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Cross-Protection Among Feline Caliciviruses

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Each of five groups of specific-pathogen-free and conventionally reared cats was infected with a different strain of feline calicivirus. Two of the strains were pathogenic, producing characteristically fever, depression, loss of appetite, buccal ulceration, and occasionally increased ocular and nasal secretion. Two of the other strains were mildly pathogenic and associated with fever or buccal ulceration or both; the fifth strain was nonpathogenic. The two pathogenic strains plus three others shown also to be pathogenic were used 3 months after the initial infection to challenge the cats in rearranged groupings. Of the 28 conventional cats challenged, six (21.4%) showed at least a febrile response, although none of the 30 specific-pathogen-free cats showed any clinical signs. After challenge, virus was recovered from throat swabs of 37 of the 58 cats (63.8%) including the six which showed symptoms, but the duration of the excretion of virus was significantly less than that seen with the initial infection. The homologous and heterotypic antibody responses correlated well with the clinical protection, or lack of it, seen on challenge. The results provide further evidence for significant cross-relationships between feline caliciviruses.

Feline caliciviruses (FCV) (feline picornaviruses) have been frequently isolated in many parts of the world; isolations have been made particularly from the respiratory tract of cats with or without clinical signs of inflammation of that tract. Preliminary serological attempts to classify the viruses suggested that numerous distinct serotypes existed (2, 3, 5). Subsequent cross-neutralization studies (14) of such isolates led to the suggestion that there were in fact considerable antigenic relationships in the group and that the isolates tested might be regarded as members of a single serotype. It was further postulated (14) that this relationship may reflect in cross-protection in cats infected with FCV.

To test this postulate, five groups of specific-pathogen-free (SPF) and conventionally reared cats were each infected under carefully controlled isolation conditions with an FCV. The clinical and immunological responses of these cats were monitored, and then at 3 months the animals were challenged with either the homologous infecting virus or a heterologous strain, and again their responses were determined.

MATERIALS AND METHODS

Viruses. The following FCV isolates were used at the passage level given in parenthesis: M-8 (5th) and N-3 (6th) were local isolates obtained respectively from a kitten with a 6-month history of respiratory illness and from a cat with ulcerative stomatitis. FCV-255 (14th) was obtained from D. E. Kahn, Ohio State University (9). F-17 (2nd after receipt), 17-FRV (25th), FC (12th), and CFI (19th) were received from Dorothy Holmes, Cornell University, from stocks prepared for the National Cancer Institute. FCV 5936 was isolated by R. A. Crandell, University of Illinois, from a cat with respiratory distress, sneezing, and oral lesions, and was used at its 3rd passage level. All passages had been made in primary, secondary, or established feline tissue culture.

Cell culture. An established line of feline cells (NLFK) was used for virus propagation, assay, and neutralizing antibody studies. Tissue culture medium routinely used was: Hanks balanced salt solution (10 times concentrated), 10%; lactalbumin supplement, 10%; Eagle vitamin solution, 10%; Eagle amino acids, 10%; 2.8% $NaHCO_3$ solution, 5%; penicillin, 50,000 IU; streptomycin, 50 mg/liter; glutamine, 293 mg/liter; and fetal calf serum, 5% for growth, and 2% for maintenance, added just prior to use.

Cats. Thirty-six SPF cats of 4 to 9 months of age were imported in individual filtered-air containers from the Laboratory Animals Centre, Carshalton, England, and from Hill Grove, England, and were immediately housed in pairs in gnotobiotic-type, flexible-film isolators. Thirty conventionally reared cats were housed together in a clean room (Bioclean) from shortly after weaning until use at 6 to 8 months, when they also were housed in pairs in the isolators. The isolators were distributed between five separate rooms. Isolator construction was a modification of that previously published (15).

