Clinical Study on Egyptian Cattle Affected With Recent Isolate of Foot and Mouth Disease Virus SAT2/2012

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Abstract

Foot and mouth disease is one of the economically important viral diseases of cattle resulting in high mortalities in young calves and reduction of production in adult animals. Egypt was endemic with serotypes O and A till march 2012 outbreak where serotype SAT2/2012 was isolated and resulted in high mortalities in young calves and adult cattle. Our study was carried out during 2012 FMD outbreak on 22 adult cattle with clinical picture suggesting FMD infection. Saliva, tongue epithelium and vesicular fluids were collected from clinically affected animals and FMD serotype SAT2/2012 was isolated from all animals. Hematological and biochemical examination was carried out on affected animals and revealed that, there's significant reduction in total protein, albumin, globulin, cholesterol and calcium. CPK level was increased in 3 samples out of 22 samples which is an indication of the degenerative effect of SAT2/2012 on the myocardium.

Postmortem and histopathological examinations were carried out on the heart of dead adult animals and compared with the heart of young calves died from FMD during 2012 outbreak. There's grayish white small foci of necrosis present mainly at heart base, these areas were friable and appeared as cooked and pale. There's hyaline degeneration and zenker's necrosis of some myocytes. There were myolysis and complete disappearance of some myocytes with presence of large numbers of mononuclear cells infiltration.

Key words: FMD, SAT2, Cattle, Myocardium, Outbreak, CPK

Introduction

Foot and mouth disease (FMD) is a contagious viral disease affecting cloven footed animals and resulted in severe economic losses (OIE, 2012). FMD virus (FMDV) belongs to genus Aphthovirus of the family Picornaviridae and is classified into seven antigenically distinct serotypes (O, A, C, SAT1, SAT2, SAT3, and Asia1) and innumerable subtypes and there's no cross protection between the different serotypes (Neeta et al., 2011)

All cloven-footed animals are susceptible to infection with FMD viruses but the facility with which different species and even breeds of animals develop clinical disease varies. This variation extends to susceptibility to infection and the routes and rates of viral excretion by different species once they are infected (Thomson, 1994). This variation is largely quantified in the case of domestic animals, Pigs are

highly susceptible (Amplifier host) followed by cattle and buffaloes (maintenance host) thensheep and goats (carrier animals) and camels with a lower susceptibility. Furthermore, it should be appreciated that in these respect different strains of FMD viruses may interact differently with different species of animals (Hedger et al., 1972; Hedger, 1976; Condy et al., 1985).

FMD is considered an endemic disease in Egypt. Prior to 2006, the only reported serotype is FMD serotype O, in 2006 outbreak the index cases occurred close to quarantine stations where animals from Ethiopia were held. Virus typing indicated FMD serotype A virus of an African topotype, So Egypt was endemic with both serotypes O and A only till February 2012, in March 2012 FMD virus serotype SAT2 was reported for the first time in Egypt in an outbreak which resulted in severe losses in cattle and buffaloes populations (Bartels and Ryan 2012; FAO 2012 and Shawky et al., 2012).

FMD in newly borne animals has high mortalities as it frequently causes necrotizing myocarditis and this lesion may also be seen in adults infected with some strains of the virus (Radostitis et al., 2007)

The present work aims to study the clinical picture of FMD 2012 outbreak, to isolate and identify the causative agent, the effect of FMDV on hematological and biochemical profile and histopatholoical changes of the myocardium in clinically infected died young and adult cattle.

Material and Methods

Animals

Thirty native breed Egyptian cattle aged 2 - 5 years were used in this study. These cattle were belonging to different localities (Sharkia, Giza, Fayoum, Gharbia, Beni Sueif) in Egypt during FMD outbreak (2012). These cattle were divided into 2 groups: 22 cattle suffering from high fever with vesicular eruptions on buccal mucosa and interdigital space suggesting FMD infection and 8 apparently healthy cattle considered as control group. All animals exposed to physical and clinical examination according to Radostits et al., (2007).

Clinical Samples

Samples including Tongue epithelia, saliva and/or vesicular fluids were collected from the 22 cattle suspected of being infected with FMD. These samples were collected on glycerol saline and at -20o C till subjected to trials of virus isolation.

