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An epizootic of highly virulent feline calicivirus disease in a hospital setting in New England

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This article reports an outbreak of 24 cases of an unusually virulent feline calicivirus (FCV) infection in a small animal hospital. The circumstances and disease signs were very similar to those recently described in an outbreak of FCV hemorrhagic disease in Northern California (Vet. Microbiol. 73 (2000) 281). The virus entered the facility through shelter cats showing upper respiratory signs. Affected cats manifested high fever, anorexia, labored respirations, oral ulceration, facial and limb edema, icterus, and pancreatitis. The infection spread rapidly among the patients by contaminated animal caretakers and hospital equipment. One case of fomite transmission from an employee to a housecat was documented. Prior vaccination, even with multiple doses of FCV-F9-based live calicivirus vaccine, was not protective. Affected cats often required extensive supportive care for 7–10 days, and the overall mortality from death and euthanasia was 32%. The strain of FCV responsible for this outbreak was genetically and serologically distinct from the FCV strain responsible for a similar epizootic and the FCV-F9 strain contained in most vaccines. Outbreaks of this type are being reported with increasing frequency, and are often associated with the practice of treating sick shelter cats in private practices. Similar to the present epizootic, outbreaks of FCV hemorrhagic disease have been self-limiting, but require prompt application of strict quarantine, isolation, personnel sanitation, and disinfection procedures.

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Introduction

Caliciviruses infect a variety of species in nature including swine, humans, rabbits, sea lions, and cats (Green et al 2000). Feline calicivirus (FCV) infections are usually inapparent or are associated with fever, limping, and mild glossal/palatine ulceration. Upper respiratory signs, when seen and unlike feline herpesvirus infection, tend to be mild. However, FCV may cause more severe disease in crowded feline populations, such as catteries and shelters (Turnquist & Ostlund 1997). Virus transmission occurs mainly by the oral route; acutely ill and carrier cats shed large amounts of virus from their oral cavities (Wardley & Povey 1977). Cats less than 4 feet apart can acquire the virus by droplet transmission (Barlough 1986). Fomite transmission by animal caregivers, caging, litter, food and water dishes, and instruments can play a significant role in hospitals. The cycle of transmission can be broken by thorough disinfection of all the surfaces and items that are frequently handled, washing or discarding the heavily soiled items, and establishing a 7–14 days quarantine of the premises, if new cases persist despite decontamination of the environment (Hurley & Bannasch 2002).

Virus isolation, using oropharyngeal swabs in viral transport medium or heparinized/EDTA blood, is relatively efficient (Arnett & Greene 1984). Subsequently, reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing of the PCR product can be employed to compare the relatedness of different isolates (Radford et al 1998). Assessment of antibody-binding patterns in these highly virulent strains can then be made, using serum neutralization assays, to assess cross reactivity with other field isolates and commercial strains of FCV.

In this study, we report a typical outbreak of atypical and highly virulent FCV disease in a private veterinary hospital. Characteristic

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features of this outbreak included high morbidity and mortality associated with icterus, edema of the face and limbs, dyspnea, bleeding tendencies, and pancreatic disease, despite solid histories of live feline rhinotracheitis, calicivirus, and panleukopenia virus vaccination. Other such outbreaks have been reported. In Pennsylvania, a shelter was depopulated to prevent the spread of a highly virulent calicivirus infection to naive feline populations after battling the outbreak unsuccessfully for 2 months (Debovy, personal communication). In Los Angeles, CA, from July to August 2002, over 50 cats in three veterinary practices were infected by a FCV isolate, FCV-Kaos, killing nearly half of them (Verdon 2002). The causative agent of the outbreak described in this study, FCV-Diva, proved to be genetically and serologically distinct from another highly virulent strain (FCV-Ari) that was associated with a similar epizootic in Northern California (Pedersen et al 2000).

Procedures

Sample collection

Initial viral isolate Refrigerated tissue samples obtained on post-mortem examination from esophagus, trachea, lung, liver, spleen, kidney, and intestine were sent on dry ice by express courier to the New York State Diagnostic Lab (Cornell Diagnostic Laboratory, Ithaca, NY).

