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Molecular Characterization, Hematological and Biochemical Studies on Foot and Mouth Disease Virus Serotype O in Buffaloes and Cows in Dakahlia Governorate, Egypt

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Abstract

The present study aimed to evaluate the hematological and serum biochemical parameters in buffaloes and cows infected with foot and mouth disease (FMD) virus. Forty buffaloes and cows (6 months - 2 years old) were used. Twenty buffaloes and cows clinically healthy and were used as control, the other twenty buffaloes and cows showed characteristic clinical signs of FMD. Blood samples were collected and serum was separated from both groups to measure the hematological and biochemical parameters. Infected buffaloes and cows showed fever, anroxia, excessive foamy salivation and ulcer formation in the mouth. Compared to the control buffaloes, erythrocytic count (RBCs), hemoglobin (Hb) and packed cell volume (PCV) in the FMD infected buffaloes were significantly decreased, while mean corpascular hemoglobin (MCH) was significantly increased. In addition, significant increase in toltal leukocyte count (TLC), neutrophils, with a significant decrease in eosinophil and lymphocyte counts were observed. Moreover, cows infected with FMD virus showed no significant changes in the erythrogram, while, significant increases in TLC and neutrophils, with a significant decrease in eosinophil and lymphocyte counts were observed when compared with the control group. There were significant decreases in the levels of serum total proteins, albumin and globulin, while serum asprtate transferase (AST), creatinine phospho-kinase (CPK), glucose and cortisol levels were significantly increased in the infected animals. Tissue and saliva specimens were collected and analyzed by using universal primer using reverse-transcription polymerase chain reaction (RT-PCR). All the identified viruses were of serotype 'O'. It is concluded that FMDV serotype 'O' is circulating among cows and buffaloes in the study area.

Keywords: FMD, RT-PCR, Cows, Buffaloes, Cortisol.

Introduction

Improving cattle and buffaloes' production in Egypt is a recognized pathway to alleviate rural poverty and improve food security. Foot and mouth disease (FMD) is a highly contagious and fatal viral disease of bi-cloven animals. The disease leads also to economic losses which include reduction in milk and meat production, reproductive inefficiencies and death in young ruminants [1]. It is characterized clinically by fever, depression, anorexi and, excessive foamy salivation with vesicles appearing on the tongue [2]. Some infected animals remain asymptomatic, but they dissiminate the virus to other animals. Initial diagnosis is usually done on the basis of clinical signs, while, confirmation of the the etiological agent is based on Reverse transcriptase-polymerase chain reaction (RT-PCR) which is reliable, rapid and highly sensitive method for early diagnosis [3]. The FMDV belongs to the genus *Aphthovirus* of the family *Picornaviridae*. There are seven serotypes (O, A, C, Asia1, SAT1, SAT2 and SAT3) and more than 65 subtypes [4]. Mixed rearing of cattle, buffaloes, sheep and goats has lead to the persistence infection and spread of FMDV [5]. The virus is RNA virus which is non-enveloped, positive sense ssRNA genome of about 8.3 kb and enclosed within a protein capsid. This protein capsid is composed of 60 copies of four different structural poly peptides (VP1, VP2, VP3) which are surface, while, VP4 is entirely internal [6-8]. The VP1 protein is highly immunogenic, whereas, VP2 and VP3 contribute to the antigenic properties of the virus [9].

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The purpose of this study was the molecular detection of FMDV in clinical samples obtained from suspected animals using RT-PCR. The hematological and biochemical effect of FMD virus on infected and control animals was also determined.

Material and Methods

Animals

A total number of forty female buffaloes and cows (6 months – 2 years old) were used in the current study. The animals were examined in Dakahlia Governorate from Belqas Center during March and April 2016 . The examined animals were divided into four equal groups, apparently healthy buffaloes and cows (n=10, each) and clinically suspected buffaloes and cows (n=10, each).

Samples

Two blood samples each of 5 mL were collected from the jugular vein of each animal. The first blood sample was taken in clean Wasserman tubes containing dipotassium salts of EDTA for hematological examination. The other sample was collected without anticoagulant in a plain centrifuge test tube, then left to clot and centrifuged at 3000 r.p.m for 20 min for biochemical analysis.

Samples from clinically suspected animals showing clinical symptoms (n=20) were collected in a transport buffer and stored at -20°C until examined. These samples included saliva and vesicular fluid.

