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Clinical Article

CONCURRENT INFECTION OF FELINE INFECTIOUS PERITONITIS AND FELINE LEUKEMIA VIRUS IN A MALE CAT

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SUMMARY

A 3.5-year-old male domestic short-haired cat brought to Teaching Veterinary Clinical Complex, Madras Veterinary College, with a history of anorexia and respiratory distress, had both pleural and peritoneal effusion on radiography. Clinical signs were suspected of feline infectious peritonitis (FIP). The collected effusion sample from the affected cat tested positive for the Rivalta test, and fine needle aspiration cytology of the thoracic cavity suggested lymphoid leukaemia. The samples were positive for both Feline Infectious Peritonitis Virus (FIPV) and Feline Leukemia Virus (FeLV) in Polymerase Chain Reaction (PCR). This is a clinical case documented for concurrent infection of Feline Infectious Peritonitis and Feline Leukemia Virus in a domestic cat at Madras Veterinary College, Chennai.

Keywords: Cats, Effusion, FeLV, FIP

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Feline infectious peritonitis (FIP) is an infectious, life-threatening disease that usually affects young cats. However, cats of all ages are vulnerable. Concurrent infection with feline leukaemia virus or feline immunodeficiency virus may aggravate the course of FIP. The Feline Corona viruses (FCoV) are positive sense-enveloped RNA viruses prevalent in domestic and wild Felidae, especially in animal shelters worldwide (Sherding, 2006). FCoV is classified into two biotypes based on pathogenicity: Feline Enteric Corona virus (FECV) and Feline Infectious Peritonitis Virus (FIPV), which are further classified into type I and type II serotypes. The feline leukaemia virus (FeLV) is a naturally occurring and widespread retro virus among domestic cats belonging to the genus Gamma retro virus (Watanabe *et al.*, 2013). Feline leukaemia virus and feline immunodeficiency virus are common causes of viral-induced immunodeficiency in cats. There are four subtypes, i.e., FeLV-A, FeLV-B, FeLV-C and FeLV-T. Subtypes of FeLV are defined based on variations in the env gene sequence, which also influences their pathogenicity. Each subtype is reported to enter cells through a distinct receptor (Brojatsch *et al.*, 1992). The FeLV-T infection has been particularly linked to immunodeficiency in cats because of its T-cell tropism (Lutz *et al.*, 2009). Many authors have reported individual infections of cats with FCoV and FeLV. Super-infection of FCoV-infected cats with feline leukaemia virus (FeLV) or feline immunodeficiency virus (FIV) has been reported as a factor inducing FIP (Soma and Ishii, 2004). Oguzoglu *et al.* (2010) reported the prevalence of concurrent FCoV and FeLV infection in cats in Turkey. However, there has been no report of co-infection in cats with FCoV and FeLV in India. This study reports the condition of cats with a co-infection of FCoV and FeLV.

A 3.5 year-old male DSH cat with a history of anorexia and respiratory distress with both pleural and peritoneal effusion on radiograph was brought to the critical care unit of Teaching Veterinary Clinical Complex, Madras Veterinary College. Whole blood samples were collected in EDTA and clot activator vials for haematology (Mindray, BC Vet 2800), and serum biochemistry analysis (A15 Biosystems, Inc). The whole blood was also subjected to a PCR study. The blood smear was stained by routine Leishman Giemsa stain for examination of blood parasites and to study the blood picture. The Pleural and Peritoneal fluid was removed out by thoracocentesis and abdominocentesis, respectively. The fluid samples were collected aseptically in 15 ml centrifuge tubes for cytology and PCR studies.

Rivalta's Test: In a 10 ml transparent reagent tube, approximately 7–8 ml of distilled water and 1 drop of 98% glacial acetic acid were added and mixed thoroughly. A drop of collected effusion fluid was carefully layered on the surface of the mixed solution. If the drop disappears and the solution remains clear, Rivalta's test is negative. If the drop retains its shape, stays attached to the surface or slowly floats down to the bottom of the tube (drop- or jelly-

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fish-like), Rivalta's test is defined as positive (Fischer *et al.*, 2012).

