PATHOGENIC DIFFERENCES BETWEEN VARIOUS FELINE CORONAVIRUS ISOLATES

Niels C. Pedersen, John W. Black, John F. Boyle, James F. Evermann, Alison J. McKeirnan, and Richard L. Ott

Depts. of Medicine, Schools of Veterinary Medicine, Univ. of Calif., Davis (Pedersen, Boyle), Washington State Univ., Pullman (Evermann, McKeirnan, Ott), and Specialized Assays, Nashville, TN (Black)

INTRODUCTION

Coronaviruses are being isolated with increasing frequency from cats. These various isolates can be divided into two major groups: 1) coronaviruses that induce a disease of cats known as feline infectious peritonitis (FIP), and 2) coronaviruses that cause a transient subclinical to severe enteritis[1,2]. The various isolates in each of these groups are morphologically and antigenically related, and probably represent strains of a common species of virus that infects cats, dogs (canine coronavirus or CCV), and swine[3,4].

The purpose of the report is to describe the coronavirus strains that have been isolated from cats. The antigenic similarities of the various isolates will be compared, and the pathogenesis of FIP virus (FIPV) and feline enteric coronavirus (FECV) infection will be discussed.

Feline Infectious Peritonitis Virus

A number of isolates of FIPV have been made throughout the world. Unfortunately, early isolates could only be propagated

P. J. M. Rottier et al. (eds.), *Molecular Biology and Pathogenesis of Coronaviruses*

in vivo by serial passage in cats, so comparisons of isolates were difficult to make. Within the last several years, however, at least 6 FIPV isolates have been cultivated in tissue culture. These include the isolate of O'Reilly and coworkers[5], the NW1 (UCD1) strain[1], the TN-409 (Black) strain[6], the Nor-15 isolate[7], the 79-1146 virus[8], and the UCD2 strain[9].

We have studied 4 of the 6 strains in our laboratory, including FIPV UCD1, FIPV-Black, FIPV-79-1146, and FIPV-UCD2. The UCD1, UCD2, and Black strains are very similar in regard to cytopathic effect (CPE), cell-associated growth, pathogenecity, and in their comparative neutralization by antiserum to various strains of FECV, FIPV, TGEV, and CCV. The 79-1146 strain, however, is clearly different in growth characteristics in tissue culture, pathogenecity, and because of its greater resemblance to CCV.

The UCD1, UCD2, and Black strains of FIPV produce slow CPE in culture. Tissue culture supernatants will rarely yield more than 4,000 to 40,000 $TCID_{100}$ of non-cell associated virus per ml. Cell sonicates will contain 10 times more infectious virus than culture supernatants. These 3 strains grow best in certain cat cell lines, such as fcwf-4 or fc-0009 (fc9) cells[1]. They grow less well on Crandell feline kidney (CRFK) cells. Foci appear slowly over a period of 36 to 96 hours and consist initially of refractile angular shaped or multinucleated cells. Primary foci enlarge slowly from the peripheries. Secondary foci of infection are seen only occasionally, indicating that infection proceeds mainly by cell to cell contact. As the foci grow, cells in the middle of the plaque slough into the media or remain adhered to the plastic in a stellate, multinucleate, or lace-like form. The infection is most readily propagated by co-passing infected with normal cells.

The 79-1146 strain of FIPV was originally isolated from a 4 day old kitten by McKeirnan and associates[8]. It was later

found to induce FIP in cats[9]. FIPV-79-1146 closely resembles canine coronavirus in its growth in cell culture. It grows well in most cat cell lines, including CRFK cells, and yields up to 10^6 to 10^7 $TCID_{100}$ of infectious virus per ml of culture supernatants. Small foci of CPE appear in cultures within 12 to 24 hours. Cells within the foci become retracted, refractile, and angular or rounded in appearance. Multinucleated giant cells are prominent. Secondary foci of infection appear very rapidly, and by 24-72 hours the entire cell sheet will be destroyed.

