International Journal of Veterinary Sciences and Animal Husbandry

**ISSN:** 2456-2912
VET 2024; 9(2): 949-953

www.veterinarypaper.com
Received: 03-01-2024
Accepted: 08-02-2024

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# Hematobiochemical alterations in feline panleukopenia affected cats

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**Abstract**
The study involved collecting fifty fecal samples from cats and kittens across various age groups to diagnose feline panleukopenia (FPL), with 30 of them testing positive using the PCR method. Subsequently, blood and serum samples from FPL-positive cats were examined. Hematological analysis indicated the presence of anemia and leukopenia characterized by neutropenia and lymphopenia in these cats. Furthermore, biochemical investigations revealed notable elevations in liver-specific enzymes such as ALT and AST, along with reductions in TP and albumin levels in FPL-affected cats compared to healthy counterparts. Additionally, kidney function tests (KFT) indicated a slight increase in serum creatinine and BUN levels in cats afflicted with Feline Panleukopenia (FPL).

**Keywords:** Cat, feline panleukopenia viral infection, hematobiochemical alteration, liver function test, kidney function test

## Introduction
Cats are the enigmatic and graceful creatures that have shared our lives for thousands of years, and continue to captivate us with their charm and mysterious behaviour. Their independent nature, agile movements, and keen senses have made them fascinating companions, evoking both joy and curiosity in the hearts of their human counterparts (Raheena *et al*., 2017) [15]. The trend of rearing and breeding cats has been developed into a flourishing pet trade facilitating more households being open to adoption of stray cats and purchasing well-maintained pedigreed cats from breeders. Cats are fascinating creatures, but like all living beings, they are susceptible to various diseases. Close congregation of naive cats has led to emergence of highly pathogenic infections like Feline Immunodeficiency virus (FIV), Feline Panleukopenia virus (FPV), Feline Calicivirus (FCV) and Feline Bartonella which causing catastrophic consequences in them. One of them is feline panleukopenia, a highly contagious viral disease caused by Feline Parvo virus which is closely related to type 2 canine parvovirus, blue fox parvovirus and mink enteritis virus (Richard, 2020) [16].
The virus has an affinity for mitotically dividing cells present in the bone marrow, lymphoid organs and intestinal crypt cells, leading to severe symptoms and potentially fatal outcomes. The virus is secreted by infected cats through their saliva, faeces, and respiratory secretions. Transmission can be aided by direct contact with these fluids or indirect interaction with contaminated things (Addie *et al.*, 1998) [1]. All ages of cats are susceptible to FPV, but kittens are particularly at risk. Kitten mortality rates are high, exceeding 90% (Parthiban *et al*., 2014) [13].
The symptoms of feline panleukopenia can vary but often include fever, lethargy, loss of appetite, vomiting, diarrhea (often bloody), dehydration, and a decrease in white blood cell count (leukopenia), which weakens the immune system. There are chances of abortion in queen cat due to feline panleukopenia infection. Kittens are particularly susceptible to severe illness and may experience neurological symptoms such as tremors, seizures, Introduction 3 and incoordination (Richard, 2020) [16]. However, studies are lacking on alteration in hematobiochemical Alteration due to feline panleukopenia virus in cats.

## Materials and Methods

### Statutory Permission to Undertaken the Research Work

Present study was undertaken as per the compendium of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Project Approval No:411/VMD/23 of (Dated-15/07/2023) College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand.

### Location and Collaboration

The Present Research Work entitled "Studies on clinical-diagnostic and therapeutic management of Feline Panleukopenia Viral Infection in Cats (Felis catus)" was undertaken over a period from November 2022 to June 2023. The research work was conducted at the Department of Veterinary Clinical Medicine in collaboration with Veterinary Clinical Complex and the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand.

### Selection of Animals

A total of 540 cats were presented at the clinic during the study period. Out of them, 50 cats were screened for FPV. Out of 50 cats, 18 cats were randomly selected for therapeutic management based on clinical signs suggestive of Feline Panleukopenia viral infection such as inappetence, vomiting, diarrhoea (haemorrhagic or nonhaemorrhagic), diarrhoeic faeces (very putrid and offensive odour), weakness, dehydration, etc. and results of immune-chromatographic (IC) test. These cats were divided into 3 groups, *viz*., Group B, C and D, each of 6 cats. During the therapeutic trial mortality was recorded in group D on initiation of protocol. These group were studied for haemato-biochemical changes in the therapeutic trial. A group of 6 healthy cats (Group A) presented at VCC for routine check-up kept as control.