Reverse Transcriptase-Polymerase Chain reaction : The pleural fluid, peritoneal fluid and blood were subjected to RNA isolation and cDNA synthesis, followed by a Polymerase chain reaction. Briefly, the sample was taken in a 1.5 mL microfuge tube. To this, 1.5 mL of RNA isoPlus (Takara, Japan) was added, mixed well and left at room temperature for 5 min, centrifuged at 12,000 RPM for 5 min at 4°C, 200 µL of chloroform was added to the supernatant, mixed well by inversion, and incubated at room temperature for 10 min. The mixture was centrifuged at 12,000 RPM for 15 min at 4°C. The upper aqueous phase was collected carefully and transferred into a fresh 1.5 ml microfuge tube (without disturbing the buffy coat). An equal volume of ice-cold isopropanol was added to the supernatant, mixed well and incubated for 10 min at room temperature. Centrifuged at 12,000 RPM for 10 min at 4°C for precipitation of RNA as a pellet. The supernatant was discarded, and pelleted RNA was washed with 1 ml of 75% ice-cold ethanol by centrifugation at about 10000 RPM for 10 min at 4°C. The supernatant was discarded, and the RNA pellet was air-dried until the ethanol had been fully vaporized. The air-dried RNA pellet was dissolved in 20 µL of Nuclease Free Water (NFW). Further, RNA concentration and purity were evaluated Spectrophotometrically using Nanodrop spectrophotometer one C (Thermo Fisher).

cDNA was synthesized from the extracted RNA using the cDNA synthesis kit: Prime Script RT reagent kit as per the manufacturer's instructions. To amplify the cDNA, a 20 µL PCR reaction mix containing 2µL5x prime script RT buffer; 1µL10 mM dNTP; 1 µLrandom hexamers; 6 µLRNA template; 1µLreverse transcriptase enzyme; nuclease-free water to make the 20 µL reaction volume. The condition was set as initial incubation at 37°C for 15 minutes and 85°C for 5 seconds, followed by incubation at 4°C. Finally, the polymerase chain reaction was carried out using the following oligonucleotide primers, which were designed from the published sequence of N-gene for FIP and gag polyprotein (gag) gene for FeLV to amplify the target by polymerase chain reaction. FP-5'-GAGGAATT ACGGGTCATC-3' and RP-5' CATTGCCAAATCAAA TCTAAAC-3' primers for feline coronavirus (FCoV), and FP-5' AACAGCAGAAGTTTCAAGGCC-3' and RP-5'-TTATAGCAGAAAGCGCGCG-3'primers for feline leukaemia virus (FeLV) were used. Cyclic conditions for amplifying the respective viruses are given in the table 1.

Amplified products were subjected to 2% agarose gel electrophoresis and visualized under the Gel documentation unit (BioRad, USA).

Physical examination of the peritoneal fluid revealed watery, slimy, and red-tinged (Fig. 1A), whereas the pleural fluid was slightly yellowish and transparent (Fig. 1B). Both fluids were viscous and stringy. It tested positive for Rivalta's test (Fig. 2). The fluid was cellular with high protein and triglycerides (1800 mg/dl) levels.

Cytology revealed increased number of leukocytes, mainly of immature lymphocytes (lymphoblast cells) suggestive of lymphoid leukaemia and few neutrophils (Fig 3a and b) RBCs revealed anisocytosis, acanthocytosis and poikilocytosis and scant number of RBCs per field. Haemogram showed severe non-regenerative anaemic change and moderate thrombocytopenia (Table 2).

The presence of FCoV and FeLV in this case was confirmed by positive RT-PCR amplification of 184bp N-gene fragment (Fig. 4) and 131bp gag polyprotein (gag) gene fragment (Fig. 5), respectively in pleural effusion and peritoneal fluid.

