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Some virological and pathological studies on Foot and Mouth disease in Egyptian buffalo

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ABSTRACT

Foot-and-mouth disease (FMD) is an endemic disease in Egypt and most parts of Africa causing huge economic losses. Control of FMD using vaccination requires information on the occurrence of various FMDV serotypes. This study aimed to determine FMDV serotypes in Qal-yobia Governorate, Egypt. A total number of 21 field samples from different localities, were collected from buffaloes at age of 6months to 3 years during 2017. Field samples have been screened for FMDV detection by r.RT-PCR using universal primers and were further subtyped using serotype-specific primers. The r.RT-PCR screening revealed that 9 samples out of 21 were positive for FMDV serotype O.9 samples were propagated on BHK-21 cell line for 3 passages revealing cytopathic effect (CPE) in the form of cell rounding and aggregations in all 9 samples. Virus neutralization test was applied on positive isolates using anti FMDV serotype O and the results confirmed that these isolates were FMDV serotype O. On the other hand, histopathological finding showed that lung was congested, emphysematous multiple alveoli with accumulation of serofibrinous exudate. The tongue exhibited multiple foci of vesicular degeneration with serous exudate and inflammatory cells. The heart showed severe zenkers necrosis with mononuclear inflammatory cells infiltration and edema. The kidney revealed swollen endothelial cell lining blood vessels with some degenerative changes and most renal tubules showing degenerative changes. Blood vessels in most organs showed severe vasculitis. Immuno peroxidase technique was done on paraffin sections showed marked strong immunopositivity reaction in basal and prickle cell layer. Therefore, control efforts should focus on reducing the circulation of FMDV among susceptible livestock with special attention towards water buffaloes.

INTRODUCTION

Foot-and-mouth disease (FMD) is a severe, highly contagious and economically devastating viral disease worldwide, which affects ani-

mals with cloven hooves including domestic and wild bovid (**Arzt et al. 2011, James and Rushton 2002**). The FMD virus (FMDV) belongs to the genus Aphthovirus, the family Pi-

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cornaviridae and the order Picornavirales (ICTV (2015)). The virus is highly mutable because its genome is composed of a linear, positive single-stranded RNA molecule with a quasi-species nature allowing the continuous evolution of new variants (Domingo et al. 2012, Domingo et al. 1992). RNA genome of FMDV consists of about 8.3 kb in size, contains capsid protein named viral protein one (VP1) (Samuel & Knowles, 2001 Ryan et al. 1989).

The causative agent, FMD virus (FMDV) exists in seven immunologically distinct serotypes, O, A, C, Asia 1, SAT (Southern African Territories) 1, 2 and 3, each with a spectrum of antigenically distinct strains (Brown 2003, Yoon et al. 2011). There is no antigenic relationship between the different FMDV serotypes; therefore, the cross-protection does not exist totally. Moreover, in many cases, the cross-protection between different subtypes of the same serotype fails to be induced (Jamal et al. 2011). Consequently, continuous updating of data regarding to the field circulating topotypes is necessary for appropriate vaccine manufacture and disease control (Jamal et al. 2014, Biswal et al. 2014).

In Egypt, FMD is endemic and considered as a major trans-boundary disease that causes great limitations on sales and exports of livestock and livestock products (Rweyemamu et al. 2008, Paton et al. 2010). Serotype O was considered the predominant serotype in Egypt because it induced all outbreaks between 1964 and 2005 excluding an outbreak in 1972 that was caused by serotype A (Knowles et al. 2007, OIE 2012). In 2006, Sharkia and many Egyptian Governorates were stroked by severe FMD outbreaks caused by serotype A (Knowles et al. 2007, Ghoneim et al. 2010).

Vaccination programs depending on locally produced bivalent vaccines, against both serotypes A and O, were applied however severe FMD outbreaks existed up to February 2012, FMDV serotype (SAT2) was the primary cause (Ahmed et al. 2012, Kandil et al. 2013). FMD epidemic in Egypt During 2012-2018, the three FMDV serotypes, O, A, and SAT2 were detected in many outbreaks among cattle and water buffaloes in Egypt (FAO 2015, WRLFMD

2020). Also there are numerous variants within each serotype of FMDV allowing them to break through the existing herd immunity (Meyer et al. 1994; Balinda et al. 2010). The changes in FMDV serotypes add up to approximately 1% per year in the VP1 gene (Abdul Hamid et al. 2011).

The buffaloes considered as the main sources of good quality milk and meat in Egypt. It has been possible to establish that the basic reproductive rates for (SAT) types FMDV in buffalo (*Synceruscaffer*) are high. Buffalo acutely-infected with the SAT types of FMDV excrete the virus by the same routes and in approximately the same quantities as acutely-infected cattle but unlike cattle, most naturally-infected buffalo do not develop obvious signs of FMD (Thomson et al. 1992). Because most infections in buffalo are in apparent, such episodes are difficult to identify. The natural host for the SAT 1–3 serotypes of FMDV is the Cape buffalo (*Synceruscafer*) in which the virus replicates and persists with minimal disease pathology (Thomson and Bastos 2002).

