	5
10	Neg	22	23	17
	p27	2	1	6
	VI	1	1	6
12	Neg	21	23	12
	p27	2	1	7
	VI	3	1	11
14	Neg	21	23	9
	p27	3	1	13
	VI	3	1	13
18	Neg	20	18	5
	p27	4	6	17
	VI	4	6	18
21	Neg	20	18	5
	p27	4	6	17
	VI	4	6	18
25	Neg	20	17	1
	p27	4	6	20
	VI	4	7	21
27	Neg	19	17	1
	p27	4	7	21
	VI	5	7	22

neg: neither antigenaemic nor viraemic  
p27: antigenaemic; VI: viraemic; #: number of cats

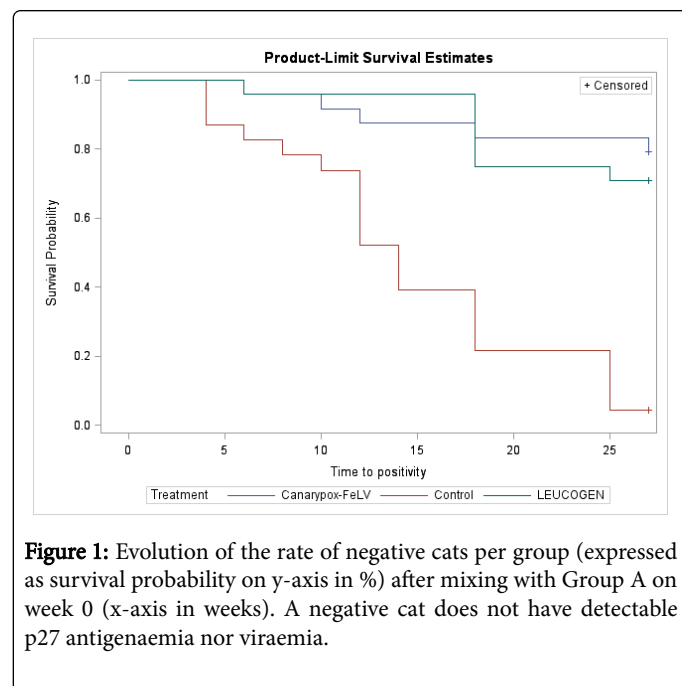
**Table 1:** Antigenaemia and viraemia status of the cats after mixing with Group A on week 0.

There was a significant difference in the proportion of persistently viraemic kittens between the different groups ( $\chi^2$  test;  $p < 0.0001$ ) but no significant difference between the two vaccine groups (Fisher's exact test;  $p = 0.5$ ).

Group	Total number	Number persistently viraemic	Proportion persistently viraemic	Preventable fraction
Canarypox-FeLV	24	4	17%	79%
Leucogen	24	6	25%	68%
Controls	23	18	78%	-

**Table 2:** Preventable fraction in vaccinated groups ( $PF = (P_v - P_c) / (1 - P_c)$ ) where  $P_v$  is the proportion of vaccinated cats which became persistently viraemic and  $P_c$  the proportion of controls which became viraemic).

The time to positive viraemia or antigenemia was significantly reduced in vaccinated groups (Log-rank test;  $p < 0.001$ ), and the difference between vaccinated groups was not statistically significant (Log-rank test;  $p = 0.58$ ).



**Figure 1:** Evolution of the rate of negative cats per group (expressed as survival probability on y-axis in %) after mixing with Group A on week 0 (x-axis in weeks). A negative cat does not have detectable p27 antigenaemia nor viraemia.

## Discussion

In the absence of identified correlate of protection, vaccine efficacy has traditionally been evaluated using experimental challenge with a virulent FeLV strain. The best practice should be to use a challenge model as close as possible to the natural conditions of infection. Using other routes of infection than the oro-nasal route or the concurrent administration of corticosteroids may introduce a bias in the assessment of vaccine efficacy. Although an oro-nasal challenge with a virulent FeLV strain is feasible as shown in our study, the intra-peritoneal route or the simultaneous administration of glucocorticoids has frequently been used to increase the efficiency of FeLV challenge [20,24,28]. However, the route of infection may have an impact on the

outcome of an infectious challenge and consequently on the efficacy of a vaccine candidate. As an example, Dunham and coll. showed that a vaccine against FIV was able to provide some protection against an intra-peritoneal challenge but not against an intra-muscular one [29]. Similarly, corticosteroids-induced immunosuppression may introduce a bias in the evaluation of vaccine efficacy. Indeed, glucocorticoids suppress cellular (Th1) immunity and promote humoral (Th2) immunity [30]. As a consequence, the impact of corticosteroids on vaccine efficacy may be different depending on the mode of action of the vaccine. Furthermore, administration of a single high dose of challenge virus to ensure a high rate of infection in controls (as required by FeLV vaccine monograph) is not representative of natural conditions and may be an unnecessary difficulty in the development of an efficacious vaccine. The natural transmission is usually the result of prolonged or repeated contacts between cats. In an attempt to address this concern, we used an experimental model which simulates natural transmission of infection and may enable a more reliable determination of the efficacy of protection that can be expected under field conditions.

