

# Electrophoresis, Hematology and Serum Biochemistry of Cats with FeLV/FCoV coinfection

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**ABSTRACT** – *This retrospective study aimed to determine serum protein electrophoresis changes, the alteration of the hematological and biochemical parameters in samples from client-owned cats with FeLV/FCoV coinfection. Serum protein electrophoresis detected changes in the electrophoretic fractions in all FeLV/FCoV+ cats. The most frequently abnormality detected by serum protein electrophoresis was decreased  $\beta_2$ -globulin levels in cats with FeLV/FCoV coinfection. Increased levels of  $\alpha_2$ -globulin with concurrently increased levels of  $\gamma$ -globulin were observed in two FeLV/FCoV+ cats. Albumin and  $\alpha_1$ -globulin levels were normal in most cats. Hyperproteinemia with concurrently increased globulin levels was frequently observed in presented study. The albumin/globulin ratio and urea levels were within the normal range in most cats. Anemia and decreased levels of hemoglobin and MCHC were the most frequent hematological abnormalities in four FeLV/FCoV+ cats. Leukogram changes were observed in four FeLV/FCoV+ cats. The results of this study suggest that serum protein electrophoresis, hematological and biochemical parameters are useful diagnostic tools for diagnosis of FeLV/FCoV coinfection. Further research using a larger number of FeLV/FCoV+ cats is required, because this study is underpowered for statistical analyses.*

**Keywords**—Electrophoresis, complete blood count, serum biochemistry, feline leukemia virus, feline coronavirus, cat

## 1. INTRODUCTION

The prevalence rates of feline coronavirus (FCoV) and feline leukemia virus (FeLV) in various parts of the world have been reported (Baneth *et al.*, 1999). Protein electrophoresis combined with hematological and biochemical parameters can be useful diagnostic tools for diagnosis and monitoring of viral diseases (Miró *et al.*, 2007; Gleich & Hartmann, 2009). To the best of my knowledge, according to published papers there have been no data on electrophoretic studies of serum proteins, hematological and biochemical analysis of cats with FeLV/FCoV coinfection. Therefore, this study aimed to determine serum protein electrophoresis changes, the alteration of the hematological and biochemical parameters of client-owned cats with FeLV/FCoV coinfection.

## 2. MATERIALS AND METHODS

### 2.1. Animals

This study enrolled 6 cats that were included in a study (Raukar, 2021a), in which the prevalence of FeLV/FCoV was determined. Blood sampling and virological laboratory methods for the detection of FeLV/FCoV coinfection have been described in this study (Raukar, 2021a), but the laboratory results of serum protein electrophoresis (SPE), complete blood count (CBC) and biochemical analysis were not presented in this previous study. Therefore, laboratory results of complete blood count (CBC), total protein, albumin, globulin, albumin/globulin ratio (A/G ratio), urea, creatinine, aspartate transaminase (AST), and serum protein fractions of FeLV/FCoV+ cats are presented in this study.

### 2.2. Ethical consideration

Ethical approval was not required (Ministry of Agriculture of the Republic of Croatia, Reference numbers: 525-6-08-3 BBŠ; 525-6-08-5 BBŠ) because this study did not include animal experiments.

### 2.3. Sampling

From each of the 6 individual client-owned cats, 1 ml and 0.5 ml of whole venous blood were aseptically sampled for SPE and biochemical analysis into tubes that did not contain anticoagulants. First blood samples were left for 30 min standing at room temperature to allow the blood to clot. After 30 min, serum was obtained by centrifugation at 4,500 rpm for 5 min. The serum was pipetted into another tube, and the samples were stored at  $-20^{\circ}\text{C}$ . From each of the 6 individual client-owned cats, blood smears of peripheral blood were prepared for the determination of leukogram. Two blood smears of FeLV/FCoV+ cats were not thin, and therefore it was not

possible to determine the differential blood count from these FeLV/FCoV+ cats. Samples were transported on dry ice with blood smears within 48 h after collection to the Clinical Pathology Platform, Faculty of Veterinary Medicine, Vienna, Austria, where diagnostic tests were conducted. From each of the 4 individual client-owned cats, 0.5 ml of whole venous blood was aseptically sampled for hematological tests into tubes with EDTA (ethylenediaminetetraacetic acid). Total erythrocyte count (TRBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT)/packed cell volume (PCV), hemoglobin (Hb), total leukocyte count (TWBC) and platelet (PLT) (thrombocyte) count were analyzed immediately after sample collection in veterinary clinic for small animals "Dr. Pezo" in Zagreb, Croatia.

