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Diagnostic Features of Clinical Neurologic Feline Infectious Peritonitis

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Feline infectious peritonitis (FIP) is a fatal Arthus-type immune response of cats to infection with FIP virus, a mutant of the ubiquitous feline enteric coronavirus (FECV). The disease may occur systemically or in any single organ system, and primary neurologic disease is a common subset of such manifestations. We examined 16 domestic cats with clinical neurologic FIP and 8 control cats with nonneurologic FIP, with the intention of identifying the ante- and postmortem diagnostic tests that most contribute to accurate diagnosis. Of the 16 cats with neurologic FIP, 15 were less than 2 years of age and all 16 originated from large multiple-cat households. The most useful antemortem indicators of disease were positive anti-coronavirus IgG titer in cerebrospinal fluid, high serum total protein concentration, and findings on magnetic resonance imaging suggesting periventricular contrast enhancement, ventricular dilatation, and hydrocephalus. Postmortem diagnosis was facilitated by FIP monoclonal antibody staining of affected tissue and coronavirus-specific polymerase chain reaction. Most cats with neurologic and ocular forms of FIP had patchy, focal lesions, suggesting that recently developed technologies described in this report may be useful for evaluation of cats with suspected FIP.

Key words: Magnetic resonance imaging; Polymerase chain reaction.

Feline infectious peritonitis (FIP) is a common and fatal infectious disease of cats, occurring most frequently in cats younger than 3 years of age in multiple-cat homes.[1] The causative agent, FIP virus (FIPV), is a macrophage-tropic mutant of the ubiquitous feline enteric coronavirus (FECV).[2–4] Clinical FIP is the result of immune-mediated host responses to macrophages infected with FIPV, and the severity of FIP appears to be determined primarily by host susceptibility and host-specific, often heritable,[5] immune responses and secondarily by virus strain. Viral strains vary in virulence, and the severity of lesions in cats inoculated with the same strain range from fatal to asymptomatic.[6]

The classic effusive form of FIP was first identified in the 1950s and is characterized by a high protein, cellular abdominal or thoracic effusion and diffuse peritonitis and pleuritis.[7] Noneffusive or "dry" FIP is typified by focal granulomatous lesions on serosal, pleural, meningeal, ependymal, or uveal membranes, extending to underlying parenchyma along blood vessels. Previous reports[8] and a review of records at U.C. Davis Veterinary Medical Teaching Hospital (VMTH) indicate that neurologic clinical manifestations occur in about one quarter to one third of cats with dry FIP (Foley and Pedersen, unpublished data). Despite previous reports that most cats with neurologic FIP also have abdominal lesions,[9] confirming an antemortem diagnosis of neurologic FIP can be very difficult. Serologic titers do not discriminate between FIPV and FECV infections because the viruses are antigenically indistinguishable. Similarly, polymerase chain reaction (PCR) on serum and feces cannot discriminate between benign FECV and fatal FIPV because mutations in several different sites can result in the transformation of FECV to FIPV.[2–4] To date, the diagnosis of FIP has involved a multifactorial approach of ranking risk and clinical factors such as age, housing history, total protein, and serum titer and eliminating other etiologic possibilities.[10]

Recently, new diagnostic methods have been developed, such as PCR for the detection of coronavirus RNA in tissues and fluids, immunoperoxidase staining for coronavirus antigen in paraffin-embedded tissues, and magnetic resonance imaging of the brain. We used a multifactorial approach (PCR, serum titers, clinical examination, magnetic resonance imaging, postmortem examination results, and immunohistochemical staining) to the diagnosis of neurologic FIP, both ante- and postmortem.

Methods and Materials

Cats

Twenty-four cats with a tentative diagnosis of FIP and 3 with neurologic presentations not associated with FIP were used in this study, including 16 cats with clinical neurologic lesions associated with FIP and 8 cats with FIP but no clinical neurologic disease (2 cats with naturally occurring effusive FIP, 1 cat with naturally occurring noneffusive FIP, and 5 specific-pathogen-free [SPF] cats with experimentally induced effusive FIP). All cats with naturally occurring FIP were obtained from cat breeders, the Society for the Prevention of Cruelty to Animals, and referring veterinarians and were raised and housed in breeder or owner facilities. The five 5-month-old SPF cats for experimental induction of FIP were bred and housed at the U.C. Davis Veterinary Retrovirology Laboratory under barrier conditions. The 3 neurologic control cats were patients at the U.C. Davis VMTH. Cat 1 was a 6-month old random-bred female stray cat with ataxia, hypermetria, and hyperesthesia, and a clinical diagnosis of congenital cerebellar hypoplasia. Cat 2 was a 4-year old intact male domestic longhair with presenting clinical signs of ataxia progressing to paralysis of the pelvic limbs. The cat was antigen positive for feline leukemia virus (FeLV) in serum and was diagnosed with spinal lymphoma on postmortem examination. Cat 3 was a 2-year old intact male domestic shorthair with a presenting complaint of seizures. Necropsy revealed infiltrative lymphoma throughout the cerebrum, cerebellum, and cranial nerves II and III. All other cats in the study were serologically negative for FeLV and feline immunodeficiency virus.

Clinical Examination

Upon presentation, cats received complete physical and neurologic examinations. Complete blood counts were performed by referring vet-

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erinarians for 6/16 cats and for all 3 non-FIP controls. Blood was obtained by jugular venipuncture, allowed to clot, and centrifuged for serum protein determination (by Shuco refractometer) and coronavirus serology. Cats were anesthetized with 10 mg/kg ketamine and 0.5 mg/kg diazepam IV for cerebrospinal fluids (CSF) sampling. The CSF was obtained by sterile puncture of the cerebellomedullary cistern. Anti-feline coronavirus IgG in serum and CSF fluid was determined with an indirect immunofluorescent antibody (IFA) test using FIPV-UCD1-infected *Felis catus* whole fetal (Fcwf)-4 cells as a substrate.[11] Sera and CSF were tested in serial dilutions of phosphate-buffered saline from 1:25 to 1:6,400.