Subsequent isolates Blood drawn from 13 acutely ill cats was shipped in EDTA tubes at room temperature by express courier service to the University of California–Davis (Center for Companion Animal Health, Davis, CA). Oropharyngeal samples were taken using special swabs (Viral Culturette, Becton Dickinson) employing Hank's balanced salt solution (HBSS) as a preservative. Four swabs were sent to University of Tennessee (ANTECH Diagnostics, Farmingdale, NY) and one swab to University of California–Davis for virus isolation.

Cytology/histopathology Abdominal fluid in EDTA was sent for fluid analysis on one case (cat 6). In addition, tissue samples of heart, lung, stomach, spleen, small and large intestines (including mesentery/omentum), liver, pancreas, kidney, urinary bladder, and skin from this animal (in formaldehyde) were submitted for histopathology. A second set of samples, including heart, lung, liver, kidney, spleen, pancreas, and small and large intestines, from cat 15 was submitted (ANTECH Diagnostics, SJ Engler, VMD).

Materials and methods

Virus isolation

EDTA blood was diluted with an equal amount of HBSS and centrifuged at 300 g for 30 min. The buffy coat was then suspended in 100 µl of HBSS and layered onto subconfluent monolayers of Crandell feline kidney (CrFK) and *Felis catus* whole fetus 4 (Fcwf-4) cells grown in 25 cm^2 flasks. Swabs were resuspended in 5 ml snap tubes with 1 ml of HBSS with 10× gentamycin and penicillin/streptomycin. After allowing the samples to settle for 2 h at room temperature, supernatant was transferred to flasks containing CrFK or Fcwf-4 cells. Cells were examined every 12 h for CPE. Seven samples yielded positive cytopathic results, consistent with calicivirus, of which two were preserved in culture for genetic and serologic studies.

Virus sequencing and genetic analysis

Two FCV isolates (cats 15 and 24) from this outbreak were sequenced from a nested PCR product, which encompassed a 234 base pair (bp) region corresponding to nucleotides 6533–6767 (hypervariable region E of capsid gene) of the FCV genome (Radford et al 1997). Microcon-50 columns (Millipore) were used to remove primers and salts, and the PCR products were then sequenced. Data were analyzed using the University of Wisconsin Genetics Computer Groups (GCG) software (GCG Program Manual for the Wisconsin package 1994). Sequences from the two FCV isolates were compared with sequences obtained from two FCV-F9 vaccine strains (Merial Purevax RCP, Norden), several low pathogenicity field isolates (FCV 2-20, FCV 2-80), and a previous isolate, FCV-Ari, causing identical clinical signs (Pedersen et al 2000). The phylogenetic relatedness of all the sequenced isolates was determined using two different algorithms, parsimony and UPGMA, as implemented with PAUP version 4.0b8a for Macintosh (Swofford 2001).

Virus neutralizing antibody assay

Virus sensitivity to neutralizing antibodies in serum was measured by a residual virus infectivity assay, employing constant amounts of

virus and anti-serum. Incubation of 100 µl (approximately 1000 $TCID_{100}$) of infectious tissue culture medium containing FCV from cat 15, FCV-Ari, and FCV-F9 was done at 37°C for 1 h with an equal volume of either a 1:20 dilution of tissue culture medium (negative control) or a 1:20 dilution of the cat serum to be tested (non-immune cat serum, cat 24 serum, FCV-Ari-infected cat serum, FCV-F9-immunized cat serum) in tissue culture medium. The titration was set up in 96 well microtiter plates containing a monolayer of subconfluent CrFk cells with 100 µl of tissue culture fluid in each well. In triplicate, 50 µl of virus/anti-serum mixture was added to the first well, mixed thoroughly, and then 50 µl was carried serially to the next 11 wells. Parallel dilutions were prepared using the same virus stock mixed with an equal volume of tissue culture medium alone. After 24 h at 37°C, the plates were observed under an inverted microscope for typical FCV CPE. The endpoint titer was determined as the last well with any detectable CPE. The difference between the average virus titer with and without serum treatment was used to calculate the virus neutralizing antibody titer of each serum sample.

Results

History of the outbreak

An infectious disease outbreak lasting 2 weeks affected 24 cats in a private small animal hospital (Table 1). FCV-Diva was identified as the causative agent. A total of 15 cats were sampled, by oral swabbing, pooled post-mortem tissues or blood draws, of which seven tested positive for calicivirus. The virus was most likely introduced to the hospital by a group of five animals from a local shelter, which were admitted between March 12 and March 19, 2001. Two of the five animals (cats 1 and 2) manifested fever, severe oral ulceration, facial edema, and poor wound healing. Cats 3, 4, and 5 were hospitalized in a separate isolation area on a floor below the main hospital area. Gloves, bleach, and phenolic disinfectant (Roccal) were used on examination surfaces and cages between cases. Of the five animals, two died in the hospital (cats 1 and 4), one was euthanatized owing to its moribund condition (cat 5), and two were discharged, returning to the shelter (cats 2 and 3).

