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RESEARCH ARTICLE

Molecular Diagnosis of Foot and Mouth Disease Virus in Cattle with Reference to Hematological and Biochemical Changes

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Abstract

The present study was carried out to clarify the effect of Foot and Mouth Disease Virus (FMDV) on cattle of different ages with references to studying the hematological parameters, biochemical aspects and cardiac biomarkers after accurate diagnosis of FMDV by reverse transcription-polymerase chain reaction (RT-PCR). Forty-five native breed Egyptian female non pregnant cattle (1-5 years old) were divided into two main groups. Group (1): 15 apparently healthy cattle as control group. This group includes animals at age 1-1.5 year (gp.1a), 2-3 years (gp.1b) and 4-5 years (gp.1c). Group (2): 30 infected cattle with the same age category as the control group (gp. 2a, gp. 2b and gp.2c) were collected from different localities in port- Said Governorate, Egypt during 2016-2017 FMD outbreak. Saliva and vesicular fluid from infected cattle were obtained for RT-PCR and blood samples for hematological and biochemical parameters estimation. The infected cattle showed fever, ropy salivation, vesicular eruptions on buccal mucosa and interdigital space. All the identified viruses were FMDV of serotype 'O' which is circulating among cows of different ages in Egypt. Biochemical results revealed a significant decrease in serum total proteins, albumin, globulins and calcium levels, with a significant increase in serum enzyme activities ALP, GGT, AST and serum levels of urea, creatinine, inorganic phosphrous, malonadiadehyde, nitric oxide, Interleukein10 (IL-10), cardiac tropinine I (cTn I) and creatine kinase MB (CK-MB) concentration. It was concluded that FMDV significantly affects the hematological and biochemical parameters of infected cattle, especially young one. The detection of cTnI is a very sensitive method for determining myocardial cell damage in the earlier stages of the disease. Moreover, RT-PCR is diagnostic biomarkers for FMD viral infection.

Keywords: FMDV, RT-PCR, cTnI, CK-MB, IL-10.

Introduction

Foot and Mouth Disease (FMD) is a highly contagious vesicular viral disease of cattle, goats, buffaloes, sheep, pigs and wild ungulates. FMD virus (FMDV) belongs to genus *Aphthovirus* of the family *Picornaviridae* [1]. There are well known seven serological types of FMDV namely O, A, C, Asia1, SAT1, SAT2 and SAT3 with more than 65 subtypes. Infection or vaccination with one serotype does not confer immunity against the others [2]. FMDV is endemic in most countries in Asia, such as India, Iran and Pakistan, as well as, in Africa, such as Egypt [3]. The disease has several outbreaks since 1950 in Egypt. The serotypes of FMDV recorded in Egypt are SAT2, A and O [4]. The disease is characterized by fever, anorexia and excessive foamy salivation with vesicles appearing on tongue, gums, cheeks, pharynx and hard palate [5,6]. Lameness is evident in animals with foot lesions. Therefore, the disease causes high economic losses in terms of meat and milk production. The purpose of the present work was to clarify

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the effect of FMDV on certain hematological and biochemical parameters in cattle of different ages, after accurate diagnosis of FMDV by using RT-PCR in cattle.

Materials and Methods

Animal grouping

Forty-five native breed Egyptian female non pregnant cattle aged 1-5 years, belonging to different localities in Port- Said Governorate (Bahr El-Baker, El kabboti El Gadid, El Radwan, El Asher and Sahl Tena) in Egypt during FMD outbreak 2016-2017 were divided into two main groups. Group (1): 15 healthy cattle were considered as control group. This group was subdivided according to the age category in to: (gp.1a) age 1-1.5 year, (gp.1b) 2-3 years and (gp.1c) 4-5 years respectively. Group (2): 30 cattle manifested characteristic clinical signs of FMDV infection (fever, vesicular eruptions on buccal mucosa and interdigital space). This group was subdivided into three subgroups according to ages as previously described in the control group. The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary

Sampling

All cattle were subjected to clinical examination [7]. Two different samples were collected including A) saliva and vesicular fluid for RT-PCR and B) blood samples for hematological and biochemical estimation. The oral mucosa and tongue were swabbed by sterile swab, and also the swab was saturated with saliva while avoiding contamination with ingested material. The swabs were then immediately placed into a 2-mL sterile cryogenic tube containing 1.5 mL of Dulbecco's Minimal Essential Medium [DMEM] (Gibco®, Invitrogen Corp., Carlsbad, CA) with glycerol, antibiotic and antimycotic (Gibco®) [8]. Two venous blood samples were collected from jugular vein of cattle. The 1st sample (about 2 mL) of blood was taken in sterile vacuum tube (with dipotassium salt of EDTA) used for hematological studies. The 2nd sample (about 10 mL) was taken in sterile vacuum plain tube without anticoagulant for serum separation for biochemical serological and oxidative profiles.

Molecular identification

Reverse transcription and Polymerase chain reaction

For RNA extraction, the tubes containing the swabs were thawed and thoroughly homogenized by vortexing, and the extraction was carried out according to the manufacturer's recommendations using Gene JET RNA Purification Kit (Thermo scientific, EU). The extracted RNA was examined firstly by RT-PCR using 5UTR universal primers 1F/1R located in the 5 untranslated regions (UTR) of the FMD virus genome generating 328 bp product regardless of the serotype [9]. The sequences of the primers are 1F: 5'- GCC TGG TCT TTC CAG GTCT -3' and 1R: 5'- CCA GTC CCC TTC TCA GATC -3'. The RT-PCR was performed using Verso ™ One Step RT-PCR Kit (Thermo scientific, EU). The thermal profile was started at 50°C for 30 min for reverse transcription; then PCR activation at 95°C for 15 min; followed by 35 cycles at 94°C for 30 sec, 55°C for 30 sec, and 72°Cfor 90 sec. Finally, the PCR reaction was completed at 72°C for 10 min. For identifying the serotype in each of the FMDV PCR positive samples, another RT-PCR was performed using serotype-specific primers for serotypes O, A and SAT2 as previously described [9,10].

PCR products were analyzed by electrophoresis on a 1.5% agarose-Tris-borate-EDTA gel containing 0.5 µg/mL ethidium bromide. DNA weight markers (GeneRuler 50bp DNA Ladder Plus, Ready-To-Use; Fermentas, Inc., Hanover, MD, USA).

Hematological studies

Red blood cells (RBCs), hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total and differential leukocytic counts were determined using automatic cell counter (Sysmex KX-21N, Japan).

Biochemical studies

Liver function

Serum alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT) and aspartate

aminotransferase (AST) activities were performed according to the methods of [11-13] respectively using test kits of Bio Med Egypt. Serum total proteins and albumin levels were estimated according to previous methods [14,15] using test kits of Bio Med – Egypt. Serum globulins level was calculated by subtracting the obtained albumin level from the total proteins level [16].

Kidney function

Serum creatinine, urea, calcium and inorganic phosphorous levels were performed using test kits of Bio Med Egypt. Serum creatinine and inorganic phosphorus levels [17], serum urea level [18], and serum calcium level [11].

Oxidant and antioxidant markers

Serum Malondialdehyde (MDA) and nitric oxide (NO) levels were measured according to previous studies [19] and [20] respectively. The estimation was colorimeterically using test kits of Bio Diagnostic, Egypt.

Interleukein10 (IL-10)

IL-10 was measured in serum following the method of Ferrari et al.[21] using test kits ELISA of Bio Diagnostic, Egypt, Catalog Number, MBS703712.

Cardiac markers

Serum cardiac tropinine I (cTn-1) and creatine kinase MB (CK- MB) were estimated by automatic analyzer TOSOH according to [22] and [23] respectively.

Statistical analysis

The obtained data in this work were statistically analyzed by independent "t" test using the MSTAT C computer program [24]. The minimum level of significance was set at $P < 0.05$.

Results

Clinical observations

As shown in Figure (1), cattle (1-1.5 years old) naturally infected with FMD showing ropy salivation, vesicle formation, while at 2-3 years old and adults (4-5 years) infected cattle showing severe ruby erosions and ulcerations on the dental pad, muzzle and interdigital space.