It has been established that, rapid control of FMD is very important to reduce dissemination of the causative virus to other non-infected regions (Nick et al. 2007). A presumptive clinical diagnosis, virus isolation and serotyping of causative agent is important for proper formulation and production of a local emergency vaccine to control severe losses among life stock (Reid et al. 2001 and Tatiane et al. 2008). The use of the polymerase chain reaction (PCR) has revolutionized the way in which viral diseases are diagnosed. Analysis of the amplified VP1 sequence could enable the classification of FMDV detected in the morbed animals (Stram et al. 1995). Real time RT-PCR can detect a small fragment of FMDV genome RNA, not just live virus. A method based on real-time RT-PCR amplification and a fluorescent probe demonstrated high specificity and sensitivity for the diagnosis of FMD (Callahan et al. 2002 and Bernd et al. 2009).

Regarding the identification of the detected FMD virus, r-RT-PCR; using specific primers; confirmed that the obtained virus isolate is A,

SAT2 and O. These findings indicate that the results of r-RT-PCR is more sensitive and supported by (Andrew et al. 2007), they showed that r-RT-PCR is more rapid and sensitive technique suitable for detection and identification of FMD virus.

This study was carried out to explore data regarding the circulating of FMDV serotypes in Qalyobia governorate, Egypt. For this reason, the different FMDV serotypes were tested by r-RT-PCR in clinically sick, apparently healthy and post mortumed buffalos in different localities across Qalyobia Governorate, Egypt. Also trials were undertaken for isolation and identification of the detected serotype on BHK21 cells. PM, histopathology and immune-histo chemical studies were done on these samples.

MATERIAL and METHOD

Samples: A total of 21 field samples [epithelial tissue, vesicular fluid and internal organs (lung-tongue-heart- kidney- spleen and liver)] were collected from diseased, and post mortumed buffalo. (Table 1) demonstrated the samples data. The samples are properly prepared according to **Kitching and Donaldson (1987)**. Each specimen is divided into two parts. One placed in 10% formalin for pathological examination (**Suvama et al. 2013**) and the second preserved at -80 0C for virological examination.

Table 1. samples collected from buffalo in Qalyobia governorate.

Governorate	age	Type of samples			Total
		Internal organs	Epithelial tissue	Vesicular Fluid	
Qalyobia	6M to 3y	10	6	5	21

PREPARATION OF TISSUE SAMPLE:

The tissue samples were prepared for virus isolation. Pieces of tissues were washed with phosphate buffered saline (PBS) PH 7.4 then homogenized to prepare 10% tissue suspension with sterile PBS, then centrifuged at 3000 rpm for 10 min. The supernatant fluid was used undiluted and stored at -80oC.

VIRUS ISOLATION:

Baby Hamster Kidney (BHK 21) cell line was used for FMD virus isolation from positive r-RT-PCR samples (**OIE Terrestrial Manual, 2021**).

VIROLOGICAL EXAMINATION

RNA extraction: RNA extraction was performed on tissue samples and vesicular fluid using **Kogenebiotech RNA extraction kit, Biotech Angen, China (REF E007)** according to the manufacturer's protocol. After extraction, RNA was stored at -70°C.

RNA amplification in real time PCR: the r-RT-PCR were performed on all samples by using common primer and probe used for detecting FMDV VP1 region in all of the FMDV serotypes (**Callahan et al 2002, OIE 2012**). The positive samples were subjected to serotyping by real time PCR using 4 sets of primers and probes specific for A, O EA3, O Pan Asia and SAT2 serotypes of FMDV (**Reid et al. 2014**). Each serotype runs in separate run. Table 2 shows the primers and probes details. Real time RT-PCR was performed on each sample using Precision OneStep™qRT-PCR Mastermix with ROX (Primerdesign. co. Uk, cat. No. OneStep -R) as manufacturer's instructions. Reaction mixture was composed of 2ul of RNA template and 18ul of a master mix in a tube. The master mix was composed of 10 ul of 2 x Precision One Step TM qRT-PCR Master-mix, 10 PM of each primer, 5 PM of probe, and sterile distilled water till reach 20

ul. The optimized cycle program of denaturation, annealing and extension temperature was as follows: initial denaturation the cycling parameters were 50°C for 2 min. 1 cycle, 95°C for 10 min. 1 cycle; then 50 cycles consisting

of 95°C for 15 sec. and 60°C for 1 min. Negative control specimen was involved. Thermocycler Stratagene MX 3005P was used for real time detection of FMDV (OIE 2012).

Table 2. Oligonucleotide sequences of used primers and probe specific for FMDV

Primer name	Sequences 5' -3'	
3D F	ACT GGG TTT TAC AAA CCT GTG A	
3D R	GCG AGT CCT GCC ACG GA	Callahan et al. 2002
3D P	FAM- TCC TTT GCA CGC CGT GGG AC - BHQ1	
A F	ACG ACC ATC CAC GAG CTY	
A R	RCA GAG GCC TGG GAC AGT	Knowles, et al. 2007
A P	FAM-CGT GCG CAT GAA ACG TGC- BHQ1	
O Pan Asia F	CCG AGA CAG CGT TGG ATA ACA	
O Pan Asia R	CCA TAC TTG CAG TTC CCG TTG T	Reid et al. 2014.
O Pan Asia P	FAM-CCG ACT TGC ACT GCC TTA CAC GGC - BHQ1	
O/ EA-3 F	CAA CAC ACG GAC GTC GCG	
O/ EA-3 R	GTT GGG TTG GTK GTG TTG TC	Gabre et al. 2017
O/ EA-3 P	FAM-GAG TTG GAC CTG ATG CAG ACC C- BHQ1	
SAT2-F	TGA AGA GGG CTG AGC TGT ACT G	
SAT2-R	CTC AAC GTC TCC TGC CAG TTT	Ahmed et al. 2012
SAT2 P	FAM- ACA GAT TCG ACG CGC CCA TCG- BHQ1	

3D= universal primer

F= forward

R= reverse

VIRUS NEUTRALISATION TEST: (VNT):

The quantitative VN micro test is performed with BHK-21 cells in flat bottomed tissue culture microtiter plate against reference "Pribright" bovine antisera to serotype O was kindly provided from Virology Department. It was done according to (OIE 2021).