Inoculation

Five SPF cats received intraperitoneal inoculation of 1–2 mL of crude centrifuged ascitic fluid containing FIPV-UCD8 passaged in SPF cats. This strain of FIP has been reported previously to produce effusive FIP when experimentally inoculated at high dose.[12] Cats were anesthetized with 10 mg/kg ketamine and 0.5 mg/kg diazepam IV immediately before euthanasia on days 10–20 postinoculation, when terminally ill with effusive FIP. CSF was obtained by sterile puncture of the cerebellomedullary cistern, and cats then were euthanized with an IV overdose of barbiturates.

Diagnostic Imaging

Magnetic resonance imaging (MRI) was performed on 4 cats using a 0.35 tesla whole body scanner (Toshiba America MRI Inc, South San Francisco, CA) fitted with a knee coil. Cats were anesthetized with 10 mg/kg ketamine and 0.5 mg/kg diazepam IV, maintained on ketamine to effect, and restrained in sternal recumbency. Each study consisted of a series of transverse slices of the entire brain. Slice thickness was 4 mm with a 1 mm interslice gap. Matrix size was 256 × 256 with a 16-cm field of view yielding a resolution of 0.625 mm/pixel. Two excitations were acquired per image. Spin-echo pulse sequences were used for all imaging studies. Proton density weighted images (pulse repetition time [TR] = 2,100 ms, echo time [TE] = 35 ms), T_2 weighted images (TR = 2,100 ms, TE = 85 ms) and T_1 weighted images (TR = 550 ms, TE = 35 ms) were obtained. The T_1 sequence was repeated after IV administration of 0.1 mmol/kg gadopentetate dimeglumine (Magnevist, Berlex Laboratories, Cedar Knolls, NJ). Because of equipment failure, T_1 images were not obtained in 1 cat.

Pathology and Case Definitions

The antemortem diagnosis of FIP was based on objective clinical criteria,[10,13] including a mucinous, yellow-tinged inflammatory exudate from the abdominal or pleural cavities containing many macrophages and neutrophils, bilirubinuria or bilirubinemia, increased serum protein concentration (>8.0 g/dL); characteristic CBC changes including leukocytosis with neutrophilia and lymphopenia, antibiotic unresponsive or cycling fever; positive CSF FIPV IgG titer (≥1:25), icterus, palpable abdominal masses (especially associated with kidneys, liver, spleen, and mesenteric lymph nodes), neurologic abnormalities, and anterior uveitis (especially with keratic precipitates). Cats exhibiting neurologic or ophthalmologic lesions were considered suspect for FIP. The diagnosis of FIP was confirmed on postmortem examination by the presence of characteristic gross and histologic lesions and by the presence of viral RNA within lesions by reverse-transcriptase (RT) PCR.

After euthanasia, fresh tissues were collected during gross postmortem examination, fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E) for histopathologic examination. Representative lesions, ascites, CSF, urine, and fecal samples were stored at −20°C for PCR. Brains were collected with attached meninges and divided into equal cerebral spheres using sterile technique. One half was stored in formalin for histopathology, and the other half was frozen for PCR. Histopathologic examination was performed on all brains to characterize lesions and confirm FIP. Histopathologic examination of brains for which MRI was available also consisted of immunohistochemical staining and spatial mapping of lesions.

Brains from the 4 cats scanned by MRI were fixed in 10% buffered formalin for approximately 10 weeks, sliced into 7–10 transverse sections, embedded in paraffin, cut at 5 (m, and stained with H&E. A representative section of paraffin-embedded brain from each cat also was stained for FIPV using a mouse monoclonal anti-FIPV spike antibody (5.2D5, N. Pedersen, U.C. Davis, CA), with a streptavidin–biotin–horseradish peroxidase system,[14] and lightly counterstained with hematoxylin. Suitable negative and positive tissue controls were included.

Molecular Techniques

RNA was purified from feces, urine, and CSF using a modified Boom method of rapid silica extraction.[15] Brain tissue, visceral granulomas from organs, and ascites were purified using the Trizol reagent protocol (GIBCO BRL, Gaithersburg, MD). Synthesis of complementary DNA was performed using murine Moloney leukemia virus reverse transcriptase (GIBCO), as described by Foley et al.[16] Specific primers c202 and 177, which flank a 355 bp fragment from the relatively conserved (ie, FECV and FIPV) 5′ region of the 7b gene of feline coronaviruses, were used for RT PCR . Amplification of the 7b PCR fragment was performed with 5 U/sample *Hsp* 92II (Promega, Madison, WI) at 37°C for 1 hour to cut contaminating double-stranded DNA before PCR, followed by routine PCR with the enzyme *Taq* DNA polymerase.[16] A coronavirus-positive sample was characterized by the presence of 355-bp bands by gel electrophoreses on a 1% agarose gel stained with ethidium bromide. If the source of RNA was a granulomatous lesion or ascites fluid, the coronavirus was then defined as FIPV.

Data Maintenance and Analysis

Data were maintained in Excel 5.0 (Microsoft Corp, Redmond, WA) and analyzed with S-Plus for Windows (StatSci, Seattle, WA) and Epi-Info 6.02 (Centers for Disease Control, Atlanta, GA). The correlation of CSF titer with serum titer was performed using Spearman's ranked (nonparametric) procedure, with the alternative hypothesis that serum titer was higher than CSF titer. A standard Pearson's correlation coefficient was obtained for compared linear values, and the one-sided null hypotheses was rejected at $\alpha = .05$.

Results

Control Cats

Of the 3 control cats with neurologic disease not due to FIP, 1 (with congenital cerebellar hypoplasia) had a serum protein concentration of 6.2 g/dL and no detectable serum anti-FIPV antibodies. No antibodies to FIPV were detected in the CSF, the total protein in CSF was 20 (μg/dL, and the CSF was PCR negative. The cat with spinal lymphoma associated with FeLV had a serum protein concentration of 7.5 g/dL and a serum anti-FIPV IgG of 1:100. The CSF had a total protein of 45 (μg/dL and no CSF antibodies detected. Results of PCR on brain and CSF were negative. The serum protein concentration of the cat with infiltrative non-FeLV-associated lymphoma was 6.2 g/dL and serum anti-FIPV IgG was 1:400. The CSF had a total protein of 22 μg/dL and no detectable coronavirus antibodies. Brain and CSF were PCR negative.