The haematology and biochemical results in this case concur with the findings of Paltrinieri *et al.* (2001). Complete blood (cell) count (CBC) and serum biochemical profile abnormalities of cats infected with FIP included non-regenerative anaemia, hypoalbuminemia and hyperglobulinemia with decreased albumin/globulin (A:G) ratio. The present findings of the case supported these results. Anaemia is a major non-neoplastic complication that occurs in a majority of symptomatic FeLV-infected cats (Gleich and Hartmann, 2009). Shelton and Linenberger (1995) reported that a pure red cell aplasia, a severe non-regenerative anemia associated with erythrocyte macrocytosis and depletion of erythroid precursors in the bone marrow are the characteristic features of FeLV infection. Further explained that is due to FeLV binds to and interferes with a heme exporter protein, which results in subsequent heme toxicosis to the developing erythrocyte. Similarly in this case severe non-regenerative anemia, macrocytosis, lymphoblastosis were observed.

FIP cases were found to be FeLV-associated, and FeLV was found in 50% of FIP-affected cats (Cotter *et al.*, 1973; Pedersen *et al.*, 1977). In experimental studies, suspected latent FIPV immune carrier cats subsequently

Table 1. Cyclic condition for amplifying the feline corona virus and feline leukemia virus

Etiological agent	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No of cycles
FCoV FeLV	95°C for 5 min	95°C for 30 sec	55°C for 30 sec	72°C for 45 sec	72°C for 10 min	36

Table 2. Haematologicalparameters of a whole blood sample collected from affected cat

Parameters	Complete Blood Count (CBC) Values	
	Presenting value	Reference range
Haemoglobin (g/dl)	3.6	9.8-15.4
PCV%	7	30-45
RBC(g/dl)	1.18	5-10
WBC (103/µl)	145	5.5-19.5
Platelets (103/µl)	111	300-800
Neutrophils%	18	45-64
Lymphocytes%	80	27-36
Monocytes%	2	0-5
Eosinophils%	0	0-4
Basophils%	0	0-1

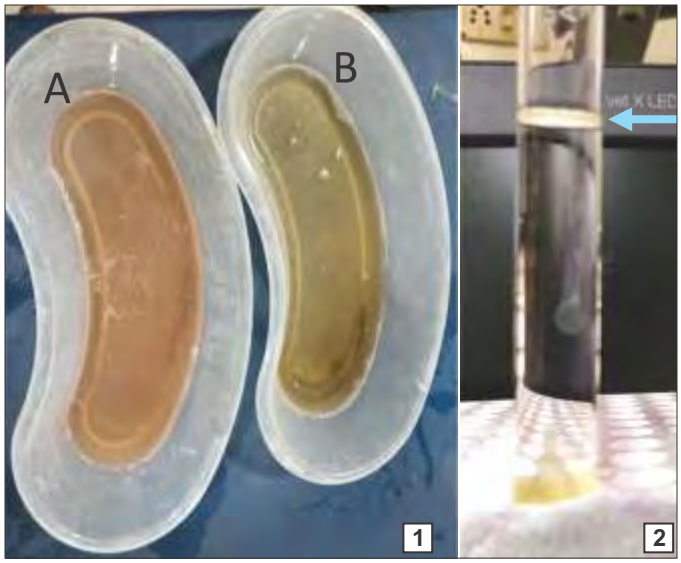
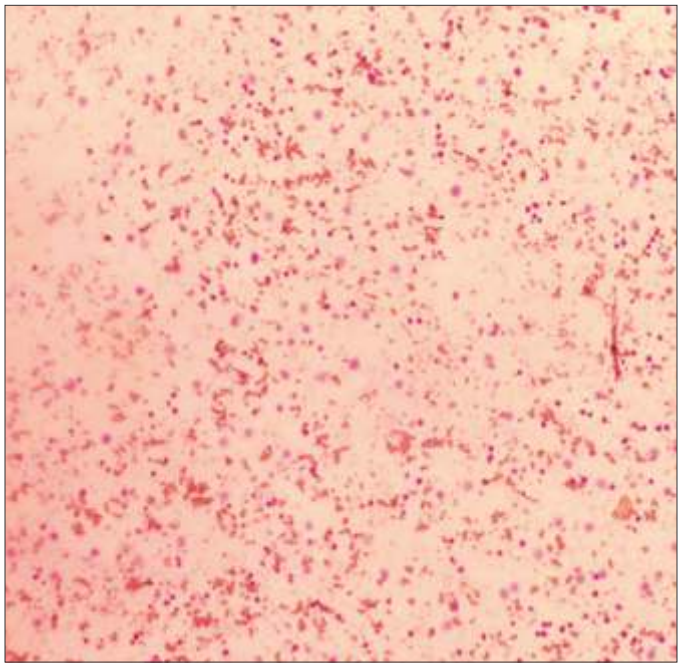


