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Investigation of the Pathological and Biochemical Characterizations in Naturally Infected Calves with Foot and Mouth Disease (FMD)

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Abstract

All cloven-hoofed animals are susceptible to foot-and-mouth disease (FMD) which is highly contagious viral illness. FMD is answerable for serious economic losses in Egypt. Despite the primary control approach being annual mass vaccination campaigns using polyvalent inactivated vaccinations, failure of vaccination has been according in several cases. The study was conducted on fifty native breed calves up to one year of age from both sexes, thirty calves suspected to be infected with FMD, and twenty clinically healthy calves were considered as controls. A total of fifty samples from organs (heart- epithelial tissue) were gathered from calves suspected to be FMD infected obtained from September 2021 to March 2022 in Sharkia and Menofeia governorates/Egypt. The current study was designed for the isolation of FMDV using BHK-21 cells. Molecular identification, through the extraction of Viral RNA, and RT-PCR were used to test samples for the FMDV virus. Diseased animals have changes in body temperature, respiration rate, and heart rate compared to controls. Moreover, murmur sounds were observed during auscultation of the heart. A hematological study revealed significant reductions in the RBCs count, hemoglobin concentration, and PCV% with leukopenia and lymphopenia in the diseased group. The serum cardiac troponin, lipase, non-esterified fatty acid, beta hydroxyl butyric acid, glucose, AST, ALT activities, and blood urea nitrogen were considerably enhanced in diseased animals. But serum insulin and amylase were significantly reduced in diseased calves. Histopathological examination of calves revealed extensive lymph histiocytic myocarditis and necrotic lesions in the pancreas, liver, and kidney. In conclusion, the early stages of FMD in calves is characterized by myocardial cell injury, elevation of blood cardiac troponin, and necrotic pancreatitis represented by atrophy of pancreatic glands and islets of Langerhans.

KEYWORDS
Foot and mouth disease, BHK; RT-PCR, Troponin, Myocarditis, Pancreatitis

INTRODUCTION

FMD is seen in both domestic and wild animals with cloven hooves as one of the most prevalent contagious transboundary animal diseases, it has a negative impact on livestock productivity and results in economic losses. (OIE, 2021). FMDV belongs to the Picornaviridae family of the genus Aphthovirus. The virus is tiny, non-enveloped, positive sense, single-stranded, and non-segmented RNA, and it is encased in an icosahedral capsid that contains roughly 60 copies of four structural viral proteins (VP1, VP2, VP3, and VP4 (Domingo *et al*., 2002). There are seven immunologically different serotypes of FMDV: SAT-1, SAT-2, SAT-3, A, O, C, and Asia. (Jamal and Belsham, 2013). Due to the endemic nature of FMD viruses as well as the introduction of exotic viral strains from the Middle East and Sub-Saharan Africa, the epidemiology of FMD in Egypt is complicated (Tekleghiorghis *et al*., 2016). An important diagnostic method for the diagnosis of FMDV is reverse transcription polymerase chain reaction (RT-PCR), which is characterized by High sensitivity and specificity characteristics. (Alsaad *et al*., 2020). Fever, appetite loss, lameness, and vesicular lesions of the foot, tongue, and teats are some of the clinical indications of FMD. The mortality rate is about 5 % in adult ruminants, but the rate may be boosted up to 50 % by heart muscle damage in young animals (Barker *et al*., 1993). In calves, myocardial inflammation is regarded as a fatal variant of FMD that does not produce the typical blister lesions seen in mature cattle. (Barker *et al*., 1993; Alexandersen *et al*., 2003). The acute myocarditis of young animals is distinguished by hyaline degeneration, necrosis of muscle fibers, and an intense infiltration mainly of lymphocytes (Alexendersen *et al*., 2003). The necrotic lesions in the heart make it incapable to pump out blood throughout the body leading to the development of a 'currant jelly clot' in the heart and its larger vasculature. Consolidated lungs were also found throughout the necropsy as described earlier (Alexandersen *et al*., 2003). Since auscultation of the chest and heart, and the incidence of sudden death in the field, may apocalypse for the clinical diagnosis of FMD, the use of some biomarkers for assessment of myocardial damage is also recommended. Diagnosis of myocardial disease in cattle is still difficult and could be extreme, based primarily on clinical exam-

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inations of the diseased animal. (Aslani *et al*., 2013). The diagnosis of FMD can be tested by hemato-biochemical, estimation of specific cardiac biomarkers like cardiac troponin-I, virus isolation, and identification (Aktas *et al*., 2015).

The primary goals of the current study are to identify recent FMD serotypes, assess the hematological and biochemical biomarker responses of local breed calves affected with myocarditis caused by FMD, and further evaluate the clinical and histopathological characteristics of afflicted and autopsied animals.

