Feline infectious peritonitis virus-associated rhinitis in a cat

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Abstract

Case Series Summary

This report describes a cat with initial respiratory signs prior to developing fulminant feline infectious peritonitis (FIP) after adoption from an animal shelter. Histologic examination of the tissues revealed typical lesions associated with FIP in the lung, liver, large intestine, and small intestine. Histological examination of the nasal cavity revealed pyogranulomatous rhinitis. Immunohistochemistry with FIPV 3-70 targeting FIP antigen in macrophages confirmed FIP and molecular analysis identified a spike protein mutation (R793S) consistent with the presence of an FIPV. Pathological changes, immunolabeling and molecular analysis provide evidence that respiratory infection by feline coronavirus is part of the spectrum of FIP-associated disease.

Relevance and novel information

This report highlights nasal pathology associated with FIP through a combination of histopathology, immunohistochemistry, and molecular characterization of the virus. Our work supports a little appreciated role of the respiratory tract in FIP.

Introduction

Coronaviruses have been implicated in respiratory and gastrointestinal disease in many animal species [1]. In cats, feline coronavirus (FCoV) consists of two biotypes, commonly referred to as feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV) [2, 3]. FECV is generally considered to be associated with a mild, self-limiting gastrointestinal infection, but can cause mild respiratory signs [4]. Initial infection with FCoV is thought to occur by oronasal exposure to the virus in fecal material or fecal contaminated fomites [4]. In some FCoV- infected cats, mutation of the viral genome leads to an alteration in the host cell tropism of FCoV from enterocytes to macrophages, leading to the systemic infection known as feline infectious peritonitis (FIP) [2, 3, 5, 6]. Feline infectious peritonitis (FIP) is one of the most important infectious diseases affecting domestic and wild cat populations. There are multiple presentations of FIP; effusive (wet), non-effusive (dry), and a mixed form, which can include a combination of effusion and pyogranulomatous lesions [4, 7]. Clinical signs can be variable in all presentations and comprise of anorexia, pyrexia, lethargy, diarrhea, and weight loss [4, 7, 8]. Early studies suggested that upper respiratory infections characterized by conjunctivitis or rhinitis preceded the development of FIP [7, 9, 10], although FCoV is not routinely considered a common respiratory pathogen in cats [4].

This case report describes a cat with respiratory signs prior to developing systemic FIP. The pathological changes with marked FIPV immunolabeling supports previous work suggest that respiratory infection is part of the spectrum of FIP-associated disease.

Case Description

An 8 week old spayed female domestic short hair cat was adopted from a shelter into a single cat home. The cat received three feline viral rhinotracheitis, calicivirus, and panleukopenia vaccinations and was not rabies vaccinated. The cat was previously dewormed and was FELV negative. The diet consisted of a commercial dry and canned food.

At 10 weeks of age, the cat presented to the general practitioner for a wellness visit. On physical examination, a mild mucopurulent discharge was noted in both eyes, and the cat was sneezing. The remainder of the examination was normal. A fecal centrifugation was performed on formed feces and no ova or parasites were detected. Tobramycin eye drops was dispensed, and one drop was administered to both eyes every twelve hours for 10 days. In addition, Lysine (Enisyl F) was dispensed and one pump was administered by mouth every 12 hours for 10 days, and Fenbendazole suspension was administered orally at 0.6 ml once daily for 5 days.

At 14 weeks of age, the cat returned to the general practitioner for evaluation of sneezing, diarrhea, a poor appetite, and a distended abdomen. On physical examination, epaxial muscle loss was noted and the abdominal distention was confirmed. A fecal centrifugation was performed on soft feces and no ova or parasites were detected. Metronidazole suspension was dispensed and 20mg was administered orally twice daily for 10 days and 25mg (0.4ml) Amoxicillin clavulanic acid 62.5mg/ml (Clavamox drops: Zoetis) was administered orally for 10 days.

