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Research Article

PLEURAL EFFUSION ASSOCIATED WITH FELINE INFECTIOUS PERITONITIS IN A KITTEN: MOLECULAR AND HISTOPATHOLOGICAL INVESTIGATION

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ABSTRACT. This study was aimed at investigating the molecular typing of coronavirus and histopathological findings of the lungs in a kitten with feline infectious peritonitis. A stray kitten, which was in respiratory distress and had not responded to a one-week course of antibiotic treatment, was referred to the Animal Hospital of Faculty of Veterinary Medicine at Atatürk University. In the physical examination, acute respiratory failure developed and did not respond emergency therapeutic intervention. Molecular and histopathological examinations were performed. Feline coronavirus type I was determined by sequence analysis of the lung and pleural fluid samples. Macroscopic findings revealed hemorrhagic fluid in the chest cavity. Fibrinoid necrosis, desquamation, edema and lymphoplasmacytic cell infiltrations were observed in the histopathological examination of the lungs. Thus, feline coronavirus type I was determined to cause severe lesions with edema, necrosis and lymphoplasmacytic cell infiltrations in the lungs and respiratory distress.

Keywords: *Feline infectious peritonitis, molecular and histopathological examination, pleural effusion.*

INTRODUCTION

Feline coronaviruses widely infect cats, especially cats in multicat households and animal shelters [1]. The significant percentage of cat population may be persistently infected with FCoVs, but generally transient infection occurs [2]. However, immune mediated lethal disease in feline infectious peritonitis develops in about 5% feline cases infected with FCoV with the high incidence in kittens and to some extent, in the old cats [3,4].

FCoVs are separated into two biotypes such as feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV) [5].

FECV usually causes subclinical infection in cats. It causes mild to severe diarrhea in young kittens [4, 6]. However, FIPV leads to vasculitis and pyogranulomatous lesions in different organs [7].

Feline infectious peritonitis (FIP) is a fatal viral infectious disease caused by a virulent biotype of feline coronavirus (FCoV) [1, 8]. FCoVs have been determined to belong to

the family *Coronaviridae*, subfamily *Coronavirinae*, genus *Alphacoronavirus* 1 [9]. FCoVs are classified as type I, which is the original FCoV, and type II, which is closely associated with canine coronavirus [10].

The strains of FCoV can cause FIP. The disease has two clinical forms: a granulomatous (dry) form and an effusive (wet) form [1]. The clinical manifestations of FIP vary from subclinical course to fatal condition [10]. Important findings in FIP cases are pyogranulomatous lesions in several organs and the accumulation of protein-rich fluid in body cavities [3]. In cases of FIP, the accumulation of fluid in the body cavities is caused by increased vascular endothelial growth factor in plasma, which is responsible for vascular permeability [11]. Granulomatous lesions are related to neutrophil survival factors (tumor necrosis factor alpha, granulocyte colony stimulating factor and granulocyte macrophage colony stimulating factor) produced by virus-infected macrophages [12].

The antemortem diagnosis of FIP is difficult because of nonspecific clinical signs, non-pathognomonic clinicopathological changes, low and medium antibody titers [13]. FIP is diagnosed by various methods, such as immunofluorescence staining of the FCoV antigen [14] and reverse transcriptase polymerase chain reaction (RT-PCR) in tissues with lesions or in effusions [15]. This study was aimed to present physical examination findings, molecular, and histopathological findings in this case.

MATERIALS AND METHODS

The animal material

The animal material of this study was a kitten referred to the Animal Hospital of Faculty of Veterinary Medicine at Atatürk University, because of anorexia, weakness, and nonresponse to treatment for 1 week. In the physical examination, acute respiratory failure was observed and did not respond emergency therapeutic intervention. Then, histopathological and molecular examination was performed.

Pathological Analyses

For histopathological examination, the lungs were fixed in 10% buffered formaldehyde solution. After routine processes, the sections of the lungs were stained with hematoxylin-eosin (H&E). The sections were examined and were photographed under a light microscope.