The various strains of FIPV are antigenically related to each other and to FECV isolates. The similarities are more pronounced when the isolates are compared by indirect fluorescent antibody than by virus neutralization assays (Table I, II). Antiserum to FECV-UCD and FIPV-Black cross reacts strongly in virus neutralization tests against FIPV-Black but weakly with FIPV-79-1146, FECV-79-1683 and CCV (Table II). Antiserum to FECV-79-1683 reacts weakly with FIPV-Black but strongly with FIPV-79-1146, FECV-79-1683 and CCV (Table II).

Table I. Indirect fluorescent antibody cross-reactions between FIPV-like (Type I) and CCV-like (Type II) feline coronaviruses. Titers are expressed as the highest inverse dilution of serum that still produced 1+ fluorescence.

	Virus Substrate	
Antiserum	FIPV-BLACK (Type I)	FECV-79-1683 (Type II)
Anti-FIPV-Black (Type I)	50,000	1,250
Anti-FECV-79-1683 (Type II)	1,500	26,600

Table II. Virus neutralization titers (inverse dilution) of serum samples collected from cats with experimentally induced feline coronavirus infection.

		Virus Neutralization Titer Against:			
Serum #	Immunizing Strain	FIPV-Black	FIPV-79-1146	FECV-79-1683	CCV
1	FIPV-Black	48	<2	<2	2
2	FIPV-Black	64	<2	<2	8
3	FECV-UCD	16	2	<2	<2
4	FECV-UCD	64	4	<2	<2
5	FECV-79-1146	4	100	400	200
6	FECV-79-1683	4	400	600	800

The CCV-like isolates of FIPV clearly represent a different strain, whereas FIPV-Black, FIPV-UCD1 and FIPV-UCD2 are very similar if not identical to each other[10]. In the field, strains similar to FIPV-Black account for the majority of cases of FIP (Table III). CCV-like strains of FIPV, such as FIPV-79-1146, are an infrequent cause of FIP in nature.

The pathogenecity of the UCD1 and Black strains of FIPV are similar on an infectious particle to particle basis[11]. They both have a relatively low infectivity for cats by oronasal instillation. Doses of virus in the range of 4,000 $TCID_{100}$ produce infection in a small number of animals, while doses of 40,000 $TCID_{100}$ or more will infect most cats. The initial febrile response, indicating the onset of fatal disease, occurs about 8-14 days after infection in coronavirus antibody free cats. Death usually occurs within 7 to 21 days from the onset of fever. If cats have been preimmunized with FECV or high passage avirulent FIPV-Black, fever is seen within 12 to 48 hours and the cats are usually moribund by day 7 to 10[1,11,12].

FIPV-79-1146 has a comparatively high infectivity by oronasal instillation, and clinical signs in coronavirus negative cats appear around day 8 to 14. Previous exposure with other related coronaviruses, however, does not shorten the incubation period. A slight fever is seen in coronavirus antibody negative and positive cats around 24-48 hours after infection with FIPV-79-1146.

Table III. Virus neutralization titers (inverse dilution) of serum samples collected from cats with naturally occurring feline infectious peritonitis.

		Virus Neutralization Titer Against:			
Serum #	FIPV Type	FIPV-Black	FIPV-79-1146	FECV-79-1683	CCV
1	I	3200	<10	<10	20
2	I	3200	<10	<10	<10
3	I	640	<20	<10	<10
4	I	1600	<10	<10	10
5	I	2400	20	<10	80
6	II	40	6400	6400	12,800
7	I	320	<10	10	20
8	I	640	<10	<10	<10
9	I	160	<10	<10	<10
10	I	500	10	<10	10
11	I	80	<10	<10	<10
12	I	160	<10	<10	<10
13	I	640	<10	<10	<10
14	I	320	<10	<10	<10
15	I	128	<4	<4	4
16	I	64	<4	<4	8
17	I	160	N.T.*	<10	<10
18	I	640	<10	N.T	N.T
19	I	320	<10	<10	<10
20	I	1600	<10	<10	10

*N.T. = not tested

The temperature returns to normal in 12 to 24 hours and the cats appear healthy until day 8-14, when a sustained fever appears. Cats infected with FIPV-79-1146, whether presensitized or not, survive longer with clinical signs than cats infected with FIPV-UCD1 or FIPV-Black.

