# Antigenic Relationship of the Feline Infectious Peritonitis Virus to Coronaviruses of Other Species\*

By

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With 2 Figures

Accepted February 23, 1978

# Summary

Utilizing the direct and indirect fluorescent antibody procedure, the antigenic relationship of the feline infectious peritonitis virus (FIPV) to 7 other human and animal coronaviruses was studied. FIPV was found to be closely related to transmissible gastroenteritis virus (TGEV) of swine. Transmissible gastroenteritis virus and FIPV were in turn antigenically related to human coronavirus 229 E (HCV-229 E) and canine coronavirus (CCV). An interesting finding in the study was that the 8 coronaviruses selected for this study fell into one of two antigenically distinct groups. Viruses in each group were antigenically related to each other to varying degrees, but were antigenically unrelated to coronaviruses of the second group. The first antigenically related group was comprised of mouse hepatitis virus, type 3 (MHV-3), hemeagglutinating encephalomyelitis virus 67 N (HEV-67 N) of swine, calf diarrhea coronavirus (CDCV), and human coronavirus OC43 (HCV-OC43). The second antigenically related group was comprised of FIPV, TGEV, HCV-229 E and CCV.

# Introduction

The family Coronaviridae is a recently characterized group of animal and human viruses (23). Coronaviruses are 60 to 220 nm in diameter, have a buoyant density in sucrose of 1.16 to  $1.23 \text{ g/cm}^3$ , are sensitive to lipid solvents, contain a large single strand of ribonucleic acid, have regularly spaced surface projections

<sup>\*</sup> This research was supported by grants from the Winn Foundation of the Cat Fanciers of America and the S.O.C.K. Organization, Lafayette, California.

that are 12 to 24 nm in length, and bud from profiles of endoplasmic reticulum into cytoplasmic vesicles in the infected cells (23). Coronaviruses cause bronchitis in chickens (5), humans (7, 10) and rats (14), acute enteric infections in baby pigs (22), calves (21) and puppies (1), hepatitis in mice (4), and encephalomyelitis and chronic vomition and wasting in swine (6, 18).

Feline infectious peritonitis (FIP) is a viral disease of cats that is characterized by peritonitis, pleuritis or disseminated granulomata (17). Feline infectious peritonitis represents an uncommon secondary form of a common inapparent or mild primary illness of cats (15). The FIP agent has strong morphologic and physical similarities to known coronaviruses (8, 16, 24, 26). A possible antigenic relationship between feline infectious peritonitis virus (FIPV) and the transmissible gastroenteritis virus (TGEV) of swine has been recently reported (13, 19, 25), which further supports the assumption that FIPV is a coronavirus. The antigenic relationship of FIPV to human and animal coronaviruses other than TGEV has not been studied, and confirmation of the antigenic relationship of FIPV and TGEV using monospecific antiserum is needed.

The purpose of this study is twofold: to confirm the antigenic relationship of FIPV to TGEV, and to demonstrate the antigenic relationship of 7 different animal and human coronaviruses to FIPV, and to each other. The viruses selected for this study were human coronavirus OC43 (HCV-OC43), human coronavirus 229E (HCV-229E), TGEV and hemagglutinating encephalomyelitis virus 67 N (HEV-67N) of swine, mouse hepatitis virus type 3 (MHV-3), calf diarrheal coronavirus (CDCV), and canine coronavirus (CCV). Antigenic comparisons were made utilizing the direct and indirect fluorescent antibody technique. This procedure has been utilized to study serologic differences between several human coronaviruses (12).

# **Materials and Methods**

#### Preparation of Specific Antiserum

Monospecific antiserum to FIPV was prepared in specific pathogen free kittens (Liberty Laboratories, Liberty Corners, NH). The cats were inoculated intraperitoneally with 0.2 g equivalents of liver suspension containing approximately 100 ID<sub>100</sub> of the UCD-1 strain of FIPV. The origin of this strain and the preparation of the inocula have been previously described (16). Serum was harvested prior to the animals' death, from 21 to 35 days after inoculation. By the indirect fluorescent antibody technique (15) this antiserum had a titer of 1:256 against FIPV.