Molecular analyses

RNA extraction and Reverse transcriptase polymerase chain reaction (RT-PCR)

Virus RNA was searched in the lung tissue and effusion fluid sent to virology laboratory for virology analysis. Samples were extracted before PCR. RNA was extracted from samples using the Virus Nucleic Acids Isolation Kit (GeneDirex, USA) according to the manufacturer's recommendations. Virus RNA was converted to cDNA by reverse transcription and PCR was performed according to the primers and optimization conditions used by Baydar et al. [16]. For this purpose, complementary DNA (cDNA) synthesis was carried out using a RevertAid first-strand cDNA synthesis kit (Thermo Fisher Scientific, USA) as described in manufacturer's protocol. Consequently, the

formation of PCR product in the expected size was analyzed using DNA gel electrophoresis

Sequencing and Phylogenetic Analyses

Sequence analysis was done after PCR. Sequencing of the amplicons of sample was conducted via service procurement (BM labosis, Ankara). The raw sequencing data were aligned using BioEdit version 7.0.5 [17] with the Clustal W algorithm. The phylogenetic map of the aligned sequences was established using MEGA version 6.0 [18].

RESULTS AND DISCUSSION

When this case arrived at the Animal Hospital of Atatürk University, physical examination findings were weakness, loss of weight, paleness of mucous membranes, and dyspnea. In this case, acute respiratory failure was observed, and the cat did not respond to emergency therapeutic interventions.

In the macroscopic examination, hemorrhagic effusion in the thoracic cavity was observed (Fig. 1).

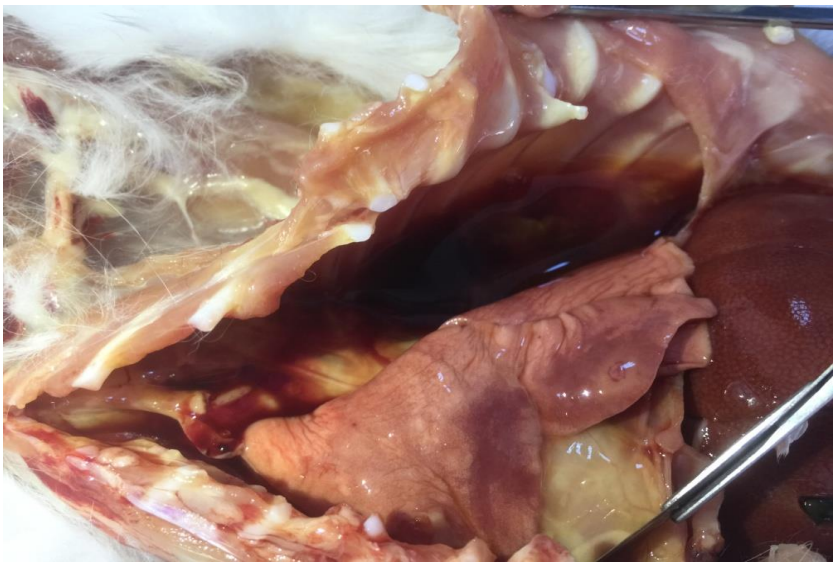

Fig. 1. *Hemorrhagic effusion in the thoracic cavity and necrotic foci in the medial and caudal lobes of the lungs.*

Histopathological findings include: intense edema in the lungs (Fig. 2a); desquamation and necrosis in the alveolar epithelium (Fig. 2b); and desquamation, necrosis and lymphoplasmacytic cell infiltrations in the bronchial epithelium (Fig. 2c).