While the shelter cats were in isolation for treatment, cats admitted to the hospital from March 15 to March 24 for routine surgical procedures began to return for evaluation of anorexia and fever of unknown origin (cats 6, 8, 9, 10, 11, 12, 13, 14). The first hospital case, cat 6, was spayed on March 15 and presented again with fever of unknown origin on March 19. The animal was admitted to the general hospital area and stayed there until March 26 for treatment of a presumed post-operative infection with subcutaneous fluids and antibiotics. Cat 7, housemate of cat 6, with no history of direct hospital contact, presented with a fever on March 26 along with the cats 8, 9, and 10, three young male cats that had been neutered the week before on the premises.

During treatment, condition of cat 6 deteriorated rapidly, developing severe facial and limb edema, abdominal effusion, icterus, and bilirubinuria. A chemistry profile result included marked panhypoproteinemia, bilirubinemia, hypochloremia, and hyponatremia. A bile acids assay (IDEXX Laboratories, Grafton, MA) supported severe hepatic disease (207 µmol/l, normal range less than 15 units). On March 29, the cat died and a post-mortem examination was performed, with tissues submitted for abdominal fluid cytology and histopathology (ANTECH Diagnostics). Abdominal fluid consisted of a sterile pyogranulomatous inflammation (ANTECH Diagnostics). The FIP serum antibody test was negative (IDEXX Laboratories) and feline infectious peritonitis (FIP) PCR on the abdominal fluid was positive (ANTECH Diagnostics). The most significant histopathological finding was severe pancreatitis. Lung tissue had lesions consistent with subacute interstitial pneumonitis. Ulcerated skin samples showed a marked segmental necrotizing dermatitis with vasculitis and thrombosis. Liver tissues exhibited chronic passive congestion and coagulative necrosis. No causative agent was identified on histopathology. Cat 7 followed a similar clinical course and was euthanized on March 31. No necropsy was performed as per the owner's request.

Owing to the highly contagious nature of the pathogen, veterinary assistance from the Massachusetts Bureau of Animal Health was requested. A veterinary epidemiologist conducted a hospital survey on April 1 to recommend measures to prevent further spread and to decontaminate the environment. At this point of time, the specific agent had not yet been isolated, but a viral cause was likely owing to persistently high fevers that were non-responsive to broad-spectrum antibiotics. Recommendations to disinfect surfaces and supplies with a 10% bleach solution and discard any contaminated objects in