Mouse anti-MHV-3 serum was produced in specific pathogen free adult Swiss white mice. Mice were inoculated intraperitoneally with a sublethal dose of a 50 per cent mouse liver suspension containing the Craig strain of MHV (MHV-3). This material was obtained from the American Type Culture Collection, Rockville, Md. Three weeks later the mice were challenged intraperitoneally with a second sublethal dose of MHV-3, followed by challenge 2 weeks later with a lethal dose of virus. Serum was harvested 3 weeks after the final challenge dose. This serum had a titer by the indirect fluorescent antibody technique of 1: 256 against MHV infected NCTC-1469 cells.

Bovine anti-CDCV antiserum was obtained from calves that had been experimentally infected with the virus. The globulin fraction of this serum was conjugated with fluorescein isothiocyanate (FITC). Conjugated antiserum was provided by Dr. C. A. Mebus, Lincoln, Nebraska. The conjugated antiserum produced maximum fluorescence in CDCV infected bovine fetal lung cells at dilutions of 1:25 or less. Swine anti-TGEV serum was obtained from a specific pathogen free sow that was experimentally infected with the Miller strain of TGEV. This serum was kindly provided by Dr. Roger Woods, Ames, Iowa. Both swine anti-TGEV and HEV serum produced maximum fluorescence by the antibody technique at di utions of 1:25 or less.

Guinea pig anti-HCV-229 E serum was provided by Dr. Harold Kaye, Communicable Diseases Center, Atlanta, Georgia. Before use, the serum was absorbed with swine testicle, human embryo fibroblasts, NCTC-1469, African green monkey (CV-1), bovine fetal lung cells and with cat liver homogenate. This serum produced maximum fluorescence by the indirect fluorescent antibody technique at dilutions of 1:25 or less.

Mouse anti-HCV-OC43 ascitic fluid was kindly provided by Dr. Harold Kaye, Communicable Diseases Center, Atlanta, Georgia. Ascitic fluid was collected from virus free mice that had been experimentally infected with HCV-OC43. This serum produced maximum fluorescence by the indirect fluorescent antibody technique at dilutions of 1:25 or less.

Canine anti-CCV globulin conjugated with fluorescein isothiocyanate was provided by Dr. L. N. Binn, Walter Reed Army Institute of Research, Washington, D.C. It was prepared from convalescent serum of puppies experimentally infected with CCV. The conjugated antiserum produced maximum fluorescence in CCV infected canine fetal thymus cells at dilutions of 1:25 or less.

### Antigen Preparations

Cryostat microtome sections of liver from FIPV infected cats were used as the antigen source of FIPV. The preparation of these slides has been previously described (15). Prior to use, the fixed liver sections were immersed for 5 minutes in  $0.1 \,\mathrm{M}$  glycine-HCl buffer, pH 2.2 to remove immunoglobulin bound *in vivo*. When this bound immunoglobulin was not removed, a false positive reaction was seen in the indirect fluorescent antibody test, especially when the second antibody was rabbit anti-cat IgG. After treating in buffer, the slides were washed immediately in phosphate buffered saline (PBS), followed by a 5 minute and 15 minute wash in PBS.

MHV-3 infected cell monolayers were prepared as follows. NCTC-1469 cells (Microbiological Associates, Bethesda, Md.), adapted to grow in Eagle's minimum essential media (MEM) and 10 per cent fetal calf serum (FCS), were grown in 8 well culture chamber slides (Lab-Tek, Microbiological Associates, Bethesda, Md.). When confluent, the cell monolayer was exposed to MHV-3 by placing 0.1 ml of a 1:100 dilution of 50 per cent infected mouse liver suspension in each well. The slides were fixed in absolute acetone when significant cytopathic effect was noticed.

Calf diarrhea coronavirus was obtained from Dr. C. A. Mebus, Lincoln, Nebraska. One-tenth ml of infectious tissue culture media was placed in each well of a culture chamber slide containing a three-fourth confluent monolayer of low passage fetal bovine lung cells. The slides were fixed in absolute acctone after 5—7 days.