Tissue culture

Primary calf kidney cell culture: It was prepared as described by El-Sayed et al. (2013**)** at the FMD Vaccine Research Department, VSVRI.

Baby Hamster kidney cell line (BHK21) Clone 13: BHK was maintained in FMD Vaccine Research Department, VSVRI, Abbasia, Cairo using Eagle's medium as described by Huang et al., (2011)

Baby mice: One hundred and ten Swiss albino baby mice (2-4 day old) were used for primary isolation of FMD virus.

Sample Preparation for virus isolation: Clinical samples (Tongue epithelia, saliva and/or vesicular fluids) were homogenized in a proper volume of tissue culture media with antibiotic, then clarified by centrifugation and filtrated via 0.22 µm millipore filter and kept at -20o C until used for virus isolation.

Isolation of FMD virus: The prepared homogenate fluids were clarified by adding chloroform and centrifugation at 7000 rpm for 10 mm at 4°C and the supernatants were aspirated for detection of FMD virus. Each sample of suspected FMDV was inoculated into:

a. Five Swiss suckling mice (100µl/mouse inoculated I/P). Death of baby mice after 24 hours of inoculation was considered non-specific, while paralysis of the hind limbs or death on the 2nd to 7th day was considered specific positive to FMDV according to El-Sayed et al. (2012).
b. Primary bovine kidney cell culture and Baby Hamster kidney (BHK-21) cell-culture were inoculated with the prepared samples and observed after 24 hrs and 48 hrs for detection of the cytopathic effect (CPE) of FMDV as described by Neeta et al. (2011).

Typing of FMD virus:

Complement fixation test (CFT): It was applied for typing of the obtained isolates of FMD virus according to Health protection Agency (2009) using reference hyper immune sera of FMDV. Seven serotypes reference guinea pig hyper immune sera were supplied by World Reference Laboratory (WRL) for FMD in Pirbright, United Kingdom.

Indirect sandwich ELISA: Typing of the isolated viruses was confirmed by indirect sandwich ELISA using ELISA kit provided by the FMD World Reference Laboratory (WRL-Pirbright, UK) as described by Alonso et al. (1992).

Detection of FMD virus genome: RNA was extracted from clinical and cell culture samples and the extracted RNAs were tested by one-step RT-PCR. Primer pair (PoR/PoF) for FMDV RNA detection was synthesized by Metabion (Germany). PoF (5´- CCT ATG AGA ACA AGC GCA TC -3´) and PoR (5´- CAA CTT CTC CTG TAT GGT CC -3´) were derived from the virus 3D polymerase (Shin et al., 2003). Total RNA was extracted from non-infected BHK cells to prepare negative controls.

Sequencing of FMD virus gene: The PCR products of field and tissue culture FMDV samples used in this study were submitted for DNA sequencing in a single sequencing reaction tube on Thermocycler (Biometra, Germany).The sequencing reactions were analyzed on an automated DNA sequencer (3130x1 Genetic Analyzers, Applied Biosystems, USA). Analysis of the sequence identity, divergence and phylogenetic relationship was performed using the clustal X method with weighted residue table provided in the MegAlign program (DNASTAR, Inc. Madison, WI, USA).

Blood samples: Blood samples were collected through the jugular vein puncture from all included animals and divided into 2 tubes, one plain for serum separation and the other with EDTA as anticoagulant for hematological studies.

Hematological analysis: Blood samples with anticoagulant were subjected for detection of cellular blood constituents according to Schalm (1986) while serum samples were examined for detection of blood indices as described by Willard et al (1989).

Serum biochemical analysis: Commercial kits were used for spectrophotometric determination of serum concentration of total protein, albumin, globulin, glucose, cholesterol, blood urea nitrogen(BUN), creatinine (Gamma Trade company, Egypt), calcium , phosphorus (Bio-Diagnostic, Giza, Egypt), AST, ALT and CPK(Specterum company , Egypt) on a selective chemistry analyzer (Abbott Alcyon 300I, USA) as described by Grunwaldt et al.(2005).

