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MOLECULAR STUDY OF FMD INFECTED EGYPTIAN BUFFALO CALVES WITH REFERENCE TO ELECTROCARDIOGRAPHY AND CARDIAC BIOMARKERS AS PROGNOSTIC TOOLS

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ABSTRACT

A total of 200 Egyptian buffalo calves aging between 9-11 months previously vaccinated with local trivalent oil inactivated vaccine (O, A and SAT-2), in different locations at Menufyia Governorate showing typical clinical signs of FMD were examined for molecular characterization of FMD virus, electrocardiographic picture (ECG) and cardiac biomarkers. The results of serological tests revealed that incidence of non-structural proteins against natural infection with FMDV was 31.5% in serum samples collected from vaccinated buffalo calves. Moreover, 51.5 % of the samples showed detection of structural proteins against trivalent vaccination. On the other hand, there were no protective antibodies against FMDV in 17 % of the examined samples. ECG of FMD infected calves showed ventricular premature depolarization with flattening of P- wave and increased QRS duration with decrease in its amplitude compared with healthy calves. In FMD infected buffalo calves, there were significant ($P < 0.01$) increases in (cTnI), (CK-MB), urea, CL and K and there was significant ($P < 0.01$) decrease in Na levels than that of healthy ones. The identified strains in FMD infected Egyptian buffalo calves were unique and different from the vaccinal and other Egyptian strains as well as they were clustered with Topotype ME-SA. Cardiac enzymes along with ECG can be used as useful prognostic tools in FMD infected Egyptian buffalo calves.

Key words: Calves, Cardiac, ECG, FMD

INTRODUCTION

Foot-and-mouth disease (FMD) is an important livestock disease of cloven-hoofed animals resulting in drastic direct economic impact including high mortality and severe productivity losses, in addition to indirect losses through importation restriction from endemic localities (Arzt *et al.*, 2011). FMD is a contagious disease and highly transmissible, moreover, a limited number of infective particles can initiate host infection. Contaminated animal products, agricultural tools, people, vehicles, and airborne transmission can contribute to the mechanical dissemination of FMD virus with higher incidence in cold seasons than others (Longjam *et al.*, 2011).

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FMD is caused by a non-enveloped icosahedral virus of genus Aphthovirus, family Picornaviridae with a single-stranded and positive-sense RNA (Racaniello, 2001). FMD virus has been classified into seven immunologically distinct serotypes (A, C, O, Asia 1, SAT 1, SAT 2, and SAT 3). The viral genome is further subdivided into P1, P2 and P3 regions. The P1 region encodes leader proteinase (Lpro) and structural proteins 1A (VP4), 1B (VP2), 1C (VP3), and 1D (VP1). The P2 region encodes non–structural proteins 2A, 2B, and 2C, while P3 region encodes 3A, 3B (VPg), 3C protease and 3D polymerase (Bergmann *et al.*, 2003; Carrillo *et al.*, 2005; Lim *et al.*, 2005).

According to the geographic distribution, the O serotype is divided into eleven topotypes: EAST AFRICA 1 to 4 (EA-1 to -4), SOUTHEAST ASIA (SEA), EUROPE-SOUTH AMERICA (EURO-SA), INDONESIA-1 and -2 (ISA-1 and -2), CATHAY, MIDDLE EAST-SOUTH ASIA (ME-SA) and WEST AFRICA (WA). Furthermore, a toptype further divides into genetic lineages and sub-lineages (Knowles *et al.*, 2016; Knowles *et al.*, 2007).

The epidemiological information of FMD in Egypt revealed that the disease is enzootic in Egypt, and outbreaks have been reported since 1950 (El-Bagoury *et al.,* 2015). FMD virus serotypes O, A and SAT2 are the most prevalent and were last isolated during autumn and winter 2016 and spring and summer 2017 at different Egyptian governorates (El-Bagoury *et al.,* 2017).

Serological detection of antibodies directed to some nonstructural protein (NSPs) of FMD virus using ELISA are useful in providing evidence of previous or current infection in the host, irrespective of vaccination status (OIE. 2009). The most reliable single NSP indicator is the poly-protein 3ABC antibodies which appear to provide conclusive evidence of previous infection, whether or not the animal have also been vaccinated so, the differentiation between infected and vaccinated animals could be detected easily (Laila and Daoud 2004).

