

Coronavirus-Like Particles in the Feces of Normal Cats*

Brief Report

By

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With 2 Figures

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Summary

Coronavirus-like particles, morphologically indistinguishable from coronavirus-like particles seen in human, canine, and simian feces, were detected by electron microscopy in the feces from both feline infectious peritonitis antibody-positive and antibody-negative cats.

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Coronaviruses have been found in a large number of mammals and birds, including mouse, rat, cat, dog, pig, cattle, human, chicken, and turkey (21). Furthermore, coronavirus-like particles have been isolated from horse (1), monkey (2), rabbit (7), pet birds (K. HIRAI, personal communication), fish (K. E. WOLF, personal communication), and ticks (20).

Feline infectious peritonitis (FIP) which is caused by a virus of Coronaviridae family, is one of the most important contagious diseases affecting domestic and wild Felidae today (4, 9, 10, 15, 19, 23). During studies on the pathogenesis and transmission of FIP virus via the oral route, fecal samples of clinically normal cats were screened for coronaviruses. Here we report the findings of coronavirus-like particles in the feces of these normal cats.

Fecal samples were collected from cats which were obtained from two cat-teries maintained as minimal disease cat (MDC) breeding colonies. The cats were housed in standard Horsfall isolation cages. A 10 percent to 20 percent suspension of each fecal sample was made using Eagle's minimum essential medium with 200 units Penicillin/ml, 200 μ g Streptomycin/ml, 100 μ g Gentamicin/ml, and no serum. After centrifugation at 3,000 rpm (1,200 \times *g*) for 10 minutes, one to 4 drops

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of this clarified fecal suspension were mixed with 20 drops of distilled water, one drop of 0.1 percent bovine serum albumin, and three to 4 drops of 4 percent phosphotungstic acid (PTA) adjusted to pH 7.0. This mixture was applied to a carbon parlodion-coated 200 mesh copper grid with a nebulizer, and examined in a Philips 201 electron microscope at 80 kV. Samples that were negative for virus particles by this method were re-examined by the following ultracentrifugation method. The 10 percent to 20 percent fecal suspension was clarified at 6,000 rpm ($3,000 \times g$) for 30 minutes, the supernatant fluid was removed and ultracentrifuged at 35,000 rpm ($80,000 \times g$) for one hour. The pellet was resuspended in one to 2 drops of distilled water, negatively stained with 2 percent PTA pH 7.0, and examined by electron microscopy (EM).

Table 1. Comparison of serum FIP titers and the presence of coronavirus-like particle in cats' feces examined by electron microscopy (EM)

Cat No.	MDC ^a colony	Age	Serum FIP titer ^b	Fecal EM	
				Initial test	Reexamination (days later)
1	B	14 weeks	<1:5	Pos	Pos (20)
2	B	14 weeks	1:25	Pos	Pos (20)
3	B	14 weeks	1:25	Pos	Pos (20)
4	B	14 weeks	1:25	Pos	Pos (20)
5	B	14 weeks	1:100	Pos	Pos (20)
6	B	14 weeks	<1:5	Pos	Pos (20)
7	A	Adult	<1:5	Neg	Neg (34)
8	A	Adult	<1:5	Neg	Neg (34)
9	A	Adult	1:100	Neg	Neg (34)
10	A	Adult	1:100	Neg	Neg (34)
11	B	Adult	1:100	Pos	Pos (34)
12	B	Adult	1:400	Neg	Neg (34)
13	B	Adult	1:1600	Pos	Pos (34)
14	B	Adult	<1:5	Neg	Neg (34)
15	B	Adult	1:400	Pos	Pos (34)
16	B	Adult	1:400	Pos	Pos (34)

^a MDC = Minimal disease cat

^b Assay by indirect immunofluorescent test using TGE virus as antigen

Indirect immunofluorescent test (IIFT) was performed for measuring serum antibody titers for FIP virus, using a canine neurofibrosarcoma cell line infected with transmissible gastroenteritis (TGE) virus, Shizuoka strain, as antigen. Table 1 summarizes the results of EM and IIFT.