Inoculation and challenge. The majority of cats

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were infected by direct intranasal instillation, using a fine dropper, of 0.2 ml of fluids containing a total of $10^{5.0}$ to $10^{5.6}$ mean tissue culture infective doses ($TCID_{50}$) of virus. The viruses used for primary infection were M-8, F-17, 255, N-3, and 17-FRV. Eight cats were infected by an aerosol of virus fluids containing 10^{6} $TCID_{50}$/ml for 1 min by a no. 40 deVilbiss nebulizer attached to a small-animal ether mask placed over the face of the cat. No essential difference could be detected in the response to these two methods of infection and thus, for subsequent challenge 12 weeks after primary infection, direct intranasal instillation was used. The viruses used for challenge were N-3. 255, FC, CFI, and 5936 at the same dose level as for primary infection. The pathogenicity of the challenge viruses, FC, CFI, and 5936, was checked by infection of two conventional and two SPF cats for each virus. The groupings of cats for infection and challenge are shown in Tables 1 and 7. Before and after inoculation and challenge all cats were observed clinically, temperatures were recorded daily, regular oropharyngeal swabs were collected, and at suitable intervals blood and nasal secretion samples were taken. The latter samples were collected after synchronous sedation with ketamine hydrochloride (Ketaset, Bristol Veterinary Products, New York) (7.5 mg/lb of body weight [1 lb equals 453.592 g]) and secretory stimulation with pilocarpine hydrochloride (0.5 mg/lb of body weight). After collection atropine sulfate was given intramuscularly at 1.0 mg/lb of body weight. Nasal secretions were filtered through a disposable filter unit (Miltex, Millipore Corp., Bedford, Mass.) and as with sera, heat inactivated at 56 C for 30 min.

TABLE 1. *Clinical and virological response of groups of cats to exposure to various feline caliciviruses*

Group	No. with clinical response	No. from which virus was recovered	Mean duration virus recovery (days)
Six conventional cats infected with M8 ..	0	5	15.6
Six SPF cats infected with M8	0	6	14.3
Six conventional cats infected with N-3 ..	4	6	16.2
Six SPF cats infected with N-3	6	4	11.5
Six conventional cats infected with F-17 .	0	6	9.3
Six SPF cats infected with F-17	6	4	2.3
Six conventional cats infected with 255 ..	2	5	5.0
Six SPF cats infected with 255	6	6	17.8
Six conventional cats infected with FRV .	2	6	12.3
Six SPF cats infected with FRV	6	5	10.0

Neutralization test. The micro-neutralization test previously described (14) was used. Briefly, sera or nasal secretion samples were diluted in phosphate-buffered saline using 0.05-ml microdiluters in 96-well disposable plates (Microtest II, Falcon Plastics, Oxnard, Calif.). Virus prediluted to a titer of $10^{3.3}$ $TCID_{50}$/ml was then added to 0.05 ml per well and the mixtures was agitated on a Micromixer (Cooke Engineering). After incubation at 37 C in 5% CO_2 for 1 h, approximately 10^{4} NLFK cells was added to each well and the plates were reincubated in a CO_2-enriched atmosphere for 3 to 4 days until controls showed complete cytopathic effect. Results were recorded as presence or absence of visible cytopathic effect in each well, and 50% end points were calculated by the method of Reed and Muench (16).

RESULTS

Clinical and virological results of infection are shown in Table 1.

Cats infected with FCV-M-8. The conventional cats used were found to have pre-inoculation antibody titers to FCV-M-8 of between 1:4 and 1:256. There was no clinical response to infection, but virus was recovered from five of the six cats. The six SPF cats in this group also had neutralizing activity against M-8 present in their sera prior to infection, but levels were minimal ($<1:4$). The group showed no symptoms after infection, but again virus was recovered from all six cats infected.

Cats infected with FCV-N-3. Only one of the conventional cats showed pre-inoculation homologous antibody, but all six showed titers of 1:2 to 1:200 to the heterologous calciviruses (M-8, 255, etc.). Four of the six conventional cats including the only one with a pre-inoculation neutralizing antibody titer (1:32) showed a febrile response ($\geq$103 F), which in three cases was biphasic, the initial rise being at 2 days and the second between days 4 and 9 post-inoculation. Symptoms in these four cats varied from occasional sneezing, through clear ocular and/or nasal discharge with tongue ulceration, to frank conjunctivitis, photophobia with purulent ocular and nasal discharge, marked lingual ulceration, transient diarrhea, and general depression. The two remaining conventional cats showed neither febrile response nor symptoms. Virus was recovered from all six cats in the conventional group and three cats were still positive at 37 days but not 57 days postinfection. Of the SPF group, none of which had pre-inoculation antibody, homologous or heterologous, two cats showed a transient fever. Tongue ulcers developed in four cats, and two of these had erosions on the hard palate also. One cat had a vesicle on the lip and another had erosion of the external

nares. Virus was recovered from four of the SPF cats.

Cats infected with FCV-F-17. All six conventional cats showed preinoculation homologous neutralizing antibody in their sera (1:2 to 1:10) and some heterologous activity also. In this group no definite febrile response was detected and no cats showed any symptoms, although virus was recovered from all cats. For the SPF group, two of which had minimal levels of homologous neutralizing activity pre-inoculation (<1:8), two cats showed a transient fever, two had lingual ulcers, one had hard-palate erosions and ulceration of the external nares, and one a hard-palate erosion only. Virus was recovered as late as 14 days post-inoculation from four of the six cats in the group.