The molecular characterization of virus isolate is very important issue for FMD control (DiMarchi *et al.,* 1986). It has been shown that viral protein1 (VP1) is highly polymorphic and the most variable among the capsid polypeptides and is considered to be the major immunogenic protein, since it contains a linear antigenic site able to induce neutralizing antibodies sufficient to protect animals against the disease (Cottam *et al.,* 2008). Nucleotide sequencing and phylogenetic analysis of the complete or partial genomic region coding for VP1 has been extensively used as a main tool for molecular epidemiological studies, the development of engineering vaccine, the establishment of diagnostic methods to trace the origin and the spread of FMD virus also for typing and subtyping of the virus (Hall 2001; Knowles *et al.,* 2009).

In calves, myocarditis is considered a fatal form of FMD that occurs without developing the characteristic blister lesions noted in adult cattle. Diagnosis of myocardial disease in cattle remains challenging and is based upon physical examination, cardiac auscultation, and the incidence of sudden death in the field. However, there are many biomarkers for myocardial injury, such as creatine kinase myocardial band (CK-MB), lactate dehydrogenase, cardiac Troponins (cTnI) and aspartate aminotransferase (Jaffe *et al.,* 1996). The best cardiac biomarkers for myocardial damage are cardiac troponins, especially (cTnI), because it has nearly absolute myocardial tissue specificity and higher sensitivity than other myocardial enzymes (Weber *et al.,* 2005). However, no more studies have particularly evaluated the contributing factors of heart affections by using electrocardiography (ECG) in FMD infected Egyptian buffalo calves. The aim of this study was focused on the molecular characterization of FMD virus, electrocardiographic picture and evaluation of some cardiac biomarkers in FMD infected Egyptian buffalo calves.

MATERIALS AND METHODS

Animals:
The procedures of the current study were carried out according to the guidelines of faculty of veterinary medicine, Benha University for using of animals under ethics approval no (BUFVTM0108).

A total of 200 Egyptian buffalo calves aging between 9-11 months previously vaccinated with local trivalent oil inactivated vaccine (O, A and SAT-2), in different locations at Menufyia Governorate showing typical clinical signs of FMD including fever, salivation, loss of appetite, depression, lameness, blisters or vesicles, erosions and ulcers in the mucosa of the mouth, tongue, lips, gums, pharynx, palate and between the claws were included in this study.

ECG examination
FMD infected buffalo calves were examined by base apex lead system II was applied as; the right forelimb electrode was placed on the right side of the neck along the jugular groove one third of the way up the neck. The left forelimb electrode was placed on the ventral midline under the apex of the heart. The ground cables were placed on the left and right stifle joints. Alligator clips moisten with alcohol were used (Hiwing 1977). 20 vaccinated healthy buffalo calves were used control.

Samples
Serum samples were collected during the period between September 2016 and July 2017 for serological and biochemical analysis including urea which was determined spectrophotometrically by using special kits according to the method that described by Patton and Crouch (1977), sodium and potassium were measured according to the method that described by Henry *et al.* (1974), and chloride was measured according to Kaplan and Pesca (1996). cTnI concentration was measured according to Carrillo *et al.* (2005), while serum level of CK-MB were measured in full automated biochemistry analyzer (Chemray 240. USSR). 20 vaccinated healthy buffalo calves were used control.

Samples of epithelial tissues were collected from tongue and buccal mucosa of 20 infected calves under standard biosafety conditions and were collected in sterilized tube that contained glycerol and phosphate-buffered saline (PBS) 1:1, pH 7.2-7.6 (Mandour *et al.,* 2014) and stored at – 80ºC until used for molecular detection of FMD antigen by using RT-PCR, sequential and phylogenetic analysis.

Serological detection of FMD antibodies and serotyping using ELISA.
Detection of IgG antibodies in calves' serum samples for detection of non-structural proteins was carried out by using FMD virus 3ABC- trapping ELISA (IZSLER, Biotechnology lab, via A. Bianchi, 9-25124 Brescia, Italy) according to manufacturer's instructions. All sera samples were subjected to indirect sandwich ELISA for serotyping of FMDV antibodies (IZSLER Biotech laboratory, pirbright institute, UK) according to manufacturer's instructions.