The virus titre of the stock virus was $10^{4.8}$ TCID₅₀/50µl calculated according to (Rweyemamu et al. 1978).

The Serum titre was calculated as antibody dilution required for 50% neutralisation of 100 TCID₅₀ units of the virus isolate.

PATHOLOGICAL EXAMINATION

Post mortem examination: was done after deaths and tissue specimens from lung, tongue, heart, kidney, spleen and liver were examined and collected and fixed in 10% neutral buff-

ered formalin for histopathological examination. They were routinely processed by slanted paraffin embedding technique. Section at 4 micron, stained with hematoxylin and Eosin (Bancroft and Gamble 2008).

Immunohistochemistry stain:

Immunoperoxidase technique was done on paraffin sections (Survana et al. 2013). Immunoperoxidase Protocol for IHC was carried on in two days according to kits' manufacture instructions (Dako, CO. and Novus Biolobius, Co.).

RESULTS

RESULT OF r-RT-PCR DETECTION

Detection of Vp1 gene of FMD virus using 3D primer and probe by r.RT-PCR revealed 9

samples out of 21 samples were positive (Figure 1).

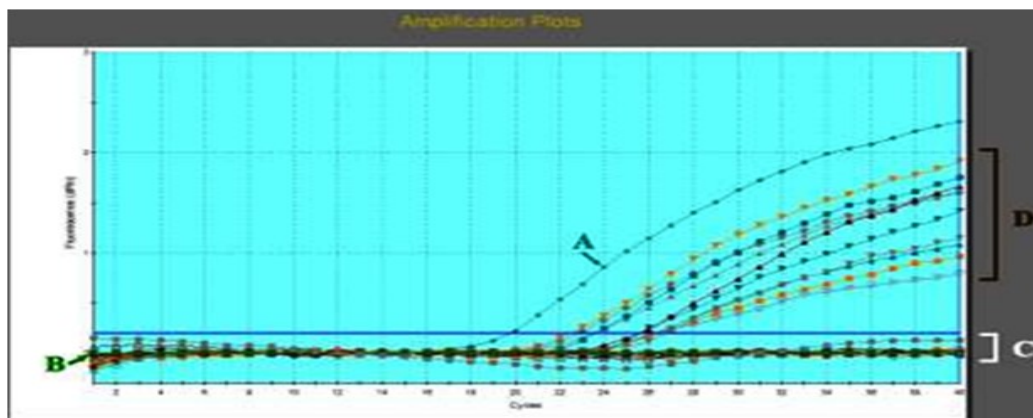


Figure 1. Amplification plot of Vp1 gene of FMD virus using 3D primer and probe by r.RT-PCR. A positive control, B negative control, C negative samples and D positive samples

Detection of serotype in these 9 samples using primer and probe to type O pan Asia , O EA, A and Sat2 by r.RT-PCR revealed that all 9 samples are type O EA (figure2).

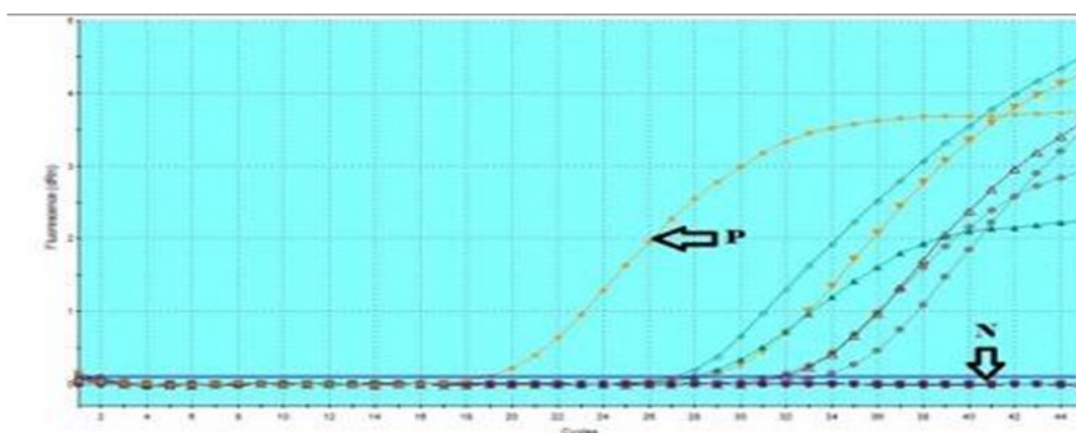


Figure 2. Amplification plot of Vp1 gene of FMD virus using primer and probe specified to FMDV serotype O EA by r.RT-PCR. P positive control, N negative control and A positive samples

VIRUS ISOLATION: 9 positive samples of FMDV detected by r. RT-PCR were inoculated in BHK 21 cell culture for three successive blind passages. Positive samples were successfully isolated by showing clear cytopathic effect in the form of cell rounding and aggregation.