Cats infected with FCV-255. Two of the conventional cats showed pre-inoculation serum neutralizing activity (1:10 and 1:20). Febrile responses occurred in only two cats and were not clearly biphasic. Only one cat showed any other signs of infection, a solitary tongue ulcer present from days 9 through 11. Virus was recovered from all cats except one between days 4 and 22 post-inoculation. By contrast, the SPF cats, none of which had pre-inoculation serum neutralizing activity, all showed some symptoms including fever (three cats); lingual ulcers particularly of the anterodorsal area (four); hard-palate ulceration (four); mild ocular and nasal discharge (two); conjunctivitis (one); and diarrhea (one). Virus was recovered for up to 5 weeks for all cats.

Cats infected with FCV-FRV. The sera of all six conventional cats had pre-inoculation neutralizing antibody against FCV-FRV of 1:2 to 1:4. No definite febrile response occurred among this conventional group, although one cat showed a lingual ulcer and one showed slight nasal discharge, tongue ulceration, and erosion of the external nares. This latter cat failed to recover from ketamine sedation at 8 weeks post-inoculation. Virus was recovered from all cats between days 2 and 16 post-inoculation. Ulceration of the external nares was a feature of infection in five of the SPF cats including the three which showed preinfection serum neutralizing activity between 1:2 and 1:3. One of the five cats had a nasal discharge and was sneezing. Two cats showed a febrile episode, and virus recovery was successful from five cats.

Cats infected with FCV-FC. A marked conjunctivitis occurred in one of the pair of conventional cats. With the SPF cats, one showed a lingual ulcer and both had ulceration of the external nares.

Cats infected with FCV-CFI. Both conventional kittens showed transient pyrexia, depression, and poor appetite, with conjunctivitis and erosions on the hard palate in one. Appetite was similarly depressed in the SPF cats, and one had a solitary lingual ulcer.

Cats infected with FCV-5936. Transient pyrexia occurred in both conventional kittens, with conjunctivitis, nasal discharge, and sneezing in one. On autopsy, at 7 days postinfection, foci of interstitial pneumonia were present in the second kitten. The two SPF kittens showed fever, anorexia, and tongue and external nares ulcers, with nasal discharge in one kitten.

Serological response to primary infection. (i) Serum. Tables 2 through 6 show the development of neutralizing antibody in each group, both against the original infecting virus and against some of the other FCV under study, that is, the homologous and heterologous response. The homologous response is shown graphically in Fig. 1 and the means between groups for the heterologous responses are shown in Fig. 2.

(ii) Nasal secretion. Many samples proved cytotoxic at low dilutions, and nasal secretion neutralizing activity was in general of low order (<1:16). Many of the conventional cats showed low-level homologous and heterologous virus neutralization in pre-exposure samples, but apart from two SPF cats in the M-8-infected group (titers of 1:4 and 1:7), none of the SPF cats showed such preinfection activity. After infection, the majority of the conventional cats showed neutralizing ability of nasal secretions but, apart from cats in the M-8-infected group, where titers reached as high as 1:200 at 4 weeks post-inoculation, titers were between 1:2 and 1:16. For the SPF animals, the peak titers reached were 1:20, but many animals failed to show detectable activity.

Clinical response to challenge at 12 weeks post-inoculation. This is summarized in table 7. There was no clinical response to the challenge infections in any cat originally infected with either M-8 or N-3 viruses. Of the cats infected initially with F-17 virus, two of the conventional group, both receiving FC as the challenge virus, showed, respectively, transient fever with a solitary lingual vesicle and a lingual ulcer without fever. Three cats, all in the conventional group, which had been infected with 255 virus gave a clinical response to challenge. Of these, one cat, challenged with virus 5936, showed fever and erosion of the external nares, and the other two cats, both challenged, as they had been initially infected, with 255 virus, showed fever, anorexia, nasal discharge,

TABLE 2. *Geometric mean serum neutralizing antibody titers ($-log_2$) for groups of cats pre- and post-exposure to feline calicivirus M-8*

Weeks post-exposure	Virus[a]									
	M-8		N-3		255		FRV		F-17	
	C	S	C	S	C	S	C	S	C	S
0	3.6	1.3	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2	8.3	6.6	1.7	1.0	3.3	3.0	4.0	2.0	4.0	2.6
4	9.2	7.9	2.0	2.3	4.0	4.0	4.3	3.0	5.6	4.0
8	8.9	9.2	4.0	3.3	4.0	5.3	NT	5.3	NT	5.0
12	9.2	8.9	4.0	4.3	3.6	5.3	NT	NT	NT	NT

[a] C, Conventional cats; S, SPF cats; NT, not tested.

