INFECTION AND IMMUNITY, Dec. 1974, p. 1307-1314

Vol. 10, No. 6
Printed in U.S.A.

Serological Relationships Among Feline Caliciviruses

R. C. POVEY

Norden Laboratories, Lincoln, Nebraska 68521, and Department of Veterinary Medicine, University of Bristol, Bristol, England

Received for publication 8 July 1974

A total of 46 strains of feline calicivirus isolates from the United Kingdom, United States, Australia, and New Zealand were used in an investigation of their serological relationships based on the serum neutralization test. Although demonstrable antigenic variation exists between these isolates, it is shown that significant in vitro cross-activity exists between all these isolates to greater or lesser extent. All isolates tested may be regarded as serological variants of a single serotype of feline calicivirus. It is postulated that this relationship would provide for considerable cross-protection during successive exposures of cats to various feline caliciviruses.

Feline caliciviruses (FCV; formerly feline picornaviruses) have been isolated on many occasions from both sick and healthy cats in many parts of the world. Most isolates have been associated with the respiratory tract, conjunctiva, and buccal cavity. A recent review has been published (9).

Serological comparisons of isolates have been made by a number of investigators using a variety of tests and nonstandardized procedures. The most widely used has been the serum neutralization (SN) test (2, 4, 5, 11, 15, 17, 18, 21; Holmes and Gillespie, 1972, cited by Gillespie and Scott [9]). The regular finding has been considerable serological differences between isolates; nevertheless, many cross-reactions occur. The complement fixation test (10, 12, 22, 23; Parker, 1972, cited by Gillespie and Scott [9]) has in general shown broad cross-reactions, as has the immunofluorescent test (8). Immunodiffusion tests (24; Chema, 1972, cited by Gillespie and Scott [9]) have tended to distinguish between isolates.

This paper presents results of extensive testing, by cross-neutralization, of many FCV isolates with antisera raised by hyperimmunizing rabbits and hamsters, and by intranasally infecting cats and harvesting postreinfection sera. The clinicopathological results of this last study have already been reported (16).

MATERIALS AND METHODS

Viruses. The following FCV isolates have been used in this study: from the United Kingdom, strains designated 67-64, 68-40, 68-1241, 68-2024, 68-80, 69-348a, 69-591, 69-609b, 69-1079e, 69-1112, 69-1345, 69-1403, 4-69, BF-69, BF-71, and A-3; from Australia, 10-66, 86-68, and 113-68B; from New Zealand, KCD; and from the United States, 17-FRV and M-8.

The source, passage history, and original reference for these isolates has been given (16; Povey, Ph.D. thesis, University of Bristol, Bristol, England, 1970).

In addition, the isolates (all from the United States) shown in Table 1, with their source and original reference, have been used.

After they were received, viruses were purified by three terminal dilutions in tissue culture.

Cells. Primary and secondary feline kidney cells and an aneuploid feline cell line were used in conventional tissue culture techniques.

Antisera production. (i) New Zealand white rabbits were inoculated intravenously with virus fluids, 0.5 ml initially, then 1.0 ml at 2 weeks, and 2.0 ml at 3, 4, and 5 weeks. Blood was collected 3 weeks after the last injection. (ii) Hamsters were inoculated by intracardiac infection of 1 ml of virus fluids (repeated weekly for a total of six injections) and were exsanguinated 3 weeks after the last injection. (iii) The method of Povey and Hale (14) was used to prepare antisera in cats. Briefly, specifical pathogen-free kittens 6 to 10 weeks of age were infected intranasally with a total of 10^4 mean tissue culture infective doses ($TCID_{50}$) of virus, and 4 weeks later were given a second similar dose. Serum was harvested after a further 2 weeks.

Antiserum treatment. After conventional separation, all sera were heat-inactivated at 56 C for 30 to 60 min. Toxicity of some sera, particularly that of rabbit origin, was much reduced by adsorbing the sera with a suspension of feline tissue culture cells overnight at 4 C, cells then being removed by centrifugation.