Postmortem specimens for Histopathological examination: Hearts from dead animals (young and adult) were subjected to careful postmortem examination and gross abnormalities were recorded and heart tissue specimens were collected, fixed in 10 % neutral buffered formalin, processed and embedded in Paraffin wax, sectioned at 4 μm and then stained with Hematoxylin and Eosin and examined microscopically to determine the histopathological changes according **to Bancroft and Gamble (2008).**

Statistical analysis: The obtained data were analyzed statistically by using SPSS program, version 16. Significant differences between the obtained values of FMD infected group and control group were indicated by P*≤ 0.05 P**≤ 0.01 P***≤ 0.00

Results and Discussion

The present study was carried out during 2012 FMD outbreak on 2 groups of animals, the first group consist of 22 cattle suffering from clinical signs suggested to be due to FMDV infection and 8 apparently healthy cattle considered as control group. All animals exposed to physical and clinical examination according to Radostits et al., (2007)

The clinical examination (table-1) revealed that 22 animals suffered from high fever depression, dullness, anorexia, salivation, lameness and had vesicular eruptions on buccal mucosa and interdigital space suggesting FMD infection. These clinical signs were in agreement with those reported by Imren et al., (1991), Smith et al., (1996) and Radostits et al., (2007). These animals showed significant increase in mean values of temperature, pulse rate and respiratory rate (P≤0.001) in comparison with mean values of control group.

Virus isolation was carried out from clinical samples (vesicular fluid, saliva and tongue epithelium). Samples were inoculated into Swiss suckling mice 2-4 days old. All inoculated mice showed paralysis of the hind limbs and death on the 2nd to 7th day in agreement with what reported by El-Sayed et al. (2012) FMDV was isolated from all 22 clinical samples. Also samples inoculated onto primary bovine kidney

and Baby Hamster kidney (BHK-21) cell-cultures. The infected cell cultures showed specific CPE within 24-48 hours post infection characterized by rounding in cells as described by Shawky et al., (2012) results were in table (2). Typing of the isolated virus was carried out using Complement fixation test (CFT) with reference hyper immune sera of FMD, Indirect sandwich ELISA, RT-PCR for detection of FMD virus genome and sequencing of FMD virus gene. All isolates revealed that the isolated serotype was SAT2 (table-3). These applied techniques were recorded as valuable methods for typing of FMD virus by Shawky et al., (2012).

Hematological analysis of FMD infected animals (table-4) revealed significant reduction in RBCs ($P \leq 0.001$) and significant increase in MCV ($P \leq 0.001$). Similar findings were recorded by Gokce et al (2004), Mohapatra et al (2005), Krupakaran et al (2009), Ghanem et al (2010) and Gattani(2011). These results could be attributed to endocrinopathy as reported previously by Radostits et al (2007).