Table 1. Summary of 24 cats involved in virulent FCV outbreak

Reference	Signalment	Vaccinations	Incubation time	Clinical signs	Calicivirus status	Outcome
Cat 1	3 Years, M, DLH	Modified live RCP 2/28/01	Unknown, index case arrived sick	Oral ulcers with necrosis, facial/limb edema, fever, icterus, epistaxis, vomiting, diarrhoea	Unavailable	Died before virus isolation
Cat 2	1 Year, M, DSH	RCP 2/28/01, 3/26/01	Unknown, arrived sick	Anorexia, fever, oral necrosis, purulent otitis, petechiae, melena	Unavailable due to shelter quarantine	Returned to shelter
Cat 3	6 Months, M, DSH	RCP 3/26/01	Unknown, arrived sick	Sneezing on arrival, oral ulcers, fever	Unavailable due to shelter quarantine	Returned to shelter
Cat 4	8 Weeks, F, DSH	None	Unknown, arrived sick	Anorexia, fever, lameness of right front leg	Unavailable, died before sampling	Acute respiratory arrest
Cat 5	8 Weeks, F, DSH	None	Unknown, arrived sick	Anorexia, fever, vomiting, hypothermia, diarrhoea	Unavailable	Euthanasia as per owner request
Cat 6	6 Months, F, S, Himalayan	RCP 12/11/00, 1/13/01	4 Days	Fever, facial/limb edema, abdominal effusion, icterus, bilirubinuria, oral ulcers	Unavailable	Died before testing
Cat 7	4 Years, F, S, Himalayan	RCP 8/2/98, 8/29/97, 7/21/97	4 Days	Fever, limb edema, icterus, bilirubinuria, oral ulcers, vomiting, abdominal effusion	Unavailable	Euthanasia as per owner request
Cat 8	5 Months, M, C	RCP 12/19/00, 1/11/01	4 Days	Fever, anorexia, oral ulcers, pneumonia	EDTA blood: negative, 4/9/01	Recovered, released
Cat 9	6 Months, M, C, DSH	RCP 3/22/01	5 Days	Fever, oral ulcers, limb edema	EDTA blood: 4/9/01, negative; 4/10/01, positive	Recovered, released from isolation
Cat 10	6 Months, M, C, DSH	RCP 3/22/01	3 Days	Fever, oral ulcers, limb edema	EDTA blood: 4/9/01, negative; 4/10/01, positive	Recovered, released from isolation
Cat 11	2 Years, F, S, DSH	RCP 11/20/99, 9/7/00	5 Days	Vomiting, lethargy, oral ulcers	Not tested	Recovered spontaneously
Cat 12	7 Years, F, S, DSH	RCP/FeLV: 9/6/94, 6/29/95, 6/12/98, 5/24/99, 9/6/00	4 Days	Fever, oral ulcers, lameness, limb edema, coagulopathy	Pooled blood negative	Treated at referral hospital, released
Cat 13	16 Years, F, S, DSH	RCP 7/5/00	4 Days	Fever, anorexia, vomiting, pancreatitis, diabetic on presentation	Oral swab and EDTA blood: 4/2/01, positive	Recovered, released from isolation
Cat 14	16 Years, M, C, DSH	RCP 8/7/97, 6/30/98	4 Days	Anorexia, fever, mild oral, ulcers, pancreatitis	Oral swab: 4/30/01, negative	Treated as outpatient
Cat 15	8 Years M, C, DSH	RCP 6/10/98	3 Days	Anorexia, fever, lameness, edema on all paws, icterus, oral ulcers	Pooled post-mortem tissue: initial virus isolation, sequenced oral swab, 4/2/01, positive virus IFA negative	Euthanasia

Table 1. *Continued*

Reference	Signalment	Vaccinations	Incubation time	Clinical signs	Calicivirus status	Outcome
Cat 16	8 Years, F, S, DSH	RCP 9/26/00	5 Days	Fever, anorexia	Not available	Euthanasia
Cat 17	8 Months, F, S, DSH	RCP 9/26/00	4 Days	Fever, anorexia, oral ulcers, limb edema	Oral swab: 4/9/01, positive; EDTA blood: 4/9/01, 4/10/01, negative	Recovered, released from isolation
Cat 18	8 Months, M, C, DSH	RCP 10/23/00	4 Days	Fever, anorexia, oral ulcers, limb edema	Oral swab: 4/9/01, positive; EDTA blood: 4/9/01, 4/10/01, negative	Recovered, released from isolation
Cat 19	16 Years, F, S, DSH	RCP 10/12/99	4 Days	Fever, anorexia, oral ulcers	EDTA blood: 4/5/01, negative	Euthanasia
Cat 20	19 Years, F, S, DSH	RCP 10/12/99	4 Days	Fever, anorexia, oral ulcers	EDTA blood: 4/5/01, negative	Euthanasia
Cat 21	1 Year, F, S, DSH	RCP 12/24/99, 1/14/00, 3/22/01	5 Days	Fever, facial and limb edema, oral ulcers, pododermatitis	EDTA blood: 4/5/01, 4/11/01, negative	Treated as outpatient
Cat 22	5 Months, F, DSH	RCP 3/22/01		Fever, oral ulcers, lameness, pneumonia, bilirubinuria	EDTA blood: 4/5/01, 4/11/01, negative	Treated as outpatient
Cat 23	5 Months, M, DSH	RCP 3/22/01		Fever, oral ulcers, lameness	EDTA blood: 4/5/01, 4/11/01, negative	Treated as outpatient
Cat 24	5 Months, F, DSH	RCP 3/22/01		Rhinitis, no oral ulcers or fever	EDTA blood: 4/5/01, positive; 4/11/01, negative, sequenced	Treated as outpatient

RCP, Purevax Merial feline Rhinotracheitis, Calicivirus, Panleukopenia vaccine; M, male; F, female; C, castrated; S, spayed; DLH, domestic long hair; DSH, domestic short hair.