SN test. Initially, studies were performed with a conventional, constant virus (32 to 320 $TCID_{50}$), varying serum (two-fold dilutions) technique with incubation of serum virus mixtures proceeding at 37 C for 1 h prior to inoculation of tissue culture tubes, with two tubes per dilution. Subsequently, a microneutralization test was developed, utilizing flat-bottomed, 96-well plates (Microtest-II, Falcon Plas-

TABLE 1. *Designation, source, and reference of additional feline caliciviruses tested*

Designation	Synonym	Source	Reference and/or source
FPL	Bolin virus	Spleen of cat with panleukopenia	(3)
CFI	FIV	Blood, throat, kitten pyrexia, depression, hyperpnoea	(6)
F-5		Upper respiratory infection	(2)
F-9		Upper respiratory infection	(2)
F-10		Upper respiratory infection	(2)
F-11		Upper respiratory infection	(2)
F-17		Upper respiratory infection	(2)
F-19		Upper respiratory infection	(2)
FJ	F-20	Spleen	(2)
FS		Stomatitis	(20)
FC		Conjunctivitis	(20)
FRI-14	(CV-14)	Clinically normal cat	(5)
FRI-29	(CV-29)	Clinically normal cat	(5)
FRI-278	(FEV-10)	Cat with mild central nervous signs	(5)
FRI-6	(AFIP-3)	Clinically normal cat	(5)
5936		Respiratory distress, sneezing, oral lesions	Crandell, personal communication, 1973
5259L		Ulcerative glossitis	Crandell, personal communication, 1973
5895		Chronic stomatitis, gingivitis	Crandell, personal communication, 1973
2279		Chronic illness: leukemia suspected	Crandell, personal communication, 1973
2260		No history	Crandell, personal communication, 1973
7058		Respiratory infection, ulceration oral mucosa	Crandell, personal communication, 1973
255		Lung, kitten pneumonia, lingual ulcer	(11)
N-3		Ulcerative glossitis, gingivitis	Norden Laboratories isolate, 1973
N-24		Mild respiratory disease	Norden Laboratories isolate, 1973

tics, Calif.). Sera were titrated in duplicate or quadruplicate in phosphate-buffered saline with 50-μliter samples of serum, diluent, and microdilutors. Virus diluted in tissue culture medium with 5% fetal calf serum to contain a calculated $10^{3.3}$ $TCID_{50}$ per ml was then added to each well by a 50-μliter dropper to give a putative 100 $TCID_{50}$ of virus per well. Plates were gently but rapidly agitated for 1 min with a mechanical mixer ("Micromixer," Cooke Engineering) and then incubated in a carbon dioxide-enriched and humidified incubator at 37 C for 1 h. A suspension of feline cells containing approximately 10^5 cells/ml was then added by automatic syringe at 0.1 ml/well. Plates were then reincubated in the carbon dioxide atmosphere at 37 C for up to 4 days.

End points in all cases were read as those tubes showing no cytopathic effect when virus control tubes or wells showed complete cytopathic effect, and 50% SN titers were calculated by the method of Reed and Muench (19).

Where two-way cross-neutralization results were available, relationships between viruses were analyzed by the method of Archetti and Horsfall (1). The geometric mean of the titer ratio (r_1), found by dividing the heterologous titer obtained with virus 2 by the homologous titer obtained with virus 1, and the ratio (r_2) found by dividing the heterologous titer obtained with virus 1 by the homologous titer obtained with virus 2, is given by the function: $r = \sqrt{r_1 \times r_2}$. Thus, the value r gives in a single figure the extent of the antigenic difference between two viruses when both agents and both antisera are used in a cross-serological reaction. A value of 1 for r of indicates no antigenic difference. A value for $1/r$ of ≥ 2 or ≤ 0.5 is usually taken to indicate significant antigenic difference.

Besides serum titrations, some comparisons were also made with the 20-antibody-units concept (13). In this case, serum of known titer would be diluted to 20 times less than its limiting dilution; for example, a serum with an SN_{50} titer of 1:600 would be diluted 1:30 and then tested against 100 $TCID_{50}$ of virus in the standard manner.

