

## Alterations in Some Haematological and Biochemical Parameters in Cattle Suffering from Foot- and -Mouth Disease

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Received: 19.02.2003

**Abstract:** The present study was designed to evaluate serum biochemical and haematological parameters and serum nitrate concentrations in cattle suffering from foot- and -mouth disease (FMD). For this purpose, 30 Swiss Brown cattle aged between 3 and 5 years were used. Twenty cattle showed characteristic clinical signs of FMD, whereas the remaining 10 were clinically healthy and used as controls. Peripheral blood samples were collected from both groups and used to establish total white blood cells (WBCs), red blood cells (RBCs), haemoglobin (Hb), and haematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH). Serum samples were also collected from these animals and used to establish the concentrations of total protein, albumin, urea nitrogen, creatinin, glucose, calcium, phosphorus, nitrate (NO<sub>3</sub>) and cholesterol and activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP). There was a significant decrease in the total number of RBCs ( $P < 0.05$ ) and a significant increase in MCV ( $P < 0.05$ ) in cattle with FMD compared to the control group. There were no significant differences in the other haematological parameters evaluated. Serum glucose concentration was significantly higher ( $P < 0.001$ ) in the FMD group than in the control group. Serum total protein ( $P < 0.05$ ), calcium ( $P < 0.01$ ), albumin ( $P < 0.001$ ) and cholesterol ( $P < 0.01$ ) levels were significantly low in the FMD group compared to those in the control group. However, there were no significant differences in the other biochemical parameters between the groups. The concentration of serum NO<sub>3</sub> was significantly high in the FMD group, suggesting that Aphtovirus induces the production of nitric oxide in vivo.

**Key Words:** Aphtovirus, biochemistry, cattle, foot- and -mouth disease, haematology, nitrate.

### Şap Hastalığına Yakalanmış Sığırlarda Bazı Hematolojik ve Biyokimyasal Parametrelerdeki Değişiklikler

**Özet:** Bu çalışma sığırlarda şap hastalığının hematoloji, klinik biyokimyasal parametreler ve serum nitrat düzeylerine etkilerini araştırmak amacıyla yapıldı. Araştırma, yaşları 3-5 arasında olan, şap hastalığına yakalanmış 20 ve sağlıklı 10 montofon ırkı sığır üzerinde yapıldı. Hematolojik olarak total eritrosit (RBC), total lökosit (WBC), hematokrit (PCV), hemoglobin (Hb), ortalama eritrosit hacmi (MCV), ortalama eritrosit hemoglobin (MCH) ve ortalama eritrosit hemoglobin konsantrasyonları (MCHC) belirlendi. Biyokimyasal olarak, serum alkalin fosfataz (AP), serum aspartat aminotransferaz (AST), serum alanin amino transferaz (ALT) aktiviteleri ile serum kreatinin, kan üre nitrojen, albumin, total protein, glikoz, kalsiyum, fosfor, nitrat (NO<sub>3</sub>) ve kolesterol konsantrasyonları ölçüldü. Şaplı hayvanlarda ortalama RBC değerlerinin sağlıklı hayvanlara göre önemli düzeyde düşük ( $P < 0,05$ ) ve MCV değerlerinin önemli derecede yüksek olduğu ( $P < 0,05$ ) belirlendi. Diğer hematolojik parametrelerde ise önemli bir değişiklik görülmedi. Şaplı hayvanlarda serum glikoz konsantrasyonunun sağlıklı gruba göre önemli derecede yüksek ( $P < 0,001$ ), buna karşın serum kalsiyum ( $P < 0,01$ ), total protein ( $P < 0,05$ ), albumin ( $P < 0,001$ ) ve kolesterol ( $P < 0,01$ ) konsantrasyonlarının önemli düzeyde düşük oldukları belirlendi. Ölçülen diğer biyokimyasal parametrelerde önemli bir değişiklik saptanmadı. Ayrıca araştırmada şap hastalığına yakalanmış hayvanların serumlarında yüksek düzeyde nitrik oksid indikatörü olan nitrat belirlendi