RNA extraction and RT-PCR

Total RNA was extracted from saliva and vesicle fluid using the QIAmp RNA extraction kit (Qiagen, USA) according to the manufacturer's instructions. The extracted RNA was reverse transcribed using the QIAGEN One Step RT-PCR Kit (Qiagen, USA) according to the manufacturer's instructions. To confirm the presence of FMDV serotypy O, 1F:5'- ACC AAC CTC CTT GAT GTG GCT 3'; 1R: 5' GAC ATG TCC TCC TGC ATC TG-3' [10] primer sets amplifying 1301 bp were used. The reaction mixture composed of 1 µL of each the forward primer and reverse primer, RNase free water (100 µL), 5x QIAGEN one step RT-PCR buffer (10 µL), dNTP Mix (2 µL), QIAGEN One Step RT-PCR Enzyme Mix (2 µL) and template RNA (10 µL) in 50 µL reaction volume. Amplification reaction was done under the following conditions; one cycle of 50°C for 30 min for reverse transcriptation, one cycle of 95°C for 15 min for pre denaturation followed by 30 cycles of 94°C for 1 min, 58°C for 1 min and 72 °C for 1.5 min for extension, and finally, one cycle of 72°C for 10 min for final extension in Techne thermocycler, UK. The PCR products were confirmed by 1.5 % agarose gelelectrophoresis after ethidium bromide staining and viewing under UV light alongsidea DNA weight markers (Bioneer, Korea).

Hematological examination

Erythrocytes, total and differential leukocytic counts were performed [11]. The packed cell volume (PCV) was estimated by the microhematocrit centrifuge and hemoglobin (Hb) was determined using the cyanmethemoglobin colorimetric method [12]. The values of Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

Biochemical examination

Serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured colorimetrically [13]. The serum bilirubin levels (total, direct and indirect) were determined [14]. Serum levels of creatinine, total proteins [15], albumin [16], blood urea nitrogen (BUN) [17], glucose [18] and cortisol [19] were also estimated. Serum protein electrophrosis was accomplished using a polyaccrylamide gel by using kits of cobasintegra company (Roche, Germany) following the manufaturers instructions. Finally, CPK determined using commercial kits from (Specterum company , Egypt) [20].

Statistical analysis

Statistical analysis was carried out using PASW Statistics (SPSS version 16.0 for Windows). An independent *t*-test was used to compare means from infected and healthy animals.

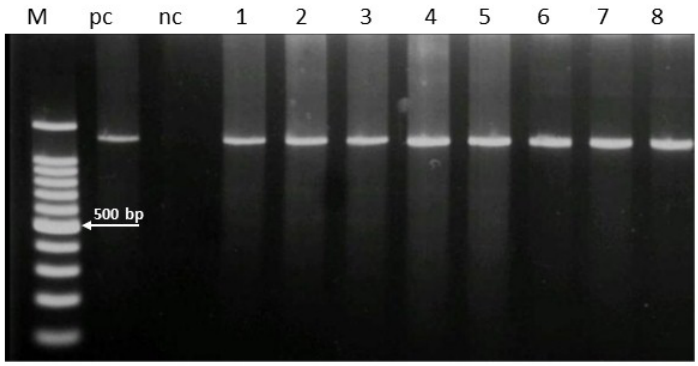

Figure 1: RT-PCR image: Lane-M, 100-bp DNA marker; Lane-PC: positive control (O/EGY/93) Lane-NC: negative control; Lane-1 to 8: FMDV isolates (serotype O) from infected cows and buffaloes 1 to 8 (1301bp).

Results

PCR Analysis

Molecular diagnosis of FMDV serotype O revealed that samples collected from all clinically suspected animals were positive by RT- PCR amplification (Figure 1). While, the apparently healthy group were negative by PCR and considered as a control group.

Clinical signs

Suspectec FMD buffaloes and cows showed fever, inappetence, anorexia, excessive foamy salivation and vesicular eruptions on buccal mucosa (Figure 2).

Hematology

The total number of RBCs, Hb and PCV in the FMD infected buffaloes were significantly decreased compared to apparently healthy buffaloes. The MCH value was significantly increased, with nonsignificant changes in MCV and MCHC in the FMD group. There were a significant increase in TLC, neutrophil count and a significant decrease in eosinophile and lymphocyte counts, with no significant differences in basophile and monocyte count between the groups (Tables 1, 2). Moreover, cows infected with FMD virus showed no significant changes in erythrogram compared with normal control. Significant increases in TLC and neutrophiles with significant decreases in eosinophiles and lymphocytes with no significant changes in monocytes when compared with normal control cows (Table 2).