RESULTS

Rabbit anti-FCV sera. The SN_{50} titers obtained with rabbit-produced antisera in a checker-board cross-neutralization against 14 FCV isolates and with control antisera produced against feline herpesvirus isolate UKA and against tissue culture fluids are shown in Table 2. An analysis of these results by the method of Archetti and Horsfall (1) is shown in Table 3, where isolates 67-64 and 68-1241, 69-348a and 69-609b, and BF-69 and 4-69

emerge as serologically identical pairs, indicating 11 separate serotypes by this criterion.

Hamster anti-FCV sera. SN_{50} titers are shown in Table 4, and when analyzed by Archetti and Horsfall's method all six viruses tested appear to be separate serotypes.

Cat anti-FCV sera. Cross-neutralization results are shown in Table 5. Analysis by the method of Archetti and Horsfall (not shown) would reveal an apparently significant relationship between 68-40 and 68-2024, 67-64 and 68-2024, 69-348a and 10-66, M-8 and 68-2024 and between M-8 and 69-348a. In all cases, however, this only holds for one of the two cat antisera produced for each virus.

The cat anti-FCV sera were then prediluted to contain 20 times their limiting concentration of antibody, as determined against homologous

TABLE 2. *Reciprocal cross-neutralization tests with some feline calicivirus isolates using rabbit anti-FCV sera*

Antisera	Reciprocal SN titer against virus strain:													
	67-64	68-40	68-1241	68-2024	69-80	69-348a	69-591	69-609b	69-1079e	69-1112	69-1345	69-1403	4-69	BF-69
67-64-RS[a]	1,024	32	1,024	64	4	—[b]	NT[c]	NT	2	—	4	2	16	16
68-40-RS	16	256	2	16	16	—	4	64	—	4	4	2	—	16
68-1241-RS	1,024	256	2,048	256	32	—	NT	NT	4	32	32	16	4	64
68-2024-RS	256	4	256	2,048	4	2	64	32	8	4	4	8	4	64
69-80-RS	64	16	64	64	2,048	4	128	64	8	16	16	64	2	256
69-348a-RS	4	32	8	64	2	2,048	64	2,048	—	NT	NT	4	4	2,048
69-591-RS	4	NT	4	—	8	16	2,048	64	2	—	—	8	32	1,024
69-609b-RS	4	—	4	8	4	2,048	32	2,048	—	—	—	4	4	2,048
69-1079e-RS	8	16	64	256	16	—	NT	NT	32	—	32	16	4	16
69-1112-RS	64	256	2,048	256	512	4	NT	NT	256	512	512	128	4	256
69-1345-RS	16	4	8	4	4	—	NT	NT	4	—	1,024	8	2	16
69-1403-RS	128	16	256	64	8	—	NT	NT	8	8	16	2,048	4	64
4-69-RS	4	4	16	64	—	4	128	64	—	4	4	16	1,024	2,048
BF-69-RS	4	16	16	4	—	4	128	128	—	4	512	16	1,024	2,048
UKA[d]-RS	—	—	2	—	—	—	—	—	—	—	—	—	—	2
TCF[e]-RS	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] RS, Rabbit serum.
[b] —, No neutralization at final serum dilution of 1:2.
[c] NT, Not tested.
[d] UKA, Feline herpesvirus (FVR).
[e] TCF, Control tissue culture fluids.

TABLE 3. *Analysis by method of Archetti and Horsfall of cross-neutralization titers obtained with rabbit anti-feline calicivirus sera*[a]

