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Recent Crisis in Egypt Livestock Industry due to Foot and Mouth Disease Virus

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Abstract: *FMD is a highly contagious viral disease that threatens the livestockindustry worldwide. The disease being endemic in many countries of Africa, Asia and South America.The disease was endemic in Egypt since 1950 and however that, the Egyptian farms have been still attacked by several outbreaks of FMDV during the last years resulted in a highly mortality rates in both young and adults. In order to investigate the morphopathologicalchanges in animals that naturally infected and died due to FMDV infection. During 2016-2017, total of 500 pm tissues samples and 100 blood and 100 serum samples collected from 170 field samples, were analyzed.Pathological examination revealed presence ofvesicular and erosive lesions developedon the tongue, interdigital space of the feet, muzzle, and teats. Multi-focal areas of lympho-plasmocytic myocarditis were seen in the heart of all calves, and similar lesions were also observed in the hearts of 66 % of adults. Detailed pathological alterationsin adult and young animals were described. Polymerase chain reaction (PCR) screening of tissue specimens using specific primers for FMDV was positive. The results provide a detailed description of the pathogenesis of the disease in cattle and buffalo livestock recently infected by FMDV outbreaks in Egypt.*

Keywords:*Foot-and-mouth disease virus; cattle; buffalo; pathological alterations, PCR*

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I. Introduction

FMD, is a highly contagious vesicular disease, genus aphthovirus, Picornaviradae family,affectinga number of wild and domestic cloven-hoofed mammals(Samuel 2001; Grubman and de los Santos 2011). FMD is an enzootic disease, seen in most large areas of the world (Barker 1993).Vesicular lesions on the tongue, feet, snout, and teats,fever and lamenessare the more characteristic lesions expressed by FMD infected animals(Donaldson 2000). The disease is notorious for its high morbidity and mortality especially in suckling animalsbut is not notable for high mortality rate in adults (Barker 1993).The mortality rate can exceed 50 %in young animals due to myocardial damage but itis aboutnearly 5 % in adult ruminants (Barker 1993). In calves, myocarditis is considered a fatal form of FMD as it is distinguished by hyaline degeneration, necrosis of muscle and can occurs without developing the characteristic blister lesions noted in adult cattle(Barker 1993 ; Alexandersen 2003).

FMD being endemic in many countries of Africa, Asia and South America(Bayry J 2001). However, outbreaksrecently occurred in countries that are normally free of FMD, including Japan, Korea, United Kingdom, France and the Netherlands (Gibbens 2001; Gibbens 2002; Muroga 2012).The disease is endemic in Egypt since 1950 (Zahran 1961), and cause a seasonal highly severe economic losses.Serotypes A and O of Foot-and-mouth disease Virus (FMDV) were the most prevalent serotypes circulating in Egypt, in addition to SAT2 serotype(EL-Bayoumy, Abdelrahman et al. 2014; Elhaig and Elsheery 2014; Abd El Moneim, Hafez et al. 2016). However, Vaccinations regime is regularly adopted by veterinary authorities; the Egyptian farms have been attacked by several outbreaks of FMDV during the last few years, which resulted in economic crisis to livestock industry as a result of highly mortality rates in both young and adult animals. So, in this study we aimed to catch the pathological alterations in cattle and buffaloes spontaneously infected with FMDVin correlation with the increased mortalities in adults; and identification of the causative FMDV strain by using RT- PCR technique based on serotype specific primers.

II. Materials and methods

2.1. Sample Collection and histopathological examination.

Clinical samples (blood, oral swabs, and post mortem tissues) were collected during FMD outbreak 2016 – 2017 from different localities in El-Beheira government.Clinical signs and all the history of diseased animals were recorded **(Table. 1).**Dead animals were immediately subjected to careful postmortem (PM) examination according to animal welfare abattoirs in Egyptand all PM findings were recorded.Suitable tissue specimens were collected from oral cavity, rumen, reticulum, omasum, abomasum, tongue, buccal tissue, dental pad, palatine tonsil, Lung, liver, spleen, heart (atria, ventricles, and interventricular septum), and lymph nodes (mandibular, parotid, retropharyngeal, tracheobronchial, mediastinal, mesenteric) for histopathological andmicrobiological examinations.A set of tissue specimens were collected and kept frozen at -80 ºC until used for RNA extraction. Another set of the tissue specimens were immediately fixed in 10% neutral buffered formalin (2–4 days at room temperature) for histopathological examinations. Fixed specimens were routinely processed through dehydration in ascending grades of ethanol and then cleared in xylene and embedded in paraffin blocks. Paraffin sections were prepared at 4 – 5µ thickness on glass slides. The sections were then stained with hematoxylin & eosin and histopathologically examined using thelight microscope (Bancroft, Suvarna et al. 2012)Severity of gross and histopathological lesions between calves and adults were evaluatedbased on previously described grading systems(Cross 1998; Kleiner DE 2005; Cheng Z 2010; Gibson-Corley, Olivier et al. 2013). The grading scale included five scores: -ve=no lesions; + =mild; ++=moderate and +++=severe.

