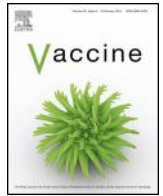




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Efficacy of an inactivated FeLV vaccine compared to a recombinant FeLV vaccine in minimum age cats following virulent FeLV challenge

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ABSTRACT

The aim of the study was to determine the efficacy of an inactivated feline leukemia virus (FeLV) vaccine (Versifel[®] FeLV, Zoetis.) compared to a recombinant FeLV vaccine (Purevax[®] FeLV, Merial Animal Health) in young cats, exposed under laboratory conditions to a highly virulent challenge model. The study was designed to be consistent with the general immunogenicity requirements of the European Pharmacopoeia 6.0 Monograph 01/2008:1321–Feline Leukaemia Vaccine (Inactivated) with the exception that commercial-strength vaccines were assessed. Fifty seronegative cats (8–9 weeks old) were vaccinated subcutaneously on two occasions, three weeks apart, with either placebo (treatment group T01), Versifel FeLV Vaccine (treatment group T02), or Purevax FeLV Vaccine (treatment group T03) according to the manufacturer's directions. Cats were challenged three weeks after the second vaccination with a virulent FeLV isolate (61E strain). Persistent FeLV antigenemia was determined from 3 to 15 weeks postchallenge. Bone marrow samples were tested for the presence of FeLV proviral DNA to determine FeLV latent infection. At week 15 after challenge with the virulent FeLV 61E strain, the Versifel FeLV Vaccine conferred 89.5% protection against FeLV persistent antigenemia and 94.7% protection against FeLV proviral DNA integration in bone marrow cells. In comparison, the Purevax FeLV Vaccine conferred 20% protection against FeLV persistent antigenemia and 35% protection against FeLV proviral DNA integration in bone marrow cells following challenge. The data from this study show that the Versifel FeLV Vaccine was efficacious in preventing both FeLV persistent p27 antigenemia and FeLV proviral DNA integration in bone marrow cells of cats challenged with this particular challenge model under laboratory conditions and provided better protection than Purevax FeLV in this experimental challenge model with highly virulent FeLV.

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1. Introduction

Feline leukemia virus (FeLV) is caused by an oncogenic retrovirus of the lentivirus family. The virus is distributed worldwide and is one of the most important viral pathogens of domestic cats [1,2]. FeLV can induce immunosuppression that leads to the development of secondary infections, hematopoietic tumors, and anaemia. FeLV is transmitted directly from cat to cat, mainly through saliva and nasal secretions but also through urine, faeces and milk, or congenitally to the fetus. There is an age-resistance to FeLV with young cats being more rapidly infected following contact exposure [3]. Exposure of cats to FeLV is commonly confirmed by detection

of p27 antigen in whole blood, plasma, or serum [4]. Following infection with FeLV, cats may become either persistently or transiently viremic. Transiently viremic cats show only a few clinical symptoms and recover from infection. Cats which are persistently viremic are characterized by continuous expression of p27 viral antigen and shed the virus which is a risk to susceptible cats. Persistently viremic cats are known to be at increased risk of succumbing to diseases associated with FeLV [1]. Another category of cats infected with FeLV are those with latent infection. While in the majority of cats with latent infections the virus is eventually eliminated, at times the infection can persist for years and can revert to a viremic state [5]. These latently infected cats can also serve as a source of infection to in-contact cats and thus prevention of latent infections is important in the control of FeLV. If latent FeLV infection is established, virus can be detected in the bone marrow by PCR [6,7]. The spleen, lymph nodes, small intestine, and

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mammary glands have also been shown to be reservoirs for latent virus [6,7]. Virus in these cats can be reactivated from latency with corticosteroids [7].

FeLV infection has been controlled by the use of vaccines with several types available [2], including those containing inactivated virions [8,9], subunits of the virions [10], or recombinant envelope protein [11]. An ideal vaccine should provide protection against both persistent and transient viremia, prevent latent infections, and prevent the development of FeLV-related diseases [8,12]. However, the protection afforded by different commercial vaccines has been shown to vary considerably [2].