All 5 experimentally infected cats developed clinica

signs of FIP within 14 days of inoculation, including fever, lethargy, and abdominal effusion. The diagnosis of FIP was confirmed by PCR on ascites fluid. The median serum titer at death was 1:25; none of the 5 cats had anti-FIPV antibodies in the CSF. The CSF was cytologically normal and PCR negative. No gross or histologic lesions suggestive of FIP were detected in brain tissue, and PCR on brain tissue was negative.

The naturally infected cats with effusive FIP were approximately 1 year old, with typical histories of weight loss, lethargy, fever, and secondary chronic upper respiratory infections. Both cats had high protein, yellow-tinged ascitic fluid; the ascites was PCR positive for both cats. The serum titers both were 1:1,600, CSF was seronegative, and CSF was PCR negative for both cats. The third naturally infected control cat had granulomatous lesions of FIP on the diaphragm and pericardium. She was a 3-year-old pregnant female, with an acute onset of inappetance and lethargy, a serum FIPV antibody titer of 1:400, serum total protein of 10.0 g/dL, and undetectable FIPV antibodies and DNA in CSF. Brain tissue was PCR negative for all 3 nonneurologic FIP cats.

Cats with Neurologic FIP

History, Signalment, and Physical Examination. Of 16 cats with chronic progressive neurologic or ocular disease associated with FIP, was a 3-year old intact male Havana Brown, 1 was an 8-year-old neutered male Birman, and the others were neutered male and female random-bred domestic shorthairs ranging in age from 4 months to 3 years. Fifteen cats were from multiple-cat households (catteries or shelter), and 1 cat (16-1) came from a private home with only 1 other cat but had been obtained from a shelter 1 year earlier. Problems included poor weight gain or weight loss, weakness, lethargy, ataxia, dementia, pica, and fever. Two cats had inappropriate elimination; 1 was incontinent (fecal and urinary). Compulsive licking (at concrete, carpeting, and other cats) was a presenting complaint in 3/16 cats.

Fourteen of 16 cats had central nervous system abnormalities, 5/16 had predominantly ocular manifestations of FIP, and 2 of these 5 had no detectable neurologic abnormalities. Neurologic abnormalities in 14 cats included hyperesthesia (physical and auditory) (7/14 cats), hyperreflexia (6/14), crossed-extensor reflex (1/14), reduced conscious proprioception on all 4 limbs (8/14), caudal paresis (1/14), dementia (6/14), and cerebellar-vestibular signs (5/14). Ocular lesions occurred in 5 cats: comprised anterior uveitis (5/5), keratic precipitates (3/5, predominantly unilateral in 2 cats), flocculent debris in the anterior chamber (1/5), retinitis (1/5), and anisocoria (1/5). The retinal lesions consisted of focal tapetal hyporeflectivity associated with granulomas. No abnormal nystagmus was observed in these 5 cats. Of the 2 cats with predominantly ocular manifestations of FIP and no other discernible neurologic deficits, 1 had mild anterior uveitis and keratic precipitates (bilaterally) and the other had unilateral keratic precipitates and anterior uveitis, and bilateral retinitis.

Abdominal abnormalities were palpated on 11/16 cats with neurologic FIP, including enlarged mesenteric lymph nodes (11/16) and irregular splenic and renal surfaces (5/15). Five of 15 cats had clinical signs of respiratory tract infections, including hyperemic conjunctivae, mucopurulent oculonasal discharge, and vesicular, proliferative faucitis. Other abnormal physical findings included generalized peripheral lymphadenopathy (1/15), low weight (13/16), and dehydration (13/16).

Clinical Pathology. CBCs were performed on 6 cats; results of 3 of these were within normal limits. Abnormal CBC findings in the remaining 3 cats were anemia (1 cat), leukocytosis (3), neutrophilia with left shift (1), neutrophilia without left shift (1), eosinophilia (1), lymphopenia (2), and lymphocytosis (1). One cat had numerous *Hemobartonella felis* organisms on the periphery of red blood cells. Serum protein concentrations ranged from 5.8 to 10.2 g/dL with a mean (±SD) of 8.6 (±1.2) g/dL (Table 1). Using an upper limit of normal of 8.0 g/dL, 13/16 cats had abnormally high serum total protein concentrations. Serum coronavirus titers of all 16 cats were positive, ranging from 1:100 to 1:3,200, with a median of 1:400. Analysis of CSF for proteins, cells, and antibodies showed increased cellularity (lymphocytes and neutrophils) in 2/16 cats and increased protein (>30 μg/dL) in 4/16. The mean CSF protein concentration was 97.3 (±2.7) g/dL. Fifteen of 16 cats had CSF titers ≥1:25, ranging from 1:25 to 1:1,600, with a median of 1:100. The correlation of CSF titer with serum titer was not statistically significant ($\rho = 0.277$, $P = .286$). The ratio of serum protein concentration to CSF protein concentration ranged from 97 to 510, with a mean of 316 (±136.41), whereas the ratio of serum titer to CSF titer ranged from 1 to 32, with a mean of 8.5 (±8.7). Serum:CSF titer was not correlated with serum:CSF protein ($t = -1.36$, $df = 14$, $P = .96$). Cat 6-109, which had bilateral ocular lesions and abdominal granulomas, had aqueous humor titers that were positive at 1:100 in the more severely affected eye and 0 in the other eye.

PCR. The presence of FIPV in tissue was confirmed by PCR in all 16 cats with neurologic or ocular FIP. Seven were PCR positive in feces (possibly FECV, not FIPV). The CSF was PCR positive for 5/16 (including the 2 with high CSF cellularity), and negative for 11/16. Brain tissue was PCR positive for 10/15 and negative for 5/15. Of the 4 cats that were scanned by MRI, 2 were PCR positive for brain and CSF and 2 were PCR negative for brain and CSF. The aqueous humor of cats with ocular lesions was PCR negative for all samples.

Magnetic Resonance Imaging. Ventricular dilatation was identified in 3 of the 4 cats examined. Areas of periventricular contrast enhancement were identified in each of the 3 cats in which contrast-enhanced studies were performed. The distribution of contrast enhancement varied and appeared to be related to the degree of ventricular dilatation. In cat 6-60, with the least ventricular dilatation, contrast enhancement was limited to the area around the 3rd ventricle. In cat 16-1, with moderate ventricular dilatation, contrast enhancement was present around the 3rd and 4th ventricles. In cat 6-49, with severe ventricular dilatation, contrast enhancement was present around the lateral, 3rd and 4th ventricles (Fig 1). The MRI findings were interpreted as being consistent with ependymitis and secondary obstructive hydrocephalus. Discrete parenchymal lesions were not identified in any of the cats.