2.2. RNA extraction and cDNA synthesis

TotalRNA extraction from (blood, oral swabs, and post mortem tissues) was done by using Qiagen, All Prep® DNA/RNA Mini kit, Germany;accordingmanufacturer's instructions. Total RNA samples were reverse-transcribed by using HiSenScript™ RH (-)cDNA Synthesis kit, NtRONBiotechnology, koria, according to the manufacturer's instructions. The obtained cDNAwas used for specific PCR by using specified primers for amplification of FMDV. The primers were synthesized by Sigma–Aldrich-Japan.

2.3. Conventional PCR analysis:

Conventional PCR for FMDV detection was performed using cDNA from collected samples using previously published specific primer(Reid, Ferris et al. 2000; Cottam, Haydon et al. 2006) for FMDV screening. All PCR reaction was carried out using an Eppendorf thermal cycler**(SENsQUEsTlabcycler).**The PCR amplification performed in a 25µ1 volume containing 4µl DNA, 2µl dNTP, 1µl of each primer (10µmol), 2.5µl 10× Ex Taq buffer, 0.25µl Ex Taq polymerase (Takara, Kyoto, Japan), and 14µl RNA, DNA free water.

The PCR condition forthe universal primer that used for detection of all FMDV serotypes was as follow: 94°C for 5 min, one cycle; 94°C for 1 min, 55°C for 1 min, followed by 35cycles at 72°C for 2 min with final extension at 72°C for 7 min, one cycle . The positive samples were used to amplify VP1 gene specific for serotype O by using the following condition: 94°C for 1 min, one cycle; 62°C for 1 min, followed by 35cycles at 72°C for 2 min with final extension at 72°C for 7 min, one cycle.

III. Results

3.1. Clinical signs

Various clinical signs with various degrees in relation to age, species were exhibited by infected animals. Young animals in both cattle and buffalo species in comparison to the adults exhibited severe clinical signs ended by mortality **(Table. 1).**Clinical signs include; fever which ranged from (39C – 42C) with severe salivation, vesicular lesions of the mouth, feet, tongue, snout and teats. Mouth lesions vary from an elevated area of hydropic degeneration on the upper surface of the tongue to red area of sub mucosal hemorrhage oral commissar, and sever ulceration that result from ruptured vesicles are found in upper lips, tips of tongue, dental pad, and upper third of the tongue. Lameness due to the foot lesion which represented by severe vesicular lesions in the digits and coronary bands ended by sever ulceration in interdigital space. Debilitating effects, rather than high mortality rates especially in young animals were also recorded **(Table. 1).**

3.2. Gross pathology

The adult cattle and buffalo showed several gross lesions asvesicles,erosions and ulcerations in the mouth and interdigital space. Oral lesions consisted of vesicular, irregular erosions/ulcers measuring 1–4 cm in diameter usually located on the torus lingua and the anterior third of tongues and larger erosion on the gingiva and lip. Foot lesions of circumferential vesicles of the coronary band with partial undermining and detachment of the bulbs of the heel, coronary bands of all feet were eroded or ulcerated, and there was severe detachment of the soles. Similar ulcers found in ruminal pillars, abomasum and intestine. Sever hemorrhagic inflammation and

Table 1: Showing lesion scoring of clinical signs, gross pathology, histopathology and PCR results of animals naturally infected with FMD outbreak.