MATERIALS AND METHODS

Ethical approval

This study was declared by the Local Committee of the (ARC-IACUC) committee Institute: Animal Health Research Institute Ethical Committee Approval Number: ARC/AH/22/10. All methods were done in accordance with the Animal Health Research Institute guidelines according to the OIE standards for use of animals in research and education.

Animals

Fifty native Egyptian calves up to one year of age were included in the study. During a disease epidemic in the Sharkia and Menofeia Governorates of Egypt, the calves were separated into 20 seemingly healthy control calves and 30 calves who had been clinically identified as having FMD infection from various farms. According to the methodology of Smith (2015), the calves were clinically checked for pulse rate, temperature, respiratory rate, and ruminal movement by auscultation to identify heart sounds and arrhythmias.

Sampling

Thirty samples were taken from calves exhibiting FMD clinical symptoms. Epithelial tissue from a recently ruptured or an unruptured tongue vesicle was used as samples, and they were placed in a transport medium made of phosphate-buffered saline (PBS) (OIE, 2012). From September 2021 to March 2022, samples from clinically diagnosed cattle were taken in the Egyptian governorates of Sharkia and Menofeia.

Viral Identification

Virus Isolation

Baby hamster kidney-21 (BHK-21) cells were injected with prepared samples the supernatants. When cytopathic effects (CPE) manifested in roughly 80% of the monolayer, inoculated cells were collected (about three days post inoculation). It was performed according to Barry *et al*. (2009) and Ferris *et al*. (2009).

Reference FMD Virus was obtained from the Virology Department, Animal Health Research Institute, Egypt, that was used as a positive control for PCR.

Polymerase chain reaction was conducted in Animal Health Research Institute, Biotechnology unit, and reference lab for veterinary quality control (OIE, 2012).

Nucleic acid extraction from samples was conducted using the QIAampminielute virus spin kit (Qiagen, Germany, GmbH).

Oligonucleotide Primers were developed from the gene for VP protein with the following sequences:

Forward primer 5'- GCCTGGTCTTTCCAG GTCT -3'

Reverse primer 5'- CCAGTCCCCTTCTCAGATC -3'. The amplicon size of the PCR product is 326 bp. It was manufactured in the laboratories of Metabion- Germany according to OIE (2012).

Analysis of the PCR Products

The PCR products were separated on 1.5% agarose gel by electrophoresis. The fragment sizes were calculated using DNA ladders from Generuler 100bp. A gel documentation system (Alpha Innotech, Biometra) took pictures of the gel, and computer software was used to analyze the information.

Blood samples

By puncturing the jugular vein, ten ml of blood were drawn from the calves, and one ml of the blood was transferred into vacuum EDTA-coated tubes for hematological analysis. The remaining blood was drawn without using an anticoagulant and allowed to coagulate for the purpose of isolating the serum. Centrifugation at 3000 rpm for 10 minutes was used to obtain serum samples, which were then stored at 20°C until analysis.

The hematological studies

Blood samples with anticoagulants were subjected to detection of cellular blood constituents according to Feldman *et al*. (2000).

Biochemical studies

Using third-generation cardiac troponin T (cTnT) produced by Roche Diagnostics, Indianapolis, GMBH, Germany, the amount of cTnT was quantitatively measured using electro-chemiluminescence technology. The Card-I-kit Combo Test was used to evaluate the level of cTnI in blood samples (AboaTech, Turku, Finland). Insulin level was detected according to Chevenne *et al*. (1998), amylase enzyme according to Winn-Deen *et al*. (2003), and lipase according to Tietz and Fiereck (1966). Serum glucose was determined according to the method described by Lott and Turner (1975). Non-esterified fatty acid (NEFA) according to Smith and Wilson (2006), and Beta hydroxyl butyric acid (BHBA) according to Young (2000). Serum liver enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated according to Murray (1984). The BUN was determined according to Patton and Crouch (1977) and the serum creatinine was estimated according to Henry (1974).

Histopathological examination

Specimens from lesions representative of FMD from freshly died calves (heart, pancreas, liver, and kidney) were fixed in 10% neutral formalin then histopathological examination; the samples were dehydrated and embedded in paraffin wax, then stained with hematoxylin and eosin and examined (Suvarna *et al*., 2013).

Statistical analysis

According to Tamhane and Dunlop (2000), the statistical analysis of the data from this study was done using the T-test.