treatment areas were followed after the site visit. FCV persists in the environment for 8–10 days and is best inactivated by contact with a 1:32 dilution of household bleach in water (Scott 1979). The hospital was closed to all feline patients for a period of 10 days. Surrounding veterinary hospitals and referral centers were contacted to alert them of the clinical course and highly contagious nature of the pathogen. All the clients who had patients in the hospital (feline and canine) during the time of the outbreak were notified and were asked if any of their pets displayed signs of illness. Animals being treated in the isolation ward were handled with gowns and gloves. All the employees were instructed on proper sanitation and isolation procedures during regular staff meetings. Bleach footbaths were set up at the door of the isolation facility and at the hospital entrance.

A cat (15) belonging to a hospital employee who had worked extensively with cat 6 developed signs of fever, anorexia, and lameness on March 28, even though the cat itself had never been brought to the hospital. This cat was treated as an outpatient with antibiotics and subcutaneous fluids, and it proceeded to develop limb edema and oral ulceration. A CBC and chemistry profile (IDEXX Laboratories) revealed severe lymphopenia (98 per microliter), hypoproteinemia, and hyperbilirubinemia. Fecal and blood cultures were negative for significant bacterial pathogens (IDEXX Laboratories). A feline coronaviral antibody test (ANTECH Diagnostics) showed a positive titer of 1:800. Cat 15 continued to decline in health and was euthanatized on March 31.

Pooled post-mortem tissues from cat 15, kept refrigerated, were shipped on dry ice for virus isolation (Cornell Diagnostic Laboratory, Ithaca, NY). After 20 h in culture, an FCV was identified. Virus fluorescent antibody tests for both feline herpesvirus and FCV were negative. Samples of the initial viral isolate were later shipped to the Center for Companion Animal Health (School of Veterinary Medicine, Davis, CA) for characterization. Histopathology results showed mild to moderate multifocal crypt and gland abscesses of the small and large intestines. Pancreatic tissue displayed mild nodular acinar hyperplasia (ANTECH Diagnostics).

The causative agent was confirmed to be a FCV on April 5. Thereafter, the efforts focused on treating the convalescing cats in the isolation facility, while preventing transmission. The animals that had been hospitalized or boarded in the general hospital area during the time when cat 6 was in the hospital continued to present with signs of infection. Cats 11, 14, 21, 22, 23, and 24 developed less severe clinical signs of fever, anorexia, and oral ulceration. Whenever possible, these and other cats were treated on an outpatient basis, so as to reduce viral load on the environment and prevent potential new exposures.

The last hospital case to present for treatment was cat 21, admitted for an ovariohysterectomy on March 21. The cat had a litter of three 5-month-old kittens (cats 22, 23, and 24), which were brought in for routine vaccination on the day of discharge from surgery. The owners were contacted to check on the health of their recently hospitalized cat on April 2. They reported that all four cats were reluctant to eat and were lethargic. After examination, the cats were treated as outpatients. The queen was most severely affected, showing lesions of an ulcerative pododermatitis that required oral antibiotic treatment. Oropharyngeal swabs and EDTA blood were collected for virus isolation characterization (Center for Companion Animal Health, School of Veterinary Medicine, Davis, CA). Tissue specimens from cat 24 yielded a calicivirus in culture, FCV-Diva.

Cats that were boarded in the area adjacent to the hospital cages also became exposed during the period when cat 6 was sick. Three of these animals (cats 16, 19, and 20) were owned by the hospital. Even though all three cats had received multiple annual boosters of a modified live rhinotracheitis, calicivirus, and panleukopenia vaccine and were moved to a remote area of the hospital to reduce the risk of transmission, they developed signs of fever, anorexia, and oral ulceration. Owing to the possibility of reverting to a carrier state once infection had passed, these cats were euthanatized. Two 8-month old boarding cats from the same household (cats 17 and 18) broke with a fever on March 30 and were moved immediately to the isolation ward for supportive care consisting of feeding tubes, oral antibiotics, and electrolytes. Bacterial blood culture and sensitivity on cat 17 were negative (ANTECH Diagnostics). Both cats tested positive for calicivirus in culture from oropharyngeal swabs, and negative for herpesvirus by immunofluorescent antibody (ANTECH Diagnostics).