Criteria		Calves (2months-2 years)				Adult	
Species		Cattle calves		buffalo calves		cattle	buffalo
Age		1_6 months	6months _2Y	1_6 months	6months _2Y	> 2 years	> 2 years
Number		36	52	22	15	27	18
Clinical signs	Fever	++	+++	+++	++	+++	+++
	Oral lesion	++	+++	+++	++	+++	++
	Foot lesion &lameness	-ve	+++	-ve	++	+++	+
	Sudden death	+++	+++	++	++	+++	++
Gross pathology	Erosion &ulcer along GIT	++	+++	+++	++	+++	++
	Myocardial necrosis	+++	+++	++	++	+++	++
	Myocardial hemorrhage	++	+++	+	++	+++	++
Histopathology of organs	Oral cavity	++	+++	+++	++	+++	++
	GIT	+++	+++	+++	++	+++	++
	Liver	+	++	++	++	+++	++
	Heart	+++	+++	+++	+++	+++	++
	Foot	-ve	+++	-ve	++	+++	++
	Lung	+++	+++	++	++	+++	++
PCR results %	+ve % by using Universal primer for FMDV	78%	67%	45%	52%	81%	44%
	+ve % by using Specific primer for serotype (O)	72%	60%	42%	45%	75%	41%

-ve = no lesions;+= mild; ++ = moderate; +++ = severe.
Sudden death + = 25%, ++ = >25-50%, +++ = >50- 75%

myocardial hemorrhage range from small petechial to sever hemorrhage with various degree of myocardial necrosis was also observed grossly (Fig 1A, B, C, and D).

Young calves have all the above mentioned lesions in oral, digestive and feet but with increased severity, represented by the increase of the size and distribution of vesicular, erosive, ulcerative lesions in that sites. Pronounced myocardial hemorrhage with the characteristic tiger heart appearance were the most pathognomonic gross lesions in all of the dead calves in the form of yellowish to grayish streaking bands in myocardium**(Table. 1).**

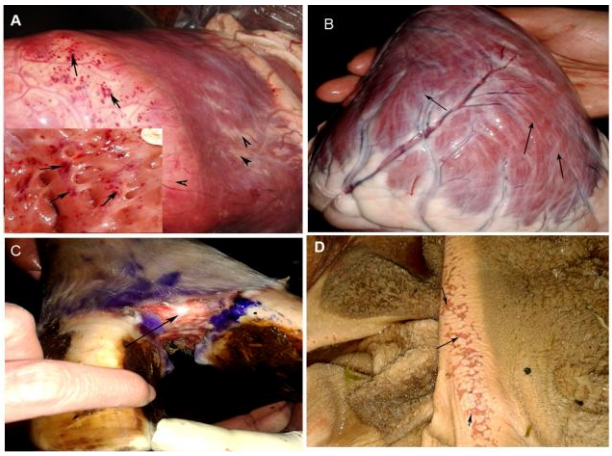

Fig.1. (A) Heart of calve 6 months old naturally infected by FMD, showing petechial hemorrhage at epicardium (arrows) with myocardium degeneration (arrowheads), the inset picture showing petechial hemorrhage in endocardium (arrows). (B) Heart of 13 months of age showing several yellow streaks (arrows) of myocardium degeneration and necrosis "tiger heart". (C) Severe ulceration with hemorrhage of hoof (arrows). (D) Focal area of ulcers of ruminal pillars (arrows).

3.3. Histopathological findings:

Histopathologicalexamination ofyoung and adult livestock wasrecorded**.**Oral cavity, foot (coronary band), omasum, and abomasum and rumen lesions revealed typical FMD lesions consisting of vesicle formation at the stratum spinosum, characterized by focal hydropic and ballooning degeneration ended by vesicular formation of varying sizes, edema, cell necrosis, and focal infiltrates of neutrophils (Fig 2 A). Intraepithelial bullae formation with superficial epithelial caps was also detected. Acanthosis with hyperkeratosis of overlying and adjacent epithelium. Erosions were seen with detached keratinocytes, amorphous eosinophilic material, neutrophils. Basal epithelium was usually preserved, but some lesions extended through the basement membrane with marked mixed inflammatory cell infiltrates in the submucosa or dermis with subsequent sloughing of necrotic tissue leading to ulcers formation. Focal myositis in the tongue, oral skin, omasum, abomasum and rumen characterized by myofiber hyaline degeneration and zenker's necrosis accompanied by sarcolemmal proliferation.The heart of adult animals showed mild to severemyocarditis (Fig 1 B,C,D), whilethe heart of the young calves is severely affected especially in younger ages in the form of moderate to severe myocarditis**(Table. 1).**Severe degeneration and necrosis of myocardium with complete lysis of some muscle fibers and replacement of these fibers by inflammatory cells **(Fig 2 A),**were also detectedin many examined cases. Inflammatory edema with pronounced infiltration with lymphocytic cell and some macrophages, myocardial hemorrhage and vasculitis were also present.The liver showed mild to moderate hydropic degeneration of hepatocytes with varying degree of coagulative necrosis andperiportal inflammation (Fig 2 B,C).The lung showedvariable degree (ranged from mild to severe) of various types of pneumonia (lymphocytic, hemorrhagic, serous, fibrinous pneumonia and bronchopneumonia). All that forms of pneumonia were seen alone or mixed with each others as serohemorrhagic or serofibrinous (Fig 2 D).