Figure 1: Characteristic clinical signs of FMDV infection in naturally infected cattle (A) Cow (at age from 1-1.5 years) showing ropy salivation. (B) Cow (at age from 1-1.5years) showing severe ruby ulcerations on the dental pad and muzzle. (C) Cow (at age from 2-3years) clarified ulceration on the dental pad and palate. (D) Adult cow (at age from 4-5 years) naturally infected with FMD showing ulcerations on dental pad.

Molecular identification

RT-PCR revealed that 30 samples were FMDV positive with the percentage 100%. All the positive samples were genotyped as serotype O in all saliva and vesicular fluids samples.

Hematological results

Regarding erythrogram as shown in Table (1) RBCs count, Hb concentration and PCV showed a significant decrease in infected cattle with FMDV (gp.2a) when compared with the normal control (gp. 1a), while, MCV, MCH and MCHC of gp.2a showed non significant change in comparison with the normal control. On the other hand, the aforementioned erythrocytic parameters showed non significant change in infected cattle (gps.2b and c) when compared with the control (gps.1b and c).

The total leukocytic count (TLC) showed a significant increase in gps.2a, b and c respectively when compared with the normal control (Table 1). The granulocyte counts were increased significantly in gps.2a, b and c respectively when compared with the normal control. The monocyte counts showed highly significant increase in infected cattle of all ages in comparison with the normal control. However, the lymphocytic count revealed non significant change in FMDV infected of all ages (1-5 years), in comparison with the normal control.

Table 1: Erythrogram and Leukogram of cattle in different groups (Mean values± SE)

	1-1.5 year		2-3 years		4-5 years	
	GP.1a	**Gp.2a**	**GP.1b**	**Gp.2b**	**GP.1c**	**Gp.2c**
RBCs ($\times10^6$/µL)	5.19 ±0.47	4.00* ±0.55	5.11 ±0.38	4.80 ±0.57	5.00±0.44	4.78 ±0.37
Hb (g/dl)	10.84±0.57	9.00* ±0.43	9.66 ±0.60	9.82 ±0.39	10.43 ±0.26	9.82 ±0.39
PCV (%)	30.00±2.21	23.15* ±2.78	22.00 ±1.71	21.80 ±3.01	22.55 ±2.86	21.70 ±2.61
MCV (fl)	57.80±5.72	57.87 ±8.00	43.05 ±1.14	45.41 ±2.47	45.11 ±1.13	45.39 ±4.27
MCH (pg)	20.88±0.43	22.50 ±7.14	18.91 ±0.58	19.85 ±2.47	20.86 ±0.36	20.54 ±1.76
MCHC (%)	36.33±1.78	38.80 ±5.82	43.90 ±1.68	45.00 ±6.53	46.23 ±1.68	45.04 ±6.53
WBCs $\times10^3$/ µL	10.97±0.47	13.32* ±1.49	9.22 ±0.48	12.55*±1.30	10.56 ±0.48	12.14* ±1.30
Granulocyte$\times10^3$/ µL	4.57 ±1.19	5.90* ±0.51	3.25 ±0.52	4.98* ±0.23	4.00 ±0.52	4.90* ±0.23
Monocytes$\times10^3$/ µL	5.30 ±0.60	5.52 ±0.82	5.00 ±0.60	5.62 ±1.12	5.45 ±0.60	5.40 ±1.12
Lymphocyte$\times10^3$/ µL	1.10 ±0.18	1.90** ±0.20	0.97 ±0.11	1.94**±0.10	1.11 ±0.11	1.88** ±0.10

*****Significant at $p<0.05$. ** Highly significant at $p<0.01$

NB: all subgroups (1a, b, and c) are negative FMDV and (2a, b, and c) are positive FMDV by RT-PCR

Biochemical results

Liver function

The serum ALP activity in the present study revealed a significant increase in gp.2a, gp.2b and gp.2c respectively of infected cattle with FMDV when compared with normal control. Moreover, serum GGT activity showed highly significant increase in all infected animals, when compared with the control. The serum activity of AST statistically showed highly significant increase in FMDV infected cattle of all ages (1-5 years) in comparison with the control (Table 2).