Table 1. *Clinical and molecular findings on cats with neurologic FIP.*

Cat No.	Protein			Titer			PCR		
	Serum (g/dL)	CSF (μ/dL)	Serum : CSF	Serum	CSF	Serum : CSF	CSF	Brain	Fecal
6-34	8.8	20	440	1 : 1,600	0	∞	Neg.	Neg.	Neg.
6-38	9.6	30	320	1 : 100	1 : 25	4	Neg.	Neg.	N/A
6-42	9.2	36	255.6	1 : 3,200	1 : 25	12	Neg.	Neg.	Pos.
6-43	8.4	26	323	1 : 400	1 : 25	16	Neg.	Pos.	Pos.
6-44	10.2	20	510	1 : 100	1 : 25	4	Neg.	Neg.	Neg.
6-47	5.8	20	290	1 : 1,600	1 : 100	16	Neg.	Pos.	Neg.
6-48	9.0	30	300	1 : 400	1 : 100	4	Pos.	Pos.	Neg.
6-49	8.8	1,120	7.86	1 : 400	1 : 25	16	Pos.	Pos.	Pos.
6-51	10.2	30	340	1 : 3,200	1 : 100	32	Pos.	Pos.	N/A
6-60	9.7	20	485	1 : 400	1 : 400	1	Neg.	Neg.	Pos.
6-62	8.6	30	287	1 : 100	1 : 25	4	Neg.	N/A	Pos.
6-72	6.8	70	97	1 : 3,200	1 : 1,600	2	Neg.	Pos.	Neg.
6-109	8.2	25	328	1 : 400	1 : 100	4	Neg.	Pos.	Neg.
8-33	9.4	20	470	1 : 400	1 : 100	4	Neg.	Pos.	Neg.
15-a1	8.2	20	410	1 : 100	1 : 100	1	Pos.	Pos.	Neg.
6-1	7.6	40	190	1 : 1,600	1 : 100	16	Pos.	Pos.	Pos.

N/A, not applicable.

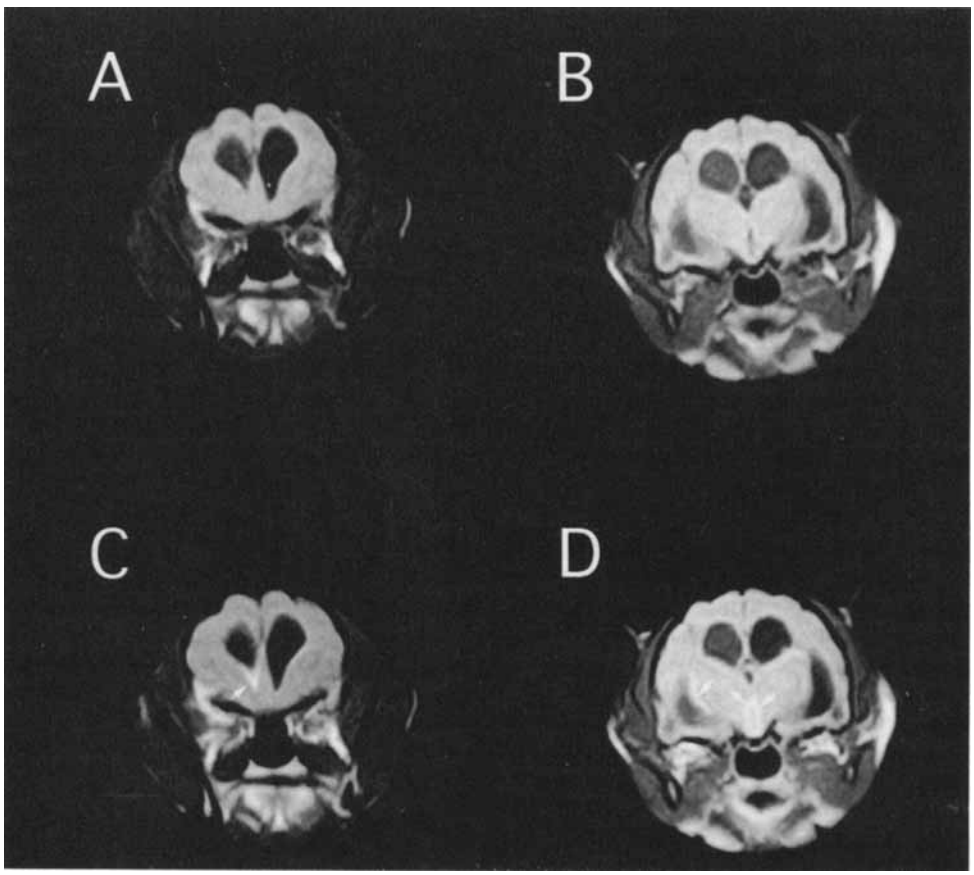

Fig 1. T1 weighted images at 2 different levels of the brain of cat 6–49 before **(A, B)** and after **(C, D)** administration of intravenous gadopentetate dimeglumine. The ventricular system is markedly enlarged and there is evidence of periventricular enhancement at the margins around the lateral and 3rd ventricles (arrows).

Table 2. Gross pathologic changes in cats with neurologic FIP.