TABLE 3. *Geometric mean serum neutralizing antibody titers ($-log_2$) for groups of cats pre- and post-exposure to feline calicivirus N-3*

Weeks post-exposure	Virus[a]									
	M-8		N-3		255		FRV		F-17	
	C	S	C	S	C	S	C	S	C	S
0	3.6	<1.0	1.0	<1.0	<1.0	<1.0	2.3	<1.0	3.0	<1.0
2	5.6	6.0	5.9	4.3	5.0	4.6	6.0	3.6	5.6	4.3
4	6.6	8.0	6.3	5.3	5.6	4.3	6.6	4.6	6.0	4.0
8	7.6	9.2	6.9	6.9	4.0	5.6	NT	6.3	NT	5.0
12	7.6	8.9	7.3	7.6	3.6	4.7	NT	NT	NT	NT

[a] C, Conventional cats; S, SPF cats; NT, not tested.

TABLE 4. *Geometric mean serum neutralizing antibody titers ($-log_2$) for groups of cats pre- and post-exposure to feline calicivirus F-17*

Weeks post-exposure	Virus[a]									
	M-8		N-3		255		FRV		F-17	
	C	S	C	S	C	S	C	S	C	S
0	2.0	<1.0	<1.0	<1.0	1.0	<1.0	2.0	<1.0	1.7	<1.0
2	5.0	5.0	<1.0	2.0	4.0	4.0	4.0	5.3	5.6	4.3
4	4.3	5.6	<1.0	2.0	4.6	5.0	4.0	6.0	6.6	4.6
8	6.6	6.0	2.3	3.3	2.6	7.0	NT	7.8	6.3	5.6
12	6.0	8.3	3.0	4.7	3.0	4.6	NT	NT	6.6	6.6

[a] C, Conventional cats; S, SPF cats; NT, not tested.

TABLE 5. *Geometric mean serum neutralizing antibody titers ($-log_2$) for groups of cats pre- and post-exposure to feline calicivirus 255*

Weeks post-exposure	Virus[a]									
	M-8		N-3		255		FRV		F-17	
	C	S	C	S	C	S	C	S	C	S
0	2.3	<1.0	1.3	<1.0	1.3	<1.0	2.0	<1.0	2.0	<1.0
2	4.6	4.6	1.7	<1.0	4.6	5.6	4.0	3.3	3.3	3.0
4	3.6	5.3	1.7	1.7	4.9	7.3	4.6	4.0	4.3	3.0
8	5.6	7.6	2.6	3.6	3.6	7.9	NT	5.0	NT	4.6
12	5.0	7.6	2.3	4.6	3.9	7.0	NT	NT	NT	NT

[a] C, Conventional cats; S, SPF cats; NT, not tested.

TABLE 6. *Geometric mean serum neutralizing antibody titers ($-\log_2$) for groups of cats pre- and post-exposure to feline calicivirus FRV*

Weeks post-exposure	Virus[a]									
	M-8		N-3		255		FRV		F-17	
	C	S	C	S	C	S	C	S	C	S
0	1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.3	<1.0	<1.0	<1.0
2	3.6	5.6	<1.0	1.7	4.6	3.3	6.3	4.9	4.0	3.3
4	4.0	5.3	1.0	2.3	5.6	4.0	7.9	5.3	3.6	3.0
8	NT	7.3	2.3	4.6	3.0	7.0	5.9	7.6	NT	5.3
12	6.6	7.7	2.3	5.6	4.3	4.3	6.6	7.0	NT	NT

[a] C, Conventional cats; S, SPF cats; NT, not tested.

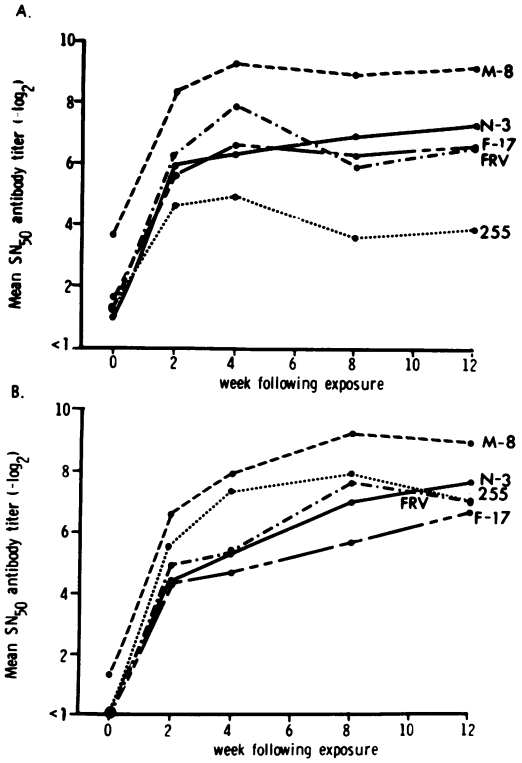

FIG. 1. *Geometric mean homologous serum neutralizing antibody titers in groups of cats (A, conventional; B, SPF) exposed to feline caliciviruses.*

