Immunogenicity and Efficacy of a Commercial Feline Leukemia Virus Vaccine

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Twenty young adult specific pathogen-free cats were randomly divided into two groups of 10 animals each. One group was vaccinated with two doses of feline leukemia virus vaccine according to the manufacturer's recommendations. All 20 cats were challenge exposed oronasally (4 times over a 1-week period), beginning 3 weeks after immunization, with a virulent subgroup A strain of FeLV (CT600-FeLV). The severity of the FeLV infection was enhanced by treating the cats with methylprednisolone acetate at the time of the last FeLV exposure. Ten of 10 nonvaccinated cats became persistently viremic compared with 0/10 of the vaccinates. ELISA antibodies to whole FeLV were present at high concentrations after immunization in all of the vaccinated cats, and there was no observable anamnestic antibody response after challenge exposure. ELISA antibodies to whole FeLV appeared at low concentrations in the serum of nonvaccinated cats after infection but disappeared as the viremia became permanently established. Virus neutralizing antibodies were detected in 3/10 vaccinates and 0/10 nonvaccinates immediately before FeLV challenge exposure, and in 8/10 vaccinates and 1/10 nonvaccinates 5 weeks later. Although vaccination did not consistently evoke virus neutralizing antibodies, it appeared to immunologically prime cats for a virus-neutralizing antibody response after infection. Active FeLV infection was detected in bone marrow cells taken 14 weeks after infection from 10/10 nonvaccinates and 0/10 vaccinates. Latent FeLV infection was not detected in bone marrow cells from any of the vaccinated cats 14 weeks after challenge exposure. (Journal of Veterinary Internal Medicine 1993; 7:34-39)

AT LEAST three new feline leukemia virus (FeLV) vaccines have appeared on the market during 1991. Two of these vaccines are conventional inactivated and adjuvanted whole virus vaccines, whereas the third is a true viral subunit vaccine containing FeLV envelope protein expressed in bacteria. Reports from each manufacturer indicate that their respective vaccine provides a high degree of protection against artificial challenge-exposure with virulent virus. The manufacturers' efficacy data has been confirmed in semi-independent studies for two of these vaccines.

The current study deals with the third of these newest FeLV vaccines, manufactured by Solvay Animal Health, Inc. This vaccine is an adjuvanted inactivated whole virus vaccine. The manufacturer has presented experimental data showing that the vaccine induces a high degree of protection to two challenge-exposure doses of Rickard-FeLV administered oronasally in the face of methylprednisolone acetate immunosuppression. Methylprednisolone largely abolishes age-related resistance to the virus and causes susceptible cats to develop persistent infections at a much higher incidence. In this study, the vaccine was tested in a similar manner but with a different strain of FeLV, CT600-FeLV. This is a subgroup A strain of FeLV that has been used in previous corticosteroid augmented challenge-exposure studies to test the efficacy of several other commercial FeLV vaccines.

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Methods and Materials

Experimental Animals

Twenty 8-month-old specific pathogen-free (SPF) cats were obtained from the breeding colony of Drs. Quinton Rogers and James Morris, Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis. The cats were healthy at physical examination and were free of all known feline viral pathogens except for feline enteric coronavirus and feline herpesvirus, type 1, by serology. The cats were divided randomly by sex and litter of origin into two groups of ten cats each and housed in animal quarters provided by the Animal Resources Services, University of California, Davis.

Virus Inoculum

The CT600, subgroup A, strain of FeLV was propagated in a permanently infected leopard cat testicle cell line (CT600 cells). A master stock of cells was frozen at low passage: infectious culture supernatants for challenge exposure were always harvested within 1–5 cell passages from the master stock. The CT600 cell cultures were grown to confluency, the medium was completely replaced, and supernatants were collected 24–48 hours later for challenge-exposure studies. The virus titer of tissue culture supernatants was titrated on Crandell feline kidney (CrFK) cells.

One-tenth milliliter from each serial tenfold dilution of infectious tissue culture supernatant was overlayered on a three-fourth confluent monolayer of CrFK cells in 96-well microtiter plates. Each dilution was tested in triplicate. A complete media change was made after 24–48 hours, and the concentration of FeLV-p27 antigen in the culture supernatants was measured at day 7 after infection by an antigen capture enzyme-linked immunosorbent assay (ELISA).