VIRUS NEUTRALISATION TEST:

The isolated virus neutralizes the FMD serotype bovine O reference antisera and the 2Log antibody titre was calculated as

$10^{2.1}$ dilution of the reference sera.

PATHOLOGY RESULT POSTMORTUM EXAMINATION:

Lung showed thickening of interlobular septum with highly congested lung tissue. The pulmonary pelura showed marked thickening with greyish white colour. **Tongue** showed absence of macroscopic lesions but found microscopic vesicles that agree with **Yilma, (1980)**. **Heart** showed whitish stripes (Tiger heart) appearances well as soft flaccid heart could be

seen.

Kidney showed congestion and haemorrhagic foci especially in the renal medulla.

Spleen appeared haemorrhagic, congested and edematous.

Liver showed some haemorrhagic foci as well as congestion of most hepatic tissue and distended gall bladder.

HISTOPATHOLOGICAL RESULTS

Lung: Lung is showing thickening in interalveolar septae with emphysematous multiple alveoli as well as marked thickening of the pelura with accumulation of serofibrinous exudates in the subpelural space. Fig. (1&5) Lung is showing sever congestion in the inter alveolar capillaries accompanied by accumulation of sero-fibrinous exudates in most alveolar spaces. Fig. (2) and in other alveolar lumen were filling by serofibrinous exudates and mononuclear inflammatory cell infiltrations. Fig.(4). Lung bronchi is showing severe degeneration and sloughing of the bronchial lining epithelium as well as severe mononuclear inflammatory cellinfiltrations in the bronchial wall. Smooth muscle cell layer is showing severe degenerative and necrotic changes with severe mononuclear cell infiltrations. Fig.(3).

Tongue: Tongue is showing multiple extended protrusions of hyperplasic proliferation of stratified squamous epithelium through submucosa Fig.(6&7). Multiple foci of vesicular degeneration though the stratified squamous epithelium especiaaly in stratum spinosum layer which filled with seroedematous fluid and multiple mononuclear inflammatory cells and some neutrophiles. Fig.(8&9) Congestion in submucosal blood vessels with infiltratededema and perivascular cuff with mononuclear inflammatory cells. Fig.(10).

Heart: Heart is showing sever zenkers necrosis in cardiac myocytes with sever mononuclear inflammatory cells infiltration and marked edema between cardiac myocytes which given the tiger heart appearance. Fig. (11&12) severe necrotizing myocarditis appear with marked disorganization of cardiac muscle fibers. Fig.(13)

Kidney: Kidney showing marked perivascular cuff off mononuclear inflammatory cells which extended to replace necrotic area of re-

nal tubules. Swollen endothelial cells lining cortical blood vessels appear with some degenerative changes of tunica media. The cortical glomeruli showing widening of bowman's space. Most renal tubules showing degenerative changes of its epithelial lining accompanied by esinophilic renal casts in the renal tubular lumens. Fig.(14&15). Swollen endothelial cells lining large corticomedullary blood vessels appear with degenerative changes of tunica media with marked edema infiltrated tunica media and surrounding those blood vessels . Fig (16)Renal medulla showing erythrocytic cast inside lumen of renal tubules. Fig.(17).

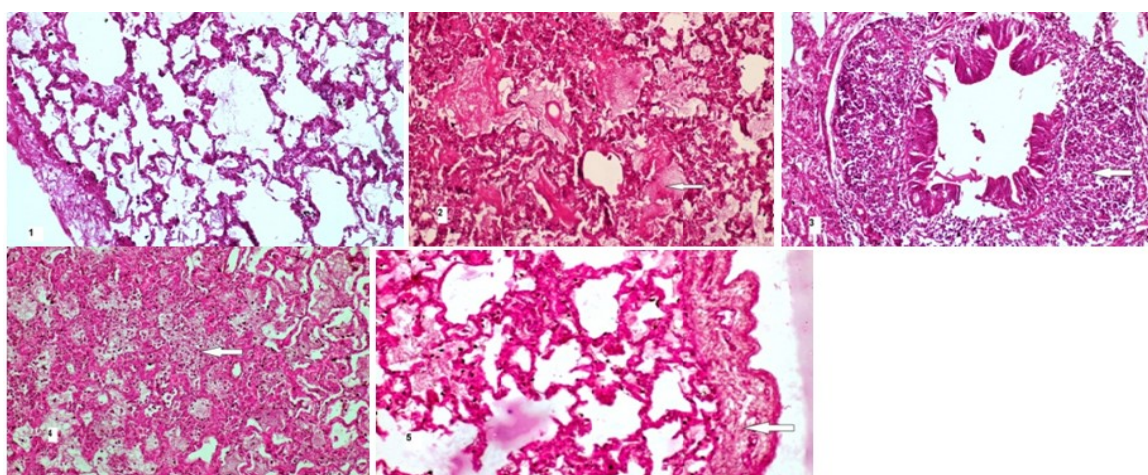
Spleen: Spleen showing severe depletion in white bulb as well as swollen endothelial cells lining splenic small blood vessels. Fig.(18&21) as well as marked swelling of endothelial cells lining splenic arteriole with marked hyaline degeneration of tunica media which infiltrated by mononuclear inflammatory cells. Some haemosidrosis could be seen in red bulb. Fig.(19). Large splenic artery showing severe degenerative changes in its endothelial lining which lead to thrombosis. Coagulative necrosis in tunica media as well as marked degenerative changes in tunica adventitia accompanied by marked dispersed edema through tunica adventitia could be detected. Fig.(20)Marked dilated and congested splenic sinusoids could be seen. Fig.(21)