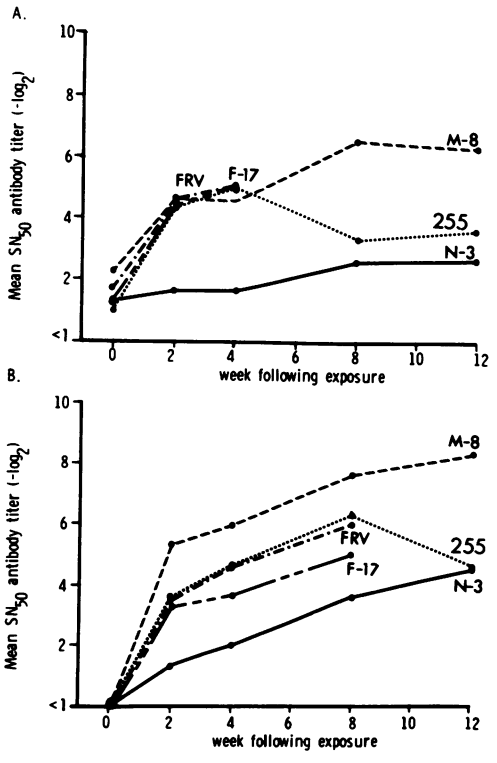

FIG. 2. *Heterologous response after exposure to feline caliciviruses. Geometric mean serum neutralizing antibody titers to each virus of all cats (A, conventional; B, SPF) except those originally infected with that virus.*

and lingual and hard-palate ulceration between days 5 and 12 post-challenge. In the group originally infected with FRV, a single conventional cat showed transient fever after challenge with N-3 virus.

Virus recovery after challenge. Virus recovery from throat swabs was successful not only from each of the six cats that showed clinical response to challenge, but also from a further 31 cats among those that had shown any symptoms after challenge infection. The mean duration of excretion was 3.1 days for the conventional cats, excluding those showing symptoms after challenge, and the mean for the SPF cats was 0.9 days. By contrast, the mean duration of excretion for those six conventional cats that showed symptoms was 14.3 days.

Serological response to challenge. Figures 3 and 4 show the serological responses in terms of neutralizing antibody in serum to the original

TABLE 7. *Grouping and response of cats to challenge with various feline caliciviruses*

Original infecting virus	Challenge virus	No. and type of cats[a]	No. showing clinical response to challenge	No. from which virus was recovered	Mean duration virus recovery (days)
M-8	CFI	1 Con+	0	1	7.0
	CFI	2 SPF	0	0	0.0
	5936	2 Con	0	2	2.0
	N-3	2 Con	0	2	5.5
	FC	2 SPF	0	2	2.0
	255	2 SPF	0	1	0.5
N-3	CFI	2 Con	0	1	3.0
	5936	2 Con	0	2	3.5
	N-3	4 SPF	0	1	0.5
	FC	2 SPF	0	0	0.0
F17	CF1	2 Con	0	1	0.5
	5936	2 SPF	0	0	0.0
	N-3	2 Con	0	2	5.0
	FC	2 Con	2	2	12.0
	FC	2 SPF	0	2	1.0
	255	2 SPF	0	2	1.0
255	CFI	2 SPF	0	2	1.5
	5936	2 Con	1	2	7.5
	5936	2 SPF	0	2	1.5
	N-3	2 SPF	0	2	4.5
	FC	2 Con	2	2	17.5
FRV	CFI	2 SPF	0	0	0.0
	5936	2 SPF	0	1	0.5
	N-3	1 Con+	1	1	13.0
	FC	2 Con	0	0	0.0
	255	2 Con	0	2	4.5
	255	2 SPF	0	0	0.0

[a] Con, Conventionally reared; +, two cats died from incidental causes, prior to challenge.

infecting virus and to the challenge virus in the 8 weeks after challenge infection.