Of the eight cats treated in the on-site hospital isolation facility, one died (cat 6) and one was euthanatized because of the severity of its condition (cat 7). Six cats, which were less ill, received extensive supportive and nursing care by feeding

Table 2. Reduction in virus titer after treatment with anti-serum

Virus	Number of FCV normal cat serum	Anti-FCV-F9 serum	Anti-FCV-Cat 24 serum[a]	Anti-FCV-Ari serum
FCV-F9	0	5691	100	165
FCV-Cat 24	0	11	56	127
FCV-Ari	0	0	45	>78 125
FCV-Cat 15	0	13	>35 511	2

[a] Immunized 2 weeks previously with FCV-F9 vaccine.

Table 3. Sequence alignments of four FCV isolates (FCV-Diva (cat 15), FCV-Diva (cat 24), FCV-Merial (FCV-F9), and FCV-Ari)

Isolates	Genomic sequence
	6585
FCV-Cat 15	ACAATACCTA ATAAGCTCAT TCCGGCTGGT GATTATGCTA TTACCAATCA
FCV-Cat 24	ACAATACCTA ATGAGCTCAT TCCGGCTGGT GATTATGCTA TTACCAATCA
FCV-Merial	ANAATTCCTG GGGAGTCGAT ACCANNTGGC GATTACACAA TCACCAATGG
FCV-Ari	ACAATCCCTG AGAAGCTGAC ACCTGCTGGC GATTACGCCA TCGTAGATGG
	6635
FCV-Cat 15	GAGTGGCAAT GATATACAAA CAAAAGAGGA ATACGAATCT GCCATGATAA
FCV-Cat 24	GAGTGGCAAT GATATACAAA CAAAAGAGGA ATACGAATCT GCCATGATAA
FCV-Merial	TACTGGCAAT GACATCACCA CGGCTACAGG ATATGACACT GTTGATNTAA
FCV-Ari	ATCAGGCAAT GACATCACAA CTAAGGATAA ATATGAAAGT GCTGATGTGA
	6685
FCV-Cat 15	TCAGCAACAA CACAAATTTC AAAAGCATGT ACATTTGTGG GTCCCTTCAA
FCV-Cat 24	TCAGCAACAA CACAAATTTC AAAAGCATGT ACATTTGTGG GTCCCTTCAA
FCV-Merial	TTAAGAACAA TACCAACTTT AAGGGCATGT ACATATGTGG TTCGCTCCAG
FCV-Ari	TCAAGAATAA CACCAATTTC AGGGGCATGT ACATTTGTGG CTCACTTCAA
	6735
FCV-Cat 15	CGAGCGTGGG GTGACAAGAA AATATCCAAC ACTGCTGT
FCV-Cat 24	CGAGCGTGGG GTGACAAGAA AATATCCAAC ACTGCA~~
FCV-Merial	CGTGCCTGGG GTGACAAGAA AATATCCAAC ACTGCA~~
FCV-Ari	AGAGCATGGG GTGACAAGAA AATATCCAAC AGTGCA~~

Sequences are from region E of the capsid gene (nucleotides 6585 and 6767 of the FCV genome).

tubes and recovered after a period of 7–10 days (cats 8, 9, 10, 13, 17, and 18). On follow-up examinations for subsequent medical care (cat 13 for diabetic regulation) and elective procedures (cat 24 for ovariohysterectomy), these cats tested negative for calicivirus on oropharyngeal swabs.

Virus neutralizing antibody assay

Reduction in virus titer after treatment with anti-serum is presented in Table 2. The FCV-F9 anti-serum did not significantly reduce the virus titer when tested against the isolate from cats 15, or FCV-Ari. Anti-serum to FCV-Ari showed low levels of neutralization against the isolate from cat 15.

Characterization of virus isolates

A total of 24 cats were studied. Cultures of oral swabs, blood, or tissues were obtained from 15 cats. FCV isolates were ultimately obtained from seven of the 15 cats. Two of these isolates (from cats 15 and 24) were selected for sequence analysis of the hypervariable region of the nucleocapsid gene. The two isolates were compared with FCV-F9 (common vaccine strain) and FCV-Ari (cause of similar epizootic in Northern California) (Table 3). The two isolates (cats 15 and 24) proved to be genetically identical, but were different from both FCV-F9 and FCV-Ari. The relatedness of the cats 15 and 24 isolate with FCV-Ari, two FCV-F9 vaccine strains (Merial, Norden), and two random field isolates (FCV 2-20, FCV 2-80) not associated with disease, was portrayed