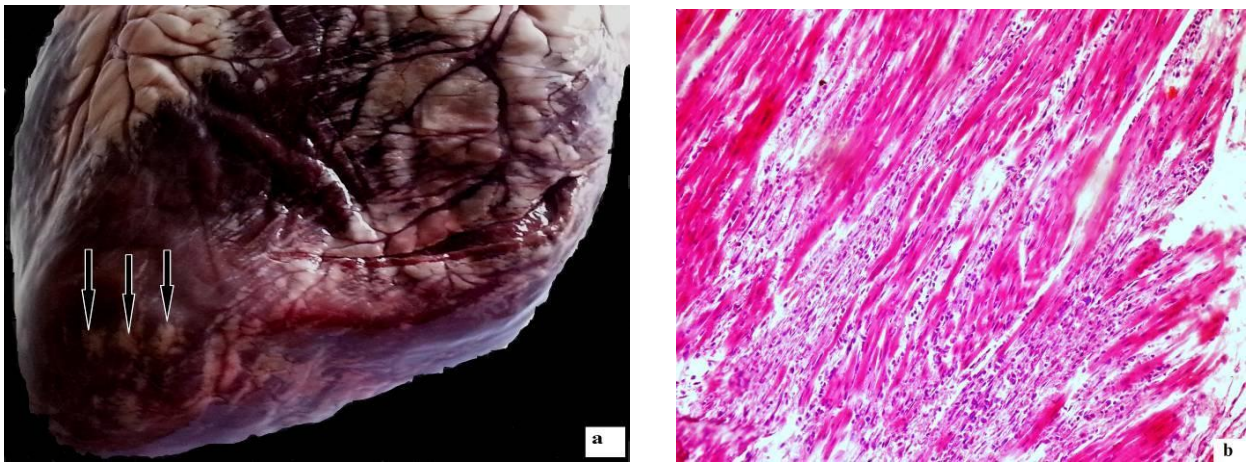

Photo (1. a): Myocardium of adult died infected cattle showing grayish white small foci of necrosis at heart base (arrows), b. micrograph of adult cattle myocardium showing hyaline degeneration and zenker's necrosis of some myocytes with myolysis and complete disappearance of some, these areas of myolysis are replaced by large number of mononuclear cells infiltration (H&E 20X).