Those cats in the groups originally infected with F-17 and 255 viruses that succumbed to challenge showed markedly lower mean serum neutralizing antibody to the original infecting virus at the time of challenge than did resistant cats in their groups. This difference is not apparent for the single cat in the FRV group that showed a transient pyrexia on challenge. However, when the antibody levels to the challenge virus are examined the differences are consistent. Thus, the cat that succumbed to challenge with FC virus had an FC antibody titer of 1.7 ($-\log_2$) compared with the mean for the FC-challenged conventional animals of 5.5. The cat susceptible to 5936 challenge had a titer to 5936 at challenge of 1.0 (mean for group, 2.8); the cats susceptible to 255 challenge had titers to 255 at challenge of 2.3 and 1.3, respectively (mean for the rest of 255-inoculated group, 6.3; mean for the rest of 255-challenged group, 5.7); and the cat susceptible to N-3 challenge had a titer to N-3 at a challenge of 3.3 as opposed to a mean for the group of 7.3.

Antibody to viruses other than those used in the original or challenge infection was also measured (results not shown) and paralleled the picture seen with those antibodies in terms of a boosting where titers were initially low but little change where titers were higher.

DISCUSSION

Recent in vitro studies (14) suggesting a cross-activity between 46 isolates of FCV have now been extended to the in vivo situation for a total of eight isolates. The serological cross-reaction obtained, and more significantly the

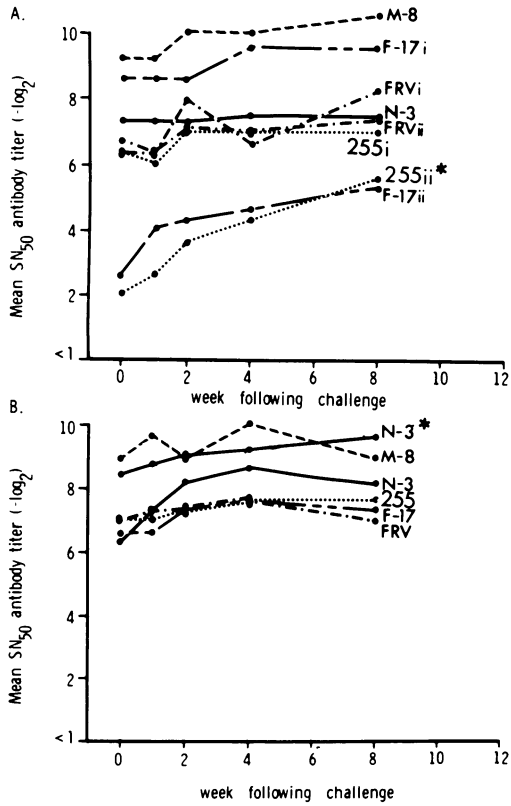

FIG. 3. *Geometric mean serum neutralizing antibody against original infecting virus in (A) conventional and (B) SPF cats after challenge with the same (*) or a heterologous feline calicivirus. Means for those cats showing clinical response to challenge (F-17ii, FRVii, 255ii) are plotted separately from those resisting challenge (F-17i, FRVi, 255i).*

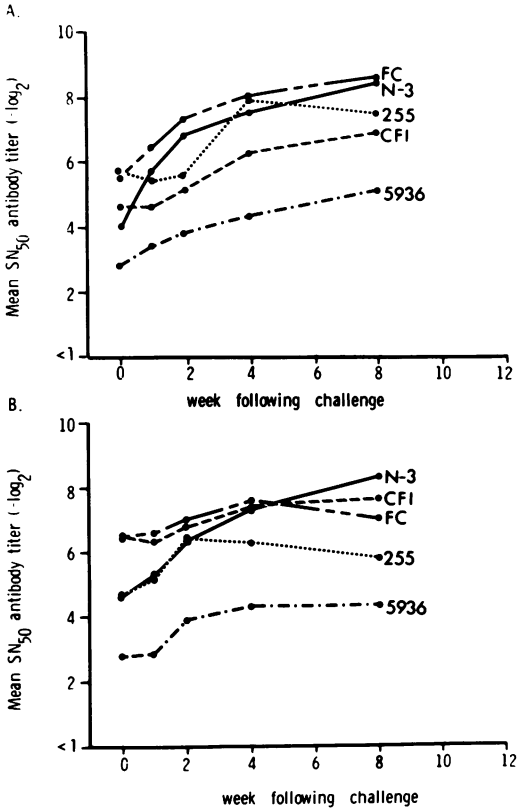

FIG. 4. *Geometric mean serum neutralizing antibody against challenge virus in (A) conventional and (B) SPF cat groups, before and after challenge.*

cross-protection achieved between the viruses, provide confirmation of these earlier results and necessitate a reconsideration of the former concept of the feline caliciviruses being a heterogeneous group with only a low-level, nonsignificant antigenic interrelationship. This earlier concept came about in part by extrapolation from the situation with the human rhinoviruses, with which the feline caliciviruses have been classified in the family of picornaviruses, although in separate genera. Even with the rhinoviruses, although numerous distinct serotypes are recognized, heterologous antibody responses do occur (7, 6). Other workers, however (17), failed to show such heterologous responses to three rhinovirus serotypes in vaccines, but the antigenicity of the vaccines was poor in two cases.