The aim of this study was to determine and compare the efficacy of an inactivated FeLV vaccine (Versifel FeLV, Zoetis) to that of a recombinant vaccine (Purevax FeLV, Merial Animal Health) in young cats using a highly virulent laboratory challenge model.

2. Materials and Methods

2.1. Animals

Fifty, healthy, specific pathogen free (SPF), 8 to 9 week old kittens (Liberty Research Inc, USA) were used in the study and randomly allocated to three treatment groups with a generalized block design blocking on date of birth and litter. Based on results from a previous study (unpublished data) at Zoetis, 20 animals were enrolled in each vaccine group to find the difference in persistent antigenaemia between 5% (Versifel® FeLV) and 45% (PureVax® FeLV) with a two-sided alpha of 0.05 and power of 80%. Ten animals in the placebo group is the minimal number of animals required by the European Pharmacopoeia.

Cats were group housed in 5 rooms which met the USDA Animal Welfare Regulations (9 Code of Federal Regulations, Chapter 1, Subchapter A - Animal Welfare) and Institutional Animal Care and Use Committee (IACUC) guidelines. All cats were negative to FeLV p27 antigen as tested by ELISA (IDEXX–PetCheck, Catalogue No. 99-09277) prior to vaccination and challenge. Cats were treated with sulfadimethoxine (27.5 mg/kg, lot no. OBYAA, Albon®, Zoetis) prophylactically for coccidiosis. Cats were observed daily throughout the study and euthanized if they became unwell during the study or at the end of the study. All male cats were castrated on study day 35. The study was carried out in compliance with national legislation and subject to local ethical review.

2.2. Vaccination

The study was designed according to the requirements of the European Pharmacopoeia 6.0 Monograph 01/2008:1321–Feline Leukaemia Vaccine (Inactivated) and licensed vaccination protocols. Animals in group T01 were placebo (saline) vaccinated controls. Animals in T02 were vaccinated with Versifel FeLV vaccine at commercial titre (lot no. L0612AS01), and animals in T03 were vaccinated with Purevax FeLV vaccine at commercial titre (lot no. L375701). Animals were vaccinated on day 0 on the right dorsal and on day 21 on the left dorsal thoracic area and immediately observed for any adverse reactions following vaccination.

2.3. Challenge

On study day 42, all animals were sedated with Tiletamine Hydrochloride (50 mg/mL), Zolazepam Hydrochloride (50 mg/mL), Telazol®, Zoetis, prior to challenge via the intraperitoneal route with 1 mL virulent Clade A FeLV isolate 61E strain (lot no. 1659-51-061004), diluted 1:10 in Minimum Essential Medium.

2.4. Serum samples

All cats were sedated prior to any blood sampling. Samples were collected on the day before first vaccination, before challenge, and weekly from 3 weeks postchallenge to the end of the study in week 15. Blood was collected in any cat that for humane reasons had to be euthanized before the end of the study. Serum samples were tested for the presence of FeLV p27 antigen by ELISA (IDEXX–PetCheck, catalogue no. 99-09277) as per manufacturer's instructions.

2.5. Bone marrow samples

A bone marrow sample was collected from each cat after euthanasia during or at the end of the study and tested for the presence of FeLV proviral DNA by real-time qPCR according to established procedure at Biological Development, Zoetis. The method is described in [10], with a slight modification in cycles used.

2.6. Data analysis

SAS Release 9.2.2 was used for the analysis. All hypothesis tests were at 0.05 significance level (two-sided). The individual animal was the experimental unit. Persistent antigenemia was the primary efficacy variable. Persistent antigenemia was defined as at least three consecutive time points positive or at least 5 time points positive postchallenge. Persistent antigenemia was analyzed using a generalized mixed linear model with the fixed effect of treatment and the random effects of room and block within room. All possible pairwise treatment comparisons were made since the overall treatment effect was significant. Frequency distributions of establishment of FeLV latent infection in bone marrow cells was calculated for each treatment.