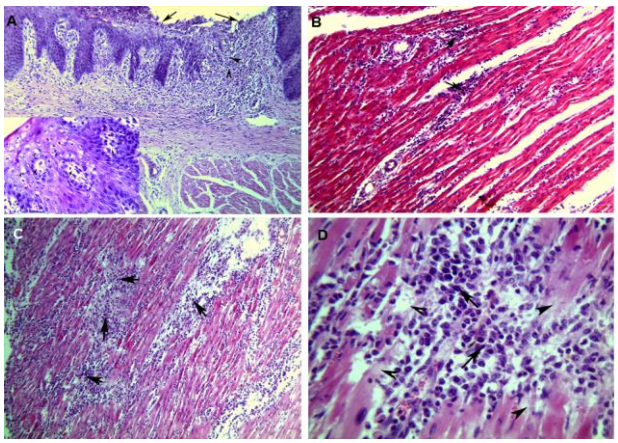

Fig.2.(A) ulcerative dermatitis of adult cow naturally infected by FMDV (arrows) with leukocytic cell infiltratios (arrowheads), the inset picture showing leukocytic cell infiltration.H&E, Bar=100 µm. (B) Mild myocarditis of myocardium with mild lymphocytic cell aggregations (arrows). H&E, Bar=100 µm. (C) Moderate myocarditis with increased number of lymphocytic cell aggregation (arrows) between degenerated muscles H&E, Bar=100 µm. (D) Higher magnifications of (c)

showing lymphocytes and mononuclear cells (arrows) with necrosed myocardial muscle (arrowheads) H&E, Bar=50 µm.

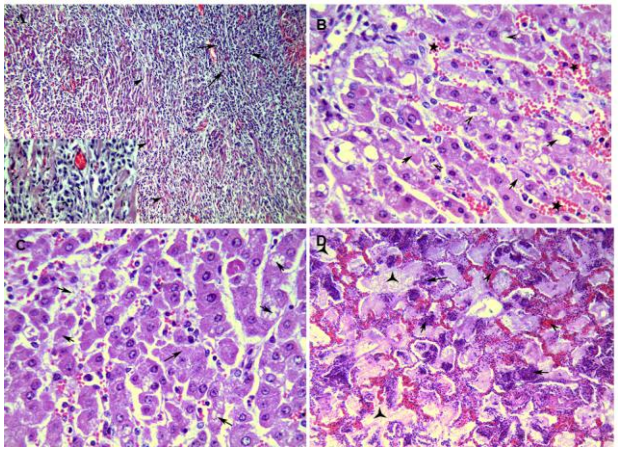

Fig.3. (A) Severe myocarditis with heavy aggregation of lymphocytes and mononuclear cells (arrows) which replaced the necrosed myocardial muscle (arrowheads), inset picture showing leukocytic cells and necrosed muscle.H&E, Bar=100 µm.(B) Liver of naturally infected buffalo by FMDV showing hydropic degeneration (arrowheads) with congestion of blood sinusoids(black stars) and mild coagulative necrosis of hepatocytes (arrows).H&E, Bar=50 µm. (C) Liver of cow naturally infected by FMDV showing focal areas of coagulative necrosis (arrows).H&E, Bar=50 µm. (D) Lung of naturally infected calve by FMDV showing congestion of perialveolar capillaries (arrowheads) with various forms of pneumonia as lymphocytic pneumonia (arrows) and fibrinous pneumonia (black stars).H&E, Bar=100 µm.