Liver: Liver showed destruction of hepatic cords accompanied by congestion of portal hepatic blood vessels and highly dilated and congested hepatic sinusoids as well as degenerative and necrobiotic changes of many hepatocytes. Fig.(22) Shrunken esinophilic remnant of hepatocytes that undergone apoptosis (Councilman body) could be seen. Portal area showing some infiltration of mononuclear inflammatory cells. Fig.(23)Liver showed marked fibrosis of portal area. Fig.(24)

IMMUNE HISTOCHEMISTRY

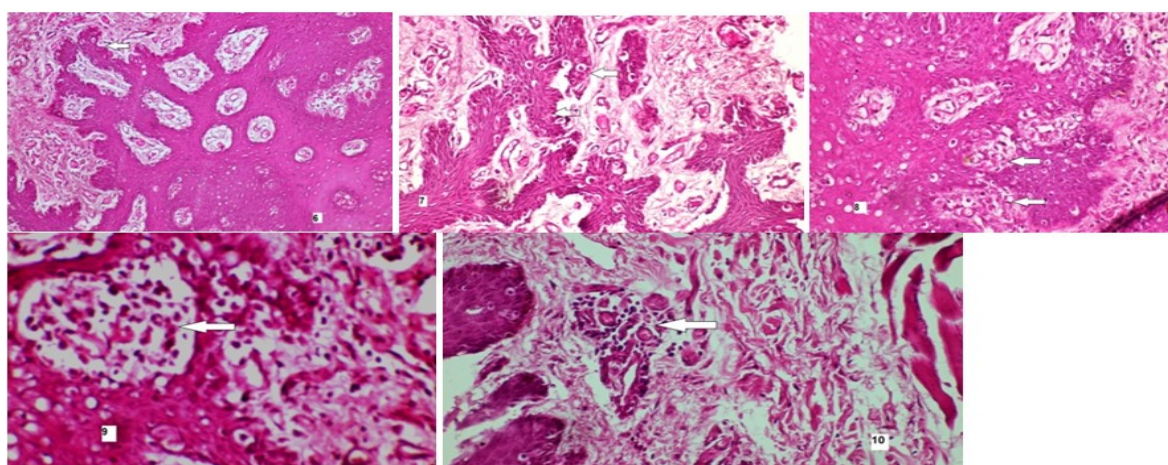
Marked strong immune-positivity reaction in basal cell layer and in the prickle cell layer especially around formed vesicles caused by effect of viral degeneration.(**Immunoperoxidase Counter Stain Mayer's haematoxylene**) Fig. (25 & 26 & 27)

lung



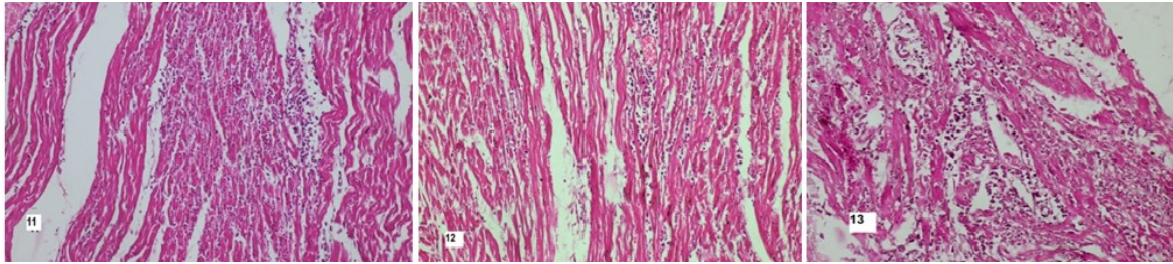
Figs(1-5): Lung is showing (1, 5) thickening in interalveolar septae with emphysematous multiple alveoli. as well as thickening of the pelura with accumulation of serofibrinous exudates in the subpelural space. (arow) (2) Sever congestion of interalveolar capillaries and accumulation of serofibrinous exudate in most alveolar spaces. (arow) (3) Severe degeneration and sloughing of the broncheal lining epithelium as well as severe mononuclear inflammatory cell infiltration in the broncheal wall. (arow) Smooth muscle cell layer is showing severe degenerative and necrotic changes with severe mononuclear cell infiltration. (4) congestion of interalveolar blood capillaries accompanied by filling of alveolar lumen by serofibrinous exudate and mononuclear inflammatory cells. (arow). H&E 1, 2, 4, 5 X 200 and 3 X 400.