The cat returned to the general practitioner two days later with hyporexia, polydipsia, lethargy, reluctance to ambulate. A moderately distended abdomen was noted on physical examination. Cytological analysis of fluid obtained via abdominocentesis revealed a high protein non-septic exudate with mixed inflammation (Table 1). A complete blood count (CBC) and chemistry panel (Tables 2-4) was performed. The complete blood count (CBC) with electronic differential revealed a slight anemia and neutrophilia (Table 2). The manual differential revealed abnormal red blood cell (RBC) and white blood cell (WBC) morphology (Table 3A-C). The RBC evaluation revealed occasional poikilocytosis, anisocytosis, microcytosis, polychromasia, with Heinz bodies (Table 3B). The WBC showed the following occasional changes in neutrophils; basophilic, vacuolated and foamy cytoplasm, swollen nucleus, indented nuclear margins and Döhle bodies (Table 3C). The chemistry panel showed mild elevations in total protein, glucose, and moderate elevations of globulin and triglycerides. A decrease in alanine amino transferase,

alkaline phosphatase, creatine kinase, and albumin were noted (Table 4). A tentative diagnosis of FIP was made based on clinical signs, fluid analysis, and laboratory abnormalities. Prednisolone oral solution 3 mg/ml was dispensed and 1.5 mg (0.5 ml) was administered twice daily.

At 15 weeks of age, the cat returned to the general practitioner for reevaluation. A feline coronavirus (FCoV) ELISA was performed and found to be positive at 1.228 (greater than 1.20 = positive, less than 0.90 = negative). Lactated ringer's fluids were administered 50 ml subcutaneously. Vitamin B12 injections were dispensed and 0.1 ml was administered subcutaneously weekly. A week later, the cat presented for an abdominocentesis prior to starting feline omega interferon. A total of 215 ml of straw-colored viscous fluid was removed. The cat's appetite was decreased. Mirtazapine 7.5 mg/ml suspension was dispensed and 1.88 mg was administered (0.25 ml) every 48 – 72 hours to increase appetite.

At 17 weeks of age, the patient presented in respiratory distress and was laterally recumbent. Physical examination revealed an afebrile temperature of 99.4°F (37.4°C), heart rate of 140 beats per min, a respiratory rate of 42 breaths per min, pale mucous membranes, and capillary refill time (CRT) > 2 sec. The cat had a negative menace, pupillary light response was present, moderately dyspneic with normal bronchovesicular sounds, and slightly bradycardic with weak femoral pulses. The cat's abdomen was distended with a fluid wave. The clinical assessment is consistent with FIP. Due to poor prognosis the client elected euthanasia and necropsy was performed. No pleural effusion was present. A with a subset of tissues fixed in 10% neutral buffered formalin, sectioned and stained with hematoxylin and eosin, and analyzed microscopically. Immunohistochemistry for the detection of FCoV was performed using monoclonal antibody FIPV 3-70 (1:1000) (Custom Monoclonals), AP-Anti-Mouse IgG and Bond Polymer Refine Red Detection (Leica Microsystems). Positive controls consisted of FIPV infected liver and negative control was isotype matched.

Histologic examination (Figure 1) revealed a diffuse pyogranulomatous rhinitis that partially obliterated the ethmoturbinates. Additional feline infectious peritonitis virus-associated lesions were found in lung,

liver, large intestine, mesenteric lymph node, and small intestine. No lesions were present in the brain or kidneys.

Respiratory samples were collected using two sterile flocked swabs from the conjunctiva, nasal cavity, and the oropharynx. The samples were pooled and were sent to the Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine for the feline respiratory panel and the pooled sample was found to be low positive for *Mycoplasma felis* (Table 5*)*. All other respiratory agents screened in panel were negative within the pooled sample (Table 5).