Marked pleomorphism occurred in the virus-like particles (Fig. 1). Roughly they can be grouped into four morphological types: 1. small (90 to 100 nm inclusive of projections) spherical particles (Fig. 2A), 2. small elongated (90 to 100×210 to 290 nm) particles (Fig. 2B), 3. medium to large (150 to 300 nm) spherical and ellipsoidal particles (Fig. 2C and 2D), and 4. pleomorphic particles (Fig. 2E and 2F). The surface projections which measured approximately 25 nm

in length were made up of spherical or teardrop-like knobs attached to the particles with thin stalks. Sometimes these thin stalks appeared to protrude outwardly beyond the knobs (Fig. 2D). In spite of a considerable variation in shape and size of the particles, lengths of the surface projections were uniform. This consistency in the length of surface projections has also been reported with avian infectious bronchitis virus (IBV), mouse hepatitis virus (MHV), and human coronavirus strain 229E (6). Morphologically this feline enteric coronavirus-like particle is indistinguishable from enteric coronavirus-like particles of humans (3, 13, 14), dogs (17), and monkeys (2). They are different in the surface projection morphology from FIP virus (8, 11, 12), human respiratory coronavirus, canine coronavirus, TGE virus of pigs, calf diarrhea coronavirus, IBV, MHV, rat coronavirus, turkey enteric coronavirus, hemagglutinating encephalomyelitis virus of pigs, and the third enteric porcine coronavirus designated CV777 (16). CAUL and EGGLESTONE (2) speculate that "there may therefore be two subgroups of coronavirus distinguishable by their projection morphology — one group possessing the classical petal-shaped projections and the other possessing projections consisting of spherical or teardrop-like knobs attached to the particle by a thin stalk". A third type of surface projection, fairly large spherical knob, has been reported for Lunde virus, a coronavirus-like agent isolated in Norway (20) associated with seabirds and the tick *Ixodes uriae*. A filiform projection without distal ends form the fourth type of projection which has been reported for FIPV (8), TGE virus (22), and porcine epidemic diarrhea type II virus (5).

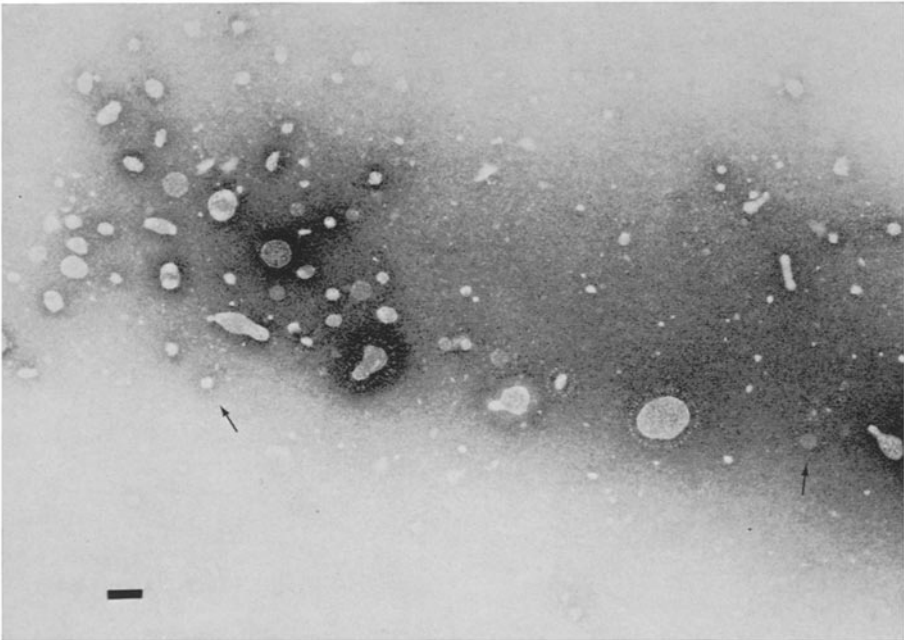


Fig. 1. Electron micrograph of negatively stained coronavirus-like particles in a typical field seen in the feline fecal sample. Small, medium, large, and pleomorphic particles are seen. Some particles appear to have lost several surface projections (arrows). Bar represents 100 nm

Table 1 shows that coronavirus-like particles were visualized in feces from both FIP antibody-positive and antibody-negative cats, although most FIP antibody-negative cats were also free from these particles. Morphologically this coronavirus-like particle appears to be different from FIP virus. It may either be

