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Induction of Immunity to Feline Caliciviral Disease

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Six specific-pathogen-free cats were exposed by aerosol to a feline calicivirus of low virulence (F-9 virus). Homotypic (anti-F-9) seroconversion occurred in all cats by postexposure day 14. The serum of one cat on postexposure day 14 and four of six cats on postexposure day 35 neutralized feline picornavirus isolate no. 255 (FPV-255), a virulent feline calicivirus. Homologous antiviral activity was detected before the appearance of heterologous (anti-FPV-255) activity and always was present in higher titer. Protective immunity was evaluated on postexposure day 35 by aerosol challenge with FPV-255. The pyrexia, depression, dyspnea, oral ulcers, and severe pneumonia produced in two susceptible specific-pathogen-free cats by exposure to FPV-255 did not occur in the cats that had been infected previously with F-9 virus. The study demonstrates that heterotypic protective immunity to feline calicivirus disease can be induced by prior infection with feline calicivirus of low virulence.

Feline caliciviruses (FCV) (16) have been isolated from the respiratory tract and visceral organs of domestic cats. Some strains, such as kidney cell degenerating virus (5), are of minimal significance as feline pathogens (2, 12, 15), whereas others induce oral ulcers and pneumonia (9, 10, 12, 14; E. A. Hoover and D. E. Kahn, J. Am. Vet. Med. Assoc., in press). The virulent FCV can produce a substantial disease problem in breeding colonies and laboratory facilities where cats are housed together.

Extensive serological cross-reactivity among FCV has been observed in tests using hyperimmune goat and rat sera (8). It is not known whether the protective immunity conferred to cats by previous FCV infection (1) is restricted to homologous virus or if the antigenic determinants shared by more than one FCV serotype can induce heterotypic protective immunity. Studies of the immunological response of the cat to FCV infection have been slowed by the limited availability of susceptible cats. The majority of random-source cats obtained from commercial animal dealers have been infected with FCV (2). This problem has been obviated by the use of specific-pathogen-free (SPF) cats that lack previous immunological experience with FCV antigens as well as subclinical infection with other feline viruses (10, 14). In this study we describe the response of SPF cats to aerosol infection with an FCV of low virulence and the heterotypic protective immunity induced to challenge with a virulent pneumotropic FCV strain.

MATERIALS AND METHODS

Viruses. FCV strain F-9 was isolated from the pharynx of a cat (3). The 24th tissue culture passage of this virus was used as the immunogen (vaccine virus) in this study. The first 10 passages of F-9 were in primary feline kidney cell cultures; subsequently the virus was propagated in a feline embryonic diploid tongue cell line, FC_1Tg (A. Kniazeff, Naval Biological Laboratory, University of California, Berkeley, Calif.). The F-9 viral stock was purified by three successive terminal dilutions and tested to assure its freedom from other viruses and bacteria. The inoculum used titered $10^{5.25}$ median tissue culture infective doses per 0.1 ml, as calculated by the Spearman-Kärber method (6).

The 5th tissue culture passage FPV-255 (11) was used to challenge the immunity of F-9-exposed (vaccinated) cats. The response of SPF cats following exposure to FPV-255 aerosols has been described (10). The challenge virus, propagated in Crandell feline kidney cell culture (4), titered $10^{5.5}$ median tissue culture infective doses per 0.1 ml.

Cats. Eight male SPF cats from the breeding colony developed in the Department of Veterinary Pathobiology were used in the study. The cats, of caesarian-derived, gnotobiotic ancestry (15), have been maintained in isolation as a closed colony free of spontaneous viral diseases since 1966. The cats were between 3 and 4 months of age and were housed in separate cages in a room isolated from other cats during the experimental period.

Animal inoculations. Aerosols of the viral inocula were generated by forcing air through a hand-operated Devilbiss no. 40 nebulizer (Devilbiss Co., Somerset, Pa.). The heads of unanesthetized cats were enclosed within an 18 cm diameter plastic chamber attached to the mouth of the nebulizer. Each

cat was exposed to the viral aerosol for 2 min. Six cats were exposed to F-9 virus at the start of the experiment, designated as postexposure day (PED) 0, and to FPV-255 on PED 35. Two cats that had not experienced F-9 infection were exposed to aerosols of the same FPV-255 inoculum. The 2 nonvaccinates served as indicators of the severity of the FPV-255 challenge.