Molecular detection of FMD virus VP1 gene by using RT-PCR
The viral RNA was extracted from epithelial tissues samples by QIAamp®Viral RNA Mini Kit (Qiagen, Hilden, Germany) following the mini spin protocol according to the manufacturer's instructions. 1 μl of the obtained RNA was used as the template in a one-step RT-PCR [Ready-To-Go RT-PCR Beads; Amersham).

The primers used were: Forward primer (FMD virus/O/FP): 5′ CCTCCTTCAAYTTACGGTG 3′ [5] and Reverse universal primer (NK61): 5′ GACATGTCCTCCTGCATCTG 3′ to amplify a target amplicon with 283 bp in length. The amplification reaction was carried out using thermal-cycler and PCR conditions were adjusted according to Li *et al.* (2011).

Virus sequencing and phylogenetic analysis
The obtained RT-PCR products were purified by Qiaquick PCR purification kit (Qiagen) according to the manufacturer's instructions. Sequence analysis was carried out to detect the nucleotide composition of the detected strain for genotypic analysis and it was carried out in both directions using the previously mentioned forward primer and reverse primers by 3730 DNA Analyzer, Applied Biosystems, USA. Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystem, UK) was used as recommended by the manufacturer's protocol.

Sequences alignment (224 bp fragments of VP1) and construction of phylogenetic tree (Neighbor-joining) to detect the genetic relatedness of the tested strain of the current study compared to other strains worldwide registered in gene bank were carried out using BioEdite software program V.5.0.9 (Goris *et al.*, 2007) and MEGA-7 software program (Kumar *et al.*, 2016).

Histopathological examination
Heart specimens from dead calves were taken and fixed in 10% buffered formalin followed by histopathological examination 18. Slaoui M., Fiette L. Histopathology procedures: from tissue sampling to histopathological evaluation (Slaoui and Fiette, 2011).

Statistical analysis
The obtained results from the experiments were expressed as mean ± SEM and were analyzed using (SPSS Statistics for Windows, version 23.0. Armonk, NY: IBM Corp). Differences were declared significant when ($P < 0.05$).

RESULTS

ECG and biochemical findings
ECG of FMD infected buffalo calves showed bradycardia with ventricular premature depolarization which characterized by prolongation of T-wave with flattening of P- wave and increased QRS duration with decrease in its amplitude compared with healthy calves. There was significant ($P < 0.01$) decrease in PR, RT and ST intervals while there was significant ($P < 0.05$) decrease in QT intervals in FMD diseased calves than that of healthy ones (Fig. 1 and table 1). There were significant ($P< 0.01$] increases in (cTnI), (CK-MB), urea, CL and K, while there was significant ($P< 0.01$) decrease in Na levels in FMD infected calves than that of healthy ones (Table 2).

Serological detection of FMD antibodies and serotyping using ELISA.
The percentage of non-structural protein of serotype O was 31.5% (63 out of 200) which reflected infection by FMD serotype O. Moreover, 51.5 % of the samples (103 out of 200) showed detection of structural protein that been created by the body immune system as a result of vaccination. Furthermore, these structural proteins were against some FMD serotypes not for all serotypes. Serotype O was the predominant as shown in Table 3. On the other hand, there were no protective antibodies against FMDV in 17 % of the examined samples (34 out of the 200) which indicated vaccination failure in this case.

Virus sequencing and phylogenetic analysis
RT-PCR successfully amplified the target gene (VP1) from all tissue samples. After direct sequencing of the 283 bp PCR products, analysis was carried out on 224-nucleotide sequences corresponding to part of the FMDVP1 encoding gene [from nucleotides 3707 to 3932 of the full length FMDV genome. Depending on the nucleotide homology between the identified strains after alignment, the results revealed presence of 5 different strains (strain 1 to strain 5) and all belongs to O strain of FMD (Fig. 2).

By comparing the sequences data of the five different identified stains in this study with vaccinal strain (Manisa/Turkey) and others Egyptian strains (Dakahlia/ 2014, Ismaalia/ 2013, El-Minia/ 2013 and Alexandria 2013) from the GeneBank using their accession numbers, it was found that there were 2

types of mutations occurred by nucleotide substitution (point mutation) and by deletion in the identified strains meaning that the identified strains were unique and different from the vaccinal and others Egyptian strains (Fig. 3). The results of phylogenetic analysis indicated that the identified strains in this study were clustered with topotype ME-SA (Fig.3).