TGEV and HEV were cultivated in swine embryonic testicle cells (National Animal Diseases Center, Ames, Iowa). TGEV (Miller strain) infected tissue culture fluid was provided by Dr. Roger Woods, Ames, Iowa. HEV (67N strain) infected tissue culture fluid was obtained from the National Animal Disease Center, Ames, Iowa. Swine testicle cells were grown in Eagle's MEM in 10 per cent FCS in 8 well culture chamber slides. When the cultures were almost confluent they were exposed to the 67N strain of HEV or Miller strain of TGEV by placing 0.1 ml of infected tissue culture fluid in each well. The slides were fixed in absolute acetone after 2 to 7 days.

HCV-229E was obtained as infected tissue culture fluid from Dr. Harold Kaye, Communicable Diseases Center, Atlanta, Georgia. Low passage human embryonic fibroblasts were grown in Eagle's MEM in 10 per cent FCS in 8 well culture chamber slides. When the cultures were almost confluent, 0.01 ml of infected tissue culture fluid was placed in each well. Slide cultures were fixed after 2 days. HCV-OC43 was obtained as a mouse brain suspension from Dr. Harold Kaye, Communicable Diseases Center, Atlanta, Georgia. High passaged African green monkey kidney cells (CV-1) were grown in Eagle's MEM with 10 per cent FCS in 8 well culture chamber slides. When nearly confluent, 0.1 ml of a 1:10 dilution of brain suspension was placed in each well. Slides were fixed in absolute acetone after 7 to 10 days.

Canine coronavirus (I-71) in tissue culture fluid was obtained from the American Type Culture Collection, Rockville, Md. Low passage dog thymus cells were cultivated in 8 well culture chamber slides. When nearly confluent, 0.1 ml of infected tissue culture fluid was placed in each well. Cultures were fixed in absolute acetone after cytopathic effect became noticeable.

## Fluorescein Isothiocyanate Conjugated Second Antibodies

Rabbit anti-mouse IgG globulin-FITC, rabbit anti-cat IgG globulin-FITC, rabbit anti-pig IgG-FITC, and goat anti-guinea pig IgG-FITC were obtained from Antibodies Incorporated, Davis, Ca. Conjugated anti-IgG globulins were free of anticoronavirus activity as determined by reacting antigen substrates with the diluted conjugates alone. Goat anti-guinea pig IgG-FITC was absorbed with cat liver homogenate and human embryo cells.

## Fluorescent Antibody Staining Procedure

Indirect fluorescent antibody staining was carried out as follows. Antigen substrate slides were overlaid with a 1:10 dilution in PBS of the appropriate antiserum and incubated at  $37^{\circ}$  C for 1 hour in a humidified chamber. The slides were immediately rinsed with PBS and then washed for 5 minutes in PBS. The slides were blotted dry and then overlaid with the appropriate anti-IgG conjugate diluted 1:20 in PBS. The slides were incubated for 1 hour at  $37^{\circ}$  C in humidified chamber and then washed in PBS. This treatment was followed by a 5 minute wash in PBS containing a 1:500 dilution of a 1 per cent stock solution of aqueous Evans blue, and a 5 minute wash in PBS. Slides were then blotted dry, and coverslips mounted with 50 per cent glycerol in PBS.

Direct fluorescent antibody staining was carried out essentially as above, except that the first antibody reaction was omitted.

#### Fluorescence Microscopy

Slides were photographed with a Zeiss reflected light fluorescent microscope, powered by a 50 watt AC mercury-vapor bulb, using exciter and barrier filters specific for fluorescein isothiocyanate. Photomicrographs were made using Ektachrome 35 mm daylight slide film, ASA 160 (Kodak Co., Rochester, NY), with 30 second exposures. All photomicrographs were prepared from black and white negatives made from the colored slides.