Cat	CNS Lesions	Other Lesions
6-34	NGL	Mild hepatic[a] lesions
6-38	Anterior uveitis, hemorrhagic meninges	Renal,[a] hepatic,[a] diaphragmatic granulomas
6-42	NGL	Mesenteric lymphadenopathy[a]
6-43	NGL (but tissue was PCR positive)	NGL
6-44	Mild swollen cerebellum, brain stem[a]	NGL
6-47	NGL	Mesenteric lymphadenopathy[a]
6-48	NGL	Mes. lymphadenopathy,[a] renal granulomas
6-49	Severe hydrocephalus/keratic precipitates	Generalized, omental miliary granulomas[a]
6-51	Anterior uveitis, hemorrhagic meninges	severe abdominal granulomas, abdominal effusion[a]
6-60	NGL	Mild renal lesions, mes. lymphadenopathy[a]
6-62	Keratic precipitates, anterior uveitis	Extensive diffuse granulomas, renal granulomas[a]
6-72	Mild swollen meninges	Renal, pericardial lesions, lymphadenopathy[a]
6-109	Thickened, hemorrhagic meninges, keratic precipitates, retinitis	Mes. lymphadenopathy, renal granulomas
8-33	NGL	NGL, kidney PCR positive
15-1a	Hemorrhagic meninges, pontine-cerebellar adhesions, cerebellar granulomas,[a] brain stem edema	NGL
16-1	NGL[a]	NGL

NGL, no gross lesions; mes. lymphadenopathy, mesenteric lymph nodes.
[a] Site of PCR-positive lesions.

Pathology. Gross pathologic findings included typical abdominal lesions of FIP in 12/16 cats (Table 2). Gross lesions ranged from mild (eg, 1 small granuloma on the renal capsular surface) to severe, generalized miliary granulomatous lesions distorting renal surfaces, disseminated throughout the omentum and gastrointestinal serosa, and invading splenic and hepatic parenchyma. Twelve of 16 cats had enlarged mesenteric lymph nodes. Four cats had grossly visible diaphragmatic and pericardial granulomas. Ocular lesions observed on physical examination were confirmed on postmortem examination. Central nervous system lesions, when grossly apparent, were limited almost exclusively to thickening and opacification of meninges, particularly on the ventral surface of the brain.

Histopathologic examination confirmed the typical granulomatous lesion of FIP in all tissues for which gross lesions were visible. In addition, distinct histologic lesions were detected in the brains of 15/16 cats with neurologic disease, including the 4 cats that were scanned by MRI (Figs 2, 3). In cat 6-34, no gross or histologic lesions of FIP were detected in brain or spinal cord, although they were observed in the liver and mesenteric lymph nodes. Lesions in the brains of the other cats with FIP consisted of meningitis, ependymitis or periventriculitis, and choroiditis of varying severity. Lymphocytes and macrophages were the predominant cell types. In some sites, the infiltrate contained abundant plasma cells, whereas in others it was mostly pyogranulomatous. In the meninges, the inflammatory cells had a predominantly perivascular distribution, forming cuffs around arteries (periarteritis) and infiltrating the wall of veins and venules (phlebitis). Pathologic evaluation of the spatial distribution of brain lesions of the 4 cats that had MRI indicated that the degree of meningeal infiltration increased towards the ventral and caudal regions

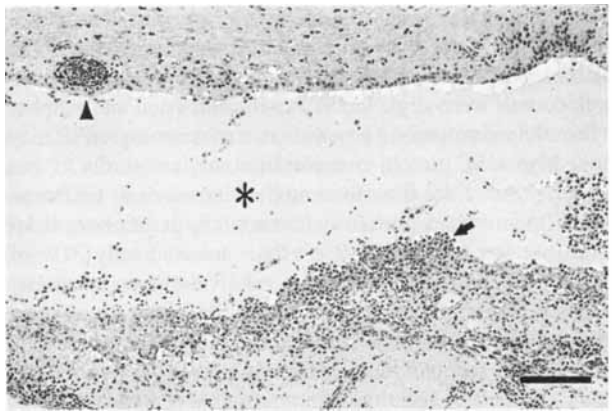

Fig 2. Cerebrum of cat 6–49, showing distention of lateral ventricle (asterisk) (which contained proteinaceous exudate), disruption of the ependyma by a lymphohistiocytic infiltrate (arrow), and mononuclear perivascular cuffing (arrowhead). Hematoxylin and eosin. Bar = 160 μm.

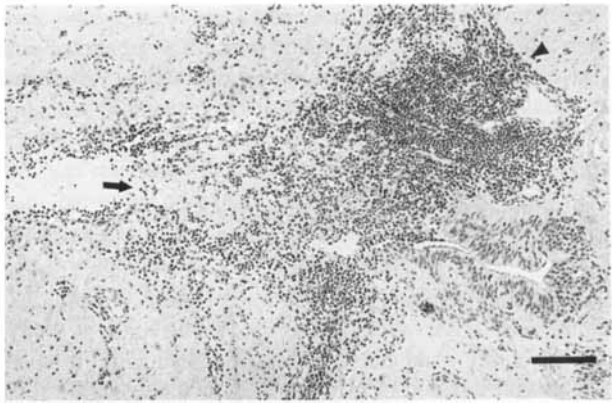

Fig 3. Midbrain of cat 6–49, showing effacement of the ventricular ependyma and lumen by histiolymphocytic infiltrate and proteinaceous exudate (arrow) and perivascular to diffuse lymphoplasmacytic periventricular infiltrate (arrowhead). Hematoxylin and eosin. Bar = 160 μm.

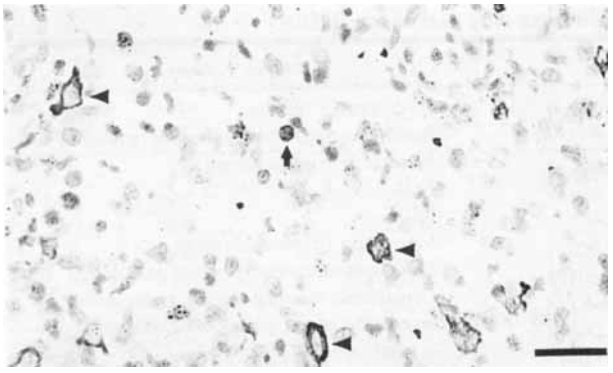

Fig 4. Cerebrum of cat 6–49, showing macrophages (arrowheads) and a lymphocyte (arrow) with diffuse cytoplasmic staining for FIPV antigen. Immunoperoxidase stain with hematoxylin counterstain. Bar = 25 μm.

of the brain and was most severe at the base of the cerebellum and the brain stem, including the medulla oblongata. In a few sites, there was moderate extension of the inflammation into the superficial neuropil and into cranial nerve roots.