Clinical observations. Cats were examined each morning and afternoon for clinical signs of illness. The rectal temperature of each cat was recorded during each observation period.

Sample collection. Swabbings of the nasal, oropharyngeal, conjunctival, and rectal mucosae were collected prior to F-9 exposure and on PED 3, 5, 7, 14, 21, 28, and 35. Swabbings also were collected daily for the first 7 postchallenge days (PCD) and on PDC 15. The method used was the same as that described previously (12).

Serum was collected prior to F-9 exposure and at weekly intervals thereafter.

Three F-9 vaccinates and the two unvaccinated cats were killed on PCD 8. The three remaining F-9 vaccinates were killed on PCD 15. Each cat was necropsied, and portions of lung, nictitating membrane, tonsil, and nasal turbinate were preserved in buffered 10% formalin for histopathologic evaluation.

Samples of tonsil, nictitating membrane, and lung were collected aseptically for viral isolation studies.

Cell culture. The Crandell embryonic feline kidney cell line (4) was used in virological and serological assays. Cultures were seeded into disposable multiple-well plastic trays (Model FB-16-24 TC Disposo Trays, Linbro Chemical Co., Inc., New Haven, Conn.) and incubated at 37 C in an atmosphere containing 5% CO_2.

Virus isolation. Swab samples and 10% (wt/vol) suspensions of tissue specimens were assayed for FCV by inoculation onto Crandell embryonic feline kidney monolayers. The presence of virus was detected by distinctive cytopathic changes in the monolayer as described previously (12).

SN test. Sera were heat-inactivated (56 C, 30 min), diluted by fourfold increments, and mixed with equal volumes of a viral suspension calculated to contain 100 median tissue culture infective doses per 0.1 ml. Each serum was tested for neutralizing antibody against both F-9 and FPV-255. The median neutralization value (SN_{50}) for each sample was determined by following previously described procedures (12).

RESULTS

Clinical observations. Small ulcerations of the palatine mucosa of two cats and ulceration of the median cleft of the nostril in two others were the only clinical evidence of illness detected following aerosol exposure to F-9 virus (Table 1). Pyrexia was not detected (Fig. 1).

After challenge with FPV-255, no clinical signs of illness were detected in four of the six cats previously exposed to F-9 virus. Mild blepharospasm and a transient rise in rectal temperature (39.4 C) were detected in one cat on PCD 1. One cat that had an ulcerative lesion of the median cleft of the nostril following F-9 exposure developed a similar lesion on PCD 7. Mean rectal temperatures of the vaccinates during the postchallenge period are plotted in Fig. 2.

Both unvaccinated cats became febrile, depressed, dyspneic, and developed ulcers of the tongue and hard palate (Fig. 2; Table 2). The illness was typical of FPV-255 infection in susceptible cats (10, 12).

Lesions. No macroscopic lesions were present in the lungs of F-9-exposed cats (Fig. 3). In two cats, mild perivascular or peribronchial collections of lymphocytes and plasma cells were observed by histopathological examination. Peribronchiolar infiltration with eosinophils or small, focal accumulations of alveolar macro-

TABLE 1. *Response of susceptible SPF cats to aerosols of F-9 virus*

PED	Clinical signs of disease			Virus isolations			
	Pyrexia	Oral-nasal ulcerations[a]	Other signs of disease	Pharyngeal swabs	Nasal swabs	Conjunctival swabs	Rectal swabs
0	0/6[b]	0/6	0/6	0/6	0/6	0/6	0/6
3	0/6	0/6	0/6	6/6	5/6	1/6	0/6
5	0/6	2/6	0/6	2/6	2/6	1/6	0/6
7	0/6	2/6	0/6	6/6	2/6	0/6	0/6
10	0/6	0/6	0/6	NT[c]	NT	NT	NT
14	0/6	0/6	0/6	2/6	2/6	0/6	0/6
21	0/6	0/6	0/6	0/6	0/6	0/6	0/6
28	0/6	0/6	0/6	2/6	0/6	0/6	0/6
35	0/6	0/6	0/6	4/6	0/6	0/6	0/6

[a] Small ulcerations of the palatine mucosa or nostril. Palatine lesions would be inapparent by routine clinical examination and cause no apparent behavioral changes.