Fig. 1a. Watery, slimy and red tinged peritoneal fluid and 1B. Transparent, pale yellowish pleural fluid

Fig. 2. Rivalta's test–Retained jelly-fish-like drop attached to the surface and floats down to the bottom of the tube



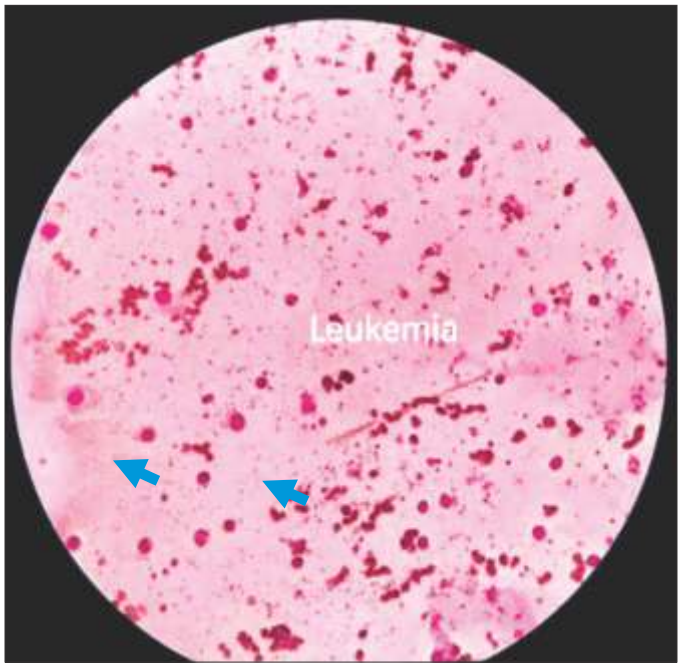


Fig. 3a & b. Cytology revealed increased number of leukocytes, mainly of immature lymphocytes (lymphoblast cells-arrow) suggestive of lymphoid leukaemia and few neutrophils

infected with FeLV often developed active FIP disease. FeLV has long been considered a common concurrent disease and predisposing factor for naturally occurring FIP in cats (Pedersen, 2022). Mutations on the FCoV genome caused by immunosuppressive events, gender, age factors and multi-cat environment might result in the generation of virulent variants in the presence of FCoV antibodies in cats, causing FIP cats.

The Rivalta test was considered successful if a precipitate formed and stayed stuck to the surface, maintained its shape (jelly fish like appearance), or gradually drifted to the bottom of the fluid as seen in our case. The Rivalta test was deemed unsuccessful if the effusion fluid drop dispersed and the solution remained clear (Fischer *et al.*, 2012). The Rivalta's test had a high positive predictive value (86%) and a very high negative predictive value for FIP (96%) in a study in which cats that presented with effusion were investigated (Hartmann *et al.*, 2003). Positive Rivalta's test results can occur in cats with bacterial peritonitis or lymphoma (Fischer *et al.*, 2012).

To confirm the presence of FCoV and FeLV in this case, positive RT-PCR amplification of N-gene and gag