### Polymerase Chain Reaction (PCR) Based Identification of Feline Panleukopenia Virus

For the molecular diagnosis, faecal samples will be directly suspended in 2 ml of Phosphate Buffer Saline (PBS) with pH 7.2. These samples will be centrifuged at 10,000 rpm for 10 minutes. The supernatant of each sample will be filtered using a 0.45
µm filter and stored at -20 °C until use. The filtered samples will be used for PCR. DNA will be extracted from faecal samples by DNA extraction kit. Confirmatory diagnosis will be done by standard PCR method for the FPV.

- Isolation of DNA from faecal sample
- Amplification of genes of FPV

### DNA extraction from the faecal sample

With slight adjustments, the virus DNA was extracted according to Antony *et al*. (2006). Sample suspended in 100 µL milli Q water were boiled for 10 minutes at 96℃. Centrifugation was employed to remove the cell debris and 3 µl of the supernatant was employed as a template DNA for PCR.

### Quantitation and quality assessment of DNA

DNA was measured with an ND-1000 Spectrophotometer using the standard that one absorbance units at 260 nm wavelength equals 50 µg DNA per ml (Nanodrop Technologies Inc., USA).

Concentration of DNA (µl per ml) = Optical Density at 260 × Dilution factor × 50

Where, 50 denotes the concentration of DNA at a single O.D.

UV absorbance at 260 and 280 nm wavelength was measured to determine sample concentration and purity. The purity of DNA was determined using an O.D. ratio of 260:280. DNA with a purity ratio of 1.8-2.0 was considered high quality.

### PCR primers for FPV

Primers specifically used for feline panleukopenia virus (Table 1) were as reported by Carreño *et al*., (2021) [4].

**Table 1:** Details of Primers used for amplification of target VP2 gene fragment of viral DNA for identification of Feline Panleukopenia virus in PCR reaction

| Name of the target organism | Primers Sequence (5'-3') | | Size of amplified products (bp) | References |
|---|---|---|---|---|
| VP2 gene | (F) | TGGTTGATGCAAATGCTTGGG | 681 | Carreño *et al*. 2021 [4] |
| | (R) | AACCAACCTCAGCTGGTCTC | | |

### PCR condition

A total 3 µL of DNA sample containing 30-50 ng/µl concentration was used as a template for PCR reaction (Table 2).

**Table 2:** Composition of reaction mixture for PCR

| Components | Quantity (µL) |
|---|---|
| 2X PCR Master mix | 12.5 |
| Forward Primer | 1.0 |
| Reverse Primer | 1.0 |
| Nuclease free water | 7.5 |
| DNA template | 3.0 |
| Total | 25.0 |

**Table 3:** Steps and conditions of thermal cycling for different primers in PCR

| Primers (Forward and Reverse) | Cycling condition | | | | |
|---|---|---|---|---|---|
| | Initial denaturation | Denaturation | Annealing | Extension | Final Extension |
| FPV F FPV R | 94 ℃<br>2 minutes | 94 ℃<br>30 seconds | 55 ℃<br>1 minute | 72 ℃<br>1 minute | 72 ℃<br>10 minutes |
| | Repeated for 40 cycles | | | | |

### Agarose gel electrophoresis

**Table 4:** The reagents for Agarose Gel Electrophoresis

| **1. Agarose** | |
|---|---|
| Agarose | 2.00 g |
| TBE (0.5X) | 100 ml |
| Ethidium Bromide | 5 µL |
| **2. Tris Borate EDTA (TBE) buffer, pH 8.3 (5x)** | |
| Tris HCL | 0.445 M |
| Boric acid | 0.445 M |
| EDTA | 10 mM |
| **3. Ethidium Bromide (1%)** | |
| Ethidium Bromide | 10 mg |
| Distilled water | 1.0 ml |
| **4. Agarose Gel Loading Buffer (6x)** | |
| Bromocresol blue | 2.5 mg (0.25%) |
| Xylene cyanole FF | 2.5 mg (0.25%) |
| Glycerol | 0.3 ml (30%) |
| Distilled water to make | 1.0 ml |

### Procedure of agarose gel electrophoresis

1. For 2% agarose gel, 2 gm of agarose powder was taken into a 250ml flask and 100 ml of 1x TAE buffer was added.
2. Solution was heated in a microwave until agarose was completely dissolved.