3. Results

3.1. FeLV p27 antigen

All cats were negative for FeLV p27 antigen prior to the first vaccination and prior to challenge. All cats from the placebo vaccinated group were antigenemic by 3 weeks post-challenge with 8 of 9 cats (89%) developing persistent antigenemia postchallenge, indicating a valid challenge model (> 80% infection in controls) to evaluate vaccine efficacy (Table 1). The back-transformed least squares mean of FeLV p27 persistent antigenemia was 92% (33% to 100% [95% confidence interval]).

In the Purevax FeLV vaccinated group, 17 out of 20 (85%) cats showed transient antigenemia postchallenge and 16 out of 20 (80%) cats developed persistent antigenemia (Table 1). The back-transformed least squares mean of FeLV p27 persistent antigenemia was 89% (33% to 99% [95% confidence interval]). The level of FeLV p27 persistent antigenemia was not significantly different ($P=0.8060$) between Purevax FeLV vaccinates and placebo (saline) vaccinates.

In the Versifel FeLV group transient viremia, as indicated by early detection of p27 in serum of cats protected from persistent antigenemia at 15 weeks after challenge, was found in 5/19 (26%) vaccinates. Only 2 out of 19 (11%) cats from the Versifel FeLV vaccinated group developed persistent antigenemia postchallenge (Table 1). The back-transformed least squares mean of FeLV p27 persistent antigenemia was 4% (0% to 47% [95% confidence interval]). Postchallenge the level of FeLV p27 persistent antigenemia was significantly lower in the Versifel FeLV vaccinates compared to the placebo (saline) vaccinates ($P=0.0034$) and to the Purevax FeLV vaccinates ($P=0.0014$).

Table 1
Number and percentages of animals positive for FeLV p27 antigen and with persistent antigenemia.

| Week post-challenge | T01 (Placebo) | | T02 (Versifel® FeLV) | | T03 (Purevax® FeLV) | |
|--|---------------------------------|----------------------------|---------------------------------|---------------|---------------------------------|----------------------------|
| | Number p27 positive animals (%) | Number tested ¹ | Number p27 positive animals (%) | Number tested | Number p27 positive animals (%) | Number tested ¹ |
| Pre-vaccination | 0 (0.0) | 9 | 0 (0.0) | 19 | 0 (0.0) | 20 |
| Pre-challenge | 0 (0.0) | 9 | 0 (0.0) | 19 | 0 (0.0) | 20 |
| 3 | 9 (100) | 9 | 2 (10.5) | 19 | 17 (85.0) | 20 |
| 4 | 8 (88.9) | 9 | 4 (21.0) | 19 | 16 (80.0) | 20 |
| 5 | 8 (88.9) | 9 | 3 (15.8) | 19 | 16 (80.0) | 20 |
| 6 | 8 (88.9) | 9 | 2 (10.5) | 19 | 14 (70.0) | 20 |
| 7 | 8 (88.9) | 9 | 3 (15.8) | 19 | 14 (70.0) | 20 |
| 8 | 8 (88.9) | 9 | 3 (15.8) | 19 | 13 (65.0) | 19 |
| 9 | 7 (87.5) | 8 | 2 (10.5) | 19 | 13 (65.0) | 19 |
| 10 | 0 (0.0) | 1 | 1 (5.3) | 19 | 0 (0.0) | 6 |
| 11 | 0 (0.0) | 1 | 1 (5.3) | 19 | 0 (0.0) | 6 |
| 12 | 0 (0.0) | 1 | 1 (5.3) | 19 | 0 (0.0) | 6 |
| 13 | 0 (0.0) | 1 | 1 (5.3) | 19 | 0 (0.0) | 6 |
| 14 | 0 (0.0) | 1 | 1 (5.3) | 19 | 0 (0.0) | 6 |
| 15 | 0 (0.0) | 1 | 1 (5.3) | 19 | 0 (0.0) | 6 |
| Number and percentage of animals with persistent antigenemia | 8/9 (88.9%) | | 2/19 (10.5%) | | 16/20 (80.0%) | |