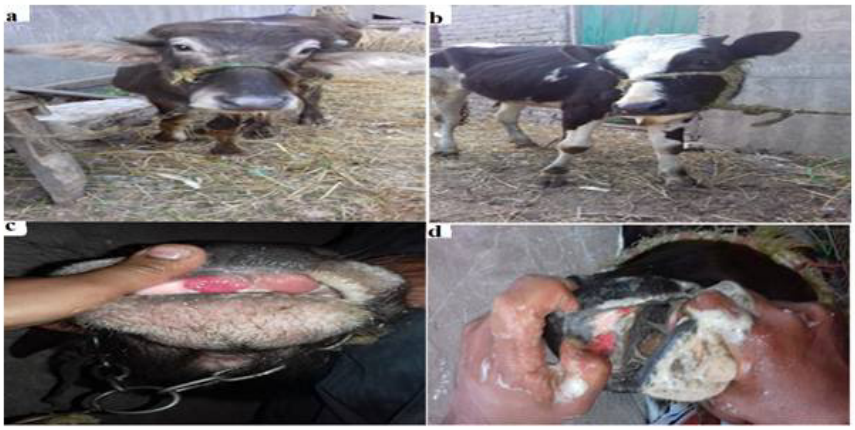

Figure 2: a) Excessive foamy salivation in infected buffalo. b) Excessive foamy salivation in infected cows. C) vesicular eruptions on buccal mucosa of infected buffalo. d) vesicular eruptions on buccal mucosa infected cows.

Biochemical examination

The serum levels of total proteins ($P < 0.05$), albumin ($P < 0.001$), globulin ($P < 0.01$), α1 and α 2 globulins ($P < 0.01$), β globulin ($P < 0.001$) and δ globulin ($P<0.01$) were significantly decreased in infected buffaloes, while the serum AST ($P < 0.001$), CPK ($P < 0.001$), glucose ($P<0.01$) and cortisol ($P < 0.001$) levels significant increase in buffloes infected with FMD virus ($P < 0.001$). No significant changes were recorded in the other biochemical parameters between control and FMD groups (Tables 1 and 2). Cows infected with FMD virus showed a significant decrease in the levels of serum total protein ($P < 0.001$), albumin ($P < 0.001$), globulin ($P < 0.01$), α-2- globulin ($P < 0.05$), β globulin($P < 0.01$) and δ globulin ($P<0.001$). The serum AST ($P < 0.01$), CPK ($P < 0.001$), glucose ($P < 0.01$) and cortisol ($P < 0.01$) were significantly increased, with nonsignificant changes in the other biochemical parameters in cows infected with FMDV compared with the control group (Table 2).

Table 1: Hematological and biochemical parameters (mean ± SD) in the FMD infected buffaloes compared with the normal control

Parameters	Control buffaloes	Infected buffaloes
RBCs ×106/µL	7.11±0.12	6.24±0.08***
Hemoglobin gm%	10.16±0.17	9.32±0.10**
Packed cell volume %	32.00±0.83	28.00±0.31**
Mean corpuscular volume fl	44.98±0.58	44.89±0.62
Mean corpuscular hemoglobin pg	14.40±0.01	14.94±0.16*
Mean corpuscular hemoglobin concentration %	32.00±0.43	33.29±0.43
Total leukocytic counts×10^3/µL	8.44±0.16	9.40[a]±0.33*
Neutrophil ×10^3/µL	3.68±0.14	5.45±0.23***
Eosinophil ×10^3/µL	0.08±0.003	0.02±0.01*
Basophil ×10^3/µL	0.00±0.00	0.00±0.00
Lymphocyte ×10^3/µL	4.44±0.08	3.68±0.13**
Monocyte ×10^3/µL	0.25±0.009	0.23±0.008
Total protein g/dl	7.51 ± 0.40	5.99 ± 0.066***
Albumin g/dl	3.064 ±0.11	2.82±0.033***
Total globulin g/dl	3.87±0.14	3.17±0.058**
α-1-globulin g/dl	0.44±0.035	0.29 ±0.009**
α-2-globulin g/dl	0.74 ±0.036	0.56 ±0.029**
β-globulin g/dl	0.93 ±0.031	0.75 ±0.019***
δ-globulin g/dl	1.75 ±0.040	1.56 ±0.012**
Alanine aminotransferase (U/L)	16.60±0.93	16.60±0.68
Aspartate aminotransferase (U/L)	71.60±0.81	89.20±1.93***
Total Bilirubin mg/dl	0.46±0.02	0.46±0.014
Direct Bilirubin mg/dl	0.3±.013	0.28±0.009
Indirect Bilirubin mg/dl	0.166±0.026	0.18±0.019
CPK (U/L)	223.45±3.07	262.26±3.83***
Glucose mg/dl	42.34±1.138	50.76±1.51**
Cortisol µg/dl	1.188±0.069	2.33±0.227***
Creatinine mg/dl	1.44±0.083	1.3000±0.20976
Urea mg/dl	23.32±0.838	23.58±1.803