Tongue



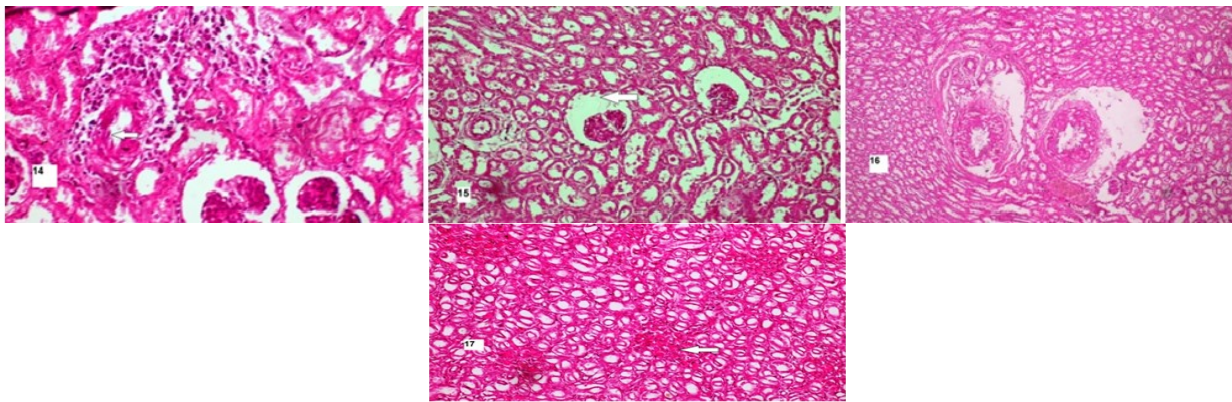
Figs(6-10): Tongue is showing (6,7) multiple foci of vesicular degeneration in tongue mucosa with protrusions of hyperplastic stratified squamous epithelium through submucosa. (arow) (8,9) multiple foci of tongue mucosa filled with serous exudate ,mononuclear inflammatory cells and some neutrophils. (arow) (10) submucosal blood vessels showing congestion and odema with perivascular cuff of mononuclear inflammatory cells . (arow). H&E 6 X 200, 7,8,10 X 400 and 9 X 600.

Heart



Figs (11-13): Heart is showing (11,12) severe Zenker's necrosis and edema between cardiac myocytes with severe mononuclear inflammatory cells infiltration. (13) marked disorganization of cardiac muscle fibers. H&E 11-13 X 400.

Kidney



Figs(14-17): Kidney showing Swollen endothelial cells lining blood vessel with degenerative changes of tunica media (14,15,16) with marked edema and infiltrated tunica media and surrounding those blood vessels (16). Kidney showing marked perivascular cuff off mononuclear inflammatory cells which extended to replace necrotic area of renal tubules (14). The glomeruli showing widening of Bowman's space (arrow) and atrophy of glomerular tuft. Renal tubules showing degenerative changes accompanied by eosinophilic casts. (15) Renal medulla showing erythrocytic cast (arrow) inside lumen of renal tubules (17). H&E 14 X 400, 15, 17 X 200 and 16 X 100.

Spleen

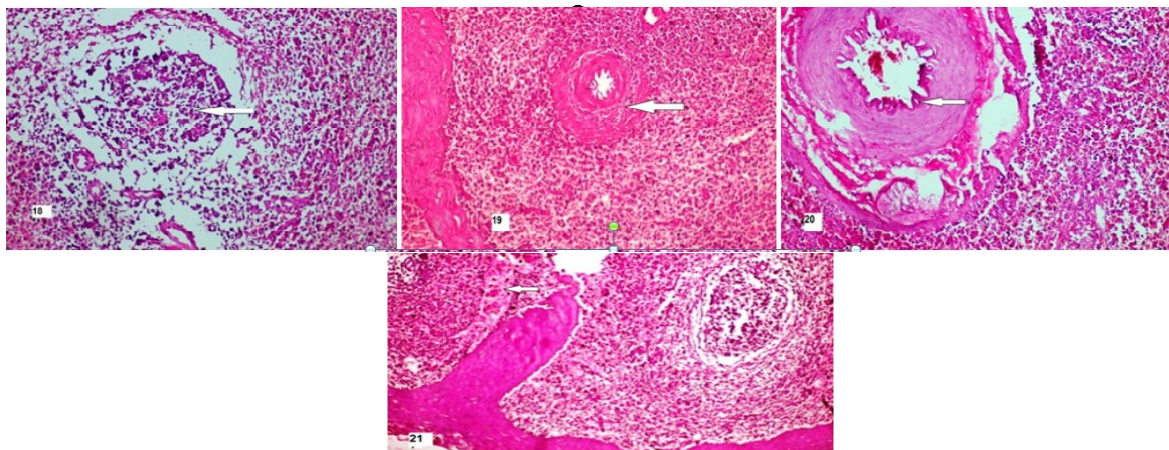
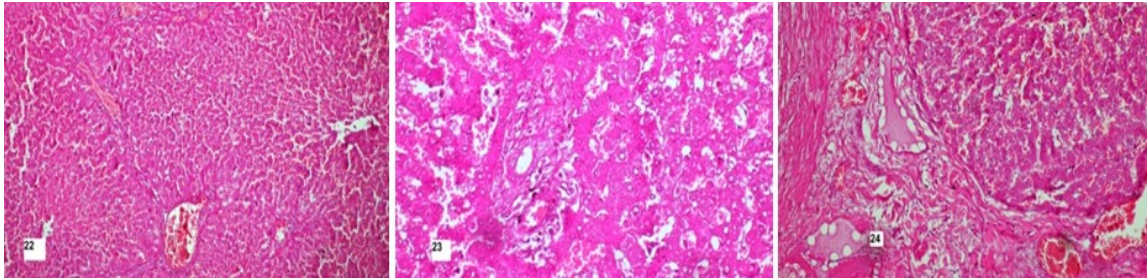