Antisera	Cross-neutralization titers against virus strain:													
	67-64	68-40	68-1241	68-2024	69-80	69-348a	69-591	69-609b	69-1079e	69-1112	69-1345	69-1403	4-69	BF-69
67-64-RS[b]	1													
68-40-RS	22.6	1												
68-1241-RS	1.4	32.0	1											
68-2024-RS	11.3	90.5	8.0	1										
69-80-RS	90.5	45.3	45.3	128.0	1									
69-348a-RS	0	0	0	181.0	724.0	1								
69-591-RS	NT[c]	NT	NT	0	64.0	64.0	1							
69-609b-RS	NT	2.0	NT	128.0	128.0	1.0	45.3	1						
69-1079e-RS	45.3	0	16.0	5.7	22.6	0	NT	0	1					
69-1112-RS	2.8	11.3	4.0	32.0	11.3	0	0	0	0	1				
69-1345-RS	128.0	128.0	90.5	362.0	181.0	NT	0	0	16.0	0	1			
69-1403-RS	90.5	128.0	32.0	90.5	90.5	0	NT	NT	22.6	32.0	128.0	1		
4-69-RS	128.0	0	181.0	90.5	0	362.0	22.6	90.5	0	181.0	362.0	181.0	1	
BF-69-RS	181.0	45.3	64.0	128.0	0	22.6	5.7	4.0	0	32.0	16.0	64.0	1.0	1

[a] Geometric mean, r, where $r = \sqrt{r_1 \times r_2}$ (expressed as $1/r$) of titer ratios.
[b] RS, Rabbit serum.
[c] NT, Not tested.

TABLE 4. *Reciprocal SN_{50} titers of hamster anti-FCV sera*

Antisera	Reciprocal SN_{50} titers against virus strain:					
	68-40	BF-69	67-64	68-2024	69-348a	69-1112
68-40-H.S.[a]	2,000 (1)[b]	<10	256	32	<10	8
BF-69-H.S.	<10 (0)	640 (1)	40	<10	40	10
67-64-H.S.	40 (22)	5 (90)	2,560 (1)	<10	40	10
68-2024-H.S.	<10 (0)	<10 (0)	<10 (0)	320 (1)	<10	<10
68-348a-H.S.	20 (0)	5 (45)	40 (32)	<10 (0)	640 (1)	<10
69-1112-H.S.	25 (113)	20 (0)	160 (45)	80 (0)	<10 (0)	1,280 (1)

[a] H.S., Hamster serum.
[b] Numbers in parentheses represent reciprocal of *r* value (1).

virus and retested against homologous (two viruses) and/or heterologous isolates in a microneutralization test employing eight wells per virus serum test. Individual wells were scored on a positive-negative basis, and four to seven wells negative for cytopathic effect is recorded (Table 6) as significant neutralization (*n*), and eight wells negative for cytopathic effect is recorded as complete neutralization (*n*). The cross-neutralization seen with this 20-antibody-units concept varies considerably between the isolates from those such as FRI-6, which is neutralized by only one antiserum, to F-17, which is significantly neutralized by all. All the antisera are able to neutralize several if not many of the viruses.

Goat anti-FCV sera. The results of SN titrations based on three separate tests are shown in Table 7. These sera were then prediluted to 20 times less than this limiting concentration (or in the case of low-titer sera, 2 to 10 times only) and tested against the range of FCV as shown in Tables 8 and 9. The antiserum prepared against FCV F-17 is particularly competent at neutralizing the other isolates, and this is the more noteworthy because, owing to its rather low homologous SN_{50} titer, it was used at only 10 times its limiting antibody concentration. Overall, the rate of significant neutralization of the 21 FCV isolates tested by the 14 goat antisera was 73.5% (excluding neutralization by homologous sera). This pattern of cross-neutralization is apparently more extensive among the American isolates than between these and the British, Australian, and New Zealand isolates, although only antisera were available to the latter, with the exception of KCD, in the United States, and two-way tests were not possible. This difference may not be due to the geographical origin of the isolates, however, but may be a reflection of the difference in production of the antisera. For instance, FCV isolates FRI-29 and FRI-6 were not significantly neutralized by 20 antibody units of cat anti-FRV sera, but were both neutralized by rabbit anti-FRV sera.

