Protection Against Feline Leukemia Virus Challenge for at Least 2 Years After Vaccination With an Inactivated Feline Leukemia Virus Vaccine*

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CLINICAL RELEVANCE

Twelve cats were vaccinated at 8 and 11 weeks of age with a commercially available inactivated FeLV vaccine (Nobivac FeLV, Intervet/Schering-Plough Animal Health). Eleven cats served as age-matched, placebo-vaccinated controls. All cats were kept in isolation for 2 years after vaccination and were then challenged with virulent FeLV to evaluate vaccine efficacy and duration of immunity. Cats were monitored for 12 weeks after challenge for development of persistent viremia using a commercial FeLV p27 ELISA. Persistent viremia developed in all 11 (100%) of the control cats, whereas 10 of 12 (83%) vaccinated cats were fully protected from persistent viremia following challenge. The results demonstrate that the vaccine used in this study protects cats from persistent FeLV viremia for at least 2 years after vaccination.

INTRODUCTION

FeLV, a member of the family Retroviridae, is an important cause of morbidity and mortality in domestic cats. FeLV is most commonly transmitted horizontally (i.e., via saliva, feces, and milk) but can also be transmitted vertically (from persistently viremic queens to fetuses).[1,2]

Most cats infected with FeLV develop transient or persistent viremia, as indicated by the presence of FeLV p27 antigen detectable by ELISA in plasma or serum.[3] Circulating virus can be cleared by the immune system (transient viremia), but some cats develop an ongo-

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ing infection characterized by persistent viremia. Cats that are transiently viremic may still harbor low levels of FeLV in circulating peripheral blood mononuclear cells, lymph nodes, or bone marrow for months to years after infection; however, in most cases, this low level of virus is not clinically relevant and these cats do not develop FeLV-associated disease.[4,5] In contrast, most cats that fail to clear the virus and become persistently viremic develop FeLV-associated disease, and 70% to 90% of persistently viremic cats die within 18 to 36 months after infection. The most common clinical outcomes in these cats are immunosuppression with associated secondary infections, anemia, and lymphoid neoplasia.[1]

The decision to vaccinate a cat against FeLV should be based on the age of the cat and the risk of exposure.

FeLV vaccines have been available in the United States since 1984 and include whole inactivated virus vaccines, a subunit FeLV antigen vaccine, and a poxvirus-vectored vaccine. Although published studies[6,7] have demonstrated wide variations in efficacy for commercially available FeLV vaccines, vaccination continues to be an important means of protecting cats against disease caused by FeLV. The vaccination guidelines issued by the American Association of Feline Practitioners (AAFP) Feline Vaccine Advisory Panel outline two overall objectives for feline vaccination: (1) vaccinate the greatest number of at-risk cats and (2) vaccinate individual cats no more frequently than necessary.[6] The decision to vaccinate a cat against FeLV should be based on the age of the cat and the risk of exposure. Cats are considered at high risk for FeLV infection if they spend time outdoors, live in FeLV-positive households, or live in households in which FeLV infection status is unknown. The incidence of FeLV infection in multicat households in the absence of preventive measures may exceed 20%.[8,9] Cats are considered at low risk for FeLV infection if they are indoor cats or live in closed multicat households with known FeLV-negative status. Although the FeLV vaccine is considered a noncore vaccine by the AAFP vaccine advisory panel (for use only in cats at risk of exposure), the panel highly recommended FeLV vaccination for all kittens because kittens are more susceptible to the development of persistent viremia after FeLV infection compared with adult cats and because unvaccinated kittens may be at increased risk for infection later in life.

The purpose of this study was to evaluate the ability of one commercially available vaccine (Nobivac FeLV, Intervet/Schering-Plough Animal Health) to protect cats from persistent viremia when challenged with virulent FeLV at least 2 years after vaccination. This adjuvanted whole inactivated virus vaccine has been shown to afford significant protection against FeLV challenge as measured by both persistent viremia and latency.[10–12] The vaccine used in this study was part of a combination vaccine also containing attenuated feline calicivirus (FCV), feline herpesvirus (FHV), feline parvovirus (FPV), and *Chlamydophila felis*.

MATERIALS AND METHODS

This study was approved by the Intervet/Schering-Plough Animal Health Institutional

Animal Care and Use Committee. Personnel administering vaccine, placebo, or challenge; testing laboratory samples; and performing clinical observations had no knowledge of treatment group assignments.