¹ For ethical reasons, to prevent development of FeLV-related symptoms, animals were euthanized postchallenge once they had confirmed persistent FeLV antigenemia results (positive for FeLV p27 antigen for 3 or more consecutive weeks).

3.2. Proviral DNA in bone marrow

The frequency distributions of animals with FeLV proviral DNA in bone marrow are summarized for all treatment groups in Table 2. Seven of 9 placebo cats (78%) were positive to FeLV proviral DNA in bone marrow cells collected on week 8 or 9 postchallenge prior to euthanasia. Only 1 of 19 Versifel (5%) vaccinated cats was positive to FeLV proviral DNA in bone marrow cells postchallenge. This cat had also shown persistent p27 antigenemia. Thirteen of 20 (65%) Purevax vaccinated cats tested positive to FeLV proviral DNA postchallenge.

In the majority of cases (43/48) results of FeLV persistent antigenemia and proviral DNA loads in the bone marrow correlated. One control animal (T01); one Versifel vaccinated animal (T02), and three Purevax vaccinated animals (T03) presented a positive result to FeLV p27 persistent antigenemia and a negative result to FeLV proviral DNA in bone marrow.

3.2.1. Adverse events and concurrent disease

Adverse events related to administration of the vaccines or control product were not observed. FeLV related clinical signs were observed in two animals (one control cat and one Purevax vaccinated cat) postchallenge and both animals were euthanized on study day 99 and 91, respectively. The control cat presented with fever, depression, low weight, moderate dehydration, enlargement of kidneys, and lethargy. The Purevax vaccinated cat showed signs of weakness and slight ataxia, dehydration, lethargy, fever, possible difficulty with urination/defecation, tachypnea, and mild gingivitis. For welfare reasons, 20 further persistently infected cats were euthanized before FeLV related clinical signs

developed. Two animals (one animal Placebo vaccinated and one Versifel vaccinated) showed clinical signs consistent with Feline Parvovirus (FPV) infection at approximately 10 days and 3 weeks postchallenge, respectively, and were euthanized and excluded from analysis. FPV infection was confirmed in both cases by histopathology and immunofluorescence on intestinal tissue samples. Testing of serum samples revealed neutralizing antibodies to FPV in all study animals at 3 weeks postchallenge whereas all cats tested seronegative to FPV prior to challenge. Subsequently it was confirmed by PCR (commercial kit for FPV) that the 61E FeLV challenge material tested positive for FPV.

4. Discussion

The risk of FeLV infection can be around 2% in healthy cats and as high as 33% in high-risk cats [13,14]. Vaccination remains a safe and effective means to prevent FeLV disease. A number of FeLV vaccines are currently commercially available, and all have been shown to reduce p27 antigenemia in various clinical models.

This study was designed to be consistent with the general immunogenicity requirements of the European Pharmacopoeia 01/2008:1321–Feline Leukaemia Vaccine (Inactivated). Kittens were at the minimum age recommended for vaccination when first vaccinated with Versifel FeLV or Purevax FeLV, and were challenged with virulent type A FeLV at approximately 3.5 months old. According to the monograph, vaccine efficacy is achieved if less than 20% of vaccinated cats develop persistent infection and for a successful challenge study more than 80% of control cats must develop persistent infection. In this study 89% of placebo vaccinated cats

Table 2
Frequency distribution of FeLV proviral DNA in bone marrow cells analyzed by Real Time qPCR.