Viral stocks were found to contain approximately 40,000 tissue culture infectious doses—100% (TCID100) of virus per ml.

FeLV Immunization

Forty vials of commercially labeled vaccine,* serial number 60100 (expiration date Jan. 9, 1993) were sent directly to this investigator by Drs. David Hines and John Cutting of Solvay Animal Health Inc. April 11, 1991. This was the first serial to be produced in excess of 10,000 doses and was used originally in the state of California and nationally for field safety trials under an experimental label.

After the vaccine* was federally licensed on March 28, 1991, the remainder of the vaccine serial was given commercial labels and distributed and sold in the state of California. The vaccine was administered to the immunized group of cats within the guidelines provided by the manufacturer: two doses at 3-week intervals, intramuscularly. Nonimmunized cats did not receive any injections.

FeLV Challenge Exposure

Cats were challenge exposed with 1 mL of CT600 cell culture medium on each of exposure days 0, 2, 4, and 7. One-half milliliter of the virus containing tissue culture fluid was instilled up both nostrils and 1/2 mL was placed into the mouth.

Cats were injected with 5 mg/kg of methylprednisolone acetate on exposure day 7. The 7-day challenge exposure period was begun 3 weeks after the second vaccination.

Monitoring FeLV Infection Status

Serum was collected from each cat at weekly intervals, beginning just before the first immunization and ending 18 weeks later. Serum was assayed for FeLV-p27 antigen by an antigen capture ELISA. FeLV-p27 antigen concentrations less than 2 μg/100 μL were recorded as negative (−), antigen concentrations from 2–5 μg/100 μL as suspicious (?), antigen concentrations from 6–10 μg/100 μL were read out as weakly positive (+), and antigen concentrations greater than 10–40 ng/100 μL or more were read out as strongly positive (+).

Latent FeLV infections were detected by a modification of a previous procedure. Heparinized bone marrow aspirates (0.05–0.2 mL) were taken 14 weeks after challenge exposure. Aspirates were diluted in 5 mL of tissue-culture media, the cells pelleted by centrifugation, and the cell pellet resuspended in 2 mL of tissue culture media. One milliliter of the bone marrow suspension was then overlaid on a freshly passed two-thirds confluent monolayer of CrFK cells in each of two wells in a 24-well microtiter plate.

Dexamethasone phosphate (1 μmol/L) was added to the medium in one of the two wells from 1 week of culture onward. All of the tissue culture medium was replaced with fresh medium at weekly intervals; old medium was saved for FeLV-p27 antigen assay. Cultures were monitored for 6 weeks.

Antibody Assays

Serum antibodies to purified whole FeLV were measured by an enzyme-linked immunosorbent assay (ELISA). Virus-neutralizing antibodies were measured by a modification of a previously described infectivity inhibition assay. Freshly trypsinized CrFK cells were resuspended to a concentration of 2 × 10⁶ cells/mL in tissue culture medium. One hundred microliters of cell suspension was then added to each well of a 96-well microtiter plate and incubated at 37°C in 5% CO₂ in air. Cat serum to be tested was diluted serially twofold in a 24-well microtiter plate with tissue culture medium to a
volume of 150 μL, with serum:medium dilutions from 1:5 to 1:160. One hundred fifty microliters of a 1:5 dilution in tissue culture medium of fresh CT600 cell supernatant (the latter containing about 40,000 TCID100/mL of CT600-FeLV) was then added to each well and incubated for 1 hour at 37°C. The plates were tilted several times to thoroughly mix diluted virus and serum. One hundred microliters of a 1:5 dilution in tissue culture medium of fresh CT600 cell supernatant (the latter containing about 40,000 TCID100/mL of CT600-FeLV) was then added to each well and incubated for 1 hour at 37°C. The plates were tilted several times to thoroughly mix diluted virus and serum. One hundred microliters of a 1:5 dilution in tissue culture medium of fresh CT600 cell supernatant (the latter containing about 40,000 TCID100/mL of CT600-FeLV) was then added to each well and incubated for 1 hour at 37°C. The plates were tilted several times to thoroughly mix diluted virus and serum.