Pathogenesis of FIPV infections - The pathogenesis of FIPV infection is complex and not completely understood. There is enough known about the disease, however, to speculate on its pathogenesis (Fig. 1). The primary route of infection is probably oral, and virus replication is thought to occur initially in the mature apical epithelium of the intestinal tract[13]. In support of this assumption, orally administered FIPV will infect the intestinal epithelium of neonatal pigs in a manner identical to TGEV[14]. Clinical signs are not associated with the initial intestinal infection of cats. In cats serologically negative for coronavirus infection, the earliest signs of illness occur from 8 to 14 days after exposure. Clinical illness appears to be associated with dissemination of virus from the mucous membranes and regional lymph nodes, probably by way of blood-borne phagocytes[15]. This is one essential difference between FIPV and FECV infections. FECV does not spread further than the intestinal epithelium and the regional lymph nodes[2]. Disseminated FIPV is found preferentially within phagocytic cells in the target tissues, which include the liver, visceral peritoneum and pleura, uveal tract of the eyes, and the meninges and ependyma of the brain and spinal cord. The form of the disease that follows dissemination is dependent on the type of immunity that develops (Fig. 1).

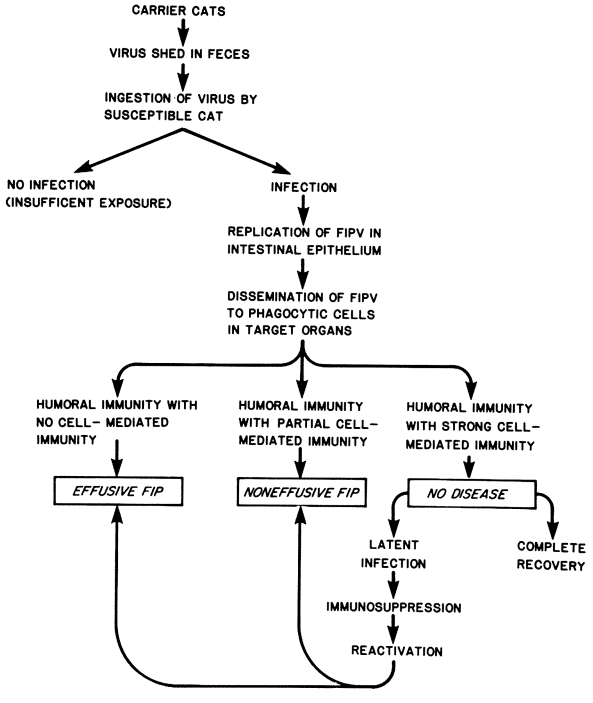

Fig. 1 - The possible pathogenesis of Feline Infectious Peritonitis as formulated from current knowledge.

FIP occurs in two distinct forms[16,17]. The first form is essentially a peritonitis or pleuritis, or both. The target tissues are the visceral peritoneum and pleura, and the omentum. Inflammation in these tissues results in a great outpouring of fluid into either or both of these body cavities, hence the name "wet" or "effusive" FIP. Meningeal and ependymal involvement is usually clinically inapparent in the effusive form of FIP. A second form of FIP was subsequently recognized by Montali and Strandberg[18]. Lesions in this form are more granulomatous in nature and localized primarily in parenchymatous organs such as the mesenteric lymph nodes, kidneys, uveal tract, and the meninges and ependyma of the brain and spinal cord. There is minimal or no exudation of fluid into the body cavities, and this form of the disease is, therefore, called "dry" or noneffusive FIP. Under experimental conditions, effusive FIP is about 2 to 3 times more common than noneffusive FIP.

Effusive FIP is characterized by a pyogranulomatous type of reaction around small venules in the target organs[19,20,21]. This vasculitis is responsible for the outpouring of protein and fibrin-rich fluid into the chest or abdomen. The lesions of effusive FIP develop simultaneously with the appearance of humoral immunity. Humoral immunity is not protective, but in fact, is actually harmful. Cell-mediated immunity appears to be the only beneficial protective response[11]. Antibody seems to enhance virus uptake by phagocytic cells, a preferred site for virus replication[1,12]. The net effect is to enhance rather than decrease the level of virus proliferation. Antibody also reacts with antigen and complement, possibly resulting in a localized arthus-like response [12,15,22-24]. The presence of circulating immune complexes is also suggested by the fluctuating complement levels and the development of glomerulonephritis in cats with FIP[25,26]. Complement mediated activation of terminal clotting

factors, coupled with vascular lesions that consume platelets and clotting factors, causes a coagulopathy in cats with effusive FIP[22].