In the FeLV vaccine monograph of the European Pharmacopeia, the efficacy test for FeLV vaccines is stringent and requires that 80% of the control cats become persistently viraemic. This is one of the reasons why natural transmission contact challenge studies are not routinely used to demonstrate the efficacy of FeLV vaccines, despite the fact that using natural routes of infection for such studies may produce results that are more likely to reflect the true efficacy of vaccines. In our study, the rate of persistent viraemia in control cats (78%) approached what is often obtained in classical oro-nasal or intra-peritoneal challenges [20]. In addition, three of the four kittens in the control group that did not become persistently viraemic showed evidence of infection by the end of the trial and it is likely that most, if not all, of these would have become persistently viraemic if the follow-up period had been extended. This demonstrates that our challenge model was rigorous compared with previous natural models, which displayed lower infection rates [31-37]. The origin of the cats, housing conditions and/or the FeLV challenge strain may explain some of these differences. The strong virulence of Glasgow-1 strain was indeed illustrated by the appearance of clinical signs strongly suggestive of FeLV infection in kittens from group A (anaemia, abdominal mass, paraparesis).

The overall rate of antigenaemic cats observed in our study is much higher than what is usually reported in the field. In some high-risk environments the proportion of antigenaemic cats can reach 10-20% [38,39], far lower than in the current study. However, those populations included cats of varying ages and it is well recognized that susceptibility to FeLV infection is higher in young kittens than in adult cats [32,40], and the cats in our study were actually all young. Our study confirmed that transmission of FeLV is very efficient in young cats living in close contact and in confinement in a restricted environment.

Results of p27 antigenemia and virus isolation on blood samples were comparable in this study. Virus isolation appeared to be slightly more sensitive than antigenaemia, although the ease of detecting antigenaemia generally makes this the method of choice to assess the FeLV status of cats in many clinical studies. In the European Pharmacopeia, persistent antigenaemia or viraemia is defined by three consecutive positive results or five positive results at any time during the follow-up period. However, the results at the end of the trial may be more relevant indicators of FeLV status and potential persistent

viraemia. This is particularly true for a natural challenge model where it generally takes longer for persistent viraemia to become established. In our study, comparable to previous reports [35], the time between mixing the groups and the first detection of viraemia varied from 4 to 27 weeks and it took more than 21 weeks to get close to 80% of the control cats persistently infected. This is likely to be the result of the overall infectious pressure increasing as new cats became persistently viraemic.

In recombinant canarypox-FeLV and LEUCOGEN<sup>®</sup> vaccinated cats, only 4 and 6 cats out of 24 in each group became persistently viraemic respectively. In both groups, one additional cat was positive by the end of the study. Overall, both vaccines significantly reduced the risk of persistent viraemia with preventable fractions of 79% and 68% respectively. While we cannot rule out the possibility that those figures would have changed if the cats had been kept in contact for a longer period [32], we believe that contact for more than 6 months can be considered as a good challenge model, especially given the infection rate seen in the control cats. A contact challenge over a 6 month period is likely to be a mix of immediate, late and repeated infections via the natural route of transmission. This suggests that the canarypox-vectored FeLV vaccine-induced immunity against FeLV was early and sustained over the 6-month follow-up period. These results are consistent with previous data demonstrating the efficacy of the non-adjuvanted canarypox-vectored FeLV vaccine against classical challenges performed 2-3 weeks or one year after vaccination [19,21,22]. Of note, the canarypox-FeLV vaccine protected more than 80% of the cats against persistent viraemia as requested by the FeLV monograph of the European Pharmacopeia.

In this study, infectious pressure increased with time while new cats became persistently viraemic and thereby additional shedders of FeLV. In that respect, the challenge model was likely more severe than in the usual field conditions where prophylaxis against FeLV relies also on the detection and isolation of FeLV positive cats to reduce the risk of exposure for non-infected animals.

## Conclusion

The natural challenge model to assess FeLV vaccines in this study proved to be a potent method to reproduce FeLV challenge, confirming that under appropriate conditions FeLV is efficiently transmitted. Despite the severity of the challenge, both vaccines provided a comparable and good protection against persistent viraemia over a 6-month contact period which led to persistent viraemia in most of the controls. This study confirmed the efficacy of a non-adjuvanted canarypox-vectored FeLV vaccine against a contact challenge mimicking the natural conditions of infection.

*\* All marks are the property of their respective owner.*

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## Conflict of Interest Statement

H. Poulet and J.C. Thibault are employees of Merial, a sanofi company.

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