*Significant at P≤0.05 **Highly significant at P≤0. 01 ***Very highly significant at P≤0.0 01.

Table 2: Hematological and biochemical parameters (mean ± SD) in the FMD infected cows compared with the normal control

Parameters	Control cows	Infected cows
RBCs ×106/µL	5.45±0.035	5.35±0.041
Hemoglobin gm%	8.88 ±0.185	8.60±0.141
Packed cell volume %	28.40±0.93	28.20±0.66
Mean corpuscular volume fl	52.12±1.77	52.67±0.92
Mean corpuscular hemoglobin pg	16.29±0.26	16.07±0.28
Mean corpuscular hemoglobin concentration %	31.43±1.37	30.55±0.72
Total leukocytic counts×10^3/µL	8.83±0.189	10.36±0.166***
Neutrophil ×10^3/µL	3.56±0.109	5.964±0.112***
Eosinophil ×10^3/µL	0.092±0.003	0.032±0.022**
Basophil ×10^3/µL	0.00±0.00	0.00±0.000
Lymphocyte ×10^3/µL	4.91±0.148	4.066±0.149
Monocyte ×10^3/µL	0.27±0.01	0.26±0.01
Total protein g/dl	5.67±0.131	4.25 ±0.05***
Albumin g/dl	2.85±0.09	2.11 ±0.02***
Total globulin g/dl	2.82±0.13	2.10 ±0.016**
α-1-globulin g/dl	0.36±0.05	0.27 ±0.02
α-2-globulin g/dl	0.64±0.02	0.57 ±0.02*
β-globulin g/dl	0.69±0.03	0.53 ±0.02**
δ-globulin g/dl	1.12±0.05	0.73 ±0.02***
Alanine aminotransferase (U/L)	20.70±0.56	21.07±0.77
Aspartate aminotransferase (U/L)	54.40±1.36	65.80±1.93*
Total Bilirubin mg/dl	0.36±.0.02	0.35±0.019
Direct Bilirubin mg/dl	0.23±0.01	0.23±0.01
Indirect Bilirubin mg/dl	0.13±0.02	0.12±0.02
CPK (U/L)	107.12±1.16	124.47±1.46***
Glucose mg/dl	60.74±1.72	69.93±0.93**
Cortisol µg/dl	1.88±0.18	3.90±0.41**
Creatinine mg/dl	1.21±0.03	1.23±0.02
Urea mg/dl	28.11±0.43	28.56±0.35

*Significant at P≤0.05 **Highly significant at P≤0. 01 ***Very highly significant at P≤0.0 01.

Discussion

FMD is one of the most infectious and vital transboundary animal diseases [21]. Clinically infected animals with FMD virus showed fever, anorexia, excessive salivation and vesicular stomatitis. These signs were reported as the characteristic signs of FMD virus [22]. In this study, serotype O of FMDV was found circulating in buffaloes and cattle. These findings coinside with other reports indicating the predominance of serotype O [10,21,22]. In infected cows there were no significant changes in RBCs, Hb, PCV, MCV, MCH and MCHC in comparison with apparently healthy ones [23], this might be due to the absence of hemorrhage and hemolysis in cows with FMD infection [24]. In infected buffaloes, there was a decrease in the level of RBCs than the control group which could be attributed to a