Plates were then incubated at 37°C in a humidified incubator in an atmosphere of 5% CO₂ in air. After 24 hours, 150 μL of culture medium was removed from each well and replaced with an equal volume of fresh tissue culture fluid. One hundred fifty microliters of tissue culture supernatant was removed from each well after 7 days of culture and assayed for FeLV-p27 antigen by an antigen capture ELISA. The average absorbance values of the triplicate test wells were calculated. Virus-neutralizing antibody activity was related to the degree that FeLV-p27 antigen elaboration was inhibited compared with control wells containing parallel dilutions of normal SPF cat serum. A strong positive reaction (+) was equal to a 75–100% reduction in FeLV-p27 antigen production. A weak positive reaction (±) correlated with a 50–75% reduction, and a negative reaction (−) correlated with a 0–50% reduction. The virus-neutralizing antibody titer was equal to the highest dilution of serum that produced a 50% inhibition of FeLV-p27 elaboration.

Results

Serum Antibody Responses

High concentrations of ELISA antibodies to whole FeLV appeared in the serum of vaccinated cats after the

![Graph](image)

**Fig. 1.** The average ± 2 standard errors. ELISA antibody concentrations to whole FeLV in sera from 10 Fevaxin FeLV* vaccinated and 10 non-vaccinated cats. The FeLV challenge exposure was completed at day 0.

| Cat #   | Pre-vaccination | Post-vaccination* | Post-challenge† |
|---------|-----------------|-------------------|-----------------|
| Vaccine cats |
| 90-550  | −               | ± 20              | ± 20            |
| 90-563  | 5               | ± 5               | ± 5             |
| 90-568  | −               | ± 10              | ± 5             |
| 90-577  | −               | −                 | ± 20            |
| 90-578  | −               | −                 | ± 5             |
| 90-663  | −               | −                 | ± 5             |
| 90-670  | −               | −                 | ± 10            |
| 90-678  | −               | −                 | ± 10            |
| 90-737  | −               | −                 | ± 10            |
| 90-775  | −               | −                 | −               |
| Control cats |
| 90-566  | −               | −                 | ± 10            |
| 90-574  | −               | −                 | −               |
| 90-643  | −               | −                 | −               |
| 90-652  | −               | −                 | −               |
| 90-666  | −               | −                 | −               |
| 90-669  | −               | −                 | −               |
| 90-681  | −               | −                 | −               |
| 90-741  | −               | −                 | −               |
| 90-771  | −               | −                 | −               |
| 90-772  | −               | −                 | −               |

* Four weeks after second immunization and 1 week after beginning challenge exposure with virulent FeLV.
† Five weeks after challenge exposure with virulent FeLV.

First immunization (Fig. 1). There was a weak anamnestic antibody response after the second immunization. No anamnestic antibody response was observed in vaccinated cats after challenge exposure with virulent FeLV. ELISA-antibodies appeared at low concentrations in the serum of nonvaccinated cats shortly after FeLV challenge exposure but rapidly declined to insignificant concentrations as the viremia became permanently established (Fig. 1).

Three of the ten vaccinated cats had detectable concentrations of virus-neutralizing antibody in their serum 4 weeks after the second immunization (1 week after challenge exposure) (Table 1). Low concentrations of virus-neutralizing antibodies were measured in the serum from 8/10 of the vaccinated cats and 1/10 nonvaccinated cats 5 weeks after challenge exposure with FeLV.

FeLV Infection After Challenge Exposure

FeLV-p27 antigenemia was observed as early as 1 week after the end of the challenge exposure period in nonvaccinated cats (Table 2). The antigenemia was somewhat variable and at low concentrations for the first several weeks in nonvaccinated cats and then persisted at high concentrations thereafter. Only one of the immunized cats became FeLV-p27 antigenemic, but only at a suspicious level for one test period. At the end of the 12-week postinfection study period, 10/10 non-vaccinates were
TABLE 2. FeLV-p27 Antigenemia in Vaccinated and Nonvaccinated Cats After Challenge Exposure with Virulent FeLV

| Cat #   | Group    | 0   | 1/2 | 2   | 3   | 4   | 5   | 6   | 8   | 10  | 12  |
|---------|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 90-550  | vaccine  | - * | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-563  | vaccine  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-568  | vaccine  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-577  | vaccine  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-578  | vaccine  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-663  | vaccine  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-670  | vaccine  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-678  | vaccine  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-737  | vaccine  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-775  | vaccine  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| 90-566  | control  | -   | +   | ±   | ±   | +   | +   | +   | +   | +   | +   |
| 90-574  | control  | -   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| 90-643  | control  | -   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| 90-652  | control  | -   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| 90-666  | control  | -   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| 90-669  | control  | -   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| 90-681  | control  | -   | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| 90-741  | control  | -   | +   | ±   | +   | +   | +   | +   | +   | +   | +   |
| 90-771  | control  | -   | +   | ±   | +   | +   | +   | +   | +   | +   | +   |
| 90-772  | control  | -   | +   | +   | +   | +   | +   | +   | +   | +   | +   |