Ependymitis was present in all cats that were scanned by MRI but was very mild in one cat (6-62). The 4th ventricle was the most consistently affected (4/4), and the lateral ventricles were the least affected (1/4). The lesions ranged from a mild ependymal infiltration to complete effacement of the ependymal lining by a thick histiocytic and lymphocytic infiltrate, periventricular vasculitis, exudation of a cell- and protein-rich fluid, and periventricular reactive astrocytosis. The cat with the most severe and diffuse ventriculitis (6-49) also had moderate hydrocephalus. Choroiditis was most severe in the choroid plexus of the 4th ventricle, especially in the areas dorsolateral to the medulla, where the architecture often was effaced by dense infiltration with lymphocytes, plasma cells, and histiocytes.

All 4 of these cats had cells with positive immunohistochemical staining for FIPV (Fig 4). These cells were mostly large macrophages, but rare small lymphocytic cells also were positive. The cells had granular or diffuse cytoplasmic staining; their nuclei did not stain. Positive cells were numerous in some areas of intense ependymitis and choroiditis; very few were found within the meningeal infiltrates. No staining was observed in vascular basement membranes or in cells of the neuropil.

Discussion

Neurologic FIP is a relatively common clinical entity that is difficult to diagnose antemortem. Approximately 35% of the referred cases of FIP we have seen in the last 2 years had predominately neurologic lesions (Foley and Pedersen, unpublished observations). The cats in the present study had presenting complaints, signalments, and histories consistent with but not specific for FIP, including age distribution less than 2 years of age,[1,10] originations from multiple-cat homes,[1] and clinical signs of fever, weight loss, and lethargy.[7,17,18] The neurologic signs observed in the present study consisted largely of paresis, hyperesthesia, seizures, ataxia, and personality changes, as have been described previously.[9,18–20] Valuable antemortem results were obtained from CSF titer, CSF PCR, and MRI, and postmortem diagnoses were facilitated by brain and abdominal lesion PCR and immunohistochemical staining of brains.

Fifteen of 16 cats with presenting complaints of neurologic or ocular disease and a diagnosis of FIP had positive CSF anti-FIFV titers, whereas none of the 8 control cats had anti-FIPV antibodies in CSF, suggesting that virus did not at any time cross the blood–brain barrier in the control cases. Unfortunately, the experimentally infected cats may have been a poor model for naturally occurring effusive FIP, as reflected by the peracute clinical course of disease and low serum titers. The 1 cat that was antibody negative in the CSF (cat 6–34) did have antemortem neurologic impairments, including abnormal reflexes and ataxia, in addition to weight loss, anorexia, and fever. Histopathology and PCR on mesenteric lymph nodes and hepatic granulomas confirmed FIP. Most assays for neurologic FIP on this cat however were negative, including CSF titer, brain and CSF PCR, and histopathology of the brain and spinal cord. The neurologic disease in this cat may have had causes other than FIP, despite the positive finding of FIP in the abdomen.

Titers of CSF of the other 15 neurologically impaired cats ranged from 1 : 25 to 1 : 1,600, suggesting that adequate serologic evaluation of CSF necessitates performing IFA testing at much lower dilutions than is routinely performed in some laboratories. The titer in CSF was not statistically correlated with serum titer and the ratio of the serum titer to the CSF titer was not correlated with serum : CSF protein ratio. If passive leakage of protein from serum into the CSF were invoked to explain the presence of anti-FIPV antibodies, it would be expected that the total protein : anti-FIPV IgG ratios would be similar in both serum and CSF. In contrast, CSF IgG titers tended to be proportionally much higher than serum titers, which suggests that anti-FIPV IgG may have been produced in the tissues of the brain in response to local replicating virus.

The cellularity and protein concentrations in CSF were variable among cats in the present study. Using a high normal concentration of CSF protein of 30 (μg/dL, 4/16 cats had abnormally high CSF protein, but only one of these cats had a marked increase in protein concentration, 1,120 (μg/dL. Similarly, cell counts and differentials in the CSF of these cats were abnormal in only 2/16 cats, and when cell counts were high, cells mostly consisted of lymphocytes and neutrophils. Cats with severe neurologic FIP may have high CSF protein concentration and cellularity,[9,19] but some authors have described modest increases in CSF neutrophil counts and protein concentration, particularly if lesions are focal.[21] The PCR on CSF detected only 31% of cats with neurologic FIP. The relatively low sensitivity could be explained by the low CSF cellularity on most cats and the paucity of virus detected histologically.

Magnetic resonance imaging was a useful adjunct diagnostic procedure, predominantly disclosing hydrocephalus and ependymitis in affected cats. The apparent severity of lesions on MRI fit better with the extent of pathologic changes seen on postmortem examination than with the severity of clinical disease. For example, MRI findings in cat 16–1 were described as moderate, whereas clinically the cat

was incontinent, unresponsive, and recumbent, with very abnormal reflexes. It is likely that this cat had more extensive lesions at least in some foci throughout the spinal cord, in addition to the brain. Ventricular dilatation was evident on MRI in 3/4 cats. Contrast enhancement with gadopentetate dimeglumine revealed subtle periventricular inflammatory lesions of FIP. Abnormalities that were detected on MRI, such as hydrocephalus and peri-ventricular contrast enhancement, corresponded well with histologic lesions. Some histologic lesions (eg, meningitis) also were present in the cat without MRI abnormalities. This finding would appear to limit the usefulness of MRI to cases with advanced ventricular involvement. The lesions detected on MRI confirmed the superficial and ventricular nature of lesions but were not specific for FIP.

Postmortem examination of cats clarified the interpretation of the results obtained antemortem. Gross pathologic examination of the brain and meninges of many cats with neurologic FIP did not disclose obvious lesions, with the exception of hydrocephalus, whereas histopathology showed microscopic superficial granulomas, as previously described.[22] Meningitis was more severe on the ventrocaudal surfaces of the brain, which has not been previously reported. The histopathologic changes in the cats of the current study were consistent with earlier descriptions of cerebral FIP lesions: lesions were generally superficial, often oriented around ventricles,[9,22–24] and often accompanied by hydrocephalus.[19,21–23,25] Previously characterized neurologic FIP lesions were pyogranulomatous or lymphoyogranulomatous.[18,21–24] In contrast, the neutrophilic component in the lesions in cats of this study was less marked than the macrophage component and usually was limited to areas with the most severe inflammation.