| Treatment Group | Integration of FeLV proviral DNA in bone marrow cells | | | | |
|----------------------|---|------|-------------------|------|-------------------------|
| | Negative | | Positive | | Total number of animals |
| | number of animals | % | number of animals | % | |
| T01 (Placebo) | 2 | 22.2 | 7 | 77.8 | 9 |
| T02 (Versifel® FeLV) | 18 | 94.7 | 1 | 5.3 | 19 |
| T03 (Purevax® FeLV) | 7 | 35.0 | 13 | 65.0 | 20 |

developed FeLV persistent antigenemia, indicating a valid challenge model to evaluate vaccine efficacy.

Almost 90% (17/19) of cats vaccinated with Versifel FeLV were protected against persistent antigenemia, indicating that the inactivated Versifel FeLV vaccine offered protection against a subsequent challenge with a virulent Clade A FeLV strain during the duration of this study. The level of FeLV p27 persistent antigenemia was significantly lower in Versifel FeLV vaccinates compared to the placebo (saline) vaccinates and to the Purevax FeLV vaccinates.

Versifel FeLV also greatly reduced latent infection in the bone marrow in this study. Only 1 out of 19 (5%) Versifel FeLV vaccinated cats had detectable levels of FeLV proviral DNA in the bone marrow postchallenge. Cats vaccinated with the recombinant Purevax FeLV vaccine were more likely to become latently infected (65%). In these cats antigenemia could not be prevented and consequently the FeLV was more likely to disseminate and insert into the bone marrow.

In the majority of cases the provirus load and the outcome of p27 persistent FeLV infection showed broad agreement for the duration of this study. A similar finding in naturally and experimentally infected cats has been reported elsewhere [15].

In five persistently infected animals, FeLV antigenemia did not cause proviral DNA integration in bone marrow cells. An explanation could be that some of the cats that were defined as persistently antigenemic, as per European Pharmacopoeia, were in fact transiently antigenemic. It seems to be the case for two Purevax FeLV vaccinated cats that were only positive in antigenemia from week 4 to 6 postchallenge. After week 6 postchallenge they were negative for antigenemia and also negative for proviral DNA at week 15 postchallenge. One Versifel FeLV vaccinated cat was positive in antigenemia only from week 3 to 8 post-challenge. After week 8 postchallenge it was negative for antigenemia and also negative for proviral DNA at week 15 postchallenge. Two other persistently antigenemic cats with discordant PCR results were euthanized for welfare reasons during week 9 or 10. It is possible that proviral DNA integration in bone marrow would have occurred after euthanasia had the cat remained in the study for longer. Other work showed an infective state where no proviral integration occurred, but this is normally seen under low infection pressure [1].

Using quantitative real-time PCR (qPCR) a study published by Torres and colleagues showed that nonantigenemic cats can be positive on viral RNA and/or DNA in blood cells and that vaccination with whole inactivated virus adjuvanted FeLV vaccines can prevent nucleic acid persistence in blood cells [16]. This study was not designed to determine nucleic acid in blood, so it is unknown whether virus could have been harbored in blood cells of cats found to be protected against FeLV challenge. Evaluation of qPCR on blood could have been instructive. However, since the clinical significance of nucleic acid-positive/antigen-negative animals is not yet fully understood, the diagnostic significance of applying FeLV qPCR on blood remains unclear.

It has been reported that the induction of virus-neutralizing antibodies to prevent viremia is of prime importance to vaccination [2,17]. Products of the viral envelope gene, such as glycoprotein (gp) 70, are thought to be major targets for FeLV antibody-mediated virus neutralization [18]. Specific humoral immunity has also been shown to correlate with lower initial proviral load in infected cats [15]. Versifel FeLV is an innovative inactivated vaccine with a novel immunostimulatory adjuvant system. The adjuvants in the inactivated vaccine enhance both the humoral and cell-mediated immune responses. This suggests that Versifel FeLV vaccination induces specific immune responses, such as virus-neutralizing antibodies, in cats that are efficiently preventing initial infection of the host target cell. Although the exact determinants of effective FeLV immunity are not fully understood, the Versifel FeLV vaccine was able to confer excellent efficacy as demonstrated by the low levels of FeLV persistent antigenemia and low FeLV proviral loads in

bone marrow. Previous results of challenge studies showed similar reductions in viremia and provirus DNA in cats vaccinated with inactivated virus vaccines [16,19].