Serum total proteins (TP) level showed highly significant decrease by 38.9% in gp.(2a), and significant decrease by 27.3% and 25.1% in gp.(2b) and gp.(2c) respectively, when compared with the normal control. The serum albumin level in FMD -infected cattle revealed highly significant decrease by 42.1% in gp.(2a) and significant decrease by 34.1%

and 36.6%, respectively in gp.(2b) and gp.(2c), when compared with the normal control. The serum globulins level showed a significant decrease in gp.(2a) by 37.02% and by 37.5% in gp.(2c) in comparison with the normal control. Moreover, gp.(2b) revealed non significant change when compared with the normal control (Table 2).

Kidney function

The serum urea level revealed highly significantly increase by 155.7%, 101.5% and 161.4%, respectively in gps.2a-c in comparison with the normal control (gps. 1a-c). The serum level of creatinine showed highly significant increase by 128.8 and 125.8%, respectively in gp.2a and gp.2b, when compared with the normal control. While, it revealed a significant increase by 28.9% in gp.2c. The serum calcium (Ca) level showed a significant decrease by 42.1%, 21.7% and 17.6% in infected cattle of all ages (gp.2a, gp.2b and gp.2c), respectively when compared with the normal control. The highest decrease was noticed in g.p.2a. However, the serum level of inorganic phophorus (IP) showed a significant increase by 87.5%, 72.7% and 69.8% in infected animals of all ages respectively in comparison with the normal control (Table 2).

Interleukein10 (IL-10)

The serum IL-10 showed very highly significant increase by 259% in gp.(2a), when compared with the normal control. It revealed highly significant increase by 187.4% in gp.(2b) and a significant increase by gp.(2c) when compared with the normal control (Table 2).

Oxidant and antioxidant markers

The serum MDA level showed a significant increase by 58.8% in gp.(2a); while gp.(2b) and gp.(2c) revealed highly significant increase by 136.8% and 232.5%, respectively when compared with the normal control (Table 2). The serum NO level revealed highly significant increase by 734.5%, 660.6% and 569.7% in FMD –infected cattle of all ages compared with the normal control (Table 2).

Cardiac markers

The serum level of cTn-1 showed highly significant increase by 7560% and 7309.1%, respectively in gp.(2a) and gp.(2b), in comparison with the normal control. Also, gp.(2c) revealed high significance by 311% if compared with the normal control (Table 2). The serum CK level showed highly statistically significant increase by 265.5%, 215.2% and 211.2%, respectively in all ages of FMD infected cattle when compared with the normal control (Table 2).

Table 2: Biochemical parameters of cattle in different groups (Mean values± SE)

	1-1.5 year		2-3 years		4-5 years	
	GP.1a	**Gp.2a**	**GP.1b**	**Gp.2b**	**GP.1c**	**Gp.2c**
ALP (U/L)	25.80±1.49	31.24*±3.90	24.14±1.49	41.75*±3.90	25.63±4.08	46.83*±15. 09
GGT (U/L)	14.00±0.91	49.40**±4.74	12.75±0.8	43.83**±5.28	13.25±0.85	43.05** ±5.28
AST (U/L)	30.53±1.39	127.38*** ±13.02	28.73±1.39	118.55***±11.97	18.99±3.21	103.65***±10.21
TP (g/dl)	8.36 ±0.16	5.10**±0.92	8.25±0.16	6.00*±0.92	8.35 ±0.22	6.25*±0.77
Albumin (g/dl)	5.20 ±0.12	3.01**±0.21	5.22 ±0.16	3.44*±0.11	5.00 ±0.19	3.17*±0.13
Globulins (g/dl)	3.16 ±0.17	1.99*±0.04	3.03 ±0.11	2.56±0.13	3.33 ±0.15	2.08*±0.11
Urea (mg/dl)	26.48 ±3.00	67.72** ±4.10	25.64±2.39	51.67**±3.02	23.23 ±2.04	60.72**±5.60
Creatinine (mg/dl)	1.70 ±0.11	3.89***±0.16	1.86 ±0.10	4.20**±0.30	1.80 ±0.05	2.32* ±0.19
Ca (mg/dl)	9.87 ±0.35	5.71* ±0.96	8.94 ±0.43	7.00* ±0.94	8.83 ±0.93	7.28* ±0.79
P (mg/dl)	4.09 ±0.17	7.67* ±0.18	4.73 ±0.70	7.17* ±0.84	4.51 ±0.52	7.66* ±0.14
IL-10 (pg/mL)	3.00±0.03	10.77***±1.11	3.88±0.03	11.15**±2.55	4.01±0.12	10.16*±1.44
MDA (mmol/L)	5.83 ±0.34	9.26* ±2.16	5.90 ±0.58	13.97** ±3.78	5.72 ±0.67	19.02**±2.88
NO (mmol/L)	1.13±0.12	9.43**±4.34	1.32 ±0.34	10.04** ±3.82	1.22 ±0.28	8.17** ±3.22
CK (U/L)	62.06±5.13	226.80***±8.54	73.75±3.13	229.50***±5.54	71.25±3.13	224.57***±6.54
cTn-I (µg/L)	0.10 ±0.02	7.66***±0.14	0.11±0.01	8.15***±0.10	0.12 ± 0.02	4.11** ±0.09