## Results

The cross-reactivity by immunofluorescence of antisera to 8 different coronaviruses is listed in Table 1. Antigenic cross-reactivity varied from nondetectable, barely detectable, weak, moderate, to very strong (equal to that produced by the homologous serum). Cross-reactivity, varying from barely detectable to very strong, was seen between MHV-3, CDCV, HEV-67N and HCV-OC43. There was no detectable antigenic cross-reactivity between these 4 viruses and FIPV, TGEV, HCV-229E, or CCV. Antiserum to FIPV reacted strongly with TGEV, and vice versa. Antiserum to both of these viruses had barely detectable to weak antigenic cross-reactivity with HCV-229E, and antiserum to HCV-229E had weak to moderate cross-reactivity with FIPV and TGEV. Antiserum to CCV did not react with any of the other 7 coronaviruses, although antiserum to FIPV and

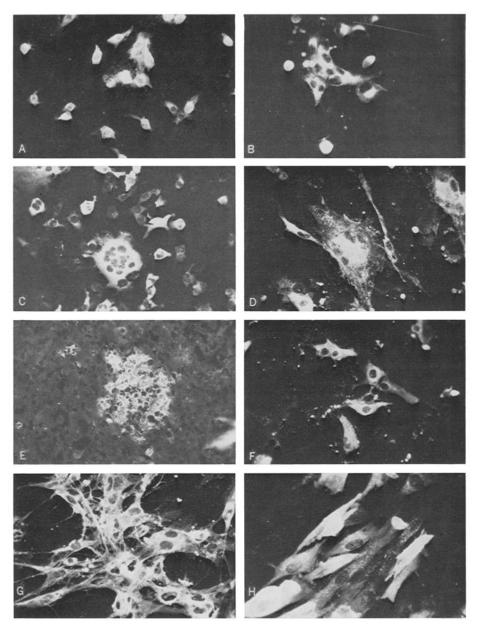


Fig. 1. A. Swine testicle cells infected with HEV. Swine anti-HEV serum, rabbit antipig IgG-FITC, ×250; B. African green monkey kidney cells infected with HCV-OC43.
Mouse anti-HCV-OC43 ascitic fluid, rabbit anti-mouse IgG-FITC, ×250; C. NCTC-1469 cells infected with MHV-3. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC, ×250; D. Primary bovine fetal lung cells infected with CDCV. Bovine anti-CDCV globulin-FITC, ×250; E. Frozen section of liver from kitten experimentally infected with FIPV. Cat anti-FIPV serum, rabbit anti-cat IgG-FITC, ×100; F. Swine testicle cells infected with TGEV. Swine anti-TGEV serum, rabbit anti-pig IgG-FITC, ×250; G. Primary whole embryonic human cells infected with HCV-229E. Guinea pig anti-HCV-229E serum, goat anti-guinea pig IgG-FITC, ×250; H. Primary fetal dog thymus cells infected with CCV. Canine anti-CCV globulin-FITC, ×400

TGEV reacted very strongly with CCV. Photomicrographs of some of the strongly positive cross-reactions are shown in Figures 1 and 2.

It was concluded from these studies that MHV-3, HCV-OC43, CDCV, and HEV-67 N are all antigenically interrelated to varying degrees, but are not related antigenically to any of the other 4 coronaviruses. Similarly, FIPV, TGEV, HCV-229 E and CCV share antigens to varying degrees with each other, but appear antigenically unrelated to MHV-3, HCV-OC43, CDCV, and HEV-67 N. In the case of CCV, however, the reactivity was largely in one direction only, in that antiserum to CCV showed no reactivity with any of the other 7 coronaviruses, whereas antiserum to FIPV and TGEV reacted strongly with CCV.

Antiserum against	Viral Substrates							
	MHV-3	CDCV	HCV- OC 43	HEV- 67 N	TGEV	FIPV	HCV- 229 E	CCV
MHV		++	+++	+++				
CDCV	±	+++	++	$\div + +$			here and	
HCV-OC43	+++		+ + +	++++				-
HEV-67N	+	++	++	+++				
TGEV			_		+++	+ + +	+	+ + +
FIPV	—			-	+++	+++	+-	+++
HCV-229E					++	+	+++	
CCV				*******				+ + +

 Table 1. Antigenic relationship of 8 different coronaviruses established by immunofluorescence

- = negative

 $\pm$  = barely detectable

+ = weak

++ = moderate

+++ = strong, equal to the reaction obtained by a dilution of serum that produced maximum fluorescence in the homologous substrate

## Discussion

These studies demonstrate conclusively that the FIP virus has antigenic similarities to known coronaviruses, namely TGE virus of swine, 229 E virus of humans, and canine coronavirus. This finding, coupled with the morphologic and