**Anahtar Sözcükler:** Aphtovirus, biyokimya, sığır, şap, hematoloji, nitrat.

### Introduction

Foot- and -mouth disease (FMD), also known as aphthous fever, is a highly contagious disease of all cloven-footed domestic and wild animals. It is caused by a

picornavirus of the genus Aphtovirus (1-3). FMD is world-wide in its distribution and is also endemic in the Kars region of Turkey. The disease is characterised by fever, blisters or vesicles, erosions and ulcers in the

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mucosa of the mouth, tongue, lips, gums, pharynx and palate. Vesicles may also be found on the coronary band, between the claws, and on the teats, especially of nursing cows or sows. Lameness is evident in animals with foot lesions and may be the only sign of infection in sheep and goats. Some strains of the virus cause necrosis of heart muscle and may result in death before lesions develop in the more common and visible locations such as the mouth or foot (4,5).

The epidemiology and aetiology of FMD have been extensively investigated (1,2). However, there are few published reports on the haematological and biochemical parameters of cattle with FMD (6,7).

Nitric oxide is a cytotoxic factor released by a variety of cells, including monocytes/macrophages (8) neutrophils (9), endothelial cells, hepatocytes (10) and thrombocytes (11). It is generated from the terminal guanidine nitrogen atom of L-arginine by nitric oxide synthase (iNOS). Nitric oxide is known to play a major role in the primary defence against several bacteria (10,12), viruses (13,14) and parasites (15,16). Studies with several viruses have shown nitric oxide to be responsible for inhibition of replication of the DNA viruses like herpes simplex virus, poxviruses and vaccinia virus (17-19). But there is no published information, however, to the best of our knowledge on the production of nitric oxide by cattle with FMD.

The aim of the present study was to determine the possible alterations in haematological and biochemical parameters in cattle with FMD. Serum nitrate ( $\text{NO}_3$ ) and nitrite ( $\text{NO}_2$ ) were used as indicators of the production of nitric oxide. Therefore serum nitrate ( $\text{NO}_3$ ) concentration was also determined to find out whether Aphthovirus induces the production of nitric oxide in vivo.

## Materials and Methods

### Cattle

Thirty Swiss Brown cattle aged between 3 and 5 years were used in this study. Of these, 20 showed characteristic clinical signs of FMD. The remaining 10 cattle, being clinically healthy, were used as controls. A routine clinical examination was carried out and the lesions were recorded. Animals without characteristic lesions for FMD were not used in the study.

### Haematology

Peripheral blood samples collected from the jugular vein into EDTA treated tubes were used to establish total white blood cells (WBCs), total red blood cells (RBCs) (20), packed cell volume (PCV %) and haemoglobin concentration (Hb) (21) manually. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Willard et al. (20).

### Clinical Chemistry

Peripheral blood samples collected into plain tubes were used to separate serum samples. These serum samples were then stored in  $-20^\circ\text{C}$  until used. Serum concentrations of calcium (Ca), phosphorus (P), urea nitrogen, creatinin, total protein, albumin, glucose and cholesterol and activity of alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using commercial kits (Abbott Alcyon) on a selective chemistry analyser (Abbott Alcyon 300I, USA).