Animals

Twenty-three specific pathogen–free cats were obtained from a commercial supplier (Liberty Research, Waverly, NY). The cats were randomly assigned to two groups (12 vaccinates and 11 controls) and were group-housed in separate rooms. Approximately 1 year before challenge, vaccinates and controls were commingled; all cats were housed together in a single room for the duration of the study. Veterinary care was provided as needed throughout the study period.

Vaccine

The vaccine used in this study consisted of a lyophilized fraction containing attenuated FCV, FHV, FPV, and *C. felis* (Eclipse 4, Intervet/Schering-Plough Animal Health) and a liquid fraction containing FeLV (Nobivac FeLV). The liquid fraction was used to rehydrate the lyophilized fraction. All antigens in the vaccine met present United States Department of Agriculture (USDA)–approved specifications for serial release.[13,14]

Cats in the control group were inoculated with a placebo consisting of a lyophilized fraction containing attenuated vaccine antigens and rehydrated with sterile diluent instead of the FeLV vaccine.

Vaccination

Cats in the vaccinated group were administered a single dose of the combination vaccine at 8 weeks of age (age range at first vaccination: 52 to 58 days) by the subcutaneous route in the interscapular region. All cats received a second dose of vaccine 21 days after initial vaccination. Cats in the control group were administered the placebo vaccine rehydrated with sterile diluent according to the same schedule and route as cats in the vaccinated group.

Challenge

Two years after the second vaccination, all cats were challenged with the A/61E strain of FeLV by the oronasal route.[15,16] The cats were sedated with ketamine/acepromazine and challenged with 1 mL of a solution (0.25 mL into each nostril and 0.5 mL orally) containing 10^5 plaque-forming units/mL FeLV-A/61E (determined by titration on Clone 81 cells[17]) on each of 2 consecutive days. To facilitate development of viremia in these older animals after challenge, cats were treated with 10 mg/kg methylprednisolone acetate (Depo-Medrol, Pfizer) 4 hours before the first challenge and again 1 week after the first challenge.

Sample Collection and Processing

Blood samples were collected in serum separator tubes from all animals 6 months after vaccination and again 2 days before challenge. Blood was also collected from each animal at weekly intervals from 3 to 12 weeks after challenge. FeLV viremia was evaluated using a commercial FeLV p27 ELISA kit (PetCheck, IDEXX Laboratories). Persistent viremia was defined as the presence of FeLV p27 antigen in serum for 3 consecutive weeks or for 5 weeks total (consecutive or not) from weeks 3 to 12 after challenge.

Statistical Analysis

The presence or absence of persistent viremia was compared between groups vaccinated with the FeLV vaccine and the placebo using Fisher's exact test. Efficacy of the vaccine was expressed as the prevented fraction: (1 – risk ratio [RR]) × 100, where RR is Pv/Pc, Pv is the proportion of FeLV vaccinates affected

with persistent viremia, and Pc is the proportion of controls affected with persistent viremia. A 95% exact confidence interval (CI) for the prevented fraction estimate was determined using the complement of the CI for the RR. Statistical analysis was performed using StatXact-6 version 6.2.0 (Cytel Software Corp, Cambridge, MA).

RESULTS

All cats were negative for FeLV p27 antigen before vaccination and challenge. No clinical signs of FeLV infection were observed after vaccination.

Persistent Viremia

After challenge, all 11 control cats (100%) developed persistent viremia as determined by detection of FeLV p27 antigen. In contrast, 10 of the 12 cats (83%) vaccinated with the Eclipse 4 + Nobivac FeLV vaccine remained free of persistent viremia after challenge (Table 1). Further, FeLV p27 antigen was not detected, even transiently, in any of these 10 cats. One control cat was removed from the study 7 weeks after challenge because of a severely aggressive temperament. This cat was already defined as persistently viremic before removal from the study (Table 1), so removal did not affect the outcome of the study.

Statistical Evaluation

The prevented fraction was 83% (95% CI, 51.6% to 98.2%), and protection from challenge was statistically significant ($P < .0001$).