Cats in this study were inadvertently exposed to FPV through intraperitoneal inoculation of contaminated FeLV-A challenge material. Natural FPV transmission occurs via the fecal-oral route, most commonly by indirect contact (carried by fomites). FPV may survive in the environment for several months and is highly resistant to some disinfectants. Thus, also under natural circumstances there is a risk for FPV coinfection in FeLV viremic cats. Cats of all ages may be affected by FPV, but kittens are most susceptible, with high mortality rates at over 90% [20]. The low number of clinically affected cats in this study is surprising considering the young age of the study animals and may be explained by the low dose the animals received. As clinical signs of FPV were seen in a control cat as well as a Versifel FeLV vaccinated cat, the susceptibility of these two cats to FPV cannot be attributed to vaccination.

Little is known about the effect of concurrent parvovirus infection on the ability of cats to respond to FeLV vaccination, however, FPV is known to cause leucopenia in infected animals therefore its impact cannot be discounted (although no leukocyte count was carried out to confirm any effect). However, as indicated, both groups were exposed to the same level therefore a comparison between the two groups is still considered valid.

It is possible that the concurrent parvovirus infection caused a lower than expected efficacy in the Purevax vaccine group since previously reported data on kittens vaccinated with a canarypox-based recombinant vaccine show profound differences in efficacy against FeLV challenge compared to this study (i.e., Poulet [21] and Tartaglia [22] demonstrated 83% [5/6 cats] and 100% [6/6 cats] protection, respectively). This could be a result of different immune responses to live versus inactivated vaccines in that the response to a live vaccine is much more cellular driven in comparison to a more antibody driven response to an inactivated product. Therefore, if the concurrent FPV infection resulted in leucopenia the response in the Purevax group could have been compromised more compared to the Versifel FeLV group. As FPV exposure is common in the field this could be considered as an indication of the benefit of the inactivated and adjuvanted Versifel FeLV compared to the live Purevax. Hence the efficacy of Versifel FeLV, even in the face of an agent that can cause immunosuppression, was robustly demonstrated.

In summary, the Versifel FeLV vaccinated cats were protected from both FeLV persistent p27 antigenemia and FeLV proviral DNA integration in bone marrow. We cannot rule out that Purevax FeLV performance was selectively affected by FPV-induced immunosuppression. However, if it was, this study shows the beneficial efficacy of Versifel FeLV even in the face of a potentially immunosuppressive agent. Additional studies would be required to investigate the effect of FPV infection on cellular and/or humoral immune responses in FeLV-vaccinated cats.

5. Conclusion

The inactivated FeLV vaccine (Versifel FeLV) protects cats against persistent and latent FeLV infection after experimental challenge with a virulent FeLV-A 61E strain under laboratory conditions. In this study Versifel FeLV was more efficacious than Purevax FeLV. The results were obtained in an artificial model comparing efficacy against a highly virulent challenge dose and concomitant exposure to FPV and therefore not necessarily reflective of vaccine efficacy following natural exposure in the field. Using FeLV vaccines such as Versifel FeLV or Purevax FeLV should be viewed as an important tool in the fight against FeLV infection. Veterinarians are encouraged to adhere to the current feline vaccination recommendations and to perform a risk-benefit assessment prior to vaccinations.

Conflict of interest

The authors are employees of Zoetis Inc, the company marketing Versifel FeLV. Zoetis provided the funding for the study. The manuscript has been read and approved by all named authors.

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