#### **2.4. Hematology**

#### **2.5. Total erythrocyte count, MCV, MCH, MCHC, hematocrit, hemoglobin, total leukocyte count and platelet (thrombocyte) count**

Total erythrocyte count, MCV, MCH, MCHC, hematocrit, hemoglobin, total leukocyte count and platelet (thrombocyte) count were performed on an automated hematology analyzer Beckman Coulter.

#### **2.6. Differential blood count**

The differential blood count was determined manually microscopically from Romanowsky (Haemaquick<sup>®</sup>, Biomed, Oberschleißheim Germany) stained blood smears.

#### **2.7. Serum biochemistry**

#### **2.8. Total protein**

Total protein content was measured using a fully selective autoanalyzer (Hitachi 911<sup>®</sup>; Roche Diagnostics, Vienna) using the colorimetric biuret method.

#### **2.9. Albumin**

Albumin was measured using a fully selective autoanalyzer (Hitachi 911<sup>®</sup>; Roche Diagnostics, Vienna) using Bromocresol Green method.

#### **2.10. Globulin**

Globulin concentration was calculated using formula: Total protein (g/dl) - Albumin (g/dl).

#### **2.11. Albumin/globulin ratio**

The ratio albumin/globulin was calculated using formula A/G ratio = albumin/globulin.

#### **2.12. Urea**

Urea was measured using a fully selective autoanalyzer (Hitachi 911<sup>®</sup>; Roche Diagnostics, Vienna) using enzymatic-kinetic UV-test.

#### **2.13. Creatinine**

Creatinine was measured using a fully selective autoanalyzer (Hitachi 911<sup>®</sup>; Roche Diagnostics, Vienna) using enzymatic color test.

#### **2.14. Aspartate transaminase (AST)**

Aspartate aminotransferase (AST) was measured using a fully selective autoanalyzer (Hitachi 911<sup>®</sup>; Roche Diagnostics, Vienna) using International Federation of Clinical Chemistry (IFCC) method.

#### **2.15. Serum protein electrophoresis**

Serum protein electrophoresis (SPE) was performed on cellulose acetate strips using the fully automated system Interlab Genio<sup>®</sup> (Menarini, Austria) following the procedure described in a study (Raukar, 2021b).

For complete blood count reference ranges were: erythrocyte count ( $5.50-10.00 \times 10^{12}/L$ ), hemoglobin concentration (100.0-150.0 g/L), hematocrit (30.0-45.0%), MCV (37.0-49.0 fL), MCH (13.0-17.0 pg), MCHC (320.0-380.0 g/L), leukocyte count ( $5.5-19.5 \times 10^9/L$ ), platelet (thrombocyte) count ( $300.0-700.0 \times 10^9/L$ ), non-segmented neutrophils (< 4.0%), segmented neutrophils (60.0-75.0%), lymphocytes (15.0-30.0%), monocytes (< 5.0%), eosinophils (< 4.0%) and basophils (< 1.0%). Reference ranges for serum biochemistry were: total protein (6.00-7.50 g/dl), albumin (2.8-3.9 g/dl), globulin (2.6-5.1 g/dl), A/G ratio (0.45-1.19), urea (20.0-65.0 mg/dl), creatinine (< 1.60 mg/dl) and AST (< 100 U/L). The following reference values for serum protein electrophoresis were: albumin (2.6-5.6 g/dl),  $\alpha_1$ -globulins (0.2-1.3 g/dl),  $\alpha_2$ -globulins (0.4-1.1 g/dl),  $\beta_1$ -globulins (0.3-1.5 g/dl),  $\beta_2$ -globulins (1.1-1.4 g/dl) and  $\gamma$ -globulins (0.6-2.6 g/dl).