The variable, generally mild symptomatology associated with at least experimental FCV infection has been noted previously (8, 15) and is supported by this work, where F-17 and FRV are mildly pathogenic and M-8 appears nonpathogenic inspite of its original isolation from a cat with chronic upper respiratory disease. In other studies (15) M-8 at a dosage level some 10-fold higher than used in this work did show some pathogenicity in terms of mild ocular discharge and lingual ulceration. N-3, 255, FC, CF1, and 5936 are significant pathogens, however, and were valid challenge viruses.

The homologous serum neutralizing antibody response to initial infection was generally rapid, being well developed at 2 weeks. This was in agreement with the finding of Olsen et al. (11) for FCV-F9. The response was particularly marked with the conventional cats infected with M-8, where there were significant preinoculation titers. It seemed to be a secondary response and presumably reflected prior exposure to an FCV, probably M-8 which was indeed originally isolated from the colony from which the majority of the conventional cats originated. Pre-inoculation antibody titers against the other FCV among the conventional cats were of low order and could still represent the heterotypic response induced by an M-8 exposure. The low-level neutralizing activity seen in the SPF sera of several cats in different groups prior to infection is probably nonspecific since there had not been any clinical or virological evidence of calcivirus infection in the SPF colony from which these cats were obtained immediately prior to use.

Olsen et al. (11), working with two caliciviruses, found that serum neutralizing antibody to the heterotypic strain developed, but at only low level, by day 35 postinfection, although heterotypic complement fixation inhibiting antibody had appeared by day 21. Except for virus N-3, where the heterotypic serum neutralizing response was delayed particularly in the conventional group, the heterologous antibody response in the present work followed very closely the development of homologous serum neutralizing, although at a generally lower order of magnitude by a factor of between two and 64. Titers to M-8 again were generally higher and this again presumably is a reflection of prior exposure to this antigen in the conventional group, together with possibly more efficient neutralization of M-8 by antibody than of the other viruses.

Present work has not elucidated the importance of nasal or other local antibody relative to serum antibody such as those found working with human rhinoviruses (4, 10, 12, 13). The neutralizing activity present in nasal secretions was of low order, if detectable at all, but did correlate somewhat with the level of serum neutralizing antibody. Titers of 1:20 were obtained in a few cats, and this peak was generally

reached 2 to 4 weeks after infection or challenge and fell rapidly. The conventional cats infected with M-8 virus were again an exception, showing titers as high as 1:200, probably reflecting both the prior sensitization of this group and also the generally higher antibody responses to M-8 antigen.

Viral shedding by the majority of cats post-challenge, in the absence of any clinical symptoms and in the presence of antibody, is not unexpected, having been noted previously with FCV challenge (15) and with other viruses, for instance human rhinovirus (4). It is quite apparent, however, that the duration of excretion after challenge (overall mean, 3.1 days) was much shorter than after initial infection (overall mean, 11.3 days). Bartholomew and Gillespie (1) reported that cats recovered from infection with FCV-CFI did not resist challenge with FCV-FJ, but their criteria for this were clinical illness and/or virus isolations, so it is not clear whether any of the cats challenged showed clinical signs.

Of the six cats that showed any symptoms after challenge, only two were significantly severe. These were cats which were challenged, as well as primarily infected, with FCV 255. With these cats, although neither had pre-inoculation antibody to FCV 255, the clinical response to the original infection had been mild and one cat did not yield virus after infection. The low serological response correlated with this, but after challenge titers rose to a level on a par with the rest of the group.

Based on the results of the challenge experiment, it would seem that serum neutralizing titer of 4.0 (1:16) or greater indicates protection against challenge with various FCV, at least at the $10^{5.3}$ $TCID_{50}$ challenge level. Further, a serum neutralizing titer of 2.6 (1:7) or less correlates with susceptibility to challenge with heterologous FCV. The protective titer of neutralizing activity in nasal secretion is difficult to quantify, but all 20 cats that had such activity detectable (mean level, 2.3 or 1:5) did resist challenge clinically although they still showed transient virus shedding (mean duration, 2.6 days).