PCR and Sanger sequencing were performed on a subset of tissues as described [11]. Briefly, 25 µl reverse transcription PCRs were performed with qScript XLT 1-Step RT PCR kit (Quantbio). PCR conditions were 20 mins at 50°C, 3 mins at 95°C and 40 cycles of 10 s at 95°C, 20 s at 55°C, 40 s at 72°C, then 10 mins at 72°C [11]. Molecular analysis of the viral spike protein showed an amino acid change from an arginine to a serine (R-S) at an essential P1 cleavage activation position at residue 793 (Figure 2) [12]. This mutation was observed in all samples, i.e. lung, liver, mesenteric lymph node, small intestine and large intestine.

Discussion

This case report illustrates a cat with pathology in the nasal cavity with immunohistochemistry staining of macrophages associated with FIP. The cat initially presented to the veterinarian for a wellness visit, where an upper respiratory tract infection was primarily observed and FIP was not considered a differential at the time. The respiratory signs resolved; however, clinical signs associated with FIP subsequently surfaced and ultimately caused the deterioration and euthanasia of the cat.

FIP has previously been observed in the nasal and oral cavities [9]. In a non-effusive case of FIP, small granulomas were observed on the frenulum of the tongue [9]. In an FIP case coinfected with toxoplasmosis, histological examination of the nasal submucosa showed a severe diffuse lymphoplasmacytic rhinitis with

perivascular aggregations of neutrophils and macrophages which immunochemistry revealed FCoV antigen within macrophages [13]. In an experimental study, when the virus was aerosolized, lesions were frequently found in the nasal turbinates, lungs, pleura and tracheobronchial lymph nodes [14].

The functionally relevant amino acid change (R793S) was found through the molecular analysis of the viral spike protein in all tissues in this cat (Figure 2). This mutation (R793S), an alteration from a charged to an uncharged amino acid, is predicted to eliminate the ability for furin to proteolytically process the S1/S2 cleavage activation site which can ultimately effect viral entry [12]. FIP has been also associated with other mutations in the spike gene (1058) [15] and the 3c gene [16, 17]. Additional sequencing of these regions is summarized in Table 6 and showed an M1058L conversion and truncated 3c as expected.

The observations and findings in this case suggest that the respiratory system is a potential route of transmission for feline coronavirus, given the significant rhinitis present in this young cat's nasal cavity. Other coronavirus such as mouse hepatitis virus (MHV), infectious bronchitis virus (IBV) in chickens, and porcine respiratory coronavirus transmit via the respiratory route prior to disseminating or targeting a specific organ system. The respiratory disease early in the course of disease in this cat may also be an early indicator of FIP. It is unlikely that an underlying immunodeficiency is the cause of the respiratory disease as it resolved and did not continue as other conditions developed. FCoV present in the nasal cavity may also suggest a role of significant hematogenous spread of the virus as the virus produces a vasculitis and the nasal cavity is extremely vascular. Examination of the nasal cavities of FIP cats at necropsy could be performed to further support the regions' role with FIP. Currently with the historical information, experimental studies and this case study, we confirm the nasal cavity is involved in the pathogenesis of FIP; however, to further elucidate the precise mechanism additional experiments are necessary. In addition, continued molecular characterization in conjunction with histological and immunohistochemical techniques in FIP pathogenesis is essential in order to uncover patterns between the virus and the disease to create methods to combat infections by earlier diagnosis in cats.

Conclusions

This case report describes a kitten with FIP where the nasal cavity was extensively involved and respiratory signs were observed early on prior to clinical signs associated with FIP. A respiratory panel was performed on pooled respiratory swabs to exclude any common pathogens associated with feline respiratory disease complex (FRDC). While the swabs were low positive for *Mycoplasma felis*, no other FRDC pathogens were detected such as feline herpes virus. While FIP is a systemic disease once it mutates, FCoV is not routinely thought of as a respiratory pathogen. This case suggests that the respiratory tract may be a mode of transmission for feline coronavirus.