3.4. RT-PCR results

\Tissues, serum and Blood samples were analyzed by PCR for the presence of viral RNA. The percentage of positivity in each species was summarized in (Table 1).

IV. Discussion

a wide variety of cloven-hoofed domesticated and wild animal species were naturally infected by FMDV, resulting in foot and mouth disease, an acute febrile disease characterized by vesicular lesions of the oral cavity and upper digestive tract, including tongue, soft and hard palate, inner sides of cheeks, feet and teats (Grubman 2004). From previous reports, FMD usually resolves without the need for treatment despite of the extensive lesions caused by the virus in a short duration in the individual animal and is seldom lethal in adults (Arzt 2011). However, recently in the last outbreaks, FMD considered one of the most feared livestock diseases not only due to the highly contagious nature, or wide dissemination but due to the adverse significant economic impact on the livestock industry represented by a highly mortality rates in both young and adults.For that reasons, we triedto provide a comprehensive overview of FMD pathological alterations in young calves, cattle, and buffalo spanning from the earliest studies to recently acquired insights that lead to the high mortality rates in both young and adults in the last outbreaks. In addition to characterization of the field isolates of FMDV and its correlation with theapparently increased virulence of virus based upon increased mortalities in adults with pathological findings.

Hydropic degeneration with vesicle formation followed by erosion and ulcers of various epithelial sites including the mouth, feet, teats, omasum, abomasum and pillars of the rumen were observed not only during the viraemic phase but it often extends beyond the period of viraemia(Alexandersen 2005; Arzt 2009).Ruptured vesicles in oral cavity, feet, teats and skin were the most prominent gross lesions seen in the dead animals at necropsy. The degree of severity based upon the size and dissemination of lesions varied from mild to moderate in adults to high severity in young calves. Gross lesions were accompanied with the microscopic lesions exhibited by the affected animals in most of the cases (Seibold 1963; Arzt 2009). Absence of the gross lesions not indicative of absence of the virus as the virus can be detected even in the absence of macroscopic lesions (Brown 1995; Murphy and Alexandersen 2010).

Death due to FMD outbreaks is a fairly common feature of FMD epizootics in young livestock, as calves, piglets and lambs, (Alexandersen 2005). Death is often attributed to myocarditis (Donaldson 1984; Gulbahar and Kabak 2007). However, the death in adults is accompanied with degeneration of the myocardium which is a rare manifestation known as ‘malignant FMD’,(Shimshony 1986; Arzt and Rieder 2011) which became not a rare nowadays. In our study we found that FMD caused high mortality in calves in addition to

increased mortality percentage in adults in contrary to earlier reports as FMD didn't result in high mortality in adult animals, than neonates(Alexandersen 2003).

RT-PCR analysis revealed positive of FMDV form all samples collected from animals showing clinical signs. Histological examinations revealedZenker's necrosis with myositis in the examined muscles of tongue, cheeks, omasum, abomasum and ruminal muscles as previously reported (Shimshony 1988), which isconsistent with myotropic nature of FMDV (Arzt and Rieder 2011).The heart showed myocardial necrosis with, hyaline degeneration, and intense mononuclear cell infiltration (Gunes 2005; Karapinar 2010). In contrary to earlier reports, many myocarditis cases have been detected in calves aged from 2 months to 2 years old and in some adult animals aged more than 2 years (Gulbahar 2007; Karapinar 2010; Aslani 2013; Kaya 2013).In which most of myocarditis were reported in calves aged one week to three months old**.**Several types of Pneumonia (serous, hemorrhagic, fibrinous, serohemorrhagic, serofibrinous and serohemorrhagic) and bronchopneumonia were predicted especially in youngcalves from 1 month to 2 years old age, with severe myocarditis and myocardial necrosis and these results were supported with the detection of high quantities of viral RNA, infectious, and viral antigen in pulmonary tissues either in the viraemicor priviraemicstage (Burrows 1981; Arzt 2010).

In conclusion, severe myocarditis with myocardial degeneration and necrosis, were the most characteristicpathological findings of FMD during the last outbreak of FMDV infection in EL-Beheiragovernorate/ Egypt. Severe pneumonia was also not uncommon findings. The risk of myocarditis in FMD is not directly related only to the agebut could also be attributed to increased pathogenicity of the recently isolated field strain circulatingamong cattle in Egyptor emerging of new viral strains due to legal and illegal trade in animals and animal products importing.

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