DISCUSSION

The serological classification of viruses, besides being pursued for taxonomical purposes, provides a basis for epidemiological studies and serves as an indicator of relationships which may or may not be valid in designing the composition of vaccines with the required protective capacities. The focus of such classification is the serotype, but unfortunately the requirements for recognition of an isolate as such vary for the different virus groups. Serum neutralization has been generally regarded as the most specific of the usual serological methods and the one most used for the identification of serotypes. The method of Archetti and Horsfall (1) is useful for viruses within those families such as the herpesviruses, where there is considerable antigenic homogeneity, but it discredits anything less than almost complete antigenic identity. Thus if the feline caliciviruses are analyzed by this method (4; this paper), they will be classified into many serotypes. However, where several FCV isolates have been compared by cross-neutralization, varying degrees of cross-reactivity (very often one-way) have been reported (2, 4, 7, 11; Holmes and Gillespie, 1972, cited by Gillespie and Scott [9]), and this cross-relationship is re-emphasized on a much larger scale in the current report, which is based on many more isolates of feline calicivirus from more parts of the world than have previously been reported.

Somewhat comparable difficulties with serotyping human rhinoviruses led to the proposed definition incorporating the arbitrary 20-anti-

TABLE 5. *Reciprocal SN_{50} titers of SPF-cat anti-feline calicivirus sera tested against 50 to 500 $TCID_{50}$ of homologous and heterologous isolates (tube neutralization test)*

Virus ($TCID_{50}$)	SPF-cat antisera reciprocal SN_{50} titers[a]													
	68-40	BF-71	69-1112	68-2024	67-64	69-348a	10-66	86-68	FRV	M-8	A-3	69-80	69-1079e	69-1345
68-40 (300)	100, 200	—[b], 10	10, 10	100, 200	—, —	—, 20	20, 20	20, —	30, —	—, 10	65, 100	NT[c]	NT	NT
BF-71 (100)	10, 30	650, 650	20, 30	65, 65	—, —	60, 20	—, —	—, —	65, 30	20, 20	20, 20	NT	NT	NT
69-1112 (50)	—, —	60, 20	1,024, 1,024	60, 20	10, —	20, 10	—, —	—, —	—, 20	20, 20	20, 20	NT	NT	NT
68-2024 (100)	10, 30	65, 65	100, 100	100, 100	20, 30	20, 20	20, 30	20, 10	30, 10	20, 20	100, 300	NT	NT	NT
67-64 (100)	—, 20	—, 10	200, 60	600, 600	10, 300	60, 100	10, 10	200, 30	100, 60	600, 60	20, 200	NT	NT	NT
69-348a (100)	—, —	10, 20	20, 10	20, 20	—, —	100, 100	65, 30	10, 10	30, 20	5, 20	20, 30	10, 10	10, 10	—, —
10-66 (500)	10, 65	10, 30	30, 65	100, 65	—, 100	100, 100	100, 200	10, 30	65, 30	30, 30	65, 65	NT	NT	NT
86-68 (100)	—, 10	—, 10	20, 20	20, 30	—, —	20, 20	30, 20	80, 80	20, 20	20, 20	20, 20	NT	NT	NT
17-FRV (100)	—, —	—, 10	20, —	20, 20	—, 30	30, 20	10, 10	20, —	200, 100	30, 20	20, 30	10, 20	10, 10	100, 60
M-8 (300)	—, 100	30, 30	30, 100	300, 100	30, —	300, 650	—, 10	65, 100	30, 10	100, 100	30, 30	NT	NT	NT
A-3 (100)	—, 20	—, —	20, 20	20, 10	—, —	—, —	—, 10	—, 10	—, —	20, 20	100, 650	NT	NT	NT
69-80 (100)	—, —	300, 10	20, 65	30, 20	—, 65	—, —	—, 20	10, 30	10, 30	200, 300	30, 200	80, 80	200, 65	20, —
69-1079e (100)	40, —	—, 10	10, —	30, 100	—, 30	—, —	20, 65	200, 20	30, 65	30, 65	100, 30	200, 20	300, 2,000	300, 200
69-1345 (100)	—, —	300, 10	20, 65	30, 20	—, 65	NT	—, 20	10, 30	10, 30	200, 300	30, 200	NT	200, —	200, 65

[a] Two cats used for each virus.
[b] –, Titer less than 1:10.
[c] NT, Not tested.