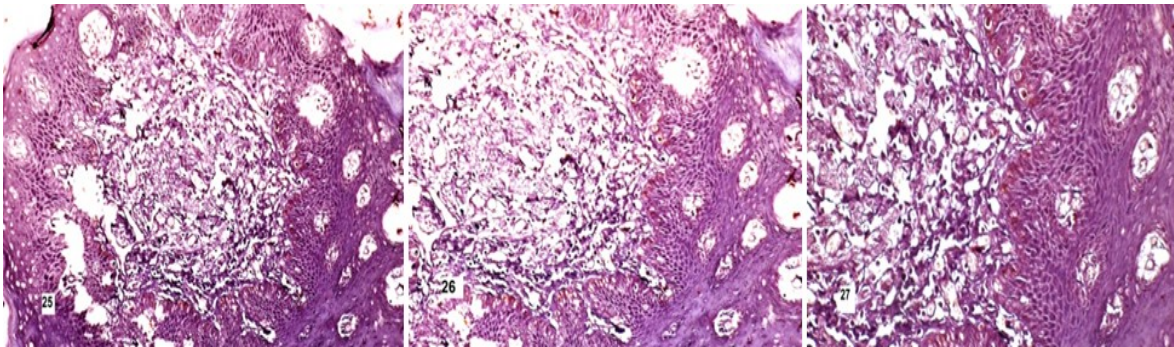
Fig.(18-21) Spleen showing (18) severe depletion in white pulp and swollen endothelial cells lining splenic blood vessels. (19) Swelling of splenic arteriole, degeneration of tunica media and hemosiderosis in red pulp. (20) Large splenic artery showing severe degenerative changes in its endothelial lining accompanied by coagulative necrosis in tunica media as well as marked degenerative changes in tunica adventitia accompanied by marked dispersed edema through tunica adventitia. (21) Splenic sinusoids dilated and congested. H&E 18 X 400 and 19, 20, 21 X 200.

Liver



Figs(22-24) Liver showing (22) destruction of hepatic cords accompanied by congestion of portal blood vessels and hepatic sinusoids . (23)Degenretive and necrobiotic changes of many hepatocytys. Shrunken esinophilicremenant of hepatocytys that undergone apoptosis (Councilman body).(24) marked fibrosis of portal area.
H&E 22X100,23X 400 and 24X200

Immune Histochemistry



Figs (25-27):marked strong immunopositivity reaction in basal cell layer and in the prickle cell layer specially around formed vesicles caused by effect of viral degeneration (Immunoperoxidase Counter Stain Mayer's haematoxylyene 25X200, 26X400 and 27X600.

DISCUSSION

Foot-and-mouth disease virus (FMDV) is one of the most dangerous viruses of ruminants and the most economically important veterinary pathogen due to its highly infectious nature, ability to cause persistent infections and long term effects on the condition and productivity of many animal species (**Knowles and Samuel 2003**). It still remains mysterious due to its diverse nature and antigenic variability (**Chakraborty et al. 2013**). So accurate and sensitive method for FMDV detection needs. Real time RT-PCR is highly sensitive and more rapid technique (**Andrew et al. 2007**) for detection and identification of FMDV. VP1 is one of the capsid protein which display epitope (s) on the virus surface to the host's immune system. VP1 is widely accepted as being the most antigenically significant of the capsid proteins and its sequence can be used to identify the serotype of FMDV (**Reid et al. 2014**). In

our study,r-RT-PCR can detect FMDV VP1 gene in 9 samples out of 21 using a universal primers and probe that can detect all FMDV serotypes (3D primers and probe) **Callahan et al. 2002**. Using different primers and probes in r-RT-PCR designed from vp1 gene that can detect different serotypes revealed that primer and probe designed to serotype O /EA-3 can detect VP1 gene in all 9 samples. In contrast, primers and probes designed to detect serotypes O pan Asia, A and Sat2 cannot detect vp1 gene in the 9 samples (**Reid et al. 2014**).

Virus isolation is the most reliable diagnostic method, but it is labour- intensive, time consuming and requires properly equipped facilities (**Reid et al. 2002**). Sometimes field viruses may require several passages before they are adapted for the cell culture and must follow by an immunoassay for identification of viral antigen (**Kitching et al. 1989**). BHK-21 is the

more suitable and sensitive cells for FMDV isolation (**OIE 2021**). In our study, FMDV isolation was done through inoculation of 9 positive samples for FMDV which previously detected by r-RT-PCR in BHK21 cell culture for three blind passages. Clear cytopathic effects in the form of cell rounding and aggregation and this agreed with (**Reid et al. 2002**). The clear CPE appeared within 48 hr (**Sahar et al. 2011 and OIE 2021**). All samples were showed clear cytopathic effect (**Clarke and Spire 1980, Nahed et al. 2011**).

neutralization test is a prescribed test for import /export certification of animals/ animal products (**EFSA 2012, Rudreshappa et al. 2012**). VN test in this study confirm the isolation of FMDV serotype O. The disease is diagnosed based on clinical science, laboratory based diagnosis with serotypes and pathological changes.