*****Significant at $p<0.05$ ** Highly significant at $p<0.01$ *** Very highly significant at $p<0.001$. NB: all subgroups (1a, b, and c) are negative FMDV and (2a, b, and c) are positive FMDV by RT-PCR

Discussion

FMD outbreak examination by most clinicians in Egypt is largely based on herd history and on clinical signs in mouth and feet, with little emphasis on laboratory facilities such as hematological and biochemical indicators that may aid the identification of FMD infected cattle.

The clinical examination in the present study manifested, ropey threads salivation and fever in addition to vesicular eruptions on buccal mucosa and interdigital space. There was a relationship between the appearance of clinical signs and age. The more severe clinical signs were observed in cattle aged 1-3years. Our results were in accordance with a previous report where vesicles at the coronary band and in the oral cavity were observed [5]. They also added that vesicles and ulcerations occurred on the mammary gland and the adult animals recovered within 8-15 days. Blistering lesions are characteristic for FMDV in adult cattle and acute severe myocardial injury in neonates [25]. Additionally, fever is also characteristic for the disease and is resulted from disturbance in heat regulatory centre, which may be resulting from FMD virus replication in the central nervous system of infected animals [26].

For the FMDV identification, RT-PCR was applied to the 30 virus isolates and all isolates were positive. RT-PCR has been used for the routine diagnosis of FMDV using highly converse universal primer for all serotypes [27]. The use of conventional RT-PCR is confirmatory diagnostic procedure in genotyping of FMDV strains using type-specific primers [28]. These results were consistent with Wekesa *et al*., [29] who reported that the most outbreaks of FMDV were caused by serotype O followed in frequency by serotype A which is endemic in many developing African and Asian countries. These results were supported by Mohammed *et al*. [30] who detected recent FMDV strains of serotypes, O and SAT-2 in Egypt using primers specific for vp 1 and 3D genes. Our results are in agreement with El-Mandrawy and Farag [31] who used tissue and saliva

specimens for the isolation of FMDV and RNA extraction then analyzed by using universal primer using RT-PCR. They reported that all the identified viruses were of serotype 'O' and concluded that FMDV serotype 'O' is circulating among cows and buffaloes in the study area.

Concerning the hematological investigation, cattle infected with FMD had a significant reduction in the RBC count, Hb concentration and PCV, while non significant changes in MCV and MCHC indicating normocytic normochromic anemia especially in animals of age 1-1.5 years, compared with the normal control group. The occurrence of anemia may be attributed to a depression of erythropoiesis [32]. Development of normocytic normochromic anemia is associated with increased inflammatory cytokines [31]. In accordance, a significant decrease in erythrocytic count, Hb content and PCV in the FMD -infected buffaloes (6 months - 2 years old) were obtained in another study [33]. On contrary, non significant change in the number of RBCs, hemoglobin concentration, PCV, blood indices (MCV, MCH &MCHC) were observed in adult male buffaloes, 2-4 years old, naturally infected with Foot and Mouth disease [34]. Concerning the leukogram, a significant leukocytosis, neutrophilia, and monocytosis were observed in FMD infected animals of all ages (1-5 years), with non significant change in lymphocyte count, in comparison with the control. The increase in the phagocytic cells (neutrophils and monocytes) could be due to tissue destruction resulting from virus infection [35]. The neutrophils and other phagocytic cells are the first line of defense against microbial and viral infections. Our results are partially consistent with those previously reported [31, 36-38].