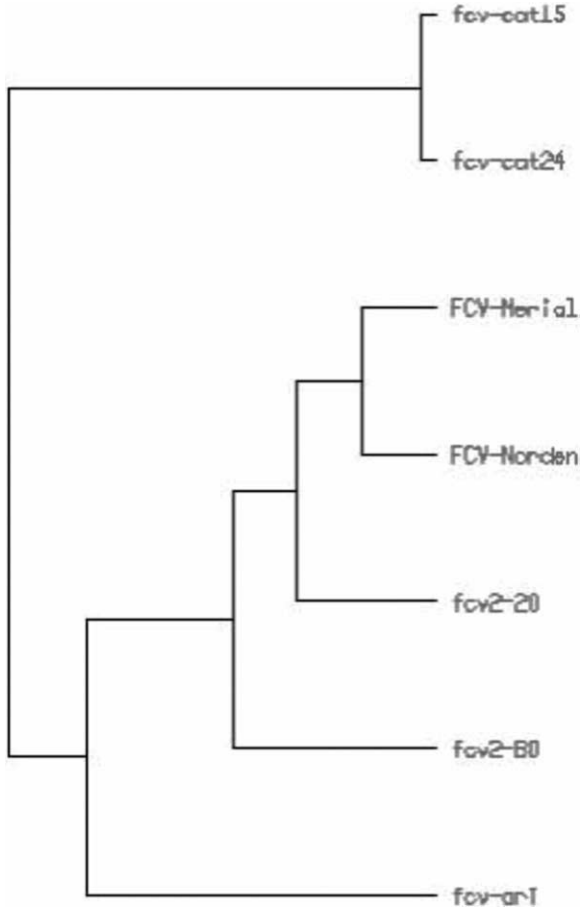

Fig 1. Multiple-sequence alignment dendrogram based on 208 bp of sequence from hypervariable E region of the capsid gene of FCV-Diva (isolates 15 and 24), FCV-Ari, FCV-F9 (Merial and Norden vaccine isolates), and two mildly pathogenic routine field isolates (FCV 2-20, FCV 2-80). Branch lengths indicate genetic distance as calculated by UPGMA. FCV-Diva isolates 15 and 24 cluster by themselves on a distant arm, while all other isolates are more closely related to FCV-F9-related vaccine strains.

as a multiple-sequence alignment dendrogram (Fig 1).

Discussion

An FCV strain, which we herein designate FCV-Diva, caused a severe disease when introduced to a private veterinary hospital, most likely through five shelter cats. The disease caused by FCV-Diva closely resembled the FCV hemorrhagic fever that occurred in a similar epizootic in Northern California by FCV-Ari (Pedersen et al 2000). The noteworthy signs in common were the severe edema (due to vasculitis, thrombosis, and necrosis) of face and limbs, pancreatitis, and pneumonia. FCV-Diva caused severe morbidity and mortality in 19 cats of varying ages and vaccination status. The virus spread among the cats by direct contact between the cats of the same household (cat 6 to cat 7, cat 21 to her kittens—cats 22, 23, and 24), infection of cats outside the hospital by a contaminated caretaker (cat 15), and it spread within the hospital, presumably as a result of virus persistence in the environment (on surfaces, hospital equipment, caretaker clothing, and hands). Airborne transmission may have played a role in some cases among boarding cats (cats 17 and 18) and hospital animals (cats 16, 19, and 20). The clinical course of cases varied, but primarily included persistent fever despite broad-spectrum antibiotics, anorexia, complete oropharyngeal ulceration, purulent otitis, facial edema, appendicular edema with intermittent lameness, and ulcerative pododermatitis. Some animals subsequently developed signs of bleeding tendencies, such as epistaxis, melena, and petechiae. Another common symptom consisted of gastrointestinal signs and serum chemistry supportive of acute pancreatitis (cats 6,13, and 14), confirmed in cat 6 on postmortem histopathology findings. Of the sick cats admitted for treatment, one died (cat 6), four were euthanized at the owner's request because of the severity of signs and poor prognosis (cats 7, 15, 19, and 20), six recovered with supportive treatment in isolation (cats 8, 9, 10, 13, 17, and 18), and one survived after extensive treatment at a veterinary referral hospital (cat 12). In our clinical experience, esophagostomy-feeding tube therapy yielded a quicker recovery than the intravenous fluid therapy. Cats experiencing signs of vasculitis and coagulopathy developed more extensive limb edema during intravenous therapy. Fevers in this outbreak were nonresponsive to antibiotics, and the surviving animals seemed to convalesce in a period of 7–10 days. Overall, 6/24 (25%) cats died with signs or were euthanized because of an extremely poor prognosis. Three additional (hospital) cats, with mild to moderate clinical signs, were euthanized to prevent potential future risk to client animals. This mortality is similar to that described for a similar epizootic in Northern California (Pedersen et al 2000).