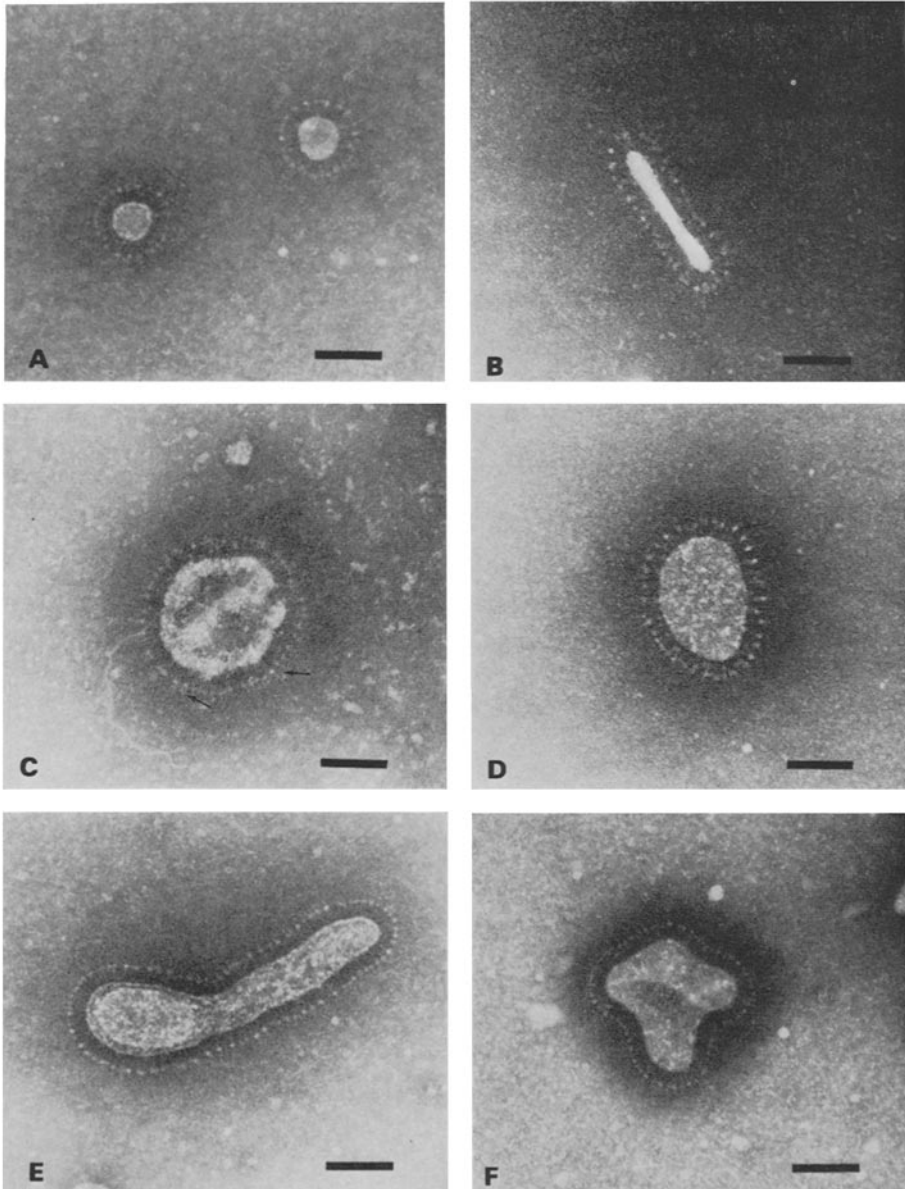


Fig. 2. Electron micrographs of negatively stained coronavirus-like particles in fecal samples. Bar represents 100 nm. *A* Small spherical particles. *B* Small elongated particle. *C* Large spherical particle. Surface projections appear to protrude outwardly beyond the knobs (arrows). *D* Large ellipsoidal particle. Typical teardrop-like surface projections are seen (arrow). *E* and *F* Pleomorphic particles

a variant of FIP virus or a second feline coronavirus. Possibility exists that this coronavirus-like particle may be connected with one or more manifestations of a new disease complex called kitten mortality complex (KMC) (18), which causes unusually high incidences of reproductive failure and death of young kittens in many catteries throughout the United States. Both MDC breeding colonies from which we obtained experimental cats have suffered from KMC.

Follow up fecal samples were still positive over a period of one month (Table 1) which suggests persistent excretion of these coronavirus-like particles.

As this is the first report of enteric coronavirus-like particles, it is unknown how widespread they are in cat populations. It is of interest from the point of view of natural history and the ecology of viruses that morphologically indistinguishable enteric coronavirus-like particles are found in cats, dogs, monkeys, and humans.

A pilot study at Cornell Feline Research Laboratory demonstrated the propagation of this enteric coronavirus-like particles in small intestinal organ cultures of the cat. Further studies on possible relationships between feline enteric coronavirus-like particles and FIP virus, transmission, and pathogenesis of this enteric coronavirus-like particles are in progress in our laboratory.

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