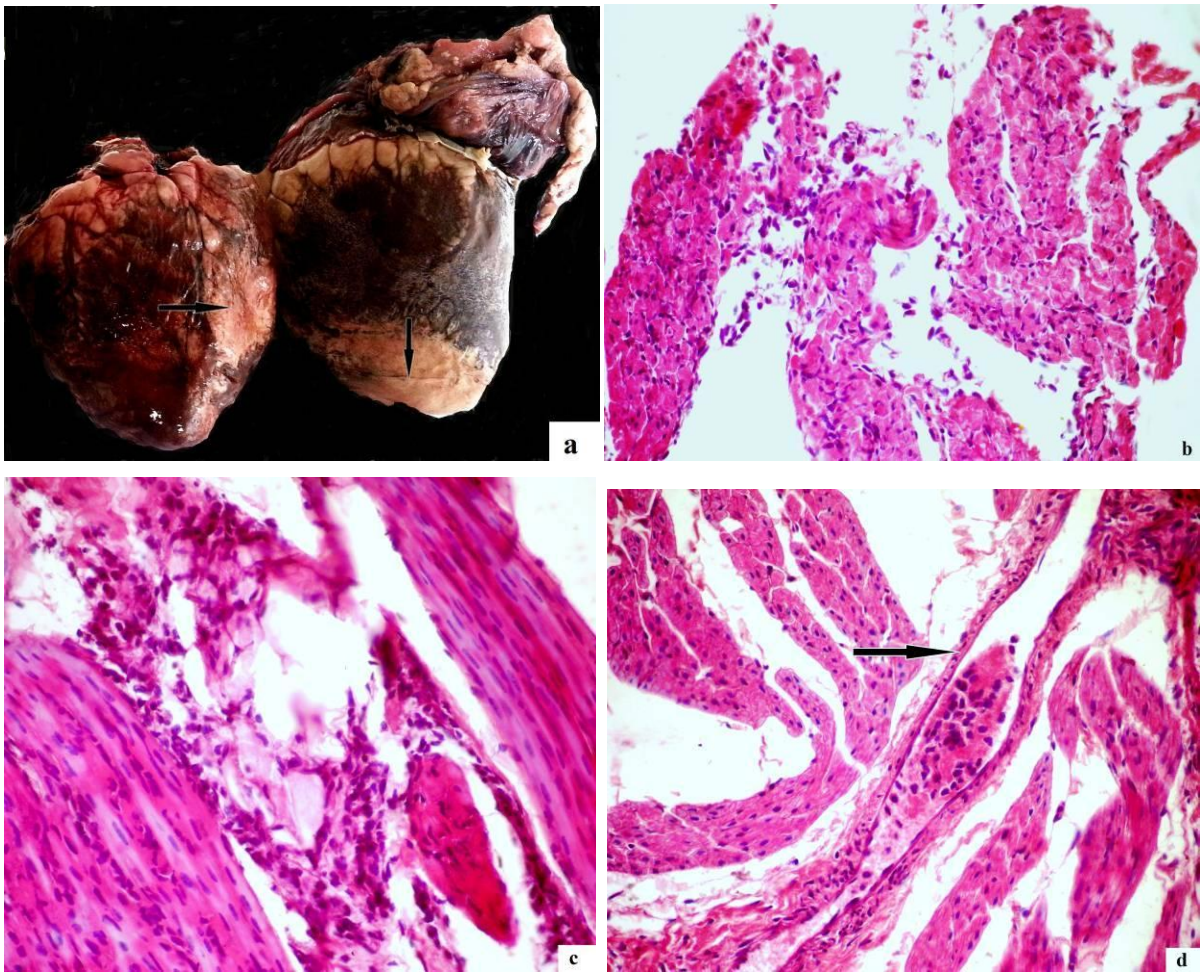

Photo (2): a- Myocardium of calves showing grayish white large area of necrosis (arrows), b- micrograph of calves' myocardium showing hyaline degeneration and zenker's necrosis of some myocytes with edema of interstitial tissue in-between muscle fibers (H&E 20X); c- micrograph of calves' myocardium, note myolysis and complete disappearance of myocytes, areas of myolysis are replaced by few number of mononuclear cells infiltration (H&E 40X); d- micrograph of calves' myocardium. Notice vasculitis and leucocytosis (arrow) with hyaline degeneration of some myocytes (H&E 20X).

Table 1: Isolation of FMD virus of Tested samples on Primary bovine Kidney cells, Baby hamster Kidney cells and baby mice

Samples	No of tested samples	Virus isolation	
		Suckling baby mice	Primary bovine & BHK cells
Tongue epithelium and vesicular fluid	22	+	+
positive control (O)	3	+	+
positive control (A)	3	+	+
positive control (SAT2)	3	+	+
Negative control	3	-	-

Table 2: Typing of FMD virus in tested cases using complement fixation test										
Tested samples	Total No.	No. of Positive	Positive %	CFT Sero typing for isolated virus						
				O	A	C	SAT1	SAT2	SAT3	ASIA1
Tongue epithelium and vesicular fluid	22	22	100	-	-	-	-	+	-	-
positive control (O)	3	3	100	+	-	-	-	-	-	-
positive control (A)	3	3	100	-	+	-	-	-	-	-
positive control (SAT2)	3	3	100	-	-	-	-	+	-	-
Negative control	3	0	0	-	-	-	-	-	-	-
CFT = Complement fixation test using reference hyper immune sera **(-)**=Negative **(+)**=Positive										

Table(3) :Physical clinical examination in the FMD group and control group.Data areexpressed as mean and mean of standard error(mean±SE)		
Parameters	**FMD group(n=22)**	**Control group(n=8)**
Temperature(c^o)	$39.9 \pm 0.17^{***}$	38.80 ± 0.15
Pulse rate (/ min)	$70.2 \pm 1.54^{***}$	52.12 ± 1.90
Respiratory rate(/min)	$26.8 \pm 1.06^{***}$	19.0 ± 0.70

Table(4) : Hematological parameters in the FMD group and control group.Data are expressed as mean and mean of standard error(mean±SE)		
Parameters	**FMD group(n=22)**	**Control group(n=8)**
RBCs($\times 10^6/\mu l$)	$4.14 \pm 0.13^{**}$	5.55 ± 0.29
PCV(%)	$33.09 \pm 0.52^{*}$	31.00 ± 0.80
Hb(g/dl)	$10.98 \pm 0.24^{*}$	10.20 ± 0.27
MCV(f)	$80.90 \pm 2.04^{***}$	56.80 ± 2.86
MCH(pg)	$27.00 \pm 0.98^{***}$	18.69 ± 0.95
MCHC(g/dl)	$33.31 \pm 0.80^{***}$	32.91 ± 0.06

Table (5): Serum biochemical parameters in the FMD group and control group.Data are expressed as mean and mean of standard error(mean±SE)		
Parameters	**FMD group(n=22)**	**Control group(n=8)**
Total protein (g/dl)	$4.32 \pm 0.17^{***}$	7.78 ± 0.33
Albumin (g/dl)	$2.17 \pm 0.11^{***}$	5.00 ± 0.27
Globulin (g/dl)	2 .14 ± 0.14	2.78 ± 0.30
Glucose(g/dl)	$73.94 \pm 2.17^{***}$	52.91 ± 3.27
Cholesterol (mg/dl)	$170.51 \pm 5.27^{**}$	192.69 ± 4.88
BUN (g/dl)	28.34 ± 0.50	28.33 ± 0.51
Creatinine (g/dl)	3.20 ± 0.11	3.12± 0.20
Calcium (g/dl)	$7.81 \pm 0.22^{**}$	9.41 ± 0.44

