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Feline Infectious Peritonitis Virus

By

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With 6 figures and 3 tables

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Introduction

Feline infectious peritonitis (FIP) was described as a separate disease entity only ten years ago (9); it is a variably progressive, usually fatal condition affecting domestic and wild Felidae, characterized by fever, anorexia, depression and ascites. Prominent pathologic changes are diffuse fibrinous peritonitis, mesothelial hyperplasia and focal necrosis in parenchymal organs (for literature see 5, 8). The infectious nature of the disease was indicated by epidemiologic observations; evidence for its viral etiology came from transmission experiments with filtrates passing through 200 nm-pore membranes (10). In thin-sections through histiocytes, macrophages and mesothelial cells from pathologic lesions virus particles were observed electron microscopically (7, 8); their etiologic rôle was established recently by animal inoculation experiments of virus grown in cat peritoneal cell cultures (4). The present study was undertaken to show by density gradient analysis and negative staining electron microscopy that the virus found in FIP-diseased cats meets most of the criteria used for classification of coronaviruses and that the disease can be reproduced using purified suspensions of the virus.

Materials and Methods

Virus

The passage history of the FIP virus strain (DAHLBERG) used in this study is given in Table I. Ascites fluid from a field case in the Netherlands which had been diagnosed on the basis of clinical findings and post mortem examina-

tion was used for infection into the abdominal cavity of random-bred short-haired cats. After death or euthanasia in a moribund state the animals were dissected and the diagnosis confirmed by macroscopic and histologic examination. — Infectious avian bronchitis virus (strain Beaudette, passage 222) was propagated in the allantoic cavity of 10 days embryonated eggs.

Tabelle 1

History of the FIP virus strain (Dahlberg) used in the present study

Passage level	Passage material	Survival time (days) of experimentally infected cats[a]	Coronavirus in electron microscope
(field case)		not known	n. d.[b]
	ascites fluid		
1		11, 23	n. d.
	ascites fluid		
2		20, 25	present
	ascites fluid, liver homogenate		
3		5	present
	liver homogenate		
4		10	present
	liver homogenate		
5		16, 17, 19, 20, 27	present

a Undiluted ascites fluids or 20 % (w/v) liver suspensions supplemented with 200 I. U. of penicillin and 200 μg. of streptomycin per ml. were used for infections by intraperitoneal inoculation; on each passage level the diagnosis of FIP has been confirmed by post mortem examination

b Not done

Purification

The detailed virus purification procedure has been published recently (3); in short, it consisted of centrifugation of a clarified ascites fluid or liver homogenate through a 25 % sucrose solution onto a 55 % cushion, precipitation of the interphase material by adding ammonium sulphate to reach 40 % saturation and separation of the resuspended material on an isokinetic sucrose gradient (11); ^{3}H-uridine-labelled Semliki forest virus served as an internal sedimentation marker (270 S). Fractions corresponding to sedimentation values between 380 and 420 S were pooled and further analyzed by isopyknic centrifugation on linear 20 % to 50 % sucrose gradients. The virus contents were estimated by electron microscopic examination.

Electron microscopy

10 μl. volumes of gradient fractions were pipetted onto carbon-coated copper grids and left for 10 min. After several rinses in distilled water the preparations were stained with a 2 % solution of potassium phosphotungstate (pH 6.0) for 10 seconds. Examination was performed at an instrumental magnification of 50,000 fold using a JEOL JEM-100 C electron microscope. Virus was quantitated by negative staining after mixing a polystyrene latex sespension (dimeter 90 nm.) of known particle concentration with equal volumes of virus preparations and determination of the particle to virion ratio (2); for each sample about 1,000 latex spherules and the corresponding number of virus particles were counted.

Results

Starting material for virus purification experiments consisted of ascites fluid and/or liver material from FIP field cases confirmed by post mortem