\* (-) = ≤2 ng/100 µL FeLV-p27
\*\* (±) = 2-5 ng/100 µL FeLV-p27
\*\*\* (±) = 6-10 ng/100 µL FeLV-p27
\*\*\*\* (±) = ≥11-40 ng/100 µL FeLV-p27

Persistently antigenemic compared with 0/10 vaccinates.

To confirm the presence of persistent infection, bone marrow aspirates were taken from all 20 cats 14 weeks after FeLV challenge exposure and co-cultivated with normal CrFK cells.

Culture supernatants of bone marrow/CrFK cell cultures from 10/10 the nonvaccinated cats and 0/10 of the vaccinated cats yielded FeLV-p27 (Table 3).

Detection of Latent FeLV Infection

Parallel bone marrow/CrFK cell cultures to those described earlier were cultured in the presence of dexamethasone to activate any latent FeLV infections that might have been present in the bone marrow cells. Any replication of FeLV by bone marrow cells would then be amplified through infection of contiguous CrFK cells. Bone marrow/CrFK cell cultures from vaccinated cats were negative for FeLV expression even after treatment with dexamethasone. In contrast, cultures from nonvaccinated and FeLV challenge-exposed cats produced the virus from the onset, indicating that all of these cats were actively infected. Cultures from latently infected cats, if there had been such animals, would have been virus-negative for the first 2-4 weeks before expressing the virus.

Discussion

The results of this study confirmed, and even improved upon, the manufacturer’s efficacy claims\(^1\) for the vaccine\(^*\). The vaccine protected 10 cats against a FeLV challenge exposure that induced persistent viremia in all ten nonvaccinates. Compared with tests by the manufacturer, the present study employed four rather than two virulent virus exposures and a different challenge strain of FeLV.

The CT600 strain of FeLV has not been tumorigenic in our hands, whereas the Rickard strain of FeLV induces thymic lymphomas in a majority of infected animals.

These findings confirmed that the challenge exposure used by the author for the testing of other commercial vaccines\(^9\) was not unreasonably vigorous and that commercial FeLV vaccines could be highly efficacious in preventing persistent FeLV viremia.

Experiments reported herein also confirmed that inactivated whole virus FeLV vaccines are effective against FeLV infection, a fact that was first reported in 1979.\(^13\) Other studies reported before 1979 suggested that inactivated whole FeLV vaccines might enhance, rather than protect against, challenge infection with virulent FeLV.\(^14\) The ability of inactivated whole FeLV vaccines to protect against FeLV infection has also been reported.\(^15,16\)

Vaccinated cats were highly resistant to both active and latent FeLV infections, which also supported the manufacturer’s claims.\(^1\) However, the CT600 strain of FeLV does not produce a high rate of latent infections compared with Rickard-FeLV.\(^11\) Therefore, the manufacturer’s tests with Rickard-FeLV challenge exposure
Table 3. Detection of Active (Without Dexamethasone Treatment) and Latent (Dexamethasone Treated) FeLV Infection in Bone Marrow cell/CrFK Cell Cultures Established from Vaccinated and Nonvaccinated Cats 14 weeks After Challenge Exposure with FeLV