Histopathological findings

Histopathological examination of the heart of dead calves revealed severe interstitial myocarditis with heavy inflammatory cellular infiltration consisting of lymphocytes, macrophage and plasma cell (Fig. 4a). Zenker's necrosis of the myocardial muscle with massive leukocytic cellular infiltrates accompanied by distortion and dissolution of the myocardial parenchyma was also observed (Fig. 4b).

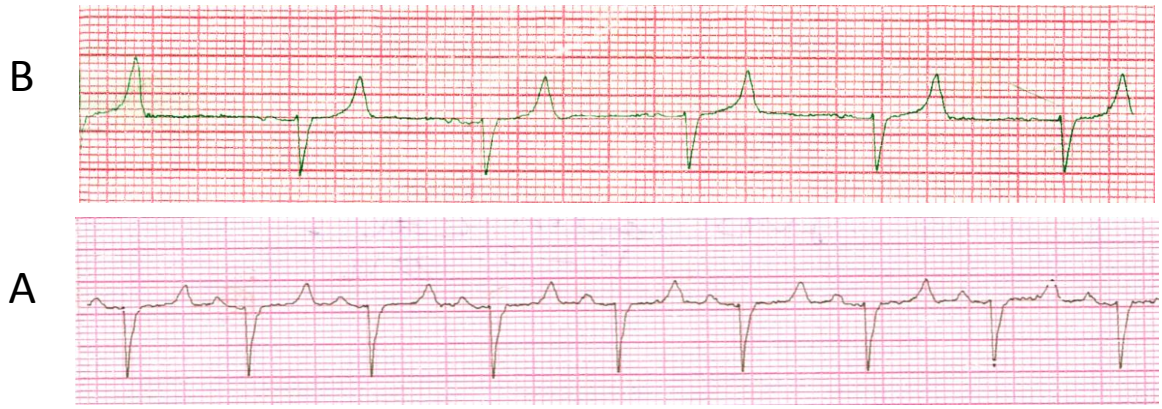

Fig. (1): ECG of FMD infected buffalo calf (B) showing bradycardia with ventricular premature depolarization which characterized by prolongation of T-wave with flattening of P- wave and increased QRS duration with decrease in its amplitude compared with healthy calves (A).

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                                  3710       3720       3730       3740       3750       3760       3770       3780       3790       3800
                                  ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
Strain 1                          CCTCCTTCAACTACGGTGCCATCAAGGCCACTCGGGTGACTGAACTGCTTTACCGCATGAAAAGGGCTGAGACATACTGCCCCCGGCCTCTTTTGGCCAT
Strain 2                          ..................................................T.....................................................
Strain 3                          ..........................................................C.............................................
Strain 4                          ..................................................T.....................................................
Strain 5                          ................G.....................................................................................
KP940473.1 (Dakahlia/2014)        ....................................................................................................
KJ210075.1 (Ismaalia/2013)        ....................................................................................................
KJ210078.1 (EL-Mania/2013)        ....................................................................................................
KJ210073.1 (Alexandria/2013)      ....................................................................................................
KY825719.1 (Manisa/Turkey/79)    ...................T..A..T.............................G.....................................

                                  3810       3820       3830       3840       3850       3860       3870       3880       3890       3900
                                  ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
Strain 1                          TCAACCGGATCAGGCTAGACACAAGCAGAAGATTGTGGCACCTGTGAAACAGCTTCTAAATTTTGACCTGCTCAAATTGGCGGGAGATGTGGAGTCCAAC
Strain 2                          ....................................................................................................
Strain 3                          ....................................................................................................
Strain 4                          ....................................................................................................
Strain 5                          ....................................................................................................
KP940473.1 (Dakahlia/2014)        ....................................................................................................
KJ210075.1 (Ismaalia/2013)        ....................................................................................................
KJ210078.1 (EL-Mania/2013)        ....................................................................................................
KJ210073.1 (Alexandria/2013)      ....................................................................................................
KY825719.1 (Manisa/Turkey/79)    ...C.....C.............................G.....................................................