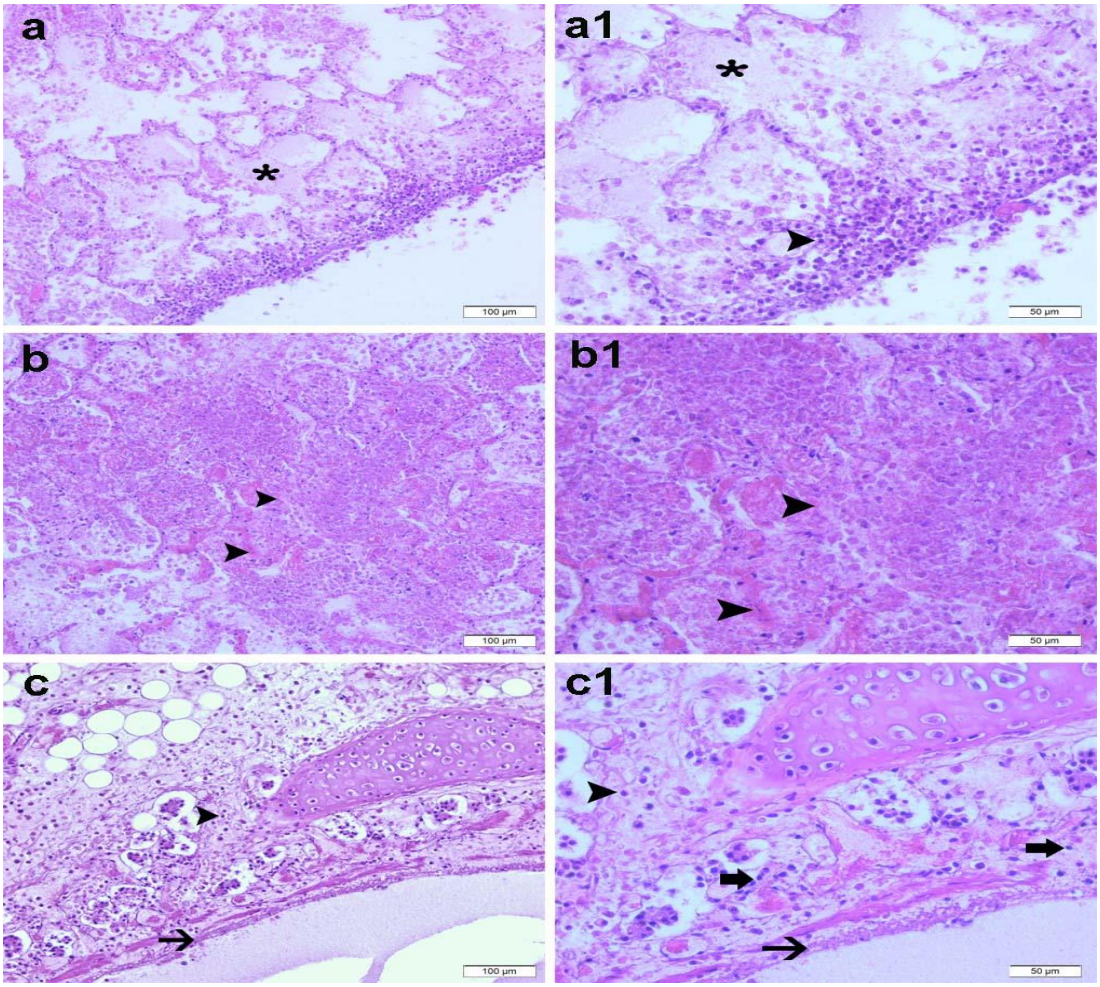

Fig. 2 *a- Intense alveolar edema in the lung (asterisk), a1: alveolar edema and lymphocyte infiltrations in the pleura; b: fibrinoid necrosis within adjacent alveolar walls (arrowheads), b1: fibrinoid necrosis (arrowheads); c: necrosis and desquamation (arrow) in the bronchial epithelium and plasma cells (arrowhead) around the bronchi; c1: higher magnification, necrosis and desquamation (thin arrow) in the bronchial epithelium and lymphoplasmacytic cell infiltrations (arrowhead, thick arrow) around the bronchi. H&E 20x and 40x magnifications.*

RNA samples extracted from the FCoV suspected case were amplified to detect FCoV using RT-PCR. Oligonucleotide primer sequences were selected from membrane protein region (M), a highly conserved gene region of FCoV. PCR amplicons were run on 1% agarose gel. Positive sample had specific DNA band.