Serum liver function tests of FMD-infected cattle showed a significant increase in serum ALP, GGT and AST activities in all infected animals at different ages in comparison with the normal control. These results suggest that FMDV probably causing damage to muscles of infected animals [37]. However, Gokce *et al*. [39] reported non significant changes in ALT, AST and ALP in cattle affected with acute FMD infection. The elevation of AST levels can be seen in several abnormalities such as liver, muscle and heart diseases [40]. Although AST is not hepatic specific it is widely used in cattle to detect liver disease in spite of its lack of specificity and its elevation in the FMD -infected cattle resulting from muscular damage [41].

Concerning the proteinogram in the present study, a hypoproteinemia with hypoalbuminemia and hypoglobulinemia were observed in FMD –infected cattle when compared with the healthy control. The hypoproteinemia observed in FMDV -infected animals is due to hypoalbuminemia and hypoglobulinemia. Similar findings and interpretations were supported by different studies [37,39]. Hypoalbuminemia may be due to the decreased feed intake, disturbed metabolism of the liver, maldigestion and malabsorption resulted from enteritis and exudation of plasma from ulcers presented on the mouth, tongue and between claws [33,39]. Further, albumin is a negative acute phase protein and its concentration decreases in inflammation [42]. The observed hypoglobulinemia in the present study may be attributed to increased interleukin- 10 levels which act as anti-inflammatory causing inhibition of immunity. These results were confirmed by Couper *et al*. [43]. Our results disagree with Eidan *et al.* [44] who, recorded significant increase in serum globulins concentrations.

Regarding kidney function evaluation, serum urea and creatinine levels showed a significant increase ($P < 0.01$) in all infected animals of different ages as compared to the apparently healthy group. The increased serum urea and creatinine levels might be due to decreased renal blood flow and renal damage. Similar results were obtained by Mansour *et al.* [45] who reported a significant increase in serum creatinine levels in guinea pig infected with FMDV. However, Nasr El-Deen [34] revealed non significant change in serum levels of creatinine and blood urea nitrogen.

The infected cattle with FMDV showed a significant hypocalcemia and hyperphosphatemia. The possible explanation for the hypocalcaemia in this study might be the significant decrease in serum protein levels and severe anorexia in cattle with FMD, resulting in a decrease in protein bounded

calcium [39,41]. Stress due to systemic infections, febrile condition and general body illness leads to the increase in cortisol level, which inhibit vitamin D and consequently depress the calcium uptake from the gut [46]. The higher serum inorganic phosphorus level in FMD -infected animals might be due to rapid respiration, higher pulse rate, tissue oxidation and acidosis due to lack of excretion as reported previously [47]. Gruenberg *et al*. [48] attributed the hyperphosphatemia to the increased salivation with the resultant dehydration and decreased renal blood flow. Moreover, the increased phosphorus level could also be a response to hypocalcaemia due to the interaction between the Ca and P homeostasis in ruminants [49].

Interleukin 10 (IL-10) is an anti-inflammatory cytokine that inhibits the activity of Th1 cells, NK cells and macrophages, which are required for optimal pathogen clearance. The serum IL-10 showed a significant increase in all FMDV –infected cattle of different ages comparatively with the control. Such increase may be attributed to inflammation in the buccal cavity and other parts in the body and /or monocytosis seen in the infected animals. Our results were consistent with Pestka *et al.* [50] who stated that IL-10 is widely recognized to contribute to the anti-inflammatory response and to the inhibition of cellular responses [51,52].

Serum concentrations of MDA and NO were significantly increased in all FMDV -infected animals at different ages. The significant increases in MDA level were in agreement with Kar *et al.* [38]. Nitrate and nitrite in serum are formed by the de-composition of NO and their concentrations in serum are used as a direct measure of NO production [53-55]. Nitric oxide has an important role in primary defense against bacteria, viruses and parasites. Moreover, Gokce *et al.* [39] demonstrated an increase in nitrate level in FMD infection, which might be resulted from increased production of *in vivo* nitric oxide by the Aphthovirus, with subsequent increase in the oxidant injury. It can be speculated that increased levels of NO found in this study might be due to the production of oxidant molecules.