Gel casting tray was used for casting and comb was placed in gel tray.

1. The Ethidium Bromide (5 µL) was added to agarose gel and poured into tray and allowed to solidify for 15-30 minutes at room temperature.
2. The comb was removed from gel and gel placed in electrophoresis unit and 1x TAE buffer (running buffer) was added till the gel got completely submerged in the buffer.
3. The PCR product (5 µl) was loaded and 2 µl of standard 100 bp Ladder was added as a reference.
4. Electrophoresis was run at 80 V for 1 hour.
5. DNA bands were visualized using UV trans-illuminator or Gel Documentation System.

### Visualization of PCR products

To verify targeted PCR amplification, five µl of PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide (at a rate of 0.5 mg/ml) at an average voltage of 80 V using a 100 bp DNA ladder. The amplified product was visualised as a single compact band of the expected size under UV illumination at 60-minute interval and the gel documentation system was used to document it.

**Fig 1:** Thermal cycler (Analytikjena)

**Fig 2:** Dry bath (Equitron)

### Haematological Analysis

Blood sample (2 ml) was collected from the cephalic or saphenous vein of FPV positive cats in sterile K3EDTA vacutainers. Haematological analysis of the sample was done by autohaemato analyzer (Abacus Junior Vet-5) from the whole blood sample for the following parameters on day 0 and 7th of therapeutic management. Haemoglobin (g/dl), Total Erythrocyte Count (x106/µl), Total Leucocyte Count (x 103/µl), Lymphocyte (x103/µl), Monocyte (x 103/µl), Neutrophils (x 103/µl), Eosinophils (x 103/µl), Basophils (x 103 µl), Platelets count (x 103/µl).

### Serum Biochemistry Analysis

Blood sample (2 ml) was collected from the cephalic or saphenous vein of FPV positive cats put under different treatment regimen in clot activator vials and serum was separated from the blood by centrifugation method. Serum biochemistry was carried out by an auto-biochemical analyzer (CKK-300) for the following parameters on day 0 and 7$^{th}$ of the therapeutic management. Total Serum Protein (g/dl), Serum Albumin (g/dl), Serum Globulin (g/dl), Alanine transaminase (ALT) (IU/L), Aspartate Aminotransferase (AST) (IU/L), BUN (mg/dl), Creatinine (mg/dl).

Blood samples were also collected from cats of healthy control group as above on day '0'for haemato-biochemical analysis to compare the findings with FPV positive cats.

### Results and Discussion

#### Polymerase Chain Reaction (PCR)

Faecal samples of 50 FPV suspected cats were subjected to PCR for further confirmation. Total 60.00% (30/50) cats were found positive and 40.00% (20/50) cats were found negative by PCR. PCR amplification produced an amplicon size of 681 bp.

Fifty fecal samples were gathered from suspected cats or kittens across various age groups, with 35 of them testing positive for feline panleukopenia (FPL) using the PCR method. Subsequently, the blood and serum samples from these 35 FPL-affected cats were included in a study to analyse hematological and biochemical profiles.

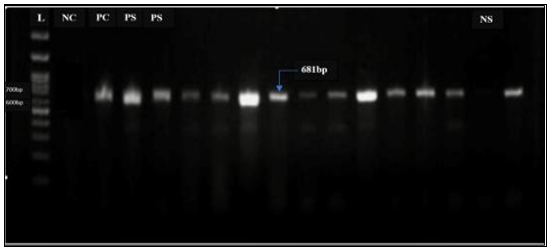


**Fig 3:** Agarose gel image of FPV showing Amplification at 681bp.

**Haematological Analysis**

Haematological examination was carried out in FPV affected cats (n=18) and compared with healthy cats (n=6) on day 0. The findings revealed that the levels of WBC (3.18±0.31×103/µl), lymphocytes (0.63±0.06×103/µl) and neutrophils (1.66±0.18×103/µl), Hb (8.59±0.33 g/dl), RBC (6.20±0.21×106/µl) and platelet count (93.11±13.87×103/µl) were highly significant decreased ($p$<0.01) and eosinophils (0.68±0.14 ×103/µl) was significantly ($p$<0.05) increased.