The phylogenetic analysis showed that the partial sequence of FCoV belonged to the alpha coronavirus subgroup. In the same analysis, FCoV was typed as type I (Fig. 3). The case could be diagnosed as FIP through clinical, histopathological and molecular findings.

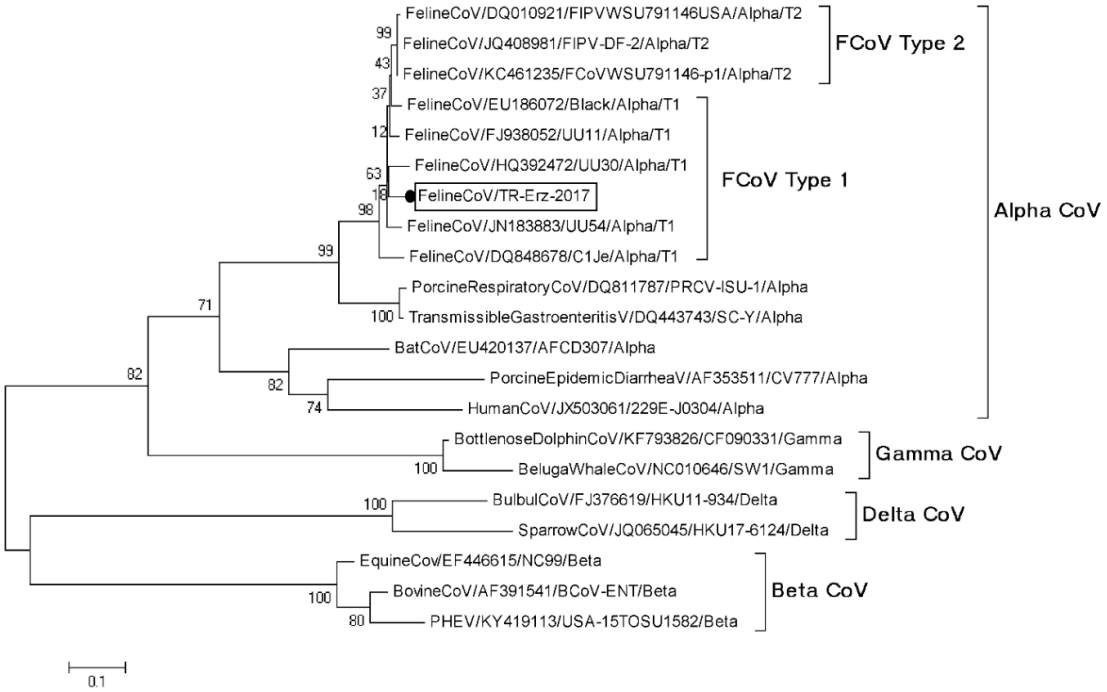

Fig. 3. *Reference coronaviruses from GenBank and the phylogenetic analysis of FCoV strain for this case. The phylogenetic relationship among different coronavirus isolates as inferred from Neighbor-Joining method in MEGA 6.0 [18]. Data for partial sequence were subjected to 1,000 bootstrap replicates. FCoV strains, Turkey/Erzurum are showed as round shape (●).*