Immunohistochemistry for FIPV provided unequivocal staining of infected cells. The mouse monoclonal antibody 5.2D5 recognizes the N protein of both FIPV and FECV; these viruses are antigenically indistinguishable in this protein.[3,26,27] A possible exception is the serotype II feline coronaviruses, which appear to be more genetically related to canine coronaviruses than to other feline coronaviruses.[3] However, the definition of an FIPV biotype is pathologic, not genetic. The FIP viruses are macrophage tropic, can survive and replicate in macrophages, and can instigate an Arthus type immune-mediated reaction resulting in fatality[6,28–30]. Thus, any feline coronavirus detected within brain tissue lesions is by definition FIPV. Positive FIPV staining was detected mostly in macrophages in intensely inflamed areas of the ependyma and choroid plexus. Macrophages free within the ventricular lumen often were strongly positive, suggesting that immunohistochemical staining of cells from CSF samples may provide valuable antemortem diagnostic information. However, the utility of this procedure would be limited to those cases with cellular CSF. A previous report of immunohistochemical staining using cat ascites-derived polyclonal anti-FIPV and BALB/c mouse-derived anti-transmissible gastroenteritis virus monoclonal antibodies described only 1 cat with positive staining in monocytes in blood vessels in the choroid plexus.[31] Positive staining of a few lymphocytes in the present study was an unexpected finding.

All FIPV-infected cats (neurologic and control) were PCR positive in at least 1 tissue, whereas only 67% of cats with apparent neurologic FIP were PCR positive in brain tissue. The most likely explanation for this was the patchy distribution of virus in the brains, as shown on immunohistochemical staining. PCR sensitivity could be increased by sampling brain tissue from multiple sites and pooling, but this approach is impractical if brain tissue must also be preserved for histopathology. Nested PCR for herpesvirus DNA in the CSF of patients with neurologic disease detected only 62% of cases of cytomegalovirus-associated encephalopathy.[32] However, PCR to detect herpes simplex virus DNA in CSF of experimentally inoculated mice was very sensitive.[33] Clumped small foci with low virus load in the brain could account for the discrepancy in sensitivity of PCR on brain compared with PCR on abdominal tissues. Abdominal tissues can usually be correctly identified postmortem as containing virus by the presence of gross lesions, whereas brain tissue usually must be sampled blindly, because gross lesions often are inapparent. Histopathologic examination revealed extensive granulomatous inflammation in some FIP-affected brains, even though monoclonal antibody staining failed to demonstrate much virus in these lesions. These results agree with findings in murine coronavirus strain JHM, which produces demyelinating lesions in infected mice.[34] Immunocompetent C57BL/6 mice cleared virus from the brain but developed severe paralysis and demyelination. In contrast, SCID mice had persistent viral loads but no neurologic impairment or detectable lesions, suggesting an immune-mediated pathogenesis similar to that of FIP. T-lymphocytes ($CD4^+$ and $CD8^+$) were required for viral clearance, whereas B cells and other cells participated in demyelination.

Numerous other infectious, neoplastic, and immunologic diseases can produce meningitis, encephalitis, and neurologic lesions in cats, although none are as frequent in occurrence as FIP.[8] Some of the more common infectious causes include feline leukemia, feline immunodeficiency virus infection,[35] rabies,[36] pseudorabies,[37] canine distemper,[38] and *Toxoplasma gondii*.[39] Rare diagnoses include paramyxovirus-like disease,[40] borna-virus-induced nonsuppurative meningoencephalomyelitis,[41,42] *Neospora caninum*,[43] *Sarcocystis* spp.,[44] fungal infections,[7] and feline spongiform encephalopathy.[45] Postmortem differentiation of FIPV infection from other infectious causes of meningitis and encephalitis is based upon the presence of vascular and superficially oriented microgranulomas. The immunohistochemical stain described in the present study was useful for cases requiring additional confirmation. Antemortem FIP diagnosis was facilitated by CSF serology, PCR, and MRI.

In conclusion, we have demonstrated the utility of MRI, PCR, and serology in the diagnosis of neurologic FIP. Many cats in this study had focal, relatively small amounts of virus in brain tissue, despite sometimes extensive histopathological changes. Additional research should be directed at understanding the feline immunologic response to FIPV. Results of such studies will clarify the nature of both viral and host adaptations. Such information also will contribute to our ability to diagnose and potentially treat other immunologically based neurologic diseases.

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References

1. Foley JE, Poland A, Carlson J, et al. Risk factors for feline infectious peritonitis among cats in multiple-cat environments with endemic feline enteric coronavirus. J Am Vet Med Assoc 1997;210: 1313–1318.

2. Poland A, Vennema H, Foley JE, et al. Feline infectious peritonitis is caused by simple mutants of feline enteric coronavirus (FECV) that arise frequently during the course of primary FECV infection. J Clin Microbiol 1996;34:3180–3184.

3. Vennema H, Poland A, Hawkins KF, et al. A comparison of the genomes of FECVs and FIPVs and what they tell us about the relationships between feline coronaviruses and their evolution. Feline Pract 1995;23:40–46.

4. Vennema H, Poland A, Foley J, et al. Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. Virology 1998;243:150–157.

5. Foley J, Pedersen N. The inheritance of susceptibility to feline infectious peritonitis in purebred catteries. Feline Pract 1996;24:14–22.

6. Pedersen NC, Black JW, Boyle JF, et al. Pathogenic differences between various feline coronavirus isolates. Adv Exp Med Biol 1983;173:365–380.

7. Pedersen N. Feline Infectious Diseases. Goleta, CA: American Veterinary Publisher; 1988.

8. Fenner W. Inflammations of the nervous system In: August J, ed. Consultations in Feline Internal Medicine. Philadelphia, PA: WB Saunders; 1991:507–517.

9. Kornegay J. Feline infectious peritonitis: The central nervous system form. J Am Anim Hosp Assoc 1978;14:580–584.

10. Pedersen NC. An overview of feline enteric coronavirus and infectious peritonitis virus infections. Feline Pract 1995;23:7–22.