It is theorized that the noneffusive form of FIP occurs in cats that develop only partial cell-mediated immunity[11]. Partial cellular immunity will limit the virus to localized sites in target organs, but is insufficient to destroy or contain the infection. The resulting granulomatous reaction surrounds small accumulations of virus-laden phagocytic cells in the center of the lesions. The granulomatous reactions seen in noneffusive FIP are, therefore, equivalent to similar reactions seen in diseases such as coccidioidomycosis, histoplasmosis, or tuberculosis.

Cats that develop strong cell-mediated immunity do not show signs of illness, or will demonstrate a transient fever and localized mesenteric lymphadenopathy. Cell-mediated immunity does not always lead to complete elimination of the virus. Virus apparently persists in the body of some cats in a walled-off form. With deterioration of immune responsiveness, usually associated with aging or diseases such as FeLV infection, the FIPV infection may become active again.

Heterotypic immunity to non-FIP-inducing coronaviruses (FECV's) may be involved in the pathogenesis of FIP. Cats with cross reacting serum antibodies are often more sensitive to intraperitoneal challenge with FIPV[1,2,12]. Cats with sensitizing heterotypic coronavirus immunity will develop effusive FIP after 24 to 72 hours, versus 8 to 14 days or more for cats without previous coronavirus exposure. This enhancement is more consistently seen when FIPV strains such as UCD1 or Black are used for the challenge. FIPV-79-1146 infection is not appreciably influenced by heterotypic immunity in one way or the other. Aerosol inoculation with FIPV-UCD1 of cats with prior

coronavirus exposure causes a severe fulminating pneumonia[15,22-24]. The enhancement of illness caused by a prior exposure to an antigenically related virus is reminiscent of dengue hemorrhagic shock syndrome of man. This similarity has been described by Horzinek and Osterhaus[27], Pedersen and Boyle[12], and Weiss and Scott[23].

Initial attempts to immunize cats using TGEV of swine have been unsuccessful[28,29]. Immunization with modified live FIPV has also failed to protect cats[11]. Kittens immunized oronasally with the modified live FIPV-Black developed both IFA and virus neutralizing antibodies. Following challenge with virulent FIPV-Black, however, the infection rate was increased, latency period reduced, and disease severity enhanced in vaccinated as compared to nonvaccinated kittens. Apparently, avirulent virus does not confer a protective type immunity, but elicits humoral immunity that is actually deleterious. The failure of avirulent FIPV to immunize might be due to its failure to persist in the body[11]. Cats have been successfully immunized against FIPV using sublethal doses of virulent virus[11]. Unfortunately, this is not of clinical relevance because the dose that immunized some cats caused fatal FIP in others.

Feline Enteric Coronavirus Infection

Feline enteric coronaviruses are the cause of inapparent to mild, infrequently severe, intestinal infections in kittens between birth and 12 weeks of age. Although they are morphologically and antigenically similar to FIPV, FECV strains do not cause FIP. To date, 2 different strains of FECV have been characterized. The first isolate, designated FECV-UCD, was described by Pedersen and coworkers[2]. A second isolate was identified by McKeirnan and associates[8], and has been designated FECV-79-1683. FECV-79-1683 was isolated from a fatal case of peracute hemorrhagic enteritis in an adult cat. Like FECV-UCD,

this strain produced mild to inapparent enteritis in specific pathogen free cats[9]. Coronavirus particles identical to those described for FECV-UCD have been identified in the stools of a cat with diarrhea by Dea and coworkers[30]. This isolate shared some antigens with calf diarrhea coronavirus. Hayashi and coworkers[13] also observed a coronavirus in the intestine of a cat with diarrhea. This virus was antigenically similar to FIPV, and was probably another FECV. Hoshino and Scott[31] have demonstrated coronavirus-like particles in the stool of normal cats, but they appear morphologically and antigenically different from other FECV isolates.

