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Evaluation of efficacy and safety of an inactivated virus vaccine against feline leukemia virus infection

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Summary: *An inactivated virus vaccine was developed for prevention of FeLV infection in domestic cats. When given in 2 doses, 3 weeks apart, to cats that were ≥9 weeks old at the time of first vaccination, the vaccine prevented persistent viremia from developing in 132 of 144 (92%) vaccinates after oronasal challenge exposure with virulent FeLV. In contrast, persistent viremia developed after oronasal challenge exposure with FeLV in 39 of 45 (87%) age-matched nonvaccinated control cats. Transient viremia, indicated by early detection of p27 by ELISA in serum of cats protected from persistent viremia at 12 weeks after challenge exposure, was found in 10 of 132 (8%) vaccinates. Cats that were aviremic 12 to 16 weeks after challenge exposure were examined for reactivation of latent FeLV infection; 4 weekly doses of methylprednisolone were administered, followed by in vitro culture of bone marrow cells. Latent infection was readily reactivated in 6 of 8 (75%) nonvaccinated control cats that had been transiently viremic after challenge exposure. However, latent infection was reactivated in only 5 of 48 (10%) protected vaccinates, and in none of 38 vaccinates in which transient viremia had not been detected. In a safety field trial, only 34 mild reactions of short duration were observed after administration of 2,379 doses of vaccine to cats of various ages, breeds, and vaccination history, for a 1.43% reaction rate. Results indicate that the aforementioned inactivated virus vaccine is safe and efficacious for the prevention of infection with FeLV.*

Feline leukemia virus is a horizontally transmitted retrovirus of cats. The virus is associated with persistent or transient viremia, and is capable of establishing latent infection in cats. Persistent viremia is strongly correlated with ultimate development of proliferative, degenerative, or neoplastic changes in cells of the hemopoietic system, resulting in immunosuppression, leukemia, anemia, or tumor development. On the other hand, infected cats that have transient viremia may develop protective immunity, resulting in elimination of cells with a chromosomally integrated FeLV genome, or cats may develop persistent, latent infection.[1] Elimination of latently infected cells is desirable to reduce the compromised neutrophil function associated with latent FeLV infection,[2] and to reduce the possibility of reactivation of virus production.

An inactivated, whole virus vaccine[a] has been developed for prevention of persistent viremia and latent infection of cats induced by FeLV. The purpose of the study reported here was to document the efficacy and safety of the vaccine.

Materials and Methods

Cats—Specific-pathogen-free male and female cats, obtained from a commercial source, were given primary vaccination between the ages of 9 and 14 weeks. Cats were gang-housed without regard to vaccination status before and after virus challenge exposure. Cats were provided commercial dry chow and water ad libitum. Cats were free of antibody to FeLV, feline calicivirus, feline rhinotracheitis virus, feline panleukopenia virus, and feline strains of *Chlamydia psittaci*.

Vaccination—The vaccine was made from cell culture fluids containing FeLV subgroup A and B viruses collected from a chronically infected feline cell line. Infective FeLV was chemically inactivated, and viral components were selectively concentrated before combining with an aqueous adjuvant. Cats were vaccinated with two 1.0-ml doses given 3 weeks apart, either IM or SC.

Challenge-exposure procedure—The Rickard subgroup-A strain of FeLV was used as the challenge virus. Vaccinates and age-matched control cats were challenge-exposed on 2 consecutive days 2 weeks after the second vaccination by the oronasal route, a natural route of exposure to FeLV.[3] One week after challenge exposure, all cats were immunosuppressed with methylprednisolone acetate,[b] administered IM at dosage of 10 mg/kg of

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[a]Fevaxyn-FeLV, Solvay Animal Health, Inc, Mendota Heights, Minn.

[b]Depo-medrol, The Upjohn Co, Kalamazoo, Mich.

body weight. Serum samples were obtained at time of vaccination, at time of challenge-exposure, and at 2, 3, 4, 6, 8, 10, and 12 weeks after challenge exposure, and cats were then evaluated for viremia. Cats not viremic 12 weeks after challenge exposure were considered protected.

Transient viremia was defined as aviremia 12 weeks after challenge-exposure in a cat that had previously been viremic. Latent infection was defined as one in an aviremic cat in which viremia could be induced by immunosuppression, or as one in which FeLV was expressed in vitro by bone marrow cells from an aviremic cat.

