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# CLINICAL AND MOLECULAR INVESTIGATION IN FELINE INFECTIOUS PERITONITIS CAT TREATED WITH GS-441524 CONTAINED DRUG

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**Abstract**

An immune-mediated disease caused by a highly virulent Feline coronavirus (FCoV), Feline infectious peritonitis, is one of the most challenging diseases in cats due to its difficulty in either diagnosis or treatment. However, the recent investigation has offered some hope with the discovery of adenosine nucleoside analog GS-441524, which has shown promising results in terms of survival rates and clinical recoveries. This study demonstrated the effectiveness of GS-441524 for FIP treatment. A-two-year-old male mix breed was admitted to Satwakita Animal Clinic, Yogyakarta and examined by responsible veterinarian. Abdominal effusion was aseptically collected for further examination in Veterinary Medicine Clinical Pathology Laboratory, Universitas Gadjah Mada. The analysis utilized RT-qPCR targeting 5'UTR confirmed FCoV infection in the cat. The cat was treated by daily administration of the nucleoside analog GS-441524 by subcutaneously injection route for 40 days. The result of this treatment proves that GS-441524 effectively cured FIP and eliminated FCoV infection in the cat. In this case report, we showed that GS-441524 was effective in this cat. Both clinical signs and FCoV RNA were significantly reduced. The results highlight the promising potential of GS-441524 for treating FIP that naturally occurs in cats.

**Keywords**: Case Report; FIP; FCoV; RT-qPCR; GS-441524

## INTRODUCTION

Feline infectious peritonitis (FIP) is caused by the feline coronavirus (FCoV), which belongs to the subspecies of alphacoronavirus-1. It is a contagious disease that affects felids worldwide. Feline coronavirus infection is quite common in cats, often causing mild symptoms like diarrhea. However, more than 10% of FCoV infections develop into FIP infections, leading to severe symptoms such as the appearance of ascites, incoordination of the body, and eventually, death (Tasker, 2018). The mutation primarily affect the coronaviral spike proteins, enabling the virus to replicate efficiently within macrophages and spread within the cat (Pedersen, 2009). Studies have shown that FIP patients are more likely to be purebred, young and sexually intact males (Rohrbach et al., 2001). FIP possess significant challenges in both diagnosis and treatment. Previous reports have outlined various methods to

detect FCoV infection, including molecular detection using nested RT-PCR targeting 3'untranslated region (3'UTR) and RT-qPCR targeting specific genes such as 5'UTR and N gene (Guan et al., 2020; Sun et al., 2021; Wasissa et al., 2021).

The average survival time without effective treatment is only 8 days after diagnosis, and most cats have to be euthanized early due to their severe condition (Ritz et al., 2007). Supportive care remains the primary approach for treating FIP, temporarily reducing clinical signs and immune-modulator to stimulate diseased patients' imunity (Pedersen, 2014). Due to the lack of an effective treatment, FIP is almost invariably fatal, with clinical mortality rate reaching as high as 90% (Rohrbach et al., 2001). However, a recent investigation has shown promising result in survival rates and clinical recoveries through the use of adenosine nucleoside analog GS-441524 (Izes et al., 2020). The previous report also showed the effectiveness of GS-441524 in treating 32 cats naturally infected with FCoV for 12 weeks period (Pedersen et al., 2019). Dickinson et al., (2020) reported that GS-441524 has the potential to treat FIP with neurological presentation. The optimal dose range for GS-441524 in cats is 5-10 mg/kg. Successful clinical recovery from FIP has been documented in previous report. This case report is the first description of the complete recovery of a cat examined through RT-qPCR, x-ray and hematology tests in Indonesia.

## RESEARCH METHODS

### Signalment and History

A two-year- old male mix breed was investigated for further examination of abdominal effusion and blood due to its high suspicion of feline infectious peritonitis (FIP), suppoted by its consistent history, clinical signs, and physical examination. The cat was admitted to Satwakita Animal Clinic, Yogyakarta and examined by a responsible veterinarian. The cat had been displaying unusual behavior for the last two weeks, followed by lethargy and abdominal distension, and tested positive for Feline Infectious Peritonitis Antibody Rapid Test. According to the owner's information, the cat population consists of 17 cats with one confirmed fatal case of FIP. All cats are kept indoors with limited access to the surrounding environment and a complete vaccination record. In the clinic, the cats presented with a reduced general condition, mild dehydration, pale mucous membrane, a body temperature of 38,5°C, and a body weight of 3,2 kg (Figure 1). The responsible veterinarian conducted Rivalta's test, hematology test and thoracoabdominal x-ray. The Rivalta's test is positive, indicated the presence of exudate-type abdominal effusion. Hematology results showed lymphocytosis (Table 1). Thoracoabdominal x-ray was performed in lateral recumbency and revealed radiopacity in the abdomen, indicating fluid accumulation in the abdominal cavity (Figure 2). The data collected about the patient and its publication was obtained with the consent of the responsible veterinarian and cat's owner.