TABLE 6. *Cross-neutralization of various feline caliciviruses by SPF cat anti-calicivirus sera containing 20 antibody units*

Virus	Cross-neutralization by cat antisera:										
	M-8	FRV	10-66	86-68	68-40	69-1112	BF-71	68-2024	67-64	69-348	A-3
KCD	n[a]	n	N[b]	n	n	n	—	—[c]	—	—	—
FPL	N	n	n	n	N	n	n	N	n	n	—
CFI	—	—	n	N	N	—	n	n	—	n	—
F-5	—	—	—	—	n	—	n	—	n	—	—
F-9	—	—	n	N	N	—	n	N	n	—	—
F-10	N	n	N	n	—	—	—	n	n	—	n
F-11	—	—	n	N	N	n	N	N	N	—	—
F-17	N	n	N	N	N	n	N	n	n	N	N
F-19	N	n	N	n	—	n	n	N	N	n	n
FC	—	—	—	N	n	—	N	N	n	—	—
FJ	—	—	n	n	N	—	N	n	—	—	—
FS	—	—	—	n	—	—	—	—	n	—	—
FRI-14	N	—	—	—	—	n	n	n	—	—	—
FRI-29	N	—	—	—	—	—	—	n	—	—	—
FRI-6	N	—	—	—	—	—	—	—	—	—	—
FRI-278	N	—	—	—	—	—	—	—	—	—	—
FPV-255	N	—	—	—	—	—	N	N	—	—	—
M-8	N	n	n	N	N	—	n	N	—	—	—
17-FRV	N	N	N	n	N	n	—	n	n	—	n

[a] n, ≥50% neutralization.
[b] N, Complete neutralization.
[c] —, <50% neutralization.

TABLE 7. *Serum neutralization titers of goat anti-feline calicivirus sera against 100 $TCID_{50}$ of homologous virus in micro-neutralization test*

Serum	Goat no.	$Log_{10} SN_{50}$			Mean	Mean SN_{50}
		Test A	Test B	Test C		
KCD	413A	2.91	2.50	3.15	2.85	1:708
FPL	4325	2.85	2.50	2.48	2.61	1:407
CFI	419A	2.80	2.70	2.47	2.66	1:457
F-5	450	1.65	1.65	1.70	1.67	1:468
F-9	437	2.35	2.20	2.70	2.42	1:263
F-10	448	1.88	1.90	2.18	1.99	1:97.7
F-11	445	1.70	1.95	1.80	1.82	1:66.0
F-17	451	2.02	1.95	2.10	2.02	1:105
F-19	441	2.80	3.15	2.60	2.85	1:708
FC	425A	3.15	2.31	2.80	2.75	1:562
FJ	408	1.90	1.70	1.73	1.84	1:69.2
FS	402A	1.80	1.65	1.60	1.68	1:47.9
FRI-14	427A	1.70	1.60	1.60	1.63	1:42.7
17-FRV	417A	NT[a]	1.10	1.00	1.05	1:11.2

[a] Not tested.

body-units concept of significant cross-reactivity: "A candidate rhinovirus was considered to be distinct if at least 20 times the limiting concentration of specific antisera which neutralized 32-320 TCD_{50} of the other serotypes (that is, 20 antibody units) failed to neutralize 32-320 TCD_{50} of the candidate virus and if at least 20 antibody units of serum to the candidate virus failed to neutralize 32-320 TCD_{50} of each of the other serotypes" (13). This definition led to the recognition of 55 serotypes of human rhinovirus from 68 candidates in the first phase, in which there were 73 candidates (14). When this concept is applied to the feline caliciviruses (Tables 6, 8, and 9), the occurrence of cross-neutralization is widespread. Comparing just two isolates in two-way cross tests, it is possible to identify apparently distinct types, for instance, KCD and FPL (Table 8), but as more isolates are compared, interrelationships occur whereby these distinguishable isolates are both neutralized by antisera to several other feline caliciviruses, and so on. Thus in an extensive comparison of feline caliciviruses, no isolate can be distinguished as a separate serotype (not neutralized by 20 antibody units of the antisera to other isolates) and vice versa. Instead, the pattern suggests a rather homogeneous group at least with regard to antigens involved in SN antibody reactions, but nonetheless without having total antigenic identity. Thus, the feline caliciviruses compared here may be regarded as serological variants of a single serotype. Because comparison of just two isolates can be misleading, in the future serological investigation of further feline calicivirus isolates, testing with the 20-antibody-units concept should be done with respect to at least two prototype isolates.