Fig. 2. A. Primary fetal dog thymus cells infected with CCV. Cat anti-FIPV serum, rabbit anti-cat IgG-FITC,  $\times 400$ ; B. Primary fetal dog thymus cells infected with CCV. Swine anti-TGEV serum, rabbit anti-pig IgG-FITC,  $\times 400$ ; C. Frozen section of liver from kitten experimentally infected with FIPV. Swine anti-TGEV serum, rabbit anti-pig IgG-FITC,  $\times 100$ ; D. Swine testicle cells infected with TGEV. Cat anti-FIPV serum, rabbit anti-oat IgG-FITC,  $\times 250$ ; E. NCTC-1469 cells infected with MHV-3. Mouse anti-HCV-OC43 asitic fluid, rabbit anti-mouse IgG-FITC,  $\times 250$ ; F. Swine testicle cells infected with HEV. Bovine anti-CDCV globulin-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Mouse anti-MHV serum, rabbit anti-mouse IgG-FITC,  $\times 250$ ; H. Swine testicle cells infected with HEV. Swine testicle cells infe

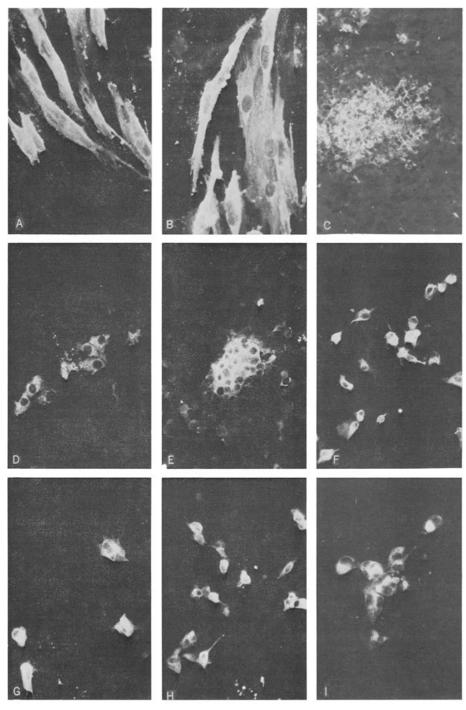


Figure 2

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physical studies of the FIP virion already published, show that the FIP agent is indeed a coronavirus.

It was interesting that the 8 coronaviruses selected for this study segregated into 2 distinct groups on the basis of antigenic cross-reactivity by immunofluorescence. Although viruses within each group were antigenically related to each other, there appeared to be no antigenic relationship of viruses from one group with viruses of the other group. The first antigenically related group was comprised of MHV-3, HEV-67N, CDCV and HCV-OC43, and the second group was comprised of TGEV, FIPV, HCV-229E and CCV. These findings confirm a number of published reports on antigenic relationships among recognized coronaviruses. On the basis of serum neutralization or complement fixation tests, antigenic relationship has been previously reported between rat coronavirus and MHV (14), HCV-OC43 and MHV (11), HEV-67N and CDCV (20), HCV-OC43 and HEV-67N (9), and CCV and TGEV (1). It has also been previously reported that HCV-229E appeared to be antigenically unrelated to MHV-3 and HCV-0C43 (11). The lack of relationship by immunofluorescence of HCV-OC43 and HCV-229E has also been reported (12).

Although the results of our studies were in agreement with most of the published literature on antigenic relationships among various coronaviruses, there were several reports that we could not confirm. We could find no antigenic relationship by immunofluorescence between MHV-3 and HCV-229 E, and HCV-OC43 and HCV-229 E. A relationship between these viruses has been previously described (3). We also found no antigenic relationship between HEV-67 N and TGEV, although a relationship using the immunoprecipitation technique has been reported (2). It has been reported that antiserum to TGEV does not react against FIPV in the fluorescent antibody test (25). In contrast, we found that antiserum to TGEV reacted strongly with FIPV. Finally, we are at a loss to explain the failure of anti-CCV globulin to react with TGEV and FIPV with immunofluorescence, especially considering the strong reaction against CCV demonstrated by both anti-FIPV and TGEV serum. Dog anti-CCV serum will apparently neutralize TGEV (1), and it is strange that this reaction was not detected with immunofluorescence.

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Received October 23, 1977