### Detection of Serum Nitrate ( $\text{NO}_3$ )

The concentration of serum nitrate, an indicator of nitric oxide, was determined according to the methods of Stevenson et al. (16) and Gokce and Woldehiwet (22). Briefly, 30  $\mu\text{l}$  of each serum sample in sterile plastic vials was incubated at room temperature for 20 min with 5  $\mu\text{l}$  of enzyme nitrate reductase (% U/ml; Sigma) and 15  $\mu\text{l}$  of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH, 1.25 mg/ml; Sigma). After incubation, 100  $\mu\text{l}$  of Griess Reagent (1% sulphanilamide and 0.1% naphthyl ethylene diamine dihydrochloride in 2.5 % phosphoric acid; Sigma) and 100  $\mu\text{l}$  of trichloroacetic acid (10% aqueous solution; Sigma) were added. Protein precipitates were removed by centrifugation at 8000 g for 5 min and 100  $\mu\text{l}$  of each supernatant, in duplicate, was transferred to the wells of 96-well flat-bottomed microtitre plates. Absorbance at 550 nm was measured immediately with a microplate reader (Tecan-Spectra, Austria). The concentrations of nitrate in serum samples were calculated by means of a standard curve derived from 2-fold dilutions of sodium nitrate (Sigma) in pooled serum obtained from uninfected

cattle, which was dialysed against phosphate buffered saline (PBS) for 24 h (16). The concentrations of nitrate in the standard used ranged from 0.97 to 1000 µM.

### Statistical analyses

All the values were expressed as mean + SD. Student's t-test was used to compare the values obtained from animals in the FMD group and the control group (23).

## Results

### Haematology

The total number of RBCs in the FMD group was significantly lower than that in the control group ( $P < 0.05$ ), while MCV values were significantly higher in the FMD group. There were no significant differences in the other haematological parameters between the groups (Table 1).

Table 1. Haematological parameters in the FMD and control groups. Data are expressed as mean and mean of the standard deviation (mean ± SD).

Parameters	FMD group (n = 20)	Control group (n = 10)
WBC ( $\times 10^3/\mu\text{l}$ )	6.00 ± 2.27	6.41 ± 1.23
RBC ( $\times 10^6/\mu\text{l}$ )	4.45 ± 1.33*	5.47 ± 0.98
PCV (%)	24.70 ± 6.27	25.10 ± 3.63
Hb (g/dl)	8.79 ± 1.69	9.07 ± 1.08
MCV (fl)	56.70 ± 8.28*	47.10 ± 9.92
MCH (pg)	20.40 ± 3.94	17.10 ± 3.94
MCHC (g/dl)	36.00 ± 3.27	36.71 ± 5.90

Significant differences in the values between the FMD group and control group are indicated by \*  $P < 0.05$ .

### Clinical chemistry

There were significant decreases in the concentrations of serum total protein ( $P < 0.05$ ), albumin ( $P < 0.001$ ), calcium ( $P < 0.01$ ) and cholesterol ( $P < 0.01$ ), while the concentration of serum glucose was significantly higher in the FMD group ( $P < 0.001$ ). No significant changes were determined in the other biochemical parameters between the groups (Table 2).

Table 2. Serum biochemical parameters in the FMD and control groups. Data are expressed as mean and mean of the standard deviation (mean ± SD).

Parameters	FMD group (n = 20)	Control group (n = 10)
AP (IU/l)	93.07 ± 39.1	73.70 ± 26.20
ALT (IU/l)	41.53 ± 12.4	54.60 ± 19.40
AST(IU/l)	97.30 ± 42.8	99.90 ± 25.30
Creatinin (mg/dl)	1.05 ± 0.34	1.00 ± 0.16
Albumin (g/dl)	3.31 ± 0.24***	4.28 ± 0.43
Urea (mg/dl)	10.61 ± 5.70	14.00 ± 6.59
Glucose (mg/dl)	67.60 ± 9.49***	43.30 ± 8.90
Total protein (g/dl)	7.39 ± 0.70*	8.10 ± 0.74
Calcium (mg/ dl)	8.15 ± 0.24**	9.18 ± 0.85
Phosphorus (mg/dl)	5.60 ± 1.94	6.81 ± 1.37
Cholesterol (mg/dl)	119.15 ± 46.20**	191.60 ± 57.90
Nitrate (µM)	12.54 ± 3.82***	4.62 ± 0.45

Significant differences in the values between the FMD and control groups are indicated by \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

### Nitrate levels

There was a significant increase in the concentrations of nitrate in serum samples obtained from the FMD group compared to those from the control group ( $P < 0.001$ ). The mean and the mean of the standard deviation of the nitrate concentrations (mean ± SD) in serum samples obtained from the FMD group was 12.54 ± 3.82 µM, whereas the concentration of nitrate in serum samples from the control group was 4.62 ± 0.45 µM.