                                  3910       3920       3930
                                  ...|....|....|....|....|
Strain 1                          CCTGGGCCCTTCTCTCTCCGACGT
Strain 2                          ........................
Strain 3                          ........................
Strain 4                          ........................
Strain 5                          ........................
KP940473.1 (Dakahlia/2014)        ............TCT.........
KJ210075.1 (Ismaalia/2013)        ............TCT.........
KJ210078.1 (EL-Mania/2013)        ............TCT.........
KJ210073.1 (Alexandria/2013)      ............TCT.........
KY825719.1 (Manisa/Turkey/79)     ............TCT.........
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Fig. (2): Sequences data of the five different detected stains in this study with vaccinal strain (Manisa/Turkey) and others Egyptian strains (Dakahlia/ 2014, Ismaalia/ 2013, El-Minia/ 2013 and Alexandria 2013). Mutation by substitution and by deletion were found.

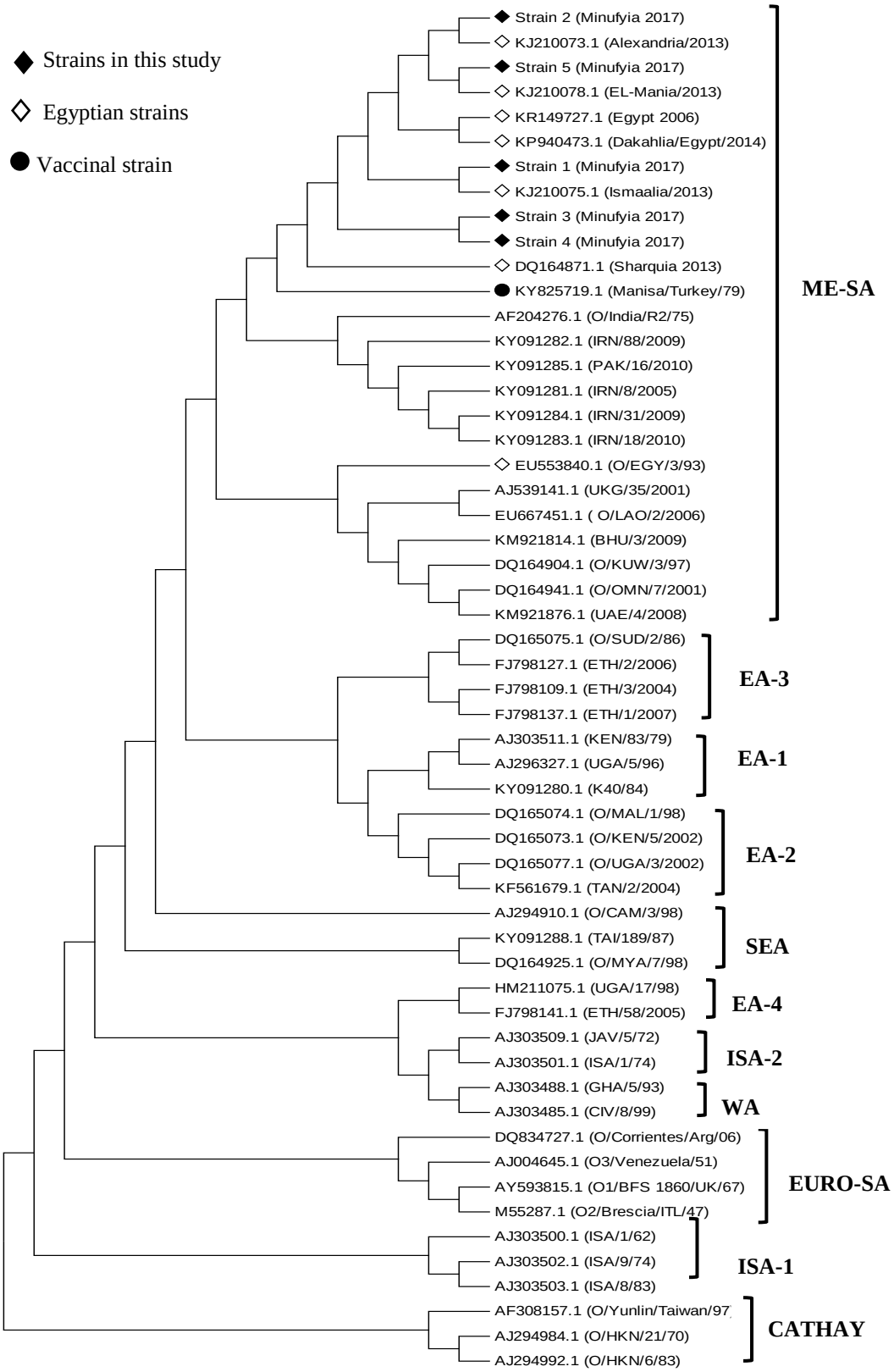

Fig. 3: Phylogenetic analysis of the FMD strains. The phylogenetic tree was based on partial sequences (224 nt). The evolutionary history was inferred using the Neigbor-joining method with bootstrap probability more than 70 %. The detected strains were clustered with topotype ME-SA.