11. Pedersen NC. Serologic studies of naturally occurring feline infectious peritonitis. Am J Vet Res 1976;37:1449–1453.

12. Hickman A, Morris J, Rogers Q, et al. Elimination of feline coronavirus from a large experimental specific-pathogen-free cat breeding colony by serologic testing and isolation. Feline Pract 1995;23:96–102.

13. Rohrer C, Suter P, Lutz H. Die Diagnositik der felinen infektioesen Peritonitis (FIP): Retrospektive und prospektive Untersuchungen. Kleintierpraxis 1993;38:379–389.

14. Peaston A, Higgins R, Naydan D, et al. Evaluation of commercially available antibodies to cytokeratin, intermediate filaments and laminin in normal cat pinna. J Vet Diagn Invest 1992;4:306–311.

15. Cheung RC, Matsui SM, Greenberg HB. Rapid and sensitive method for detection of hepatitis C virus RNA by using silica particles. J Clin Microbiol 1994;32:2593–2597.

16. Foley JE, Poland A, Carlson J, et al. Patterns of feline coronavirus infection and fecal shedding from cats in multiple-cat environments. J Am Vet Med Assoc 1997;210:1307–1312.

17. Greene C. Clinical Microbiology and Infectious Diseases of the Dog and Cat. Philadelphia, PA: WB Saunders; 1985.

18. August J. Feline infectious peritonitis: An immune-mediated coronaviral vasculitis. Vet Clin North Am Small Anim Pract 1984;14: 971–984.

19. Pedersen N. Feline infectious peritonitis and feline enteric coronavirus infections. Part 2: Feline infectious peritonitis. Feline Prac 1983;13:5–19.

20. Greene CE, McDermott M, Jameson PH, et al. *Bartonella henselae* infection in cats: Evaluation during primary infection, treatment and rechallenge infection. J Clin Microbiol 1996;34:1682–1685.

21. Tamke P, Petersen M, Dietze A, et al. Acquired hydrocephalus and hydromyelia in a cat with feline infectious peritonitis: A case report and brief review. J Can Vet 1988;29:997–1000.

22. Slauson D, Finn J. Meningoencephalitis and panophthalmitis in feline infectious peritonitis. J Am Vet Med Assoc 1972;160:729–734

23. Krum S, Johnson K, Wilson J. Hydrocephalus associated with the noneffusive form of feline infectious peritonitis. J Am Vet Med Assoc 1975;167:746–748.

24. Legendre A, Whitenack D. Feline infectious peritonitis with spinal cord involvement in two cats. J Am Vet Med Assoc 1975;160 729–734.

25. Summers B, Cummings J, de Lahunta A. Feline infectious peritonitis. In: Veterinary Neuropathology. St. Louis, MO: Mosby Year-Book; 1995:119–121.

26. Pedersen N. The history and interpretation of feline coronavirus serology. Feline Pract 1995;23:46–52.

27. Wesseling J, Vennema H, Godeke GJ, et al. Nucleotide sequence and expression of the spike (S) gene of canine coronavirus and comparison with the S proteins of feline and porcine coronaviruses. J Gen Virol 1994;75:1789–1794.

28. Olsen C. A review of feline infectious peritonitis virus: Molecular biology, immunopathogenesis, clinical aspects, and vaccination. Vet Microbiol 1993;36:1–37.

29. Pedersen NC, Evermann JF, McKiernan AJ, et al. Pathogenicity studies of feline coronavirus isolates 79-1146 and 79-1683. Am J Vet Res 1984;45:2580–2585.

30. Stoddart C, Scott F. Intrinsic resistance of feline peritoneal macrophages to coronaviruses correlates with virulence. J Virol 1989;63: 436–440.

31. Tammer R, Evensen O, Lutz H, et al. Immunohistological demonstration of feline infectious peritonitis virus antigen in paraffin-embedded tissues using feline ascites or murine monoclonal antibodies. Vet Immunol Immunopathol 1995;49:117–182.

32. Fox J, Brink N, Zuckerman N. Detection of herpesvirus DNA by nested polymerase chain reaction in cerebrospinal fluid of human immunodeficiency virus-infected persons with neurologic disease: A prospective evaluation. Infect Dis 1995;172:1087–1090.

33. Boerman R, Arnoldus E, Raap A. Polymerase chain reaction and viral culture techniques to detect HSV in small volumes of cerebrospinal fluid. An experimental mouse encephalitis study. Virol Methods 1989;25:189–198.

34. Houtman J, Fleming J. Dissociation of demyelination and viral clearance in congenitally immunodeficient mice infected with murine coronavirus JHM. Neurovirology 1996;2:101–110.

35. Hurtrel M. Comparison of early and late feline immunodeficiency virus encephalopathies. AIDS 1992;6:399–406.

36. Innes J, Saunders L. Comparative Neuropathology. New York, NY: Academic Press; 1962.

37. Fankhauser R, Fatzer R, Steck F, et al. Morbus Aujesky bei hund und katze in der Schweiz. Schweiz Arch Tierheilkd 1975;117: 623–629.

38. Appel M, Sheffy B, Percy D, et al. Canine distemper virus in domesticated cats and pigs. Am J Vet Res 1974;35:803–806.

39. Dubey J, Beattie C. Toxoplasmosis of Animals and Man. Boca Raton, FL: CRC Press; 1988.

40. Cook R, Wilcox G. A paramyxovirus-like agent associated with demyelinating lesions in the CNS of cats. Proc Am Assoc Neuropathol 1981:89.

41. Lundgren AL, Ludwig H. Clinically diseased cats with nonsuppurative meningoencephalomyelitis have borna disease virus-specific antibodies. Acta Vet Scand 1993;34:101–103.

42. Nowotny N, Weissenbock H. Description of feline nonsuppur-

ative meningoencephalomyelitis ("staggering disease") and studies of its etiology. J Clin Microbiol 1995;33:1668–1669.

43. Dubey J. A review of *Neospora caninum* and *Neospora*-like infections in animals. J Protozool Res 1992;2:40–52.

44. Dubey J, Higgins R, Barr B, et al. *Sarcocystis*-associated meningoencephalomyelitis in a cat. J Vet Diagn Invest 1994;6:1118–1120.

45. Gruffydd-Jones T, Galloway P, Pearson G. Feline spongiform encephalopathy. J Small Anim Pract 1991;33:471–476.