These results could be explained by feline panleukopenia's predilection for lymphocyte and lymphatic tissues. The virus typically targets cells that divide quickly, including those in lymphoid organs, the intestines, and bone marrow. The mortality of haemopoietic progenitor cells of various leukocyte types, particularly in bone marrow and other lymphoproliferative organs including the thymus, lymph nodes, and spleen, is attributed to this discovery, which has gained widespread support. Due to these, the enormous demand for leukocytes in the inflammatory gastro intestinal tract is not adequately met (Addie *et al*., 1998) [1].

The levels of Eosinophils (0.68±0.14) and Basophils (0.06±0.02) in the affected group did not exhibit significant changes. This finding aligns with previous studies by Mosallanejad *et al*. (2009) [11], Bayati (2016) [3], Porporato *et al.* (2018) [14], and Radhy and Zenad (2020) [19], which observed neutropenia and lymphopenia in cats affected by feline panleukopenia (FPL). Parrish (1995) [12] suggested that panleukopenia or lymphopenia in FPL-affected cats could be attributed to the replication of the FPL virus in bone marrow progenitor cells, leading to cell death and potentially reducing neutrophil numbers.

There were predominant haematological alterations were anaemia and thrombocytopenia. Interleukin-6 is released as a result of parvoviral enteritis, which inhibits the release of hepcidin and reduces the production of ferroportin, both of which have an impact on erythropoiesis (Klainbart *et al*., 2017). [8] Anaemia may result from FPV infections because to abnormal iron uptake and metabolism in the inflamed intestine, gastrointestinal haemorrhage, and diminished RBC life (Barrs., 2019) [2].

Thrombocytopenia as predominant haematological finding in FPV infected cats Kruse *et al.* (2010) [9], Klainbart *et al*. (2017) [8] and Bakde *et al*. (2020) [17]. In addition to being a variable component of feline panleukopenia in cats who develop DIC, thrombocytopenia can also be brought on by a direct damage to the bone marrow and can coexist with leukopenia in the early stages of infection (Green, 2012) [7].

**Table 5**: Mean values of haematological parameters in FPL affected and apparently healthy cats

| Parameters | Group A (Healthy Cats) (N=6) | FPV affected cats (N=18) | "p" value |
|---|---|---|---|
| Hb (g/dl) | 11.38±0.41 | 8.59±0.33** | 0.001 |
| RBC (× 106/µl) | 8.33±0.39 | 6.20±0.21** | 0.001 |
| WBC (× 103/µl) | 12.96±0.62 | 3.18±0.31** | 0.001 |
| Lymphocyte (×103/µl) | 2.30±0.31 | 0.63±0.06** | 0.001 |
| Monocyte (×103/µl) | 0.42±0.05 | 0.34±0.05 | 0.325 |
| Neutrophils (×103/µl) | 10.23±0.49 | 1.66±0.18** | 0.001 |
| Eosinophils (×103/µl) | 0.05±0.02 | 0.68±0.14* | 0.02 |
| Basophils (×103/µl) | 0.001±0 | 0.06±0.02 | 0.08 |
| Platelets count (×103/µl) | 282.17±8.46 | 93.11±13.87** | 0.001 |