## Discussion

In the present study, characteristic clinical signs of FMD were observed in all the cattle in the FMD group. These clinical signs were in agreement with those in previous reports (4,24,25). A significant reduction in the total number of RBCs and an increase in MCV values were observed in the FMD group. These findings may indicate anaemia (26). However, haemorrhage and haemolysis were not observed in cattle with FMD and the other haematological parameters were normal in this study. The significant reduction in RBCs and elevation in MCV may be attributed to endocrinopathy as reported previously (4), but to what extent endocrinopathy occurs in cattle with FMD requires further study.

Reductions in serum albumin and total protein concentrations have been reported to be associated with

hepatic and renal damage, starvation, and enteropathies resulting in protein loss, parasitic infestation and chronic organ diseases (27), but there were no changes in AST, AP, creatinin or urea, an indicator of liver and kidney functions, indicating abnormal hepatic and renal functions. Possible causes of the decreases in serum albumin and total protein concentrations observed in the study may be associated with lesions on the oral mucosa and interdigital regions. It is known that protein requirement increases in the presence of any lesions on the body (28). It is also known that consumption of protein increases in animals with diabetes mellitus (29), as was detected in cattle with FMD (7). Therefore, diabetes mellitus may be another reason for the decrease in protein concentrations observed in this study. Low albumin and protein concentrations may also be due to alterations in pancreatic  $\beta$ -cell functions that might have developed during the clinical course of FMD as reported by Barboni et al. (30).

In the FMD group, serum concentrations of glucose increased significantly, while calcium concentrations of serum significantly decreased compared to those in the control group. It is well documented that an increase in glucose concentration is a common finding in cattle affected by the stress of a systemic disease (31). Kaneko et al. (28) also reported that the increase in blood glucose concentration was in response to hypocalcaemia because of interference with the secretion of insulin from the pancreas. An adequate amount of calcium ions in extracellular fluids is required for insulin secretion in response to glucose and other secretagogues for insulin. This may explain the increase in glucose concentrations observed in this study and it may be associated with the

diabetes mellitus reported in cattle with FMD by Sahal et al. (7).

The low calcium concentration in this study may be associated with inappetence and hypoproteinaemia as reported elsewhere (28,32). Therefore, in the study there was a significant decrease in serum protein levels and severe anorexia in cattle with FMD, which may be the possible explanation for the hypocalcaemia observed.

A significantly low level of cholesterol was observed in cattle with FMD. It is known that conditions such as pancreatic dysfunction that decrease cholesterol synthesis will also decrease insulin synthesis, resulting in hypocalcaemia, hypoproteinaemia and hyperglycaemia (28). This may explain the low cholesterol levels observed in the study as pancreatic  $\beta$ -cell dysfunction was reported in cattle with FMD (30).

A significantly high level of nitrate, an indicator of nitric oxide production, was detected in serum samples obtained from cattle with FMD. The results of this study suggest that Aphthovirus, the causative agent of FMD, induces the production of nitric oxide in vivo. The production of nitric oxide is known to be induced by various viruses and it inhibits the replication of several viruses (17-19). However, further studies are needed to determine the role of nitric oxide produced by cattle infected with Aphthovirus.

In conclusion, biochemical and haematological alterations may indicate the development of anaemia and pancreatic dysfunction in cattle suffering from FMD. This study also indicated that Aphthovirus induces the production of nitric oxide, in vivo. These findings provided information on our understanding of the clinical pathology and pathogenesis of FMD in cattle.

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