**Figure 1A. Picture of the cat on day 0 (day of first presentation in the clinic), 1B. on day 40, six weeks of treatment.**

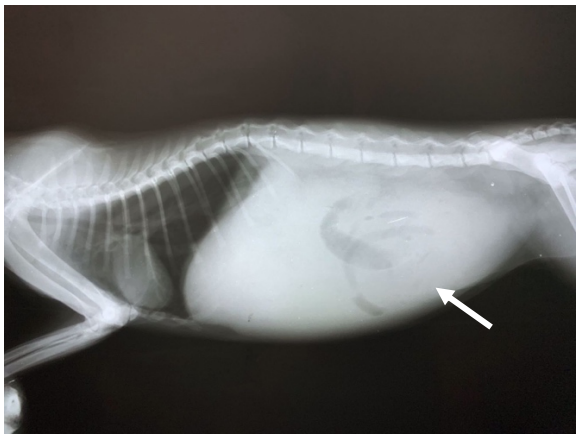

**Figure 2. Thoracoabdominal X-Ray Lateral View Showing Radiopacity Of The Abdomen Indicating Fluid Accumulation**

**Table 1. Haematology results on day 0 and day 40 of treatment**

| Measurement | Reference interval | Result (day 0 treatment) | Interpretation | Result (day 40 treatment) | Interpretation |
|---|---|---|---|---|---|
| RBC ($10^6$/µl) | 4,6-10 | 7,21 | Normal | 8,9 | Normal |
| Hb (g/dL) | 9,3-15,3 | 10,7 | Normal | 12,7 | Normal |
| HCT (%) | 28-49 | 31,1 | Normal | 33,7 | Normal |
| MCV (fL) | 39-52 | 43,2 | Normal | 39,7 | Normal |
| MCH (pg) | 13-21 | 14,8 | Normal | 14,2 | Normal |
| MCHC (g/dL) | 30-38 | 34,4 | Normal | 37,6 | Normal |
| WBC ($10^3$/µl) | 5,5-19,5 | 16,9 | Normal | 12,1 | Normal |
| Lymphocyte ($10^3$/µl) | 0,8-7 | 7,5 | Lymphocytosis | 4,5 | Normal |

| Measurement | Reference interval | Result (day 0 treatment) | Interpretation | Result (day 40 treatment) | Interpretation |
|---|---|---|---|---|---|
| Monocyte ($10^3$/µl) | 0,0-1,9 | 1 | Normal | 0,9 | Normal |
| Neutrophil ($10^3$/µl) | 2,1-15 | 8,4 | Normal | 6,7 | Normal |
| Eosinophil ($10^3$/µl) | 0-1,5 | 0,26 | Normal | 0,19 | Normal |
| Basophil ($10^3$/µl) | Rare | 0 | Normal | 0 | Normal |
| PLT ($10^3$/µl) | 100-514 | 272 | Normal | 419 | Normal |

*Reference interval (Weiss and Wardrop, 2010)

**Improvement of Clinical Signs, Thoracoabdominal X-ray, and Laboratory Abnormalities during Treatment.**

To gain definitive diagnosis, abdominal effusion was aseptically collected for cytology analysis and molecular detection in Veterinary Medicine Clinical Pathology Laboratory, Universitas Gadjah Mada. Cytology analysis of abdominal effusion revealed cloudy exudate contained macrophages and neutrophils with eosinophilic proteinaceous background (Figure 3). Definitive diagnosis of FIP was based on consistent history, clinical signs, laboratory abnormalities, and molecular detection of FCoV using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) targeting 5' untranslated region (5'UTR) and positive if the cycle threshold was <35 and melt curve was 80 (±0,5)°C. Molecular analysis was performed using the primer listed in Table 2. The result of molecular analysis before treatment is listed in Table 3.

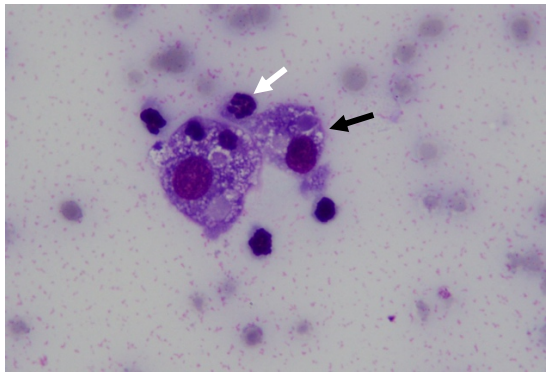

**Figure 3. Cytology Analysis of Abdominal Effusion Contained Macrophages (Black Arrow) And Neutrophils (White Arrow) With An Eosinophilic Proteinaceous Background**

**Table 2. The Primer That Were Used In This Investigation**

| Target | Primer | Nucleotide | Reference |
|---|---|---|---|
| 5'UTR | FCoV-SYBR-F | GAGGAATTACGGGTCATC | Sun *et al.* (2021) |
| | FCoV-SYBR-R | CATTGCCAAATCAAATCTAAAC | |

The cat was treated by daily administration of the nucleoside analog GS-441524 by subcutaneously injection route, for 40 days. Due to abdominal effusion manifestation, a dose of supposedly 6 mg/kg (according to the manufacturer) was chosen. From the fourth day of treatment onward, the cat's appetite improved and it started to gain weight. Six weeks of treatment (day 40), the cat had reached a weight of 3,8 kg, and a body temperature of 38,6°C (Figure 1B). Hematology results were found at normal levels (Table 1). A thoracoabdominal x-ray performed in lateral recumbency showed a decrease in fluid accumulation in the abdominal cavity (Figure 4). Molecular analysis showed an increase of cycle threshold after 40 days of treatment (Table 3).