The histopathological examination of lung revealed the same by **Ranjan et al. 2016** which found the main viral replication in the pulmonary alveolar septae, as well as tongue is showing multiple foci of vesicular degeneration though the stratum spinosum of epithelial layers lining the tongue mucosa that agree with **Alexandersen et al 2003** with multiple extended protrusions of hyperplastic proliferation of stratified squamous epithelium through submucosa **Feng et al. 2018** has found that Nucleoside diphosphate kinase 1 (NME1) is well-known as a tumor suppressor that regulates p53 function to prevent cancer metastasis and progression. NME1 suppresses viral replication in FMDV-infected cells. as so p53 where not under regulation which explain the extended protrusions of hyperplastic proliferation of stratified squamous epithelium.

Tongue is showed multiple foci of vesicular degeneration though the stratified squamous epithelium especially in stratum spinosum layer which filled with seroedematous fluid and multiple mononuclear inflammatory cells and some neutrophils. That micro vesicles not appeared on the tongue by macroscopic examination, which could be explained by **Yilma, 1980**. Heart is showing severe Zenker's necrosis in cardiac myocytes with severe mononuclear inflam-

matory cells infiltration and marked edema between cardiac myocytes which given the tiger heart appearance that explained by **Nazeen et al. 2016**. Severe necrotizing myocarditis appear with marked disorganization of cardiac muscle fibers. That agree with **Shimshony et al. 1986**. High Fatality Syndrome were due to severe myocardial necrosis **Rhyan et al. 2006**.

Kidney showed marked perivascular cuff off mononuclear inflammatory cells which extended to replace necrotic area of renal tubules. Swollen endothelial cells lining cortical blood vessels appear with some degenerative changes of tunica media. The cortical glomeruli showing widening of Bowman's space. Most renal tubules showing degenerative changes of its epithelial lining accompanied by eosinophilic renal casts in the renal tubular lumens. Renal medulla showing erythrocytic cast inside lumen of renal tubules. Most renal tubules showing degenerative changes in its lining epithelium. Those histopathological changes in kidney come in agree with **EL-Amir et al. 2014**.

Spleen showed severe depletion in white pulp which coinciding with severe depletion of T cells in spleen founded by **Diaz-San Segundo et al. 2006** as well as swollen endothelial cells lining splenic small blood vessels. As well as marked swelling of endothelial cells lining splenic arteriole with marked hyaline degeneration of tunica media which infiltrated by mononuclear inflammatory cells. Some haemorrhage could be seen in red pulp. Large splenic artery showing severe degenerative changes in its endothelial lining which mainly lead to thrombosis which agree with **Hynes 2002** Coagulative necrosis in tunica media as well as marked degenerative changes in tunica adventitia accompanied by marked dispersed edema through tunica adventitia could be detected.

Liver showed destruction of hepatic cords accompanied by congestion of portal hepatic blood vessels and highly dilated and congested hepatic sinusoids as well as degenerative and necrotic changes of many hepatocytes. Shrunken eosinophilic remnant of hepatocytes that undergone apoptosis (Councilman body) could be seen. Portal area showing some infil-

teration of mononuclear inflammatory cells. Liver showed severe congestion of portal hepatic blood vessels accompanied by as well as marked fibrosis of portal area. That could be agree with **Zhang and Alexandersen 2004**.

Monaghan et al. 2005 Brown et al. 2006 O'Donnell et al. 2009 detected that epithelial sites that are not FMDV permissive that were found in close association with blood vessels in various tissues that explain the histopathological changes in blood vessels in most organs. Immune Histochemistry: marked strong immunopositivity reaction in basal cell layer and in the prickle cell layer specially around formed vesicles caused by effect of viral degeneration (Immunoperoxidase Counter Stain Mayer's haematoxyline) that explained by **Arzt et al. 2009 and Pacheco et al. 2009**. They found the highly active virions of FMD virus concentrated in epithelial cells around the periphery of degenerated necrotizing vesicles. **Zhang and Alexandersen 2004**. found that the load of FMD viral RNA was in tongue tissue greater 1000 times than the load of FMD RNA in other tissues. That explains the height positive reaction of immunohistochemistry on the tongue.

Genetic susceptibility or resistance to FMDV in large animals. Infection of susceptible mouse strains can lead to a lethal systemic infection in adults, with virus replicating in all major organs, including the heart, lung, kidney, liver and spleen that explained by **Salguero et al. 2005 and Sanz-Ramos et al. 2008** that agree with the severs histopathological changes in our research.

Buffalo were considered as carrier for FMD viral particles in pharyngeal region for more than 5 years (**Arzt et al., 2011**).

In conclusion, the study demonstrates that real-time RT-PCR currently used for FMD provides an extremely sensitive and rapid additional procedure for improved laboratory diagnosis of FMD. The RT-PCR generated results in less than one day from test commencement, in contrast to up to four days to define some positive and all negative samples by combined use of classical method for virus isolation. This is an important feature when

definitive diagnostic results are required in a short timescale during emergencies. Study demonstrates that the most circulating FMDV in Qalyobia Governorate from serotype O-EA-3. Continuous surveillance, at molecular and immunological levels, of FMDV serotypes is needed for the effectiveness of any adopted control strategy targeting FMD including vaccination. From histopathological changes in tongue mucosa of buffalo revealed that FMDV is an oncogenic virus that need more research about the severity of FMD-serotype O-EA3 infection in buffaloe and the current vaccine effectiveness.

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