[b] Numerator, Number positive; denominator, total.

[c] NT, Sample not taken.

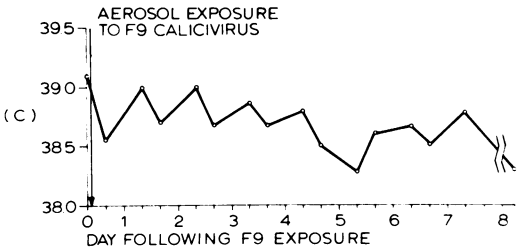

FIG. 1. *Mean rectal temperatures of SPF cats after aerosol exposure (vaccination) to F-9 FCV. Pyrexia was not detected.*

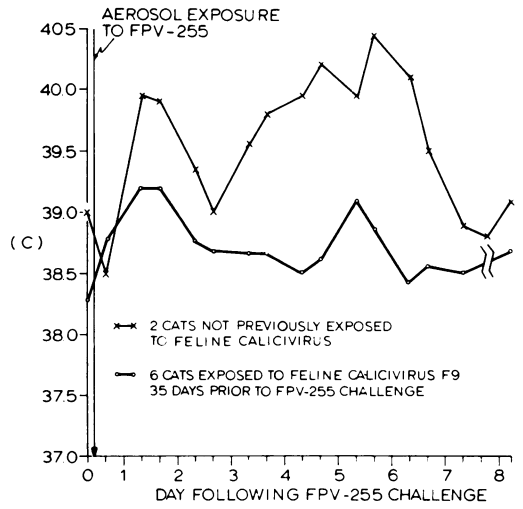

FIG. 2. *Mean rectal temperature of cats previously exposed to F-9 virus aerosols and susceptible SPF cats after challenge with FPV-255.*

phages were present in two cats. Infiltrations of neutrophils were observed in tonsilar crypt epithelium of four cats and nictitating membrane of three cats. The results are summarized in Table 2.

Gross lesions of pneumonia were present in both nonvaccinates. The lesions involved virtually the entire apical and cardial lobes in one cat (Fig. 4). Histopathologic examination revealed severe fibrinopurulent pneumonia with extensive inflammatory edema or alveolar necrosis. The process was typical of FPV-255-induced pulmonary lesions studied previously (9, 10). Ulcerative lesions of the tonsilar crypt epithelium, tongue, and palatine mucosa (Table 2) also were similar to those described previously in FPV-255-infected SPF cats (10).

Virus isolation. Cytopathogenic agents were not cultured from nasal, conjunctival, pharyngeal, or rectal swabbings collected prior to F-9 viral exposure.

FCV was isolated from swabbings collected after exposure of cats to F-9 virus (Table 1). Virus was isolated from pharyngeal swabbings with greatest frequency, and these were the only samples to contain virus after PED 14. FCV was isolated from pharyngeal swabbings of four cats collected on PED 35, just prior to challenge with FPV-255.

The frequency of FCV isolation from swab samples collected from F-9-exposed and unvaccinated cats after FPV-255 challenge is listed in Table 3. FCV was isolated from 75% of the swabbings collected from the F-9-exposed cats on PCD 3. Virus was isolated less frequently from specimens collected subsequently. A simi-

TABLE 2. *Response of F-9-exposed (vaccinated) and unvaccinated SPF cats after aerosol challenge with FPV-255*[a]

Group	Cat no.	PCD killed	Clinical signs of disease	Lesions		
				Lung	Tongue and palate	Nostril
F-9 exposed cats	1	8	None	−	−	−
	2	8	Nasal ulceration (PCD 7)	−	−	+
	3	8	Mild blepharospasm and transient, mild pyrexia (PCD 1)	±		
	4	15	None	±	−	−
	5	15	None	−	ND	ND
	6	15	None	−	−	−
Unvaccinated cats	1	8	Pyrexia (PCD 1 to 6); ulcers of tongue and palate; depression; dyspnea	+++	++	−
	2	8	Similar to above	++++	++	−

[a] −, Lesions not detected; ±, peribronchiolar infiltration of eosinophils or small, focal accumulations of macrophages within alveoli; +, lesions present, graded + to +++ according to severity; ND, sample not evaluated microscopically.