TABLE 8. *Cross-neutralization results for the 14 "NCI" feline caliciviruses against goat-produced antisera*

Virus (100 $TCID_{50}$)	Cross-neutralization by antisera:[a]													
	KCD	FPL	CFI	F-5[b]	F-9	F-10[b]	F-11[b]	F-17[b]	F-19	FC	FJ[b]	FS[b]	FRI-14[b]	FRV[c]
KCD	N[d]	—[e]	n[f]	—	—	n	—	N	—	—	n	N	N	—
FPL	—	N	—	—	N	N	n	n	—	—	—	N	—	—
CFI	n	N	N	—	—	N	—	N	n	n	N	N	n	N
F-5	n	N	n	N	N	n	n	N	n	N	N	N	N	—
F-9	—	n	n	—	N	n	—	N	—	n	n	N	N	—
F-10	n	n	N	—	N	N	—	N	n	n	—	N	N	n
F-11	N	n	N	n	—	N	N	N	n	n	n	N	N	n
F-17	n	—	n	—	n	n	n	N	—	n	n	N	n	n
F-19	n	—	N	n	N	N	n	N	N	N	n	N	N	N
FC	n	N	N	—	N	N	—	N	n	N	N	N	N	n
FJ	n	n	N	—	N	N	—	N	n	N	N	N	N	n
FS	—	n	N	N	n	n	—	N	—	N	n	N	n	—
FRI-14	—	n	—	n	n	N	n	n	n	N	N	N	N	n
17-FRV	N	—	n	—	n	n	N	n	n	N	n	N	n	N

[a] Twenty times limiting dilution against homologous virus except where stated.
[b] Ten times limiting dilution against homologous virus.
[c] Two times limiting dilution against homologous virus.
[d] N, Complete neutralization.
[e] —, <50% neutralization.
[f] n, ≥50% neutralization.

TABLE 9. *Cross-neutralization of various feline caliciviruses by 14 goat-produced antisera*

Virus (100 $TCID_{50}$)	Cross-neutralization by antisera:[a]													
	KCD	FPL	CFI	F-5[b]	F-9	F-10[b]	F-11[b]	F-17[b]	F-19	FC	FJ[b]	FS[b]	FRI-14[b]	FRV[c]
N-3	—[d]	—	N[e]	n[f]	n	n	N	N	N	N	n	—	N	—
FRI-278	N	—	N	n	n	—	—	n	—	—	—	—	—	—
FPV-255	—	—	N	n	n	N	N	N	N	N	n	—	N	—
FRI-6	—	—	N	—	n	N	N	N	n	N	N	—	N	N
N-24	—	—	N	—	N	—	N	N	N	n	n	—	N	N
FRI-29	N	—	N	n	n	N	n	N	n	N	—	—	N	N
M-8	N	—	N	N	n	n	N	N	N	N	N	N	N	N

[a] Twenty times limiting dilution against homologous virus except where stated.
[b] Ten times limiting dilution against homologous virus.
[c] Two times limiting dilution against homologous virus.
[d] —, <50% neutralization.
[e] N, Complete neutralization.
[f] n, ≥50% neutralization.

To pursue elegant epidemiological studies and to achieve pedantic sub-classification of these viruses would require a more specific tool than serum neutralization. However, with regard to the far more significant question of to what extent infection with one variant of feline calicivirus can protect a cat against subsequent infection with other variants, these results present an optimistic outlook. The investigation of the in vivo situation is the subject of current work.