Detection of FeLV viremia—The presence of FeLV core protein p27 was used as an indication of FeLV in serum (viremia) or cell culture fluids. Viral p27 was detected, using a commercially available ELISA.[c]

Latency studies—In vivo reactivation of latent FeLV infection in aviremic cats was attempted by treatment of cats once a week for 4 weeks with 10 mg of methylprednisolone/kg given IM, beginning between 12 and 16 weeks after challenge exposure. Serum was obtained weekly and examined for viral p27. Cats that were still aviremic after 4 weekly doses of corticosteroid were subsequently examined further by an attempt to reactivate FeLV infection from bone marrow cells cultured in vitro. Bone marrow culture was done, using a modification of the method of Pedersen.[2] At 16 to 18 weeks after challenge exposure, a femoral bone marrow specimen was aspirated into RPMI 1640 medium containing 25 U of heparin/ml. After vigorous pipetting to break up aggregates, the bone marrow cells were filtered through cotton gauze. Cells were cultured at 37 C and 5% CO_2 in air in T-25 flasks, using 5 ml of RPMI 1640 medium supplemented with 15% horse serum, 50 μM 2-mercaptoethanol, 10^{-7} M hydrocortisone and 5 μg of gentamicin/ml. Initial cell density was 2×10^6 nucleated bone marrow cells/ml. Two cultures were established for each cat. After 7 days of culture, half the medium in each T-25 flask was replaced. At 14 and 21 days, all medium was replaced. Each culture was tested for p27 by ELISA on days 7, 14, 21, and 28.

Safety field trial—Vaccine was distributed to participating veterinarians in 10 states. The vaccine was administered IM or SC to healthy cats representing various ages, breeds, vaccination histories, and both genders. For primary vaccination, cats ≥9 weeks old were given 2 doses, 3 weeks apart. Cats previously vaccinated with a FeLV vaccine were given a single booster dose. Cats were observed for any immediate reaction after vaccination by the participating veterinarian, and owners were requested to report any signs of reaction they observed after the cat returned home.

[c]Petchek, IDEXX Corp, Portland, Me.

Table 1—Protection from challenge exposure-induced persistent and transient viremia in vaccinated cats

Vaccine No.*	Persistent viremia (No./total)		No. of transiently viremic vaccinates
	Vaccinates	Controls	
1	0/18	4/4	0/18
2	0/11	4/4	1/11
3	1/10	5/5	1/9
4 and 5	2/13	4/5	0/11
6	1/6	4/5	1/5
7 (IM)†	2/44	18/22	3/42
7 (SC)†	6/44	...	4/38
Total	12/144 (8%)	39/45 (87%)	10/132 (8%)

*Each number was a separately prepared vaccine. †Route of vaccine administration.

Results

Prevention of persistent viremia—Persistent viremia was prevented by 7 separate lots of the vaccine (Table 1). In 6 separate experiments, 144 vaccinated and 45 nonvaccinated age- and gender-matched control cats were challenge-exposed oronasally with FeLV. All 45 control cats were viremic 4 weeks after challenge exposure, but viremia was transient in 6 of these cats, for a value of 87% persistent viremia at 12 weeks after challenge-exposure. In contrast, 132 of 144 vaccinates were not viremic 12 weeks after challenge exposure, 92% protection from persistent viremia. Furthermore, only 10 of the 132 (8%) vaccinates protected from persistent viremia were transiently viremic after challenge exposure. All vaccinates that were viremic became so by 4 weeks after challenge exposure. Vaccine 7 was more efficacious when administered IM (95% protection) than when given SC (86% protection).

Protection from latent infection—Because the proportion of cats with latent infection has been shown to decrease with time,[1] attempts to reactivate latent FeLV infection in cats aviremic 12 weeks after challenge exposure were initiated within 16 weeks after challenge exposure. Furthermore, the Rickard strain of FeLV used as the challenge virus in these studies has a high propensity to establish latent infection.[1] Thus, these investigations were conducted in a situation in which the probability of establishing and detecting a latent infection was high. Indeed, latent infection was readily detected in 6 of 8 (75%) transiently viremic control cats (Table 2). In contrast, latent infection was not detected in 38 protected vaccinates that had never been viremic. Latent infection was, however, detected in 5 of 10 transiently viremic vaccinates. Thus, examination of all transiently viremic vaccinates from the studies (Table 1) indicated 50% rate of latent infection. Two persistently viremic cats were tested to ensure adequacy of the detection procedures.