With regard to heart function assessment, both serum cTnI concentrations and CK-MB activity were significantly increased in the FMD -infected cattle at different ages as compared to control one. Serum cardiac troponins are the earliest appearing biochemical markers during myocardial damage [56]. Myocardial degeneration was considered the main cause for serum CK, AST and LDH elevation [40,57]. Our results were consistent with other studies [7, 31,38].

Conclusion

From the present study, it could be concluded that vesicular lesions and RT-PCR are diagnostic for FMD. RT-PCR used to detect the specific serotype of FMDV (serotype O), so it helps in reaching to specific vaccine. FMDV significantly affects the hematological and biochemical profile of infected cattle, especially young one. The detection of cTnI is a very sensitive method of determining myocardial cell damage in the earlier stages of the disease either in young or adult cattle.

Conflict of interest

The authors declare no conflict of interest.

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الملخص العربي

التشخيص الجزيئي لفيروس مرض الحمى القلاعية في الأبقار مع الإشارة إلى التغيرات الدموية و البيوكيميائية

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تمت هذه الدراسة لمعرفة تاثير فيروس مرض الحمى القلاعية على خلايا الدم وكيمياء الدم و ذلك بعد تشخيصها باستخدام تفاعل البلمرة المتسلسل النسخى العكسى فى الابقار ذات الأعمار المختلفة. اجريت هذه الدراسة على عدد 45 من اناث ابقار من السلالات المحلية اعمارها من سنة الى 5 سنوات والتى قسمت الى مجموعتين رئيسيتين كالتالى: المجموعة الأولى: شملت عدد 15 من الأبقار الغير مصابه استخدمت كمجموعة ضابطة و تم تقسيمهم إلى ثلاث مجموعات حسب العمر. (gp.1a) من عمر 1- 1.5 سنة، (gp.1b) من عمر 2-3 سنوات و المجموعة (gp.1c) من عمر 4- 5 سنوات. شملت المجموعة الثانية 30 من الأبقار المصابة من نفس الفئة العمرية مثل المجموعة الضابطة (gp. 2a, gp. 2b and gp.2c). قد تم أخذ عينات من اللعاب وذلك لتستخدم في اختبار البلمرة المتسلسل النسخى العكسى ,كما تم أخذ عينات من الدم والمصل لإجراء صورة دم كاملة مع بعض الفحوصات الكيميائية . كانت الحيوانات المصابة بالحمى القلاعية تعانى من ارتفاع فى درجة حرارة الجسم مع وجود قرح في الفم واللثه و بين الظفرين مصاحبة بنزول اللعاب من الفم . جميع الفيروسات التي تم التعرف عليها كانت فيروس الحمى القلاعية من السلالة"O" الذي ينتشر بين الأبقار من مختلف الأعمار في مصر. كشفت النتائج البيوكيميائية عن انخفاض معنوي فى مستويات البروتين الكلى والالبيومين و الكالسيوم ، مع زيادة ملحوظة فى نسبة الاسبرتيت امينوترانسفريز والجاما جلوتاميل ترانسفيريز, الكرياتنين, اليوريا, الفسفور الغير عضوي ، المالونلدهيد ، النيتريك أوكسيد ، انترلوكين10 ، التروبنين (cTn I) وتركيز الكرياتين كيناز (CK-MB)MB. نستخلص من هذا البحث أن فيروس مرض الحمى القلاعية له تأثير ضار على صورة وكيمياء الدم فى الابقار خاصة الاعمار صغيرة السن. يعد الكشف عن التروبنين (cTnI) طريقة حساسة للغاية لتحديد تلف خلايا عضلة القلب في المراحل المبكرة من المرض. علاوة على ذلك تفاعل البلمرة المتسلسل النسخى العكسى من المؤشرات الحيوية التشخيصية للإصابة بالعدوى الفيروسية.