### **3. RESULTS**

#### **3.1. Hematology**

##### **3.2. Erythrocytes**

Of the four FeLV/FCoV+ cats in which erythrocytes were measured, two cats (50.0%) had normal number of erythrocytes, and two cats (50.0%) had decreased number of erythrocytes ( $3.12 \times 10^{12}/L$ ;  $5.23 \times 10^{12}/L$ ).

##### **3.3. Hemoglobin**

Of the four FeLV/FCoV+ cats in which hemoglobin was measured, three cats (75.0%) had decreased values of hemoglobin which varied from up 72 g/L to 87 g/L, and one cat had normal values.

##### **3.4. Hematocrit**

Of the four FeLV/FCoV+ cats in which hematocrit was measured, two cats (50.0%) had normal values of hematocrit, and two cats (50.0%) had decreased levels of hematocrit (17.6%; 24.7%).

### **3.5. MCV**

Of the four FeLV/FCoV+ cats in which MCV was measured, two cats (50.0%) had normal values of MCV, and two cats (50.0%) had increased levels of MCV (49.6 fL; 56.6 fL).

### **3.6. MCH**

Of the four FeLV/FCoV+ cats in which MCH was measured, three FeLV/FCoV+ cats (75.0%) had normal values of MCH, and one cat had increased levels of MCH (26.1 pg).

### **3.7. MCHC**

Of the four FeLV/FCoV+ cats in which MCHC was measured, three FeLV/FCoV+ cats (75.0%) had decreased values of MCHC which varied from up 287 to 304 g/L, and one cat had increased levels of MCHC (461 g/L).

### **3.8. Platelets**

Of the four FeLV/FCoV+ cats in which platelets were measured, two cats (50.0%) had normal number of platelets, and two cats (50.0%) had decreased number of platelets ( $194 \times 10^9/L$ ;  $260 \times 10^9/L$ ).

### **3.9. Leucocytes**

Of the four FeLV/FCoV+ cats in which leucocytes were measured, three FeLV/FCoV+ cats (75.0%) had normal number of leucocytes, and one cat had decreased number of leucocytes ( $5.3 \times 10^9/L$ ).

### **3.10. Non-segmented neutrophils**

Of the four FeLV/FCoV+ cats in which non-segmented neutrophils were measured, four cats had normal non-segmented neutrophils.

### **3.11. Segmented neutrophils**

Of the four FeLV/FCoV+ cats in which segmented neutrophils were measured, two of four FeLV/FCoV+ cats (50%) had segmented neutrophils within the physiological range, one had increased (95.0%) and one had decreased values (37.0%).

### **3.12. Lymphocytes**

Of the four FeLV/FCoV+ cats in which lymphocytes were measured, two of four FeLV/FCoV+ cats (50%) had normal lymphocytes values, one had increased (51.0%), and one had decreased values (5.0%).

### **3.13. Monocytes**

Of the four FeLV/FCoV+ cats in which monocytes were measured, two of four FeLV/FCoV+ cats (50%) had normal monocytes values, and two (50%) had increased values (6.0%; 8.0%).

### **3.14. Eosinophils**

Of the four FeLV/FCoV+ cats in which eosinophils were measured, two of four FeLV/FCoV+ cats (50%) had normal eosinophils values, and two (50.0%) had increased values (5.0%; 8.0%).

### **3.15. Basophils**

Of the four FeLV/FCoV+ cats in which basophils were measured, all four FeLV/FCoV+ cats had basophils values within the normal range.

### **3.16. Serum biochemistry**

#### **3.17. Total serum protein**

Four of six FeLV/FCoV+ cats (66.7%) had increased total protein levels which varied from up 8.46 to 10.24 g/dl, whereas the remaining two had normal levels.