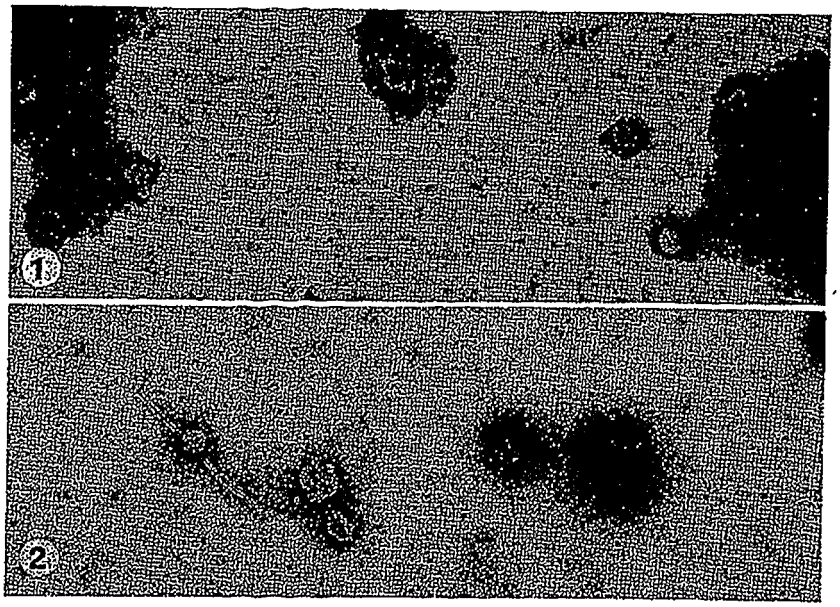

Fig. 1. Electron microscopic appearance of sucrose gradient 1.17 g./ml.-material of a liver preparation from a cat which had died from FIP; note size heterogeneity and pleomorphism of virions

Fig. 2. Latex particles (diameter 90 nm.) included as a concentration and size standard. × 50,000

examination on one hand and of liver homogenates from experimentally infected animals at different passage levels (Table 1) on the other hand. Particles of a characteristic morphology (Figs. 3—5) were regularly detected by electron microscopy, when fractions of the 400 S-region of the isokinetic sucrose gradient (3, 11) were centrifuged to isodensity at 1.17—1.18 g./ml. (Fig. 1). Using the latex quantitation technique (Fig. 2) virus particle concentrations approaching 5×10^9 (corresponding to 186 virions per 1,000 latex spherules) were estimated at this density whereas significantly fewer and/or less well-preserved structures were encountered in the adjacent fractions. Coded preparations of liver material from four normal, clinically healthy cats were examined as a control; no structures resembling the particles described were detected.

Table 2

Results of electron microscopic examination and animal inoculation (4th in vivo passage) using selected fractions of isopyknic sucrose gradients

Density (g / ml)	Coronavirions in electron microscope	Results of kitten inoculation	Coronavirions in liver preparation of experimental kittens
≤ 1.11	none observed	survival	n. d.[a]
1.15	none observed	survival	n. d.
1.16	none observed	survival	none observed
1.17 - 1.18	present	death	present
1.18	present, damaged	death	present
≥ 1.19	none observed	n. d.	n. d.