Phosphorus (g/dl)	8.55 ± 0.57***	4.66 ± 0.28
AST(u/l)	103.95 ± 4.37	101.68 ± 5.18
ALT (u/l)	24.36 ± 1.40	20.93 ± 2.31

Table (6) The effect of infection with FMD on serum CPK level in cattle

Number of animal	CPK (U / L)	Normal range
1	613.27	35-280 according to **Jackson,et al (2002)** Note : Animals numbers 1, 5, 6 had high levels of CPK in comparison with normal range recorded by **Jackson,et al (2002)**
2	116.65	
3	279.97	
4	123.32	
5	389.96	
6	309.96	
7	168.31	
8	96.65	
9	228.31	
10	118.32	
11	101.65	
12	148.31	
13	64.99	
14	61.66	
15	99.99	
16	33.33	
17	153.31	
18	59.99	
19	89.99	
20	71.65	
21	124.98	
22	125.58	

In addition, serum biochemical analysis of infected cattle (table-5) showed significant reduction in total protein, albumin , globulin, cholesterol and calcium levels ($P \leq 0.001$) coming in parallel with what mentioned by Gokce et al (2004),Mohapatra et al (2005) , Krupakaran et al(2009) , Ghanem et al (2010) and Gattani (2011). Hypoprotenemia and hypoalbuminemia could be a result from sever anorexia and off food due to oral lesions as mentioned by Kaneko et al (1997). It is known that protein requirement and protein catabolism increases in the presence of infection or any lesion in the body as recorded by Meyer et al (1998). In our study significant reduction in cholesterol level may be due to dysfunction of pancreas B-cell as suggested by Gokce et al (2004). Hypocalcaemia could be attributed to sever anorexia and hypoproteniemia in affected cattle resulting in decrease protein bounded calcium as stated by Gokce et al (2004). Hyperglycemia and hyperphosphatemia recorded in our result were similar to those reported by Gokce et al (2004), Mohapatra et al (2005), Krupakaran et al (2009), Ghanem et al (2010) and Gattani (2011). Hyperglycemia is a common finding and well documented in cattle affected with FMD as recorded by Szopa et al (1993), Elitok et al (1999), Yeotikar (2003) and Gokce et al (2004).

The present results showed that there were no significant changes in BUN, Creatinine, ALT and AST levels as indicator of liver and kidney functions revealing that FMDV did not affect liver and kidney functions. CPK was measured in diseased animals where three animals out of 22 had high level of CPK (table-6) which is an indicator of myocardial degeneration and that support the high mortality in adult animals during 2012 outbreak due to the degenerative effect of FMD SAT2 on the myocardium as demonstrated by Radostistis et al., (2007).

Post mortem examination of myocardium of diseased died adult cattle showed grayish white small foci of necrosis present mainly at heart base, these areas were friable and appeared as cooked and pale (photo-1.a). Myocardium of calves revealed large pale area of necrosis; this area was soft and friable (Photo-2.a)**.** Similar findings were described by Gulbahar et al., (2007)

Histopathological examination of the myocardium of diseased died adult cattle showed hyaline degeneration and zenker's necrosis of some myocytes in which sarcoplasm was more esinophilic with loss of cross striation, karyopykosis and karyolysis. There were myolysis and complete disappearance of some myocytes with presence of large numbers of mononuclear cells infiltration (Photo-1.b). Calves' myocardium revealed hyaline degeneration of some myocytes with presence of edematous fluid in-between muscle fibers and few mononuclear cells infiltration between degenerated myocytes. There were vasculitis and leucocytosis in some blood vessels (Photo-2, b, c &d). These results revealed that FMDV serotype SAT2 have degenerative effect on the myocardium of some adult cattle as well as young calves. These findings come in agreement with those described by Radostitis et al., (2007)

Conclusion

The causative agent Foot and mouth disease during 2012 outbreak in Egypt was FMD virus serotype SAT2 resulted in high morbidity and mortality in both young and adult animals. Foot and Mouth disease serotype SAT2 induced degenerative changes in the myocardium of some adult animals which could be one of the important causes of mortality in adults. So, it could be recommended that the national policy should be directed toward animal protection against FMD must include serotype SAT2.

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