#### **3.18. Albumin**

Four of six FeLV/FCoV+ cats (66.7%) had normal albumin levels, one had increased levels (4.46 g/dl), and one had decreased levels (2.75 g/dl).

#### **3.19. Globulin**

Three of six FeLV/FCoV+ cats (50.0%) had increased globulin levels which varied from up 6.74 to 6.96 g/dl, whereas the remaining three (50.0%) had normal levels.

#### **3.20. Albumin/globulin ratio (A/G)**

Five of six FeLV/FCoV+ cats (83.3%) had normal A/G ratio, and one had decreased levels (0.41).

#### **3.21. Urea**

Five of six FeLV/FCoV+ cats (83.3%) had normal values of urea, and one had increased values (140.0 mg/dl).

#### **3.22. Creatinine**

All six FeLV/FCoV+ cats had creatinine values within the physiological range.

#### **3.23. Aspartate transaminase (AST)**

All six FeLV/FCoV+ cats had AST values within the normal range.

#### **3.24. Serum protein electrophoresis**

##### **3.25. Albumin**

Four of six FeLV/FCoV+ cats had normal albumin values (66.7%), and two had decreased values (1.9 g/dl; 2.3 g/dl).

##### **3.26. $\alpha_1$ -globulins**

Four of six FeLV/FCoV+ cats (66.7%) had  $\alpha_1$ -globulins within the physiological range, whereas the remaining two had decreased levels (0.1 g/dl).

### **3.27. $\alpha_2$ -globulins**

Four of six FeLV/FCoV+ cats (66.7%) had normal  $\alpha_2$ -globulin levels, whereas two had increased values (1.5 g/dl; 1.6 g/dl).

### **3.28. $\beta_1$ -globulins**

The  $\beta_1$ -globulin levels of all six FeLV/FCoV+ cats were within the physiological range.

### **3.29. $\beta_2$ -globulins**

Five of six FeLV/FCoV+ cats (83.3%) had decreased levels of  $\beta_2$ -globulin which varied from up to 0.5 to 0.8 g/dl, whereas one had normal values.

### **3.30. $\gamma$ -globulins**

Three of six FeLV/FCoV+ cats (50.0%) had increased  $\gamma$ -globulin levels which varied from up to 2.8 to 5.4 g/dl, whereas the remaining three (50.0%) had normal levels.

### **3.31. Other characteristics**

Monoclonal peak in the  $\alpha_2$ -globulin region and a polyclonal peak in the  $\gamma$ -globulin region of the electrophoretogram were detected in one FeLV/FCoV+ cat.

### **3.32. Health status**

Five of six FeLV/FCoV+ cats (83.3%) had clinical signs suggestive of FeLV infection. Clinical signs of FCoV infection or feline infectious peritonitis (FIP) were not observed in all six FeLV/FCoV+ cats.

## **4. DISCUSSION**

In this study decreased  $\beta_2$ -globulin levels was the most frequently detected SPE abnormality in cats with FeLV/FCoV coinfection.

Increased levels of  $\alpha_2$ -globulin with concurrently increased levels of  $\gamma$ -globulin were observed in two FeLV/FCoV+ cats, one of which had a monoclonal peak in the  $\alpha_2$ -globulin region and a polyclonal peak in the  $\gamma$ -globulin region of the electrophoretogram, whereas another cat had increased segmented neutrophil values. These findings indicate that these cats may have had an acute immune response to infection or inflammation (Miró *et al.*, 2007; Flo *et al.*, 2019).

Decreased albumin and increased urea levels were observed in one FeLV/FCoV+ cat. These results indicate that FeLV infection may have induced kidney dysfunction in this cat (Hoffman-Lehmann *et al.*, 1997; Arjona *et al.*, 2000).