a Not done

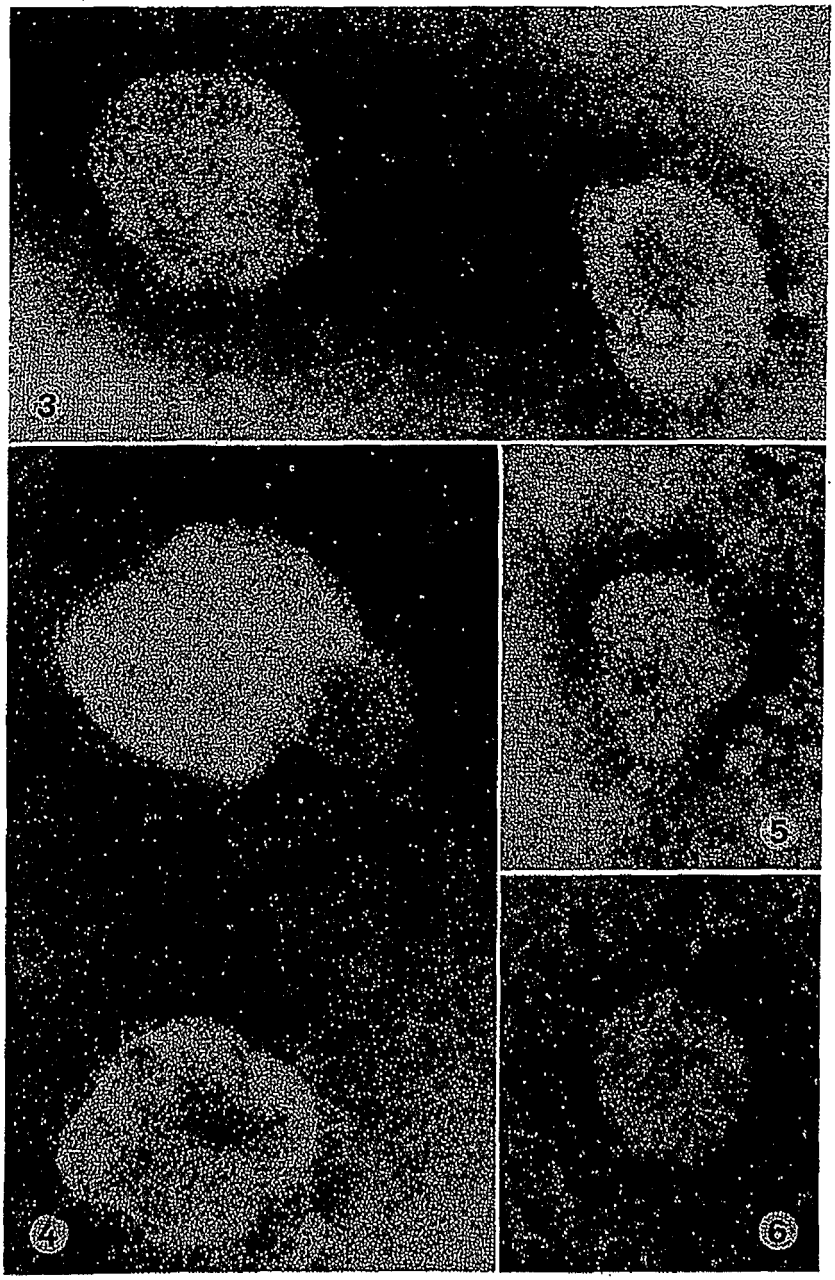

Fig. 3. FIP virus, particles purified from cat liver; note "pits" in one virion (right)
Fig. 4. Damaged FIP virions showing stain penetration (below) and "bleb" (above); note absence of projections on the protrusion
Fig. 5. Virion from ascites fluid of a FIP field case after fixation with 0.2 % formaldehyde
Fig. 6. Avian infectious bronchitis virion included for comparison of particle morphology
× 250,000

In order to demonstrate the etiological rôle of the observed particles for FIP, a litter of 8-weeks-old kittens was infected by intraperitoneal inoculation with gradient fractions having densities between 1.11 and 1.18 g./ml.; as summarized in Table 2, fatal infection was restricted to materials banding at 1.17 to 1.18 g./ml. This result could be reproduced in a second, independent experiment.

The detailed morphology of FIP virus is shown in Figs. 3—5. The overall size of the roughly spherical, sometimes rather pleomorphic particles ranged from 90 to 160 nm. with an average of 125 nm. (n = 76). The virion surface was covered with regularly arranged projections 12 to 15 nm. in length. In our preparations these were filiform rather than bulbous, which was also true for avian infectious bronchitis virus serving as a morphological coronavirus reference in these experiments (Fig. 6). Only about 15 % of the particles in the 1.17—1.18 g./ml.-fraction excluded negative stain from their interior; virions partially penetrated by phosphotungstate (40 %) showed irregular patterns of higher electron density (Figs. 3, 4) and the presence of membranous envelopes; in some of these, 6 nm.-pits resembling those after antibody-complement interaction (10) are visible. "Bleb"-artifacts devoid of surface projections can be discerned in some particles (Fig. 4). The fragility of the virions is further illustrated by the observation that about 45 % of the particles were encountered in different degrees of disruption. Ring-like structures measuring about 11 nm. in diameter were frequently present, especially in samples from liver homogenates (Figs. 3, 4); they probably represent ferritin molecules.

Discussion

The experimental evidence for regarding the coronavirus-like agent described earlier (3) as the causative virus of FIP is based mainly on our observation that its appearance and frequency in latex-standardized electron micrographs correlated with the results of infectivity tests in kittens. Although no virus quantitation was performed by biologic assay it can be stated that fatal infectivity was limited to gradient fractions with densities between 1.17 and 1.18 g./ml. (Table 2). In organ materials of kittens which had succumbed to the infection the typical virions were visualized whereas no such particles could be detected in preparations of an animal which had been inoculated with an

Table 3

Properties of FIP virus as compared with those of established coronaviruses

Virion properties	FIP virus	References	Coronaviruses (reference 6)
Size (negative staining)	90 - 160 nm, round, non rigid	present report	60 - 220 nm, round, non rigid
Surface projections	12 - 15 nm long, bulbous and filiform	present report, 3	12 - 24 nm long, bulbous
Substructure (thin section)	doughnut-shaped nucleoid (50 - 55 nm), enveloped by unit membrane	4, 7	inner (55 nm) and outer shells, sometimes separated by electron lucent space
Buoyant density (sucrose)	1.17 - 1.18 g / ml	present report	1.16 - 1.23 g / ml
Sedimentation coefficient	about 400 S	present report, 3	374 - 416 S
Intracellular localization	cytoplasm budding into smooth endoplasmic reticulum; no budding at plasma membrane	4, 7, 10	cytoplasm budding into endoplasmic reticulum, no budding at plasma membrane

adjacent gradient fraction. In organ homogenates from field cases of FIP these structures have been found whereas all attempts to prepare similar particles from apparently healthy cats have proved unsuccessful.