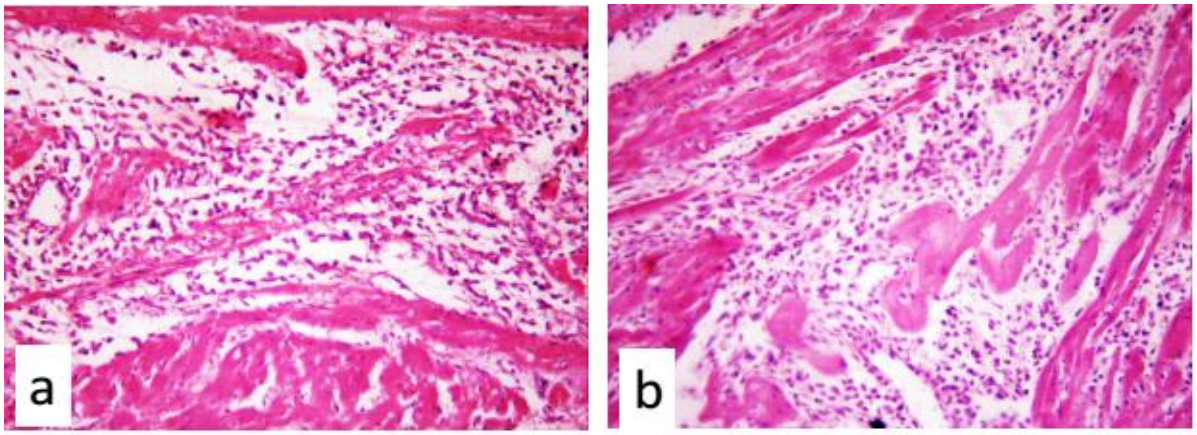

Fig. 4a: Heart of dead calf showing severe interstitial myocarditis with heavy inflammatory cellular infiltration consisting of lymphocytes, macrophage and plasma cell. (H&E x200).

Fig. 4b: Heart of dead calf showing zenker's necrosis of the myocardial muscle with massive leukocytic cellular infiltrates accompanied by distortion and dissolution of the myocardial parenchyma. (H&E x200).

Table (1): ECG traces in healthy and FMD infected Egyptian buffalo calves.

Animal	P-wave		QRS complex		T-Waves		PR-Interval	RT-Interval	ST - Interval	QT - Interval
	Amplitude	Duration	Amplitude	Duration	Amplitude	Duration				
Healthy calves	0.19 ± 0.01	0.08 ± 0.04	0.86 ± 0.04	0.04 ± 0.01	0.41 ± 0.01	0.08 ± 0.01	0.28 ± 0.01	0.48 ± 0.02	0.43 ± 0.02	0.23 ± 0.01
FMD infected calves	0.06 ± 0.01**	0.28 ± 0.17**	0.77 ± 0.01*	0.07 ± 0.01**	0.65 ± 0.03**	0.17 ± 0.01**	0.17 ± 0.01**	0.26 ± 0.01**	0.20 ± 0.01**	0.35 ± 0.02*

Means (± SE) are significantly different when ($P<0.05$)* and ($P<0.01$) **.

Table (2): Biochemical parameters in healthy and FMD infected Egyptian buffalo calves.

	cTnI	CK-MB	Na	K	Cl	Urea
Diseased	8.1± 0.6**	4.35 ± 0.18**	129.13 ± 2.40**	4.74 ± 0.10**	144.26 ± 2.51**	41.77 ± 0.99**
Control	0.8 ± 0.4	2.53 ± 0.2	145.14 ± 1.30	3.52 ± 0.31	112.63 ± 2.32	25.42 ± 1.17

Means (± SE) are significantly different when ($P<0.01$) **.