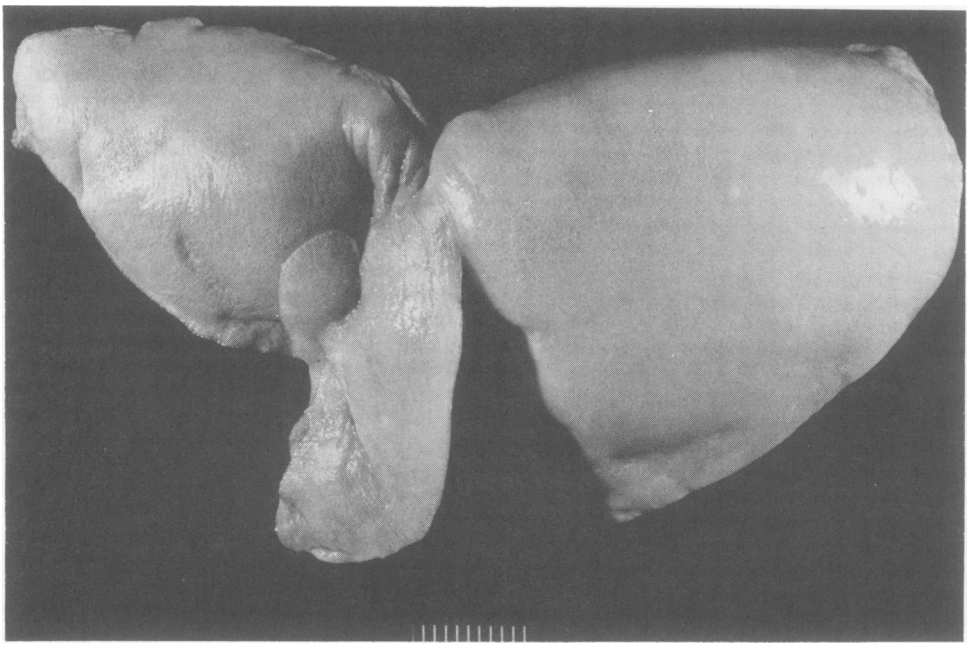

FIG. 3. *Left lobes of the lung of an F-9-vaccinated cat killed 8 days after aerosol challenge with FPV-255. No pulmonary lesions are present.*

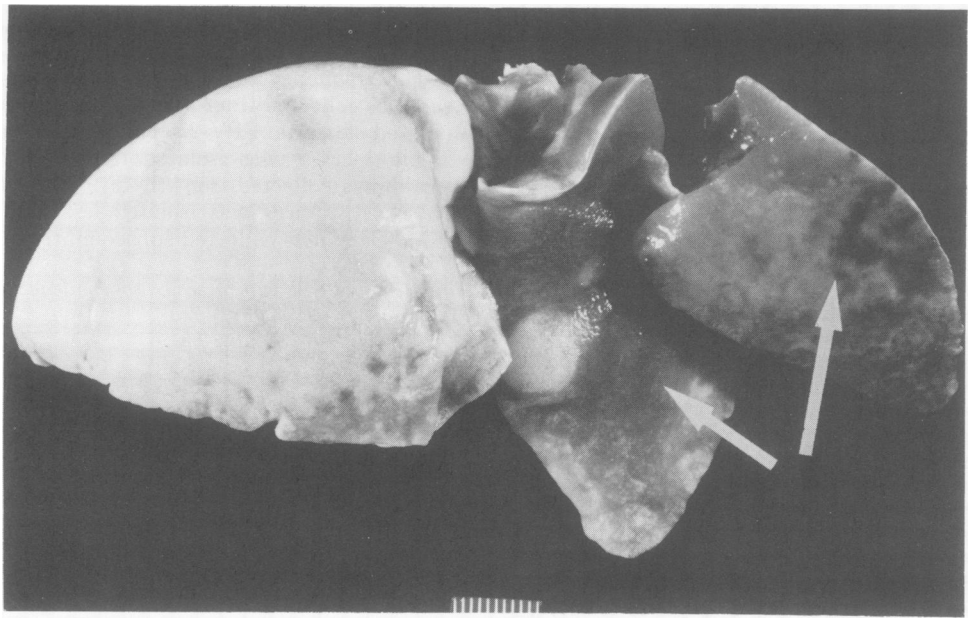

FIG. 4. *Right lobes of a susceptible SPF cat killed 8 days after aerosol challenge with FPV-255. There is extensive pneumonia involving the anterior and middle lobes (arrows).*

lar reduction in the frequency of FCV isolation from samples collected from the unvaccinated cats after FPV-255 challenge did not occur.