ACKNOWLEDGMENTS

The technical assistance of C. J. Hale, S. Payne, and J. Ingersoll during various phases of this study is gratefully acknowledged. I thank C. S. Grunsell (University of Bristol) and W. H. Beckenhauer (Norden Laboratories, Lincoln, Neb.) for their constant support and the use of their facilities.

This study was made possible by funds from the Wellcome Trust and Norden Laboratories.

LITERATURE CITED

1. Archetti, I., and F. L. Horsfall. 1950. Persistent antigenic variation of influenza A virus after incomplete neutralization *in ovo* with heterologous immune serum. J. Exp. Med. **92**:441-462.
2. Bittle, J. L., C. J. York, J. W. Newberne, and M. Martin. 1960. Serologic relationship of new feline cytopathogenic viruses. Amer. J. Vet. Res. **21**:547-550.
3. Bolin, V. D. 1957. The cultivation of panleukopenia virus in tissue culture. Virology **4**:389-390.
4. Burki, F. 1965. Picornaviruses of cats. Arch. Gesamte Virusforsch. **15**:690-696.
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. Crandell, R. A., and S. H. Madin. 1960. Experimental

studies on a new feline virus. Amer. J. Vet. Res. **21**:551-556.
7. Crandell, R. A., W. H. Niemann, J. R. Ganaway, and F. D. Maurer. 1960. Isolation of cytopathic agents from the nasopharyngeal region of the domestic cat. Virology **10**:283-285.
8. Gillespie, J. H., A. B. Judkins and D. E. Kahn. 1971. Feline viruses. XIII. The use of the immunofluorescent test for the detection of feline picornaviruses. Cornell Vet. **61**:172-179.
9. Gillespie, J. H., and F. W. Scott. 1973. Feline viral infections. Advan. Vet. Sci. Comp. Med. **17**:163-200.
10. Hersey, D. F., and F. D. Maurer. 1960. Immunological relationship of selected feline viruses by complement fixation. Proc. Soc. Exp. Biol. Med. **107**:645-646.
11. 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.
12. Kamizono, M., S. Konishi, M. Ogata, S. Kobori. 1968. Studies on cytopathogenic viruses isolated from cats with respiratory infections. Jap. J. Vet. Sci. **30**:197-206.
13. Kapikian, A. Z., et al. 1967. Rhinoviruses: a numbering system. Nature (London) **213**:761-763.
14. Kapikian, A. Z., et al. 1971. Rhinoviruses—extension of a numbering system. Virology **43**:524-526.
15. McEwan, P. J., and J. A. R. Miles. 1967. An electron microscope study of viruses associated with upper respiratory tract infections in cats. Proc. Univ. Otago Med. Sch. **45**:21-23.
16. 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.
17. Povey, R. C., and R. H. Johnson. 1970. Observations on the epidemiology and control of viral respiratory disease in cats. J. Small Anim. Pract. **11**:485-494.
18. Prydie, J. 1966. Viral diseases of cats. Vet. Rec. **79**:729-738.
19. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty per cent end points. Amer. J. Hyg. **27**:493-497.
20. Sinha, S. K. 1958. Feline viruses. Calif. Vet. **11**:18-20.
21. Studdert, M. J., M. C. Martin, and J. E. Peterson. 1970. Viral diseases of the respiratory tract of cats: isolation and properties of viruses tentatively classified as picornaviruses. Amer. J. Vet. Res. **31**:1723-1732.
22. Takahashi, E., S. Konishi, and M. Ogata. 1971. Studies on cytopathogenic viruses from cats with respiratory infections. II. Characterization of feline picornaviruses. Jap. J. Vet. Sci. **33**:81-87.
23. Tan, R. J. S. 1970. Serological comparisons of feline respiratory viruses. Jap. J. Med. Sci. Biol. **23**:419-424.
24. Tan, R. J. S., and J. A. R. Miles. 1971. Further studies on feline respiratory virus diseases. I. Vaccination experiments 2. Immunodiffusion tests. N. Z. Vet. J. **19**:12-18.