Table (3): Serotyping of FMDV antibodies of 200 Egyptian buffalo calves vaccinated with local vaccine **(**ND=Non-detected)

Type of antibodies	**No. of samples**	**%**	**Serotyping of FMD Antibodies**
Against natural infection (Non- structural proteins)	63	31.5%	O
Against trivalent vaccine (Structural proteins)	40	20%	O, A
	43	21.5%	O, SAT2
	20	10%	O
ND	34	17%	ND

DISCUSSION

FMD is considered one of the important infectious contagious viral diseases affecting cloven hoofed animals producing a drastic economic loses (Knight-Jones and Rushton 2013). In Egypt, FMD has taken an enzootic form and many outbreaks had occurred since 1950 and onwards. FMDV type O was the most prevalent until serotype A appeared in 2006 and serotype SAT2 in 2012, however**,** the serotype O still the most predominant serotype until now (Ahmed *et al.,* 2012; Knowles *et al.,* 2007). Vaccination of cattle and other susceptible species against FMD considered the only way for controlling the disease in Egypt, moreover, the identification of the causal agent and its serotype was imperative for choosing and formulating an effective vaccine (Ghanem and Abdel-Hamid 2010). Another method for controlling the disease is determination of its topology and understanding its epidemiology using molecular diagnostic technique through analysis of VP1 sequence then phylogenetic analysis thereby, tracking its transmission and sources (DiMarchi *et al.,* 1986; Knowles *et al.,* 2007). In September 2016, there was a new destructive FMD outbreak that struck a critical income source of the rural community in Egypt.

ECG is the clinical method of choice to evaluate cardiac problems associated with the production and conduction of electrical stimuli. It is also a useful tool in evaluating electrolyte disturbances. Ventricular premature beats characterized by abnormal amplitude and duration of ORS complexes and T waves are indicative of myocardial diseases (Mohammad *et al.,* 2013; Radostits OM *et al.,* 2007). FMD associated with excessive salivation resulting in acidosis which characterized by low level of arterial pH and reduced plasma bicarbonate concentration following the loss of bicarbonate in saliva. Extracellular and intracellular buffering and respiratory compensations minimize the change in blood pH until the kidney can excrete sufficient amount of hydrogen ion to correct the acid-base imbalance. Acidosis causing movement of potassium ion out the cell into the extracellular space and enhances the movement of hydrogen ion into the cell resulting in hyperkalemia (Hall 2001) which characterized clinically by cardiac arrhythmia and this agree with the obtained electrocardiographic results of this study. Our results also revealed hyponatremia, and hyperkalemia which were similar to those results obtained by Gattani *et al.* (2011). On the same hand, the decrease in chloride could be achieved by hyper salivation which facilitates sodium and chloride loss (Gattani *et al.,* 2011; Mahmoud and Neamat-Allah 2016) and changes in pancreatic β-cell function which occurred during the clinical course of FMD as reported by Shawky *et al.* (2013). Significant increase in (cTnI) and (CK-MB) which are considered as cardiac biomarker in large animals, providing a sensitive and persistent indicator of myocardial injury (Radostits OM *et al.,* 2007). It has been reported that serum (cTnI) concentration is an earlier marker of myocardial damage after virus infection (Lim *et al.,* 2005). Histopathological findings were included myocardial degeneration, necrosis, infiltrations of muscle fiber with mononuclear cells especially lymphocytes and few plasma cells (Aktas *et al.,* 2015) which may indicate to myocarditis (Aslani *et al.,* 2013; Kaya *et al.,* 2013).

The incidence of non-structural protein serotyping O of FMDV was 31.5% in serum samples collected from vaccinated buffalo calves. The vaccine should induce the immune system to produce the protective antibodies against all serotypes of FMD virus (Hegde *et al.,* 2016). 17 % of vaccinated buffalo calves had not any protective antibodies against FMD virus. This means that these buffalo calves are more susceptible for any serotype of FMD virus infection. While, the 51.5 % have some serotype of FMD virus antibodies this means that not all vaccinated buffalo calves have the three serotypes of antibodies against FMD virus, although the local vaccine should provide the animal with protective antibodies against O, A and SAT2 serotype of FMD virus. The results of the current study indicated that the local vaccine did not promote the immune system and is not enough to control against FMD virus.