Virus was isolated from the lungs and tonsils of both nonvaccinates and from the nasal turbinate of one cat. In the F-9-exposed cats, FCV could not be isolated from lung samples, but it was cultured from three of six tonsils, one of six nasal turbinates, and one of six nictitating membranes.

SN tests. Neutralizing antibody to either F-9 or FPV-255 was not detected in 1:4 dilutions of

serum from cats prior to their initial viral exposure. Anti-F-9 (homotypic) activity was present in four of six sera on PED 7. Homotypic antibody was detected in all sera from F-9-exposed cats collected on PED 14 and at subsequent sampling periods. Geometric mean SN_{50} values for the group of F-9-exposed cats are plotted in Fig. 5.

Anti-FPV-255 (heterotypic) neutralizing activity was detected in four of the six sera on PED 35. Heterotypic antibody was first detected in the serum of one cat on PED 14 (SN_{50} = 1:4), and was detected in the serum of a second on PED 21 (SN_{50} = 1:8). Serum of all six cats sampled on PCD 7 neutralized FPV-255. Geometric mean SN_{50} values of anti-FPV-255 activity are plotted in Fig. 5. The deviations of the geometric means of heterotypic titers from those of anti-F-9 titers were significant ($P \leq 0.025$) on PED 7, 14, 21, 28, and 35. Following FPV-255 challenge, the differences were not significant (Student's t test [7]).

An increase in neutralizing titer to F-9 virus followed challenge with FPV-255 but was less dramatic than the rise in anti-FPV-255 titer (Fig. 5).

Serum samples from nonvaccinates collected

TABLE 3. *FCV isolation from swab samples collected from F-9-exposed (vaccinated) and unvaccinated SPF cats after aerosol challenge with FPV-255*

PCD	F-9 vaccinates				Unvaccinated cats			
	Pharyngeal swabs	Nasal swabs	Conjunctival swabs	Rectal swabs	Pharyngeal swabs	Nasal swabs	Conjunctival swabs	Rectal swabs
0	4/6[a]	0/6	0/6	0/6	0/2	0/2	0/2	0/2
1	5/6	3/6	4/6	0/6	2/2	1/2	2/2	0/2
2	5/6	4/6	3/6	1/6	2/2	2/2	2/2	0/2
3	6/6	4/6	4/6	4/6	2/2	2/2	2/2	0/2
4	6/6	5/6	3/6	0/6	2/2	2/2	2/2	0/2
5	3/5	3/5	2/5	2/5	2/2	2/2	2/2	1/2
6	6/6	4/6	2/6	0/6	2/2	2/2	2/2	0/2
7	6/6	2/6	0/6	2/6	2/2	2/2	2/2	0/2
15	3/3	0/3	0/3	0/3	NT[b]	NT	NT	NT

[a] Numerator, Number positive; denominator, total.
[b] NT, Sample not taken.