| Cat #    | Without Dexamethasone | With Dexamethasone |
|----------|-----------------------|--------------------|
|          | 1 | 2 | 3 | 4 | 5 | 2 | 3 | 4 | 5 | 6 |
| 90-550   | - | - | - | - | - | - | - | - | - | - |
| 90-563   | - | - | - | - | - | - | - | - | - | - |
| 90-568   | - | - | - | - | - | - | - | - | - | - |
| 90-577   | - | - | - | - | - | - | - | - | - | - |
| 90-578   | - | - | - | - | - | - | - | - | - | - |
| 90-663   | - | - | - | - | - | - | - | - | - | - |
| 90-670   | - | - | - | - | - | - | - | - | - | - |
| 90-678   | - | - | - | - | - | - | - | - | - | - |
| 90-737   | - | - | - | - | - | - | - | - | - | - |
| 90-775   | - | - | - | - | - | - | - | - | - | - |
| 90-566   | + | + | + | + | + | + | + | + | + | + |
| 90-574   | + | + | + | + | + | + | + | + | + | + |
| 90-643   | + | + | + | + | + | + | + | + | + | + |
| 90-652   | + | + | + | + | + | + | + | + | + | + |
| 90-666   | + | + | + | + | + | + | + | + | + | + |
| 90-669   | + | + | + | + | + | + | + | + | + | + |
| 90-681   | + | + | + | + | + | + | + | + | + | + |
| 90-741   | + | + | + | + | + | + | + | + | + | + |
| 90-771   | + | + | + | + | + | + | + | + | + | + |
| 90-772   | + | + | + | + | + | + | + | + | + | + |

FeLV infection was monitored by FeLV-p27 antigen release into the culture medium.

might be more appropriate for measuring the vaccines ability to prevent latent infections. Latent infections from virus strains such as Rickard-FeLV occur mainly in cats that have undergone a transient primary viremia. This is also in agreement with the findings of other researchers. The vaccine appeared to be immunogenic, as measured by its ability to induce near maximal concentrations of ELISA antibodies to whole FeLV after a single immunization. However, the vaccine only induced virus neutralizing antibodies in 3/10 twice immunized cats. There was a good secondary virus neutralizing antibody response in 8/10 cats after challenge exposure, indicating that the vaccine had immunologically primed the cats for a virus neutralizing antibody response after infection.

This study supported previous observations on the role of antibodies in protection against persistent FeLV viremia. First, FeLV vaccines do not need to induce detectable concentrations of virus neutralizing antibodies to be protective. Ten of ten vaccinated cats were solidly protected against challenge exposure, although only 3/10 of these vaccinated animals had serum virus-neutralizing antibodies after immunization. Second, cats that are destined to become persistently viremic usually make much lower concentrations of antibodies of all types than cats that resist persistent viremia. Third, a small proportion of cats that become persistently viremic may develop low and transient virus-neutralizing antibody titers during the earlier stages of infection. These observations tend to temper previous beliefs that virus-neutralizing antibodies are solely responsible for protection. Finally, the efficacy of FeLV vaccines is best measured by challenge exposure and not by its ability to make any particular type or concentration of antibody.

FeLV vaccines have been tested for efficacy by a number of different types of challenge-exposure conditions; several different virus strains have been used, the ages of cats have varied between and within studies, FeLV exposure has involved direct contact as well as artificial means, both oronasal and parenteral routes of infection have been used, and some studies have used methylprednisolone acetate treatment to augment infection.

However, the most important question is not how various challenge exposures compare with each other but how each of them compares to the type of exposure that occurs in the field. Unfortunately, the clinical outcome of field exposure also varies greatly depending on the age of the animal, the duration and magnitude (dose) of exposure, route of exposure (transplacental, lactogenic, oral, or parenteral by bites), the degree of associated stress, the level of maternal immunity, etc. Although no single experimentally administered challenge-exposure regimen can mimic all field situations, the method of experimental infection should incorporate the most crucial features of natural field exposure. At a minimum, the challenge exposure should be administered to cats with developed immune systems (post-weaning age) and by the most common natural route (mucosal). There is no evidence that corticosteroid aug-
mentation provides any more or less rigorous exposure than non-steroid augmented infections. However, the practice may be useful because it decreases the numbers of animals needed to demonstrate efficacy and it may mimic the most stressful exposure situations seen in the field.

Although veterinarians differ on the relevance of a particular challenge-exposure regimen, surprisingly little concern is placed on the duration of vaccine immunity. Neither the present study, nor that of the manufacturer, tested the duration of immunity evoked by the primary series of FeLV immunizations. Challenge exposure in this study, and other studies on commercial FeLV vaccines that have been reported in the literature, has followed the last immunization by a few weeks and no long-range protection experiments have been reported. Although manufacturers recommend that cats be reimmunized yearly, this period has been based more on historical protocol than scientific studies.

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