The results of molecular detection of the virus using RT-PCR indicated that the detected serotype in this outbreak was O that is the most predominant (Ahmed *et al.,* 2012). While the sequencing of the detected strain targetting the VP1 protein showed that there were two types of mutation occurred by nucleotide substitution (point mutation) and by deletion in the detected strains (Bergmann *et al.,* 2003; Ding *et al.,* 2013) when compared with the vaccinal and others Egyptian strains. These kinds of mutations cause change in a sequence which subsequently can cause a change in primary, secondary and even tertiary protein structural, so this change in a sequence may be strongly related to antibody escape policy of FMD from host immune mechanism and appearance of outbreaks of FMD even in vaccinated animals (Domingo *et al.,* 2002).

Neighbor-joining tree analysis of the detected strains in this study when aligned and compared with Egyptian strains and 11 topotypes of the FMD serotype O and their lineages spread in the globe was carried out and the results demonstrated that the detected strains were clustered with topotype Middle East-South Asia (ME-SA**)** (Barker *et al.,* 1993).

Vaccination constitutes an important control policy for FMD in affected areas with advanced eradication programs, as well as in free regions that decide to use immunization as a control measure after a recent introduction of the disease (Beck and Strohmaier 1987). In Egypt, the local commercial (trivalent O, A and SAT2) inactivated vaccines were used for rapid control of the disease that supplied by the FMD Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo (Domingo *et al.*, 2002; Soltan *et al.*, 2017).

According to the results of the current study, we can conclude that the identified strains in FMD infected Egyptian buffalo calves were unique and different from the vaccinal and other Egyptian strains as well as they were clustered with Topotype ME-SA Panasian viruses. Cardiac biomarkers as serum (cTnI) and (CK-MB) along with ECG can be used as useful prognostic tools in FMD infected Egyptian buffalo calves.

Conflict of interest

Authors declare no conflict of interest.

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دراسه جزيئيه علي عجول الجاموس المصريه المصابه بالحمي القلاعيه ومرجعية رسام القلب الكهربائي والدلالات الحيويه القلبيه كاداوات انذار

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اجمالي ٢٠٠ من العجول الجاموس المصريه يتراوح اعمارها بين ٩-١١ شهر تم تحصينها مسبقا بالتحصين المحلي ثلاثي العترات (O,A and SAT-1) من اماكن مختلفه من محافظة المنوفيه ظهر عليها علامات الاصابه بالحمي القلاعيه تم فحصها بالفحص الجزيئي لخصائص فيروس الحمي القلاعيه وكذلك عمل رسم قلب كهربائي وقياس بعض الدلالات الحيويه الخاصه بالقلب ومقارنتها بالحيوانات غير المصابه. اظهرت النتائج ان ٣١,٥% من الحيوانات اوضحت وجود بروتين غير تركيبي ضد العدوى الطبيعيه لفيروس الحمي القلاعيه في حين نسبة ٥١,٥% اظهرت وجود بروتين تركيبي ضد التحصين والذي كان مختلفا عن تلك الخاصه بالعتره (O) السائده. علي الجانب الاخر لوحظ عدم وجود اجسام مضاده لفيروس الحمي القلاعيه في ١٧% من العينات. رسام القلب الكهربائي اوضح وجود موجات من اللاقضبيه غير الناضجه للبطين مع تفلطح في موجة P وزياده في عمر موجة QRS مع نقص في قوتها بالمقارنه برسام القلب الكهربائي للحيوانات المحصنه غير المصابه مع وجود زيادة معنويه في التروبنين والكرياتين كاينار القلبي واليوريا والكلور والبوتاسيوم في حين وجد نقص معنوي في الصوديوم في العجول المصابه عن مثيلتها في العجول المحصنه غير المصابه. من خلال الدراسه نخلص الي ان العتره التي تم التعرف عليها في العجول الجاموس المصريه المصابه بالحمي القلاعيه فريده وتختلف عن عترات التحصين المستخدم وهناك تشابه مع عترة الشرق الاوسط ME-SA بالاضافه الي ان رسام القلب الكهربائي ودلالات القلب قد تكون اداه تعريفيه بمأل مرض الحمي القلاعيه في العجول الجاموس المصريه.