DISCUSSION

Intervet/Schering-Plough Animal Health currently markets Nobivac FeLV as an aid in the prevention of diseases associated with FeLV infection; the vaccine works by preventing persistent viremia in cats exposed to virulent FeLV. Nobivac FeLV contains cell culture–derived FeLV subgroups A and B. The virus has been chemically inactivated and combined with a proprietary aluminum-free adjuvant designed to enhance the immune response. The vaccine provides significant protection against both persistent viremia and latency in artificially immunosuppressed cats. Protection against latency was determined in previous studies with this vaccine.[10,11] The vaccine has also been shown to aid in the prevention of lymphoid tumors in cats after challenge with virulent FeLV.[18]

The vaccine used in this study was a complex combination containing five antigen components. This represents an opportunity for other components to interfere with the ability of the FeLV vaccine to induce protective immunity, but no such interference was observed in this study.

USDA guidelines[13] for licensure of FeLV vaccines suggest that at least 80% of the nonvaccinated control animals should develop persistent viremia after challenge and that at least 75% of the vaccinated animals should be protected from persistent viremia. The European Pharmacopeia requires that at least 80% of nonvaccinated control animals become persistently viremic following challenge, while at least 80% of vaccinated animals must be protected from persistent viremia.[19] In this study, 100% of nonvaccinated cats developed persistent viremia after challenge with the use of an immunosuppressive agent (methylprednisolone acetate), which was used to overcome the cats' natural age-related resistance to infection by FeLV. The severity of the challenge is underscored by the fact that every nonvaccinated cat was positive for FeLV p27 antigen at each sampling period after challenge. In contrast, 10 of 12 vaccinated cats (83% prevented fraction) were completely protected from development of persistent or transient viremia after challenge according to the testing methodology

TABLE 1. FeLV p27 Antigen ELISA Results in Cats Challenged With Virulent FeLV

		ELISA Results at Weeks Postchallenge									
Vaccine ID	***Animal ID***	*Week 3*	*Week 4*	*Week 5*	*Week 6*	*Week 7*	*Week 8*	*Week 9*	*Week 10*	*Week 11*	*Week 12*
Vaccinates[a]	JAA1	–	–	–	–	–	–	–	–	–	–
	JAB1	–	–	–	–	–	–	–	–	–	–
	JAB2	–	–	–	–	–	–	–	–	–	–
	JAB5	–	–	–	–	–	–	–	–	–	–
	JAC5	+	+	+	+	+	+	+	+	+	+
	JAD3	–	–	–	–	–	–	–	–	–	–
	JAE5	–	–	–	–	–	–	–	–	–	–
	JAH3	–	–	–	–	–	–	–	–	–	–
	JAI4	–	–	–	–	–	–	–	–	–	–
	JAI5	–	–	–	–	–	–	–	–	–	–
	JAM2	–	–	–	–	–	–	–	–	–	–
	JAM3	+	+	+	+	+	+	+	+	+	+
Controls[b]	JAA2	+	+	+	+	+	+	+	+	+	+
	JAB4	+	+	+	+	+	*removed from study*				
	JAC1	+	+	+	+	+	+	+	+	+	+
	JAC4	+	+	+	+	+	+	+	+	+	+
	JAC6	+	+	+	+	+	+	+	+	+	+
	JAC7	+	+	+	+	+	+	+	+	+	+
	JAE1	+	+	+	+	+	+	+	+	+	+
	JAI1	+	+	+	+	+	+	+	+	+	+
	JAK3	+	+	+	+	+	+	+	+	+	+
	JAM6	+	+	+	+	+	+	+	+	+	+
	JAN3	+	+	+	+	+	+	+	+	+	+

+ = cat was positive for FeLV viremia as measured by p27 ELISA.
– = cat was negative for FeLV viremia as measured by p27 ELISA.
[a]Vaccinated with Eclipse 4 + Nobivac FeLV.
[b]Vaccinated with a lyophilized fraction of feline calicivirus, feline herpesvirus, and *Chlamydophila felis* combination vaccine.

used in this study and accepted by the USDA for demonstration of persistent viremia.[13]

Although other FeLV vaccines have been shown to be protective for up to 1 year,[20–22] this study is significant because it is the first time a commercial FeLV vaccine has been shown to induce immunity for a minimum of 2 years after a primary course of vaccination. Because the vaccine does not need to be administered annually to ensure protection of at-risk cats, adverse events associated with more frequent vaccinations are less likely. Differences in production and composition of vaccines from different manufacturers make it difficult to extend these findings by inference to other commercial vaccines, especially because significant differences in efficacy have been reported for commercially available FeLV vaccines.[7,12]

CONCLUSION

Results from this study demonstrate that the vaccine used in this study (Nobivac FeLV) protects cats from persistent FeLV viremia for at least 2 years after vaccination.

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