**Table 3. The results of RT-qPCR before and day 40 treatment**

| | Cycle threshold |
|---|---|
| Day 0 (before treatment) | 26,91 |
| Day 40 of treatment | 29,06 |

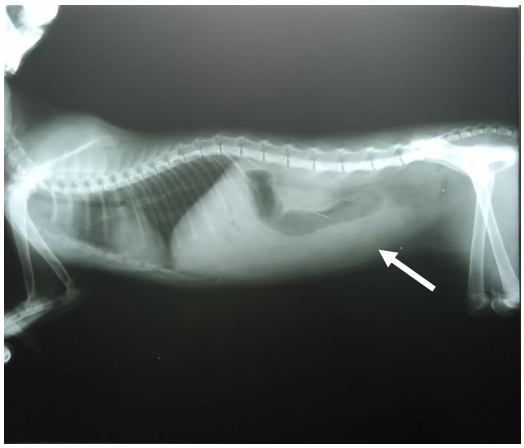

**Figure 4. Thoracoabdominal X-Ray Was Performed In Lateral Recumbency Showed A Decrease Of The Accumulation Fluid In The Abdominal Cavity**

## RESULT AND DISCUSSION

This case report describes a cat that participated in investigating the efficacy of parenteral antiviral drug to treat FIP. The cat describe here was included in the study as it fulfilled the inclusion criteria of (1) clinical symptoms that appeared lead to FIP, (2) positive test result for FCoV molecular diagnosis (RT-qPCR), (3) there's one fatal case FIP confirm in its population. After initial presentation with apathy, lack of appetite and abdominal effusion, the cat showed a swift response to treatment, with rapid improvement of clinical and laboratory parameters leading to full. The cat was treated with 6 mg/kg of the nucleoside analog GS-441524 by subcutaneously injection route. The considerations for using GS-441524 in this case are (1) GS-

441524 has been shown to be effective in curing FIP in some cases, as in study conducted by Krentz *et al.*, (2022), (2) investigation in feline infectious peritonitis cat treated with GS-441524 has never been done in Indonesia, that it can be a good information for veterinarians, (3) cat's owner support this investigation.

GS-441524 a 1'cyano-substituted adenine C-nucleoside ribose analogue is a small molecule that exhibits potent antiviral activity against a number of RNA viruses, including the zoonotic severe acute respiratory syndrome (SARS) coronavirus (Cho *et al.*, 2012). GS-441524 is the targeted antiviral drug to be evaluated for the treatment of cats with FIP in the past two to three years. This drug inhibited viral replication in two very different manners, either by terminating viral RNA transcription or blocking viral polyprotein cleavage (Murphy *et al.*, 2018). The main abnormality of FIP in the cat presented in this case report was abdominal effusion. The appearance of abdominal effusion is one of the clinical symptoms in wet FIP cases. Wet FIP cases accounts for 80% of all FIP cases, so this tendency is one of the characteristics of FIP. The classical effusion of wet FIP results from mainly from acute damage to the vessel walls and leakage of plasma into the interstitial spaces and eventually into body cavities. This can occur because activated monocytes will spread the virus and cause vasculitis. Monocytes will migrate and attach to blood vessels and cause the formation of focal infiltrates in the blood vessel walls (Kipar *et al.*, 2005).

GS-441524 treatment over a total period of 40 days was remarkably safe. No longterm abonormalities were observed in viral RNA level, thoracoabdominal x-ray and haematology test. Decrease of viral RNA level is indicated by cycle threshold which increase after 40 days of treatment. Similarly with Pedersen *et al.*, (2019) that viral RNA level decreased by 2-5 days after GS-441524 treatment. Abdominal effusion rapidly decreased starting around 14 days post-treatment, and rapidly disappear around six weeks. Cat presented with elevated lymphocyte counts, which droped to average level within six weeks of treatment. Immediate pain reactions in injection site were manifested by vocalization, occasional growling, and postular changes lasting for 40-60 seconds, but there is no scars in injection sites.

## CONCLUSION

This is the first case report describing clinical and molecular investigation in a cat cured of FIP. In this case report, we showed that GS-441524 was effective in this cat. Both clinical signs and FCoV RNA were significantly reduced. GS-441524 holds great promise in the treatment of naturally occurring FIP. This case report will be essential for future efforts in comersialization of this drug in Indonesia.

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