The causative agent was first isolated from post-mortem frozen tissue of cat 15; shelter cats could not be sampled for calicivirus testing and comparison owing to quarantine instituted by the Massachusetts Bureau of Animal Health. Therefore, the definitive origin of the virus could not be determined. Calicivirus infection was definitively confirmed in hospital cases by virus isolation from oropharyngeal swabs in four cats (cats 13,

15, 17, and 18) and from EDTA blood in three cats (cats 9, 10, and 24). Of these confirmed positive cats, two were successfully sequenced, compared in a multiple-sequence alignment analysis, and were tested in a virus neutralizing antibody assay. Phylogenetic analysis using Parsimony and Bootstrap methods yielded trees with identical topology in both the cases. Parsimony analysis identified a single, most-parsimonious tree of length (=115), indicating that FCV from cats 15 and 24 clustered together and were clearly distinct from FCV-Ari and FCV-F9. The branch separating FCV isolates from cats 15 and 24 from the other taxa had a bootstrap value of >98% (1000 replicates), strongly supporting the conclusion that these two were distinct.

Virus neutralizing antibody assays showed no significant reduction in virus infectivity when FCV-F9 serum was tested against FCV-Diva isolates and FCV-Ari. This would suggest that FCV-Ari and FCV-Diva are related. However, low levels of neutralization by FCV-Ari anti-serum against FCV-Diva samples suggest that the FCV-Ari strain from California is antigenically, and by inference genetically, distinct from FCV-Diva. FCV strains vary greatly in genetic sequence, mainly through variations in the mutable C and E regions of the capsid gene. Although variable, most isolates can be placed into two major subgroups when referenced against the FCV-F9 vaccine strain (Radford et al 1997, Radford et al 2001). One group will be more than 90% related, while the second group will be less than 80% related. Serologic differences have been linked mainly to the E region of the capsid gene (Geissler et al 2002). Although FCV-Ari and FCV-Diva are similar in their high virulence, they differ genetically in the E region and even belong to different subgroups. FCV-Ari is more FCV-F9-like, while FCV-Diva is significantly different. Although the two viruses differ in virus neutralization patterns and genetic sequence in the hypervariable E region, a more thorough study is required to determine the genetic origin of their virulence, which may be shared.

In conclusion, we report on another highly virulent strain of FCV (FCV-Diva), against which present vaccine strains are ineffective. Although genetically distinct, these highly virulent strains cause identical disease under very similar circumstances. Crowded, high-stress environments, such as catteries and shelters, seem to select for these highly contagious and virulent pathogens. Once selected, these strains spread rapidly by cat-to-cat and cat-to-human-to-cat contacts, regardless of the age, overall health, and vaccination status of the affected felines. Veterinarians must be aware that FCV infections have the potential to cause severe disease and must take measures to treat all symptomatic animals on an outpatient basis. When visiting shelters and examining sick animals, appropriate decontamination measures must be taken to avoid transporting the virus back to cats in other households or hospitals. The type of disinfectant used in an FCV outbreak is important, because the virus has no envelope and is, therefore, not destroyed by substances, such as quaternary ammonium compounds (Eleraky et al 2002). Chlorine dioxide and potassium peroxymonosulfate are highly effective against FCV, as is 3% sodium hypochlorite in tap water (Eleraky et al 2002). Quarantine measures and client education are essential in limiting the spread of these highly virulent FCV strains to the larger populations. Fortunately, this outbreak, as well as the earlier one caused by FCV-Ari, tended to be self-limiting with reasonable control measures. Therefore, these mini-epizootics undoubtedly involve a number of co-factors. These outbreaks identify the need for further research to uncover the molecular basis for increased viral pathogenicity of natural FCV strains and to aid in the development of vaccines that provide consistent protective immunity.

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