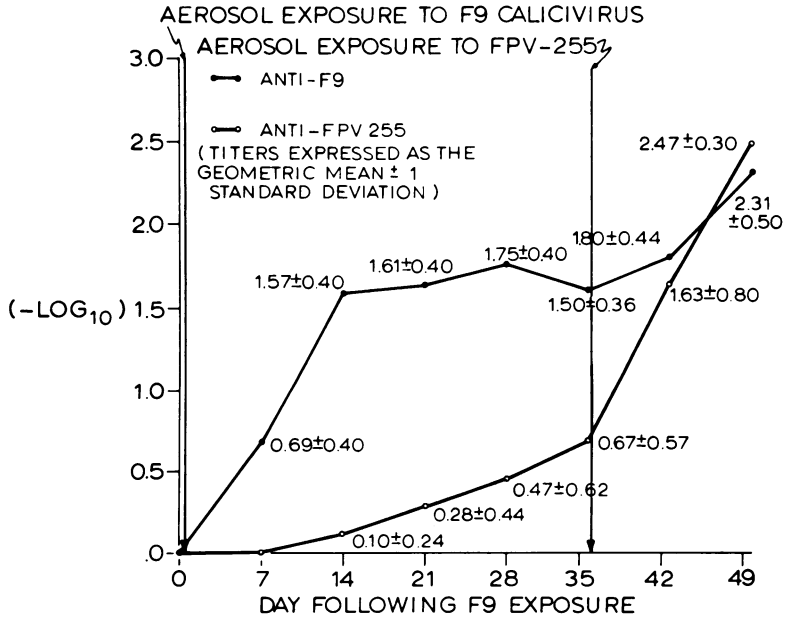

FIG. 5. *Geometric mean neutralization titers of sera from cats sequentially exposed to aerosols of F-9 and FPV-255 caliciviruses.*

7 days after challenge with FPV-255 neutralized homologous virus (SN_{50} = 1:16 and 1:32), but not F-9 virus.

DISCUSSION

The one study of FCV immunity that has been reported determined that once cats had recovered from FCV-induced disease they remained asymptomatic after re-exposure to the same viral strain (1). Protective immunity could not be demonstrated when convalescent cats were exposed to a heterologous strain of FCV. We have observed that convalescent FPV-255-infected cats are protected from illness upon subsequent exposure to the same virus (D. E. Kahn and E. A. Hoover, unpublished data). Our present findings indicate that protective immunity to a highly virulent FCV also can be induced by previous infection with a serologically distinct FCV strain of minimal virulence (F-9 virus). The contrast in responses of susceptible and vaccinated cats was striking. Although FCV was isolated from swab samples collected from both groups following FPV-255 exposure, vaccinates developed no clinical illness and few lesions, whereas pyrexia, depression, dyspnea, ulcers of the oral mucosa, and pneumonia occurred in the nonvaccinated cats.

The F-9 virus used as an immunogen in this study did not induce signs of respiratory illness. The virulence of this F-9 viral inoculum was reduced compared to a lower tissue culture passage level of the same virus (E. A. Hoover and D. E. Kahn, in press). Further attenuation may be necessary to produce an immunogen that no longer produces ulcerative lesions of the nostril.

Serological classification of various FCV strains remains to be resolved. Comparison of 12 FCV strains by reciprocal neutralization tests using hyperimmune goat antisera indicated broad antigenic overlap among the viruses. In complement-fixation tests employing antisera produced in gnotobiotic rats, eight FCV strains were resolved into seven serologically distinguishable groups (8). Our study indicates that whereas F-9 and FPV-255 may share some common antigenic determinant(s), they are not identical viruses. Homologous antiviral activity was detected before the appearance of heterologous activity and always was present in higher titer. The homologous antibody detected during the early stages of F-9 infection does not fix guinea pig complement but is detectable by a complement-fixation-inhibition test (13). Complement-fixing antibody to F-9 virus was detectable by PED 35 (13). This suggests that the initial species of antibody formed following FCV does not fix complement but is viral strain specific. Later the cat forms antibody that fixes complement and neutralizes heterologous virus (FPV-255).

We are conducting reciprocal neutralization tests using sera from cats collected during the first several weeks of FCV infection. The objective is to determine whether such sera are less cross-reactive than the goat and rat antisera that have been employed previously (8). The development of highly specific immune sera would assist materially in the serotyping of FCV strains.

Correlation between serological antiviral activity and immunological protection of the convalescent FCV-infected cat when challenged with an antigenically related virus remains to be investigated. Should there be a high correlation, it may prove feasible to substitute serological procedures for animal trials in determining which FCV strains cross-protect. It remains to be determined whether the protective immunity observed to FPV-255 induced by prior F-9 infection resulted from a fortuitous pairing of FCV strains, or whether this immune state extends to other virulent FCV as well. If the protective immunity can be shown to be a generalized phenomenon in FCV infection, immunoprophylaxis may prove to be a feasible method for the control of this prevalent viral disease of the cat.

Information concerning the development of the feline caliciviral vaccine used in this study will be published separately (J. L. Bittle and W. J. Rubic, manuscript in preparation).

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