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Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

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Ethical Approval

This study involved the use of client-owned animal(s) only and followed internationally recognized high standards ('best practice') of individual veterinary clinical patient care. Ethical Approval from a committee was not therefore needed.

Informed Consent

Informed Consent (either verbal or written) was obtained from the owner or legal guardian of all animal(s) described in this study for the procedure(s) undertaken.

Table 1. Summary of the fluid analysis performed at 14 weeks of age

Source of Fluid	Abdominal fluid
Color of Fluid	Light Reddish – Tan
Turbidity	Moderate
Viscosity	Slight
TNNC (Coulter)	16.2 (X 10^3/mm^3)
Specific Gravity	1.030
Total Protein	5.8 (g/dL)

Table 2. Summary of the complete blood counts (CBC) – electronic differential performed at 14 weeks of age

	Electronic Differential
Hematocrit	**23.5** (26.0 – 47.0 %)
Hemoglobin	**7.5** (RI 8.5 – 15.3 g/dL)
RBC	5.82 (4.60 – 10.20 10^6/uL)
MCV	40.3 (RI 39.0 – 54.0 fL)
MCH	12.9 (RI 11.8 – 18.0 pg)
MCHC	32.1 (RI 29.0 – 36.0 g/dL)
RDW-CV	18.1 (RI 16.0 – 23.0%)
Platelet	237 (RI 100 – 518 10^3/uL)
MPV	13.4 (RI 9.9 – 16.3 fL)
WBC	16.45 (RI 5.50 – 19. 50 10^3/uL)
Neutrophils	**13.52** (RI 3.12 – 12.58 10^3/uL)
Lymphocyte	2.43 (RI 0.73 – 7.86 10^3/uL)
Monocyte	0.28 (RI 0.07 – 1.36 10^3/uL)
Eosinophils	0.21 (RI 0.06 – 1.93 10^3/uL)
Basophils	0.01 (RI 0.00 – 0.12 10^3/uL)
% Neutrophils	**82.2** (RI 38.0 – 80.0%)
% Lymphocyte	14.8 (RI 12.0 – 45.0 %)
% Monocyte	1.7 (RI 1.0 – 8.0%)
% Eosinophils	1.2 (RI 1.0 – 11%)
% Basophils	0.1 (RI 0.0 – 1.2%)

Table 3. Summary of the complete blood counts (CBC) – manual differential performed at 14 weeks of age

A)

	Manual Differential
WBC	16.45 (RI 5.50 – 19.50 10^3/ul)
Plasma Color from HCT	Trace Lipemic
Total Plasma Solids	7.4 (RI 6.0 – 7.5 g/dL)
PCV (Spun HCT)	**23** (RI 24.0 – 45.0 %)
Platelet Estimate/HPF	Appears normal (RI 6 – 36/HPF)
Neutrophils	**14642** (RI 2500 – 12500)
Bands	0 (RI 0 – 300)
Trans. Bands	0 (RI 0 – 1)
Lymphocyte	**987** (RI 1500 – 7000)
Monocyte	0 (RI 0 – 800)
Eosinophils	329 (RI 0 – 1500)
Basophils	0 (RI (0 – 300)
Atypical Lymphocytes	0 Lymphs with cleaved nucleus (RI 0 – 1)
Reactive Lymphocytes	**494** Large Lymphs with basophilic cytoplasm (RI 0 – 1)
Lymphoblasts	0 (RI 0 – 1)
Metamyelocytes	0 (RI 0 – 1)
Myelocytes	0 (RI 0 – 1)
Other Cells	0 (RI 0 – 1)
NRBC	0 (RI 0 – 2/100WBC)

B) RBC Morphology – Abnormal

Size – Anisocytosis	Occasional 1+
– Microcytic	Occasional
Color – Polychromasia	Rare
Shape – Poikilocytosis	Occasional
Shape – Heinz Bodies	Occasional