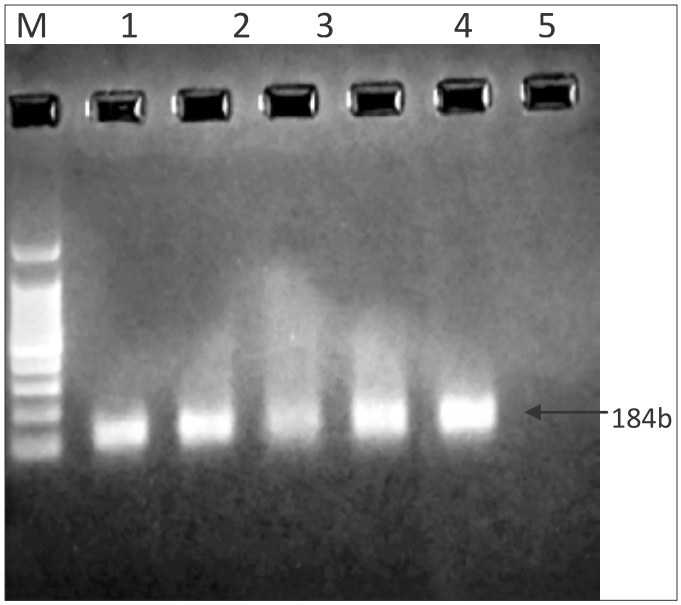


Fig. 4. Amplified product of FCoV, M-100bp Ladder, 1,2-Pleural effusion, 3,4-Peritoneal fluid, 5-positive control, 6-Negative control

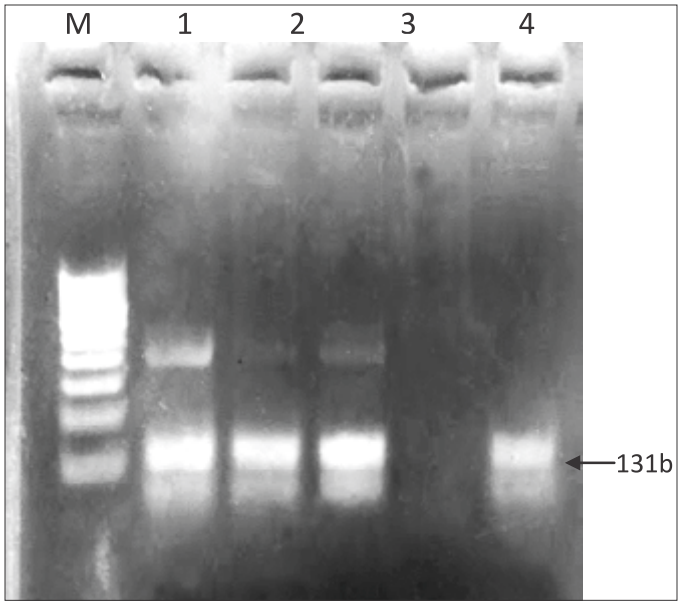


Fig. 5. Amplified product of FeLV, M-100bp Ladder, 1-Pleural effusion, 2-Peritoneal fluid, 3-blood, 4-Negative control, 5-Positive control

Table 3. Biochemical parameters of serum sample collected from affected cat

Parameters	Biochemistry Values			
	Reference range	Serum	Pleural effusion	Abdominal effusion
Blood Urea Nitrogen mg/dl	14-36	49.86	-	-
Creatinine mg/dl	0.6-2.4	1.01	-	-
Total Protein g/L	8.2-10.8	7.4	4	4.2
Albumin g/L	2.4-8.2	1.7	0.9	1
Alanine Transaminase IU/L	8.3-53	46	-	-
Alkaline Phosphatase IU/L	9.2-40	16	-	-
Gamma Glutamyl Transferase IU/L	1.8-12	1	-	-
Total Bilirubin mg/dl	1.2-2.1	0.51	0.77	1.09
Direct Bilirubin mg/dl	0.04-0.16	0.42	0.66	0.59
Calcium mg/dl	8.2-10.8	8.19	-	-
Phosphorus mg/dl	2.4-8.2	8.19	-	-
Glucose mg/dl	80-120	109	96	99
Cholesterol mg/dl	120-130	119	66	60

polyprotein (gag) gene, respectively, supported our findings of concurrent infection. Oguzoglu *et al.* (2010) reported the prevalence of concurrent FCoV and FeLV infection in cats in Turkey. Simons *et al.* (2005) found that RT-PCR is one of the definitive diagnostic tools for FIP owing to its low rate of false-positive results (high specificity). In India, the concurrent infection of cat with FCoV and FeLV has no earlier reference other than this report.

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