In serum biochemistry, the most frequently observed changed biochemical parameters included increased total protein levels in most FeLV/FCoV+ cats with clinical signs suggestive of FeLV infection. One of these cats had increased albumin level, but normal globulin level and A/G ratio. These findings indicate that hyperproteinemia is caused by dehydration (Beer *et al.*, 2000). Hyperproteinemia with concurrently increased globulin levels were frequently observed in cats with clinical signs. These results indicate that hyperproteinemia was caused by hyperglobulinemia. The A/G ratio was reduced in one FeLV/FCoV+ cat, as a result of hypoalbuminemia and hyperglobulinemia.

Anemia was the most frequent hematological abnormality in FeLV/FCoV+ cats in this study. The most frequently observed changed red blood cell (RBC) parameters included decreased levels of hemoglobin and MCHC indicating hypochromic anemia (Furman *et al.*, 2014). Decreased number of erythrocytes and decreased levels of hematocrit were frequently observed in presented study, which is indicative of anemia (Furman *et al.*, 2014). Thrombocytopenia was also frequently observed in FeLV/FCoV+ cats in this study. Leukopenia was observed in one FeLV/FCoV+ cat with anemia, which is indicative of secondary infections and immunodeficiency (Arjona *et al.*, 2000). Anemia, leukopenia and thrombocytopenia observed in FeLV/FCoV+ cats with clinical signs suggestive of FeLV infection in presented study may be due by FeLV infection (Arjona *et al.*, 2000; Hartmann & Gleich, 2009). Anemia in 50.0% of the FeLV+ cats and leukopenia in 46.2% of the FeLV+ cats were observed in study in Spain (Arjona *et al.*, 2000). In study in Germany (Hartmann & Gleich, 2009) thrombocytopenia was found in FeLV+ cats.

Increased or decreased segmented neutrophils, lymphocytes, monocytosis and eosinophilia were observed in FeLV/FCoV+ cats with clinical signs suggestive of FeLV infection in this study. The alterations in the segmented neutrophils and lymphocytes were observed in two FeLV/FCoV+ cats. One of these cat had eosinophilia. The alteration of the leukogram observed in FeLV/FCoV+ cats may be due by FeLV infection (Arjona *et al.*, 2000; Collado *et al.*, 2012). Studies in Spain (Arjona *et al.*, 2000; Collado *et al.*, 2012) and Germany (Gleich & Hartmann, 2009) reported the alterations in the lymphocytes, neutrophils, monocytosis and eosinophilia in FeLV+ cats.

## **5. CONCLUSION**

SPE abnormalities were observed in all FeLV/FCoV+ cats. The most frequently abnormality detected by SPE was decreased levels of  $\beta_2$ -globulin in FeLV/FCoV+ cats. Increased  $\alpha_2$ -globulin and  $\gamma$ -globulin levels were

observed in two cats. Biochemical changes were observed in most FeLV/FCoV+ cats. The main biochemical changes were hyperproteinemia and hyperglobulinemia. Anemia, decreased levels of hemoglobin and MCHC were the most frequent hematological abnormalities in four FeLV/FCoV+ cats. Leukogram changes were observed in four FeLV/FCoV+ cats. The results of this study suggest that SPE, hematological and biochemical parameters are useful diagnostic tools for diagnosis of FeLV/FCoV coinfection. Further research using a larger number of FeLV/FCoV+ cats is required, because this study is underpowered for statistical analyses.

### Conflict of Interest Statement

The author declares that there is no conflict of interest.

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### LIST OF ABBREVIATIONS

FeLV: feline leukemia virus

FCoV: feline coronavirus

FIP: feline infectious peritonitis

SPE: serum protein electrophoresis

A/G: albumin/globulin ratio

AST: aspartate transaminase

EDTA: ethylenediaminetetraacetic acid

TRBC: total erythrocyte count

MCV: mean corpuscular volume

MCH: mean corpuscular hemoglobin

MCHC: mean corpuscular hemoglobin concentration

Hb: hemoglobin

HCT: hematocrit

PCV: packed cell volume

TWBC: total leukocyte count

PLT: platelets

IFCC: International Federation of Clinical Chemistry

RBC: red blood cell