It was documented by anamnesis report that this case did not respond to the antibiotic treatment. This finding complied with the report of Tsai et al. [19], who explains that FIP cases with anorexia and fever do not respond to antibiotics. The clinical findings related to FIP — such as weight loss, pale mucous membranes, fever, abdominal distention with ascites, dyspnea with pleural effusion, jaundice, multifocal neurological signs, abdominal pain, and uveitis — have been reported in other studies [1,14,16,20,21]. In this case, weight loss, pale mucous membranes, and dyspnea with open-mouth breathing were determined in the physical examination. The hemorrhagic fluid appearing in the thoracic cavity was determined to comply with the report of Pedersen [15]. In addition, mucosal paleness was observed, which might be attributed to anemia, as many cases with FIP indicate the development of anemia [14,19,20]. The fluid accumulation in the chest cavity compromises the lung function and leads to pulmonary atelectasis, hypoventilation, and dyspnea [22]. Effusion in the thoracic cavity can sometimes occur [15]. Similarly, in another study where the etiology was assessed in pleural effusion in cats, positivity ratio was determined to be 8.5% in FIP cases (26/306). In addition, the cases with FIP have been reported to be younger than the cases with cardiac disease and neoplasia [23,24]. Thus, FIP can be suspected in younger cats with pleural effusion. Some other diseases where pleural effusion occurs in cats are congestive heart failure, neoplasia, pyothorax, idiopathic chylothorax, and toxoplasmosis [22]. These diseases can be determined using tests such as effusive fluid cytology, culture, total protein, lactated dehydrogenase levels, total nucleated cell counts, triglyceride and cholesterol levels, hematocrit levels, serum

albumin, globulin, radiography, echocardiography, fine needle aspiration biopsy and core biopsy, hematology, biochemistry, urinalysis, and various kits [22].

RT-PCR has been shown to be an excellent specificity for the diagnosis of FIP and has been recommended when sensitivity is higher in body cavity effusion samples than in peripheral blood mononuclear cells and serum [25]. In the molecular analysis of effusion fluid and the lung tissues of this case, FCoV was determined as type I and genus *Alphacoronavirus*. Type I FCoVs have been determined to predominate in the cat population in the other countries [4,26,27,28,29]. In addition, Amer et al. [4] have reported that 97.5% of local isolates are type I FCoV and these isolates have high sequence homology and phylogenetic similarity with several FCoV isolates from Europe, South East Asia and USA. There are no serological or morphological discriminations for FECV and FIPV [3].

Benetka et al. [19] verified FIP caused by FCoV types I and II. In addition, it was reported that in healthy cats carrying FCoV, FIP might develop, and that in cats with FIP, FCoV type I was predominant. Furthermore, FCoV type II development in the result of FCoV type I and CCoV recombination was reported in the FIP cases at 14%. In a study performed in Japan, it was reported that FCoV type 1 was predominant (98%) in cases with FIP, and that virus neutralization test could be used for the serological discrimination of FCoV [30]. Thus, our study can lead future studies for FCoV type analyses and the determination of prognosis.

It has been shown that natural FCoV infection causes intestinal infection with viral shedding and either systemic infection without clinical signs or FIP. In a study where FCoV RNA loads in haemolymphatic tissues of healthy, long-term FCoV-infected cats and cats with FIP were investigated, it was determined that there was a significant increase in viral loads in cats with FIP and a decrease in the viral clearance in FCoV–infected cats without disease signs [31]. Pathological examinations related to FIP have described fibrinous and granulomatous serositis, protein-rich serous effusions, and/or pyogranulomatous lesions in various organs [3]. Granulomas with and without areas of necrosis, focal and perivascular lymphoplasmacytic cell infiltrations and granulomatous to necrotizing vasculitis have also been shown [32]. The macroscopic examination of this case detected hemorrhagic effusion in the thoracic cavity. In addition, intense edema, desquamation, necrosis, and lymphoplasmacytic cell infiltrations in the lungs were determined. FCoV may cause severe systemic infection as FIP. Thus, feline coronavirus type I was determined to cause severe lesions with edema, necrosis and lymphoplasmacytic cell infiltrations in the lungs and respiratory distress.

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Ethical Statement. This study does not present any ethical concerns.

Conflict of Interest. The authors declare no potential conflicts of interest with regard to this research, authorship, and/or publication of this article.

Authors Contributions. B.H.: Writing, M.O.T.: Data Collection, Processing, H.A.: Data Collection, Processing, S.A.: Data Collection, Processing, S.Ç.: Data Collection, Processing, K.E.Y: Data Collection, Literature Search

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