reduction of the process of erythropoesis [24]. The normocytic normochromic anemia could be resulted from endocrinopathy occurring secondary to viral infection [25] or due to decreased feed intake as a consequence of mouth ulceration. The results of total and differential leukocytic count revealed a significant increase in total leukocytic count with the presence of a highly significant neutrophilia, esinopenia, lymphopenia and no significant change in monocytic count in infected buffaloes and cows. Such increase in the phagocytic cells (neutrophils) could be due to tissue destruction [26]. The lymphopenia may be resulted from the infection of T and B cells with Foot and mouth disease virus in short periods of time post infection, corresponding with the peak of viremia and thus causes a transient immunosuppression [27]. Serum biochemical analysis of infected buffaloes and cows showed significant decrease in total proteins, albumin and globulin. The hypoprotenemia and hypoalbuminemia may be a consequence of off food due to oral lesions [28]. It is clear that protein requirements and protein catabolism increases in case of infections or any lesions in the body [29]. The current finding of protein electrophoresis in cows and buffaloes infected with FMDV revealed significant decrease in total globulins and gamma globulins, this may be explained by the increase in the level of cortisol which suppress the activity of the immune system by inhibiting the lymphoid mitosis and reducing the immune cell number and function [30].

There were non significant changes in the serum ALT activity, BUN and Creatinine levels in the present results indicating normal liver and kidney functions revealing that FMDV had no effect on liver and kidney functions. Moreover, a highly significant increase in the activity of AST, CPK, glucose and cortisol hormone was observed in infected buffales and cows. The increased activity of AST may due to the vesicular stomatitis, muscular or cardiac lesions [31]. Higher rectal temperature due to fever in infected animal induces stress condition which might have accelerated the transaminase activity. The serum level of glucose was significantly increased in diseased animals compared to control one, indicating hyperglycaemia [32]. The hyperglycaemia may be due to destruction of beta cells of islets of Langerhans resulting from FMD virus multiplication which leads to defect in insulin production [33]. FMD infected animals revealed elevation in the serum CPK, which indicated myocardial degeneration, that support the high mortality in young animals due to the degenerative effect of FMD on the myocardium [34].

Conclusion

It could be concluded from the presented study that FMDV serotype O is circulating in the study area. The virus had more seriously effect on hematological, biochemical parameters and stress marker in buffaloes more than cows.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربي

التوصيف الجزيئي، دراسات الدم والكيمياء الحيوية على فيروس الحمى القلاعية النمط المصلي O في الجاموس والأبقار بمحافظة الدقهلية-مصر

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[1]قسم الباثولوجيا الإكلينيكية- كلية الطب البيطري- جامعة الزقازيق

[2]قسم الفيرولوجيا- كلية الطب البيطري- جامعة الزقازيق

الهدف من هّذه الدراسة هو تقييم العوامل البيوكيميائية للدم والمصل في الجاموس والأبقار المصابة بفيروس الحمى القلاعية. تم استخدام عشرون من الجاموس والبقر (٦ أشهر - ٢ سنة) كمجموعة ضابطة . وكانت المجموعة الاخرى مصابة بفيروس الحمى القلاعية.تم تجميع عينات دم وتم فصل مصل الدم من المجموعتين لقياس التغيرات فى صورة الدم والعوامل البيوكيميائية. أظهرت النتائج ان الجاموس والأبقار المصابة بمرض الحمى القلاعية ظهر عليها بعض الاعراض من بينها زيادة فى افراز اللعاب، ارتفاع درجة الحرارة ، فقدان الشهية وقرحة في الفم. بالمقارنة مع المجموعة الضابطة. أظهرت النتائج أن المجموعة المصابة انخفاض فى عدد كرات الدم الحمراء، الهيموجلوبين وحجم كرات الدم الحمراء المتكدسة في الجاموس المصاب بفيروس الحمى القلاعية بشكل ملحوظ. وبالإضافة إلى□لك، لوحظت زيادة كبيرة في عدد خلايا الدم البيضاء . وعلاوة على□لك، لم تظهرالأبقار المصابة بفيروس الحمى القلاعية تغييرات في صورة الدم. بالنسبة للتغيرات البيوكيميائية كانت هناك انخفاضات معنوية في مستويات البروتين الكلي، الزلال والجلوبيولين. كما أن مستويات الجلوكوز والكورتيزول زادت بشكل ملحوظ في الحيوانات المصابة.مع عدم وجود تغيرات فى انزيمات الكبد. تم جمع عينات الأنسجة واللعاب وتحليلها باستخدام االختبار البلمرة المتسلسل كانت جميع العينات الموجبة التي تم تحديدها تنتمي إلى النمط المصلي (O) وخلصت الدراسة إلى أن النمط المصلي (O) يصيب كلا من الأبقار والجاموس.