Safety of the vaccine, as indicated by detection of adverse reactions—Forty-two veterinarians in 10 states administered 2,379 doses of vaccine to cats

Table 2—Activation of latent FeLV infection after challenge exposure of vaccinated and control cats

	No. of cats examined	No. of serum samples p27 pos	No. of bone marrow samples p27 Pos	No. with latent infection
Vaccinates	48	1	4	5 (10%)*
Transiently viremic†	10	1	4	5 (50%)
Never viremic	38	0	0	0 (0%)
Transiently viremic controls	8	4	2	6 (75%)
Viremic controls	2	2	2	...

*Number in parentheses is the percentage of cats examined. †Only 10 of 132 (8%) of protected vaccinates had transient viremia.

*Table 3—Vaccine performance in a field safety trial involving 2,010 cats and 2,379 doses of vaccine**

Adverse reactions	Duration	No.	Concurrent vaccination	Reaction rate
Local				
Soreness	24 to 48 hours	3	3	0.13%
Swelling	<2 days	3	2	0.13%
Systemic				
Lethargy	24 to 48 hours	23	17	0.96%
Fever	24 to 48 hours	3	3	0.13%
Emesis/diarrhea	12 hours	1	1	0.04%
Drooling	0.5 hour	1	1	0.04%
Total		34	27	1.43%

*Primary vaccination involved 389 cats (n = 2 doses each), and 1,641 cats were given a booster vaccination.

of multiple breeds, ages, and vaccination histories. Three-hundred sixty-nine cats were given 2 doses as primary vaccination and 1,641 cats were given a single booster dose; 97.9% of the vaccinations were given SC. Sixty-four percent of the vaccinations were given concurrently with another licensed vaccine. Reported adverse reactions other than those of an immediate local nature were determined (Table 3). Thirty-four mild, short-term reactions were reported, for a reaction rate of 1.43%. Twenty-seven of the 34 (79%) adverse reactions were associated with concurrent administration of another licensed vaccine at a separate site, making an unambiguous interpretation of the role of the vaccine in these reactions difficult.

Discussion

The vaccine provided consistent protection from persistent viremia and transient viremia after FeLV challenge exposure that induced persistent viremia in 87% of age-matched control cats. The 92% protection from persistent viremia afforded by the vaccine was obtained after oronasal challenge exposure, a natural route of FeLV transmission. Thus, the challenge-exposure model used in these studies resulted in FeLV infection in a manner that is natural for at-risk cats, and requires the vaccine to interrupt a normal pathogenic process. In addition to using a natural route of challenge exposure with FeLV, vaccinates and controls cohabitated in random manner during the entire duration of the study (Table 1). Therefore, because all control cats were viremic by 4 weeks after challenge exposure and, thus, were capable of shedding FeLV, the vaccinates potentially had multiple exposures to FeLV over the remainder of the observation period. However, persistent or transient viremia in all vaccinates was established by 4 weeks after challenge exposure. Vaccinates that were not aviremic at 4 weeks subsequently became viremic, which indicates that vaccinates protected from initial oronasal instillation of challenge virus may also have been protected from natural contact exposure over the remaining 8 to 12 weeks of observation.

In addition to affording 92% protection from persistent viremia, the vaccine also provided a high degree of protection from transient viremia. Only 8% of vaccinated cats aviremic by 12 weeks after challenge exposure were ever viremic. The importance of protection from transient viremia is indicated by the observation that latent FeLV infection was detected only in cats that had been transiently viremic (Table 2). Latency was not observed in 38 vaccinates that had never been viremic, but was found in 50% of vaccinates that had transient viremia. Therefore, because only transiently viremic vaccinates were likely to have latent infection, it follows that 92% protection from transient viremia and 50% protection from latency in transiently viremic vaccinates indicates 96% protection from latency overall. The 75% incidence of latency in controls was not unexpected because the Rickard strain used as the challenge virus has been shown to induce high incidence of latent infection.[1] Because the incidence of latency decreases over time,[1] our results are difficult to compare with those of a study on prevention of latency by another FeLV vaccine.[4] That study was conducted 2 to 4 years after challenge exposure.[4] Moreover, that study did not document ability to detect latent infection in age-matched control cats.

A field study involving administration of 2,379 doses of vaccine resulted in a 1.43% reaction rate. The 34 reported reactions were all mild and of short duration. In agreement with a recent study for another FeLV vaccine,[5] concurrent administration of another feline vaccine (either multivalent feline vaccines, rabies vaccine, or both) increased the incidence of reactions. Local immediate reactions reported as discomfort of short duration were of a frequency and severity that was expected with any vaccination administered SC.

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