C) WBC Morphology – Abnormal

Basophilic Cytoplasm	Occasional neutrophil
Döhle Bodies	Occasional neutrophil
Foamy Cytoplasm	Few neutrophil
Ind Nuc Margins	Occasional neutrophil
Swollen Nucleus	Few – Moderate neutrophil
Vacuolated Cytoplasm	Rare lymph/Occasional neutrophil
Ruptured WBC's	Occasional – few

Table 4. Summary of the chemistry profile performed at 14 weeks of age

Test	
Chloride	116 (RI 107 – 121 meg/L)
Sodium (Na^+)	148 (RI 143 – 162 meg/L)
Potassium (K^+)	4.5 (RI 4.1 – 5.9 meg/L)
NA^+/K^+ Ratio	32.8
Carbon Dioxide	23.3 mEg/l
Cholesterol	118 (RI 63 – 132 mg/dl)
Triglycerides	**68** (RI 15 – 49 mg/dl)
ALT	**6** (RI 11 – 35 U/L)
GGT	4 (U/L)
AST	25 (RI 9 – 34 U/L)
ALP	**18** (39 – 124 U/L)
Total Bilirubin	0.20 (RI 0.10 – 0.80 mg/dl)
Creatine Kinase	**102** (RI 185 – 894 U/L)
Glucose	**117** (RI 59 – 102 mg/dL)
Phosphorus	7.9 (RI 6.9 – 9.5 mg/dL)
Total Protein	**7.3** (RI 5.4 – 6.8 g/dL)
Albumin	**1.7** (RI 2.5 – 3.6 g/dL)
Globulin	**5.6** (RI 2.9 – 3.2 g/dL)
A/G Ratio	0.3
Calcium	8.9 (RI 8.9 – 10.9 mg/dL)
BUN	24 (RI 19 – 34 mg/dL)
Creatinine	0.55 (RI 0.40 – 0.90 mg/dL)
BUN/Creatinine Ratio	43.6

Table 5. Summary of the respiratory panel performed at 14 weeks of age

Respiratory Agent	Result	Methodology
Bordetella	Not Detected	PCR
Chlamydia	Not Detected	PCR
Influenza Virus	Not Detected	PCR
Mycoplasma cynos	Not Detected	PCR
Mycoplasma felis	**Low Positive**	PCR
Pneumovirus	Not Detected	PCR
Streptococcus zooepidemicus	Not Detected	PCR
Calicivirus	No Virus Isolated	Virus Isolation
Herpesvirus	No Virus Isolated	Virus Isolation

Table 6. Summary of the molecular analysis performed on the viral genome in various samples

	spike S1/S2 sequence	spike pos. 1058	3c gene
Brain	ND*	ND*	ND*
Kidney	SRRSR**S**S	L	Truncated
Large intestine	SRRSR**S**S	L	Truncated
Liver	SRRSR**S**S	L	Truncated
Lung	SRRSR**S**S	L	Truncated
Mesenteric LN	SRRSRSS	L	Truncated

* = not determined

= individual sequences were premature stop codon and frameshift

Figure Legends

Figure 1. Nasal Histopathology (a) Ethmoturbinates are replaced by extensive inflammation (low magnification). (b) The region of the cribriform plate is obscured by dense sheets of pyogranulomatous inflammation (low magnification). (c) High magnification illustrates the replacement of the cribriform plate by pyogranulomatous inflammation. (d) FIP immunohistochemistry reveals extensive immunolabeling in macrophages within the inflammation at the level of the cribriform plate (low magnification).

Figure 2. **Molecular analysis of the spike gene**. A 156 base pair region of the feline coronavirus spike gene is shown and represented in single amino acid code, with variant residues and amino acid positions noted. The activation site between the S1 and S2 domains (S1/S2) is indicated and boxed amino acid positions are based on that for FCoV RM spike (Genbank accession # ACT10854.1) as a prototype sequence. Sequences were analyzed using Geneious Prime 2020.05.

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