**$p$<0.01= highly significant and *$p$<0.05= significant

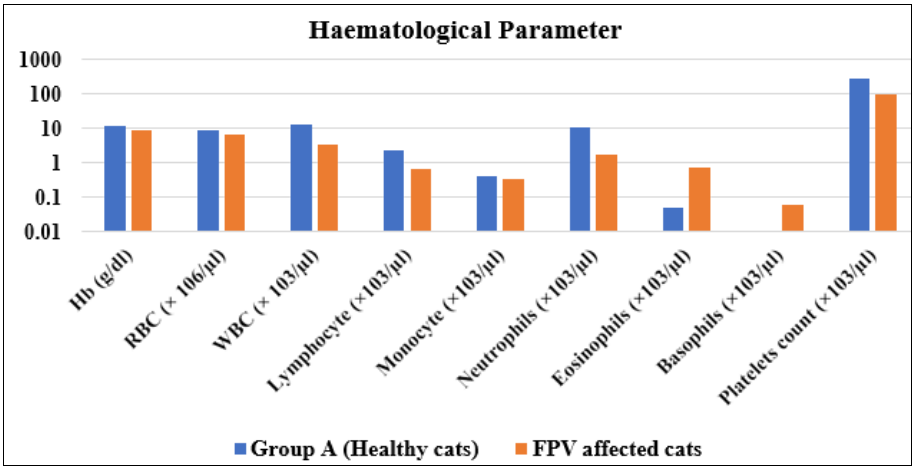


**Fig 4:** Haematological observation in healthy and feline panleukopenia affected cats on days 0 (Mean ± SE)

**Serum biochemical Analysis**

The concomitant serum biochemistry examinations revealed that the level of ALT (85.35±2.91 IU/L) and AST (56.90±2.99 IU/L) were highly significant increased ($p$<0.01). Increased levels of ALT and AST can be attributed to extreme haemolysis of RBCs in FPV affected cats (Chapman and Hostutler, 2013) [5] Cowell (2004) [6] who reported that liver is crucial in the process of toxin detoxification, cats with GI tract infections had greater levels of all liver enzymes than usual.

Serum total protein (4.96±0.03 g/dl), albumin (2.25±0.03 g/dl) and globulin (2.77±0.02 g/dl) were highly significant decreased ($p$<0.01). Severe hypoalbuminemia in cats affected with FPV has been linked to deranged protein uptake coupled with excessive loss into gastrointestinal tract due to epithelial mucosal injuries (Mayur *et al.* (2016) [10]. Cowell (2004) [6] reported a decline in albumin and globulin levels due to severe diarrhoea as a result of leakage of these enzymes via the injured intestine.

The level of BUN (27.25±0.17 mg/dl) and creatinine (1.23±0.04 mg/dl) highly significant increased ($p$<0.01) in FPV affected cats in comparison to healthy cats. Cowell (2004) [6] suggested that a slight increase in BUN and creatinine occurs in animals affected with GI tract disorder.

**Table 6:** Mean values of serum biochemistry parameters in FPL affected and apparently healthy cats

| Parameters | Group A (Healthy Cats) (N=6) | FPV affected cats (N=18) | "p" value |
|---|---|---|---|
| ALT (IU/L) | 51.55±4.02 | 85.35±2.91** | 0.001 |
| AST (IU/L) | 28.00±4.06 | 56.90±2.99** | 0.001 |
| Total Protein (g/dl) | 5.96±0.03 | 4.96±0.03** | 0.001 |
| Albumin (g/dl) | 3.03±0.03 | 2.25±0.03** | 0.001 |
| Globulin (g/dl) | 2.92±0.02 | 2.77±0.02** | 0.003 |
| Creatinine (mg/dl) | 0.98±0.04 | 1.23±0.04** | 0.004 |
| BUN (mg/dl) | 20.26±1.69 | 27.25±0.17** | 0.002 |

In the **$p$<0.01= highly significant and *$p$<0.05= significant

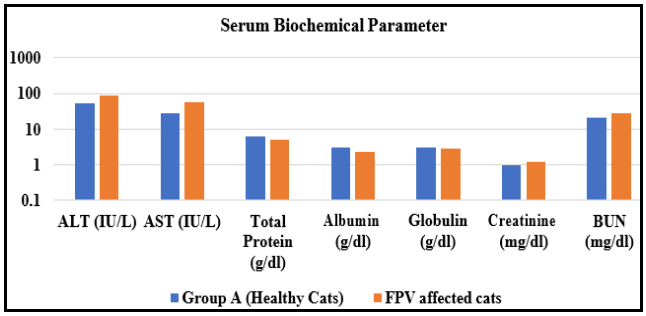


**Fig 5:** Serum biochemical observation in healthy and feline panleukopenia affected cats on days 0 (Mean ± SE)

## Conclusion

In summary, the hematological analysis of cats affected by Feline Panleukopenia (FPL) demonstrated leukopenia, anaemia, thrombocytopenia, neutropenia, and lymphopenia. Conversely, the biochemical parameters indicated elevated levels of liver-specific enzymes and renal function parameters, along with decreased levels of serum proteins, when compared to corresponding values observed in apparently healthy cats. These findings underscore the systemic impact of FPL on both hematological and biochemical profiles, highlighting the severity of the disease and its implications on organ function and overall health.

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