Repeated attempts to grow FECV-UCD in cell culture have failed. The virus is currently maintained by in vivo passage in kittens. FECV-79-1683 grows readily in cell culture, and in regard to cytopathic effect, cell tropism and level of free virus production, it is similar to FIPV-79-1146. It also seems to be more closely related to CCV than to FECV-UCD.

Pathogenesis

The target tissue for FECV-UCD infection is the mature columnar epithelium of the small intestine[2]. Virus replication also occurs to a lesser extent in the tonsils and mesenteric lymph nodes. Clinical signs occur when a large percentage of the apical intestinal villous epithelium is damaged. Signs began between 2 and 7 days after oronasal infection. Vomiting is the first sign observed, preceding diarrhea by 12 to 48 hours. The diarrhea is seldom severe and lasts for 48 to 96 hours. Fever, when it occurs, is mild. Many recovered cats will become asymptomatic virus shedders.

FECV-79-1683 causes an almost identical disease syndrome to FECV-UCD, although perhaps milder in nature. Following oral infection, virus replication is seen in the mature apical

columnar epithelium of the small intestine, mesenteric lymph nodes, tonsils, and to a lesser extent in the lungs. Virus shedding, as detected by cell culture infectivity of fecal supernatants, is only apparent for the first 13-16 days. Recovered cats, however, remain infectious to susceptible contact animals for a much longer period of time.

Classification of Feline Coronaviruses

We propose that feline coronaviruses be classified using the following critera: 1) morphological, structural, and antigenic relationship to TGEV and CCV, 2) type of disease that they cause, e.g. FIP, enteritis, etc., 3) growth characteristics in cell culture (ease of isolation in cell culture, ease of adaptability to various cell-lines, cell-associated growth characteristics), and 4) degree of relatedness to CCV in virus neutralization tests. In such a scheme, the 5 characterized isolates can be categorized as listed in the following outline:

I. Feline Coronaviruses

- A. Non TGEV-like (theoretical existence)
- B. TGEV-like
  - 1. FIP inducing
    - a. Type I (difficult to grow in cell culture, grow best in selective cell lines, cell associated growth, antiserum to these strains reacts weakly in virus neutralization with heterologous strains such as CCV).
      - 1) FIPV-UCD1
      - 2) FIPV-UCD2
      - 3) FIPV-Black

b. Type II (easily isolated in cell culture, grows in many different cell lines, produces large amounts of non-cell associated virus, antiserum to these strains reacts strongly in virus neutralization with heterologous viruses such as CCV).

1) FIPV-79-1146

2. Non-FIP inducing

a. Enteritis causing agents

1) Type I (criteria as listed above for type I FIPV strains)
   a) FECV-UCD
2) Type II (criteria as listed above for type II FIPV strains)
   a) FECV-79-1683

REFERENCES

1. Pedersen NC, Boyle JF, Floyd K: Infection studies in kittens utilizing feline infectious peritonitis virus propagated in cell culture. Am J Vet Res 42:363-367, 1981a.

2. Pedersen NC, Boyle JF, Floyd K: An enteric coronavirus infection of cats, and its relationship to feline infectious peritonitis. Am J Vet Res 42:368-377, 1981b.

3. Horzinek MC, Lutz H, Pedersen NC: Antigenic relationship among homologous structural polypeptides of porcine, feline, and canine coronavirus. Infect Immun 37:1148-1155, 1982.

4. Pedersen NC, Ward J, Mengeling WL: Antigenic relationship of the feline infectious peritonitis virus to coronavirus of other species. Arch Virol 58:45-53, 1978.

5. O'Reilly KJ, Fishman LM, Hitchcock LM: Short communication: Feline infectious peritonitis; isolation of a coronavirus. Vet Rec 104:348, 1979.

6. Black JW: Recovery and in-vitro cultivation of a coronavirus from laboratory-induced cases of feline infectious peritonitis (FIP). VM SAC 75:811-814, 1980.

7. Evermann JF, Baumgartner L, Ott RL, et al: Characteristics of a feline infectious peritonitis virus isolate. Vet Path 18:256-265, 1981.

8. McKeirnan AJ, Evermann JF, Hargis A, et al: Isolation of feline coronaviruses from two cats with diverse disease manifestations. Feline Pract 11(3):16-20, 1981.