The typical morphology of the virion in negatively stained suspensions, its buoyant density and sedimentation coefficient are in agreement with the observation of other authors that the particles encountered in thin sections through pathological FIP specimens resemble coronaviruses (4, 7, 8); a comparative listing of FIP virus and Coronaviridae properties is given in Table 3.

Although information on the virion is still incomplete — which is true for most other established coronaviruses — we propose that FIP virus should be tentatively classified as a Coronaviridae family member; serologic studies to determine possible antigenic relationships with other family members are in progress.

Summary

From ascitic fluids and liver homogenates of natural and experimentally induced cases of feline infectious peritonitis (FIP) virus particles have been purified by ammonium sulphate precipitation and sucrose gradient centrifugation; they appear as coronavirus-like on the basis of their morphology (round, non-rigid, about 100 nm. in diameter, surface projections), sedimentation behaviour (about 400 S) and buoyant density in sucrose (between 1.17 and 1.18 g./ml.). Using gradient-purified virus material the disease could be reproduced in experimental kittens and the virus recovered from them whereas it was absent from surviving animals. Based on these results it is proposed to classifiy FIP virus as a new member of the Coronaviridae family.

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Zusammenfassung

Virus der infektiösen felinen Peritonitis
I. Reinigung und Elektronenmikroskopie

Aus Ascitesflüssigkeiten und Leberhomogenaten von natürlichen und experimentell induzierten Fällen von Feliner Infektiöser Peritonitis wurden mittels Ammoniumsulfatpräzipitation und Rohrzucker-Dichtegradientenzentrifugierung Viruspartikeln gereinigt. Sie ähneln Coronaviren hinsichtlich ihrer Morphologie (sphärisch, verformbar, etwa 100 nm Durchmesser, Oberflächenprojektionen), ihres Sedimentierungsverhaltens (etwa 400 S) und ihrer Schwebedichte in Rohrzucker (zwischen 1,17 und 1,18 g/ml). Mit gereinigtem Material aus Gradienten konnte die Erkrankung in Versuchskatzen reproduziert und das Virus in diesen wiederum demonstriert werden, während es in den überlebenden Tieren nicht nachweisbar war. Auf Grund dieser Ergebnisse wird vorgeschlagen, das FIP-Virus als ein neues Mitglied der Familie Coronaviridae zu klassifizieren.

Résumé

Virus de la péritonite infectieuse du chat
I. Purification et microscopie électronique

Des liquides ascitiques et des homogénats de foie de cas péritonites infectueuses félines, enduits de façon naturelle et expérimentale, on a purifié des particules de virus au moyen de précipitation sulfat d'ammonium et de centrifugation en gradients de sucrose; ils resemblent les coronavirus vue leur morphologie (sphérique, non rigide, environ 100 nm de diamètre, projections de surface), leur coefficient de sédimentation (environ 400 S) et leur densité de flottaison en sucrose (entre 1,17 et 1,18 g/ml). Utilisant du matériel viral purifié par gradients de densité, on a reproduit la maladie chez les chats expérimentaux et démontré le même virus dans ces animaux, tandis qu'il était absent chez les animaux survivants. Vue les résultats mentionnés ci-dessus il est proposé de classifier le virus FIP parmi la famille des Coronaviridae.

Resumen

Virus de la peritonitis infecciosa del gato
I. Purificación y microscopía electrónica

De los líquidos ascíticos y homogenados de hígado de casos naturales e inducidos por vía experimental de peritonitis felina infecciosa, se purificaron partículas virales mediante precipitación con sulfato amónico y centrifugación con gradientes de sucrosa. Las mismas se asemejan a los virus Corona en cuanto a su morfología (esféricas, deformables, de un diámetro de unos 100 nm, con proyecciones en la superficie), su coeficiente de sedimentación (alrededor de 400 S) y su densidad de flotación en sucrosa (entre 1,17 y 1,18 g/ml). Partiendo de un material purificado en los gradientes, se logró reproducir la enfermedad en gators de experimentación, recuperándose el virus en estos animales, mientras que no se pudo evidenciar en aquellos que sobrevivieron. A la vista de los resultados mencionados, se propone clasificar el virus de la peritonitis felina infecciosa (FIP) como un miembro nuevo de la familia de los Coronaviridae.

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