The likely persistence of the antibody response is impossible to forecast, but there is no tendency for decline of titers up to 8 weeks post-challenge.

It would have been desirable to include in the experiment some FCV isolates from other parts of the world besides the United States, but importation licenses were impossible to obtain at the time. The extensive cross-neutralizing activity previously reported (14), however, which included results using isolates from several countries, would indicate that a valid extrapolation could be made of the in vivo cross-protection obtained with the isolates currently tested, to incorporate most, if not all, feline caliciviruses. Work will continue to test this hypothesis.

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LITERATURE CITED

1. Bartholomew, P. T., and J. H. Gillespie. 1968. Feline viruses. 1. Characterization of four isolates and their effect on young kittens. Cornell Vet. **58**:248–265.
2. Bittle, J. L., C. J. York, J. W. Newberne, and M. Martin. 1960. Serologic relationship of new feline cytopathogenic viruses. Am. J. Vet. Res. **21**:547–550.
3. Burki, F. 1965. Picornaviruses of cats. Arch. Gesamte Virusforsch. **15**:690–696.
4. Buscho, R. F., J. C. Perkins, J. L. S. Knopf, A. Z. Kapikian, and R. M. Chanock. 1972. Further characterization of the local respiratory tract antibody response induced by intranasal instillation of inactivated rhinovirus 13 vaccine. J. Immunol. **108**:169–177.
5. Crandell, R. A. 1967. A description of eight feline picornaviruses and an attempt to classify them. Proc. Soc. Exp. Biol. Med. **126**:240–244.
6. Fleet, W. F., R. G. Douglas, Jr., T. R. Cate, and R. B. Couch. 1968. Antibody to rhinovirus in human sera. II. Heterotypic responses. Proc. Soc. Exp. Biol. Med. **127**:503–509.
7. Hamre, D., A. P. Conelly, Jr., and J. J. Procknow. 1964. Virologic studies of acute respiratory disease in young adults. 3. Some biologic and serologic characteristics of 17 rhinovirus serotypes isolated Oct. 1960–June 1961. J. Lab. Clin. Med. **64**:450–460.
8. Hoover, E. A., and D. E. Kahn. 1973. Lesions produced by feline picornaviruses of different virulence in pathogen-free cats. Vet. Pathol. **10**:307–322.
9. Kahn, D. E., and J. H. Gillespie. 1970. Feline viruses. X. Characterization of a newly-isolated picornavirus causing interstitial pneumonia and ulcerative stomatitis in the domestic cat. Cornell Vet. **60**:669–683.
10. Knopf, H. L. S., J. C. Perkins, D. M. Bertran, A. Z. Kapikian, and R. M. Chanock. 1970. Analysis of the neutralizing activity in nasal wash and serum following intranasal vaccination with inactivated type 13 rhinovirus. J. Immunol. **104**:566–573.
11. Olsen, R. G., D. E. Kahn, E. A. Hoover, N. J. Saxe, and D. S. Yohn. 1974. Differences in acute and convalescent-phase antibodies of cats infected with feline picornaviruses. Infect. Immun. **10**:375–380.
12. Perkins, J. C., D. N. Tucker, H. L. S. Knopf, R. P. Wenzel, R. B. Hornick, A. Z. Kapikian, and R. M. Chanock. 1969. Evidence for protective effect of an inactivated rhinovirus vaccine administered by the nasal route. Am. J. Epidemiol. **90**:319–326.
13. Perkins, J. C., D. N. Tucker, H. L. S. Knopf, R. P. Wenzel, A. Z. Kapikian, and R. M. Chanock. 1969. Comparison of protective effect of neutralizing anti-

body in serum and nasal secretions in experimental rhinovirus type 13 illness. Am. J. Epidemiol. 90:519–526.
14. Povey, R. C. 1974. Serological relationships among feline caliciviruses. Infect. Immun. 10:1307–1314.
15. Povey, R. C., and C. J. Hale, 1974. Experimental infections with feline caliciviruses (picornaviruses) in specific-pathogen-free kittens. J. Comp. Pathol. 84:245–256.
16. Reed, L. J., and H. Muench. 1938. A simple method for estimating fifty percent end points. Am. J. Hyg. 27:493–497.
17. Stott, E. J., C. Draper, P. B. Stones, and D. A. J. Tyrrell. 1969. Absence of heterologous antibody responses in human volunteers after rhinovirus vaccination. Arch. Gesamte Virusforsch. 28:89–92.