9. Pedersen NC: unpublished observations, 1983.

10. Black JW: Unpublished observations, 1983.

11. Pedersen NC, Black JW: Attempted immunization of cats against feline infectious peritonitis using either avirulent live virus or sublethal amounts of virulent virus. Am J Vet Res 44:229-234, 1983.

12. Pedersen NC, Boyle JF: Immunologic phenomena in the effusive form of feline infectious peritionitis. Am J Vet Res 41:868-876, 1980.

13. Hayashi T, Watabe Y, Nakayama H, Fujiwara K: Enteritis due to feline infectious peritonitis virus. Jpn J Vet Sci 44:97-106, 1982.

14. Woods RD, Cheville NF, Gallagher JE: Lesions in the small intestine of newborn pigs inoculated with porcine, feline, and canine coronaviruses. Am J Vet Res 42:1163-1169, 1981.

15. Weiss RC, Scott FW: Pathogenesis of feline infectious peritonitis: Nature and development of viremia. Am J Vet Res 41:382-390, 1981a.

16. Holmberg CA, Gribble DH: Feline infectious peritonitis: Diagnostic gross and microscopic lesions. Feline Pract 3(4):11-14, 1973.

17. Pedersen NC: Feline infectious peritonitis: Something old, something new. Feline Pract 6:42-51, 1976.

18. Montali RJ, Strandberg JD: Extraperitoneal lesions in feline infectious peritonitis. Vet Pathol 9:109-121, 1972.

19. Feldman BM, Jortner BS: Clinicopathologic conference: Feline systemic proliferative and exudative vasculitis. J Am Vet Med Assoc 144:1409-1420, 1964.

20. Hayashi T, Goto N, Takahashi R, Fujiwara K: Systemic vascular lesions in feline infectious peritonitis. Jpn J Vet Sci 39:365-377, 1977.

21. Ward JM, Munn RJ, Gribble DH, Dungworth DL: An observation of feline infectious peritonitis. Vet Rec 83:416-417, 1968.

22. Weiss RC, Dodd WJ, Scott FW: Disseminated intravascular coagulation in experimentally induced feline infectious peritonitis. Am J Vet Res 41:663-671, 1980.

23. Weiss RC, Scott FW: Antibody-mediated enhancement of disease in feline infectious peritonitis: Comparison with dengue hemorrhagic fever. Comp Immunol Microbiol Infect Dis 4:175-189, 1981b.

24. Weiss RC, Scott FW: Pathogenesis of Feline Infectious Peritonitis: Pathologic changes and immunofluorescence. Am J Vet Res 42:2036-2048, 1981c.

25. Jacobse-Geels HEL, Daha MR, Horzinek MC: Isolation and characterization of feline C3 and evidence for the immune complex pathogenesis of feline infectious peritonitis. J Immunol 125:1606-1610, 1980.

26. Jacobse-Geels HEL, Daha MR, Horzinek MC: Antibody, immune complexes, and complement activity. Fluctuations in kittens with experimentally induced feline infectious peritonitis. Am J Vet Res 43:666-670, 1982.

27. Horzinek MC, Osterhaus ADME: The virology and pathogenesis of feline infectious peritonitis. Brief review. Arch Virol 59:1-15, 1979.

28. Toma B, Duret C, Chappuis G, Pellerin B: Echec de L'immunisation contre la peritonite infectieuse feline par infection de virus de la gastro-enterite transmissible du porc. Rec Med Vet 155:799-803, 1979.

29. Woods RD, Pedersen NC: Cross-protection studies between feline infectious peritonitis and porcine transmissible gastroenteritis viruses. Vet Microbiol 4:11-16, 1979.

30. Dea S, Roy RS, Elazhary MASY: Coronavirus-like particles in the feces of a cat with diarrhea. Can Vet J 23:153-155, 1982.

31. Hoshino Y, Scott FW: Coronavirus-like particles in the feces of normal cats. Arch Virol 63:147-152, 1980.

Support for this work was obtained from the Robert H. Winn Foundation for Cat Research of the Cat Fancier Association, Red Bank, NH, Save our Cats and Kittens (SOCK) Corp., Walnut Creek, CA, and the Ralston Purina Co., St. Louis, Mo.