RESULTS

Clinical findings

Depending on the calves' ages, the clinical symptoms varied

somewhat. Anorexia, cardiac arrhythmia, fever that occasionally reached 41°C, and the unexpected death of some animals without any prior clinical symptoms. The sick calves exhibit indications of lethargy, inactivity, panting while mouth breathing, being unable to suckle, and recumbent. Sever salivation with vesicles, erosions, and ulcers at different sites of the buccal cavities and inter-digital spaces of the hoof. 30 diseased calves showed high temperature (>40 °C), tachypnea (>50 breath / min), tachycardia (> 100 beats/ min), murmur sounds.19 diseased calves showed oral lesions. 7 diseased calves showed foot lesion. 4 diseased calves showed both oral and foot lesions. This was compared to 20 control calves that are free from previous symptoms.

Tissue culture

Propagation of suspected samples on the BHK-21 cell line resulted in showing characteristic cytopathic effect (CPE) for three successive passages 24-48 h post-inoculation (Fig. 1).

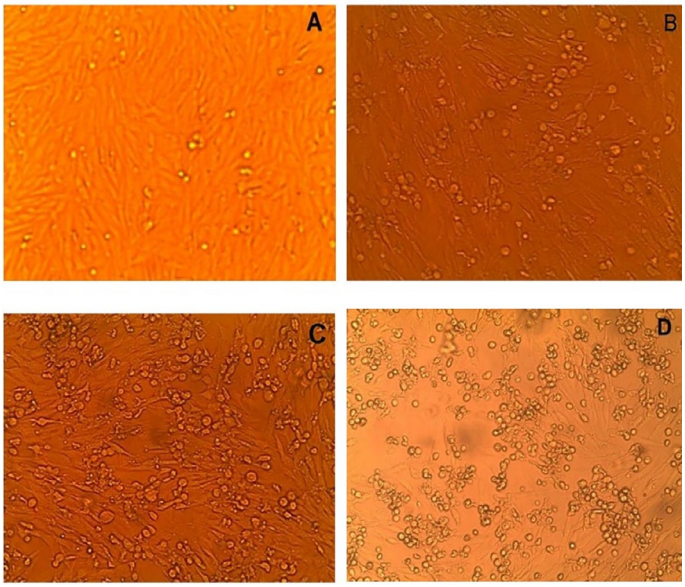

Fig. 1. (A) Normal control BHK-21 cell line. (B) FMD infected BHK-21 cell (stage 1: initiation of infection and cell rounding, and flattening started, photo taken after 12 hours). (C) FMD infected BHK-21 cell (stage 2, almost 100% cell infected, photo taken after 24 hours of infection). (D) FMD infected BHK-21 cell (stage 3) (D).

Molecular characterization of FMDV by PCR

FMDV samples (n.=10) tested for the presence of VP1 (target gene) at expected size: 326 (Fig. 2).

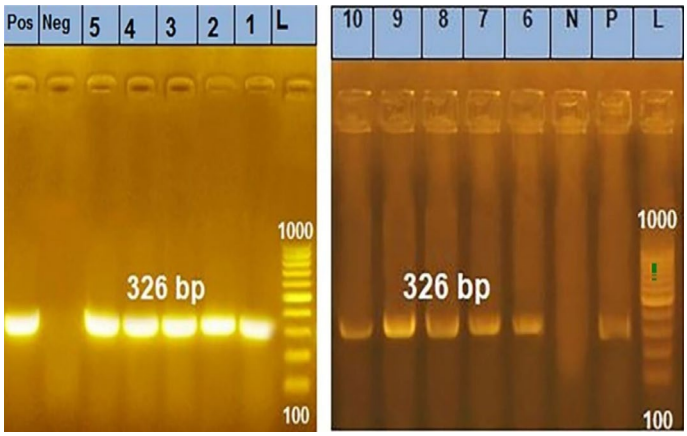

Fig. 2. Detection of FMDV by PCR (agarose gel electrophoresis of the PCR products) The specific primers set amplified a DNA fragment of 326 bp equivalent to the expected amplification product (amplicon) size from FMDV. Lanes: (L) 100 bp DNA ladder. (Pos): positive PCR products from FMD virus reference strain. (Neg): negative control (no primers). Lanes (1, 2, 3,4,5,6, 7, 8, 9, 10): +ve samples.

Hematological findings

Diseased calves revealed significant decreases in RBCs count, Hb concentration and PCV% compared with the control group. Moreover, the leukogram of diseased calves revealed significant leukopenia and lymphopenia (Table 1).

Table 1. Hematological parameters (Mean values± S.E) of healthy control and diseased calves.

Parameters	Control calves (n=20)	Diseased calves (n=20)
RBCs (10^6/UL)	7.12±0.14	5.24±0.16***
Hb (gm/dl)	11.48±0.10	9.22±0.17***
PCV %	37.38±0.18	28.04±0.21**
TLC (10^3/UL)	8.66±0.19	7.09±0.23***
Neutrophil (10^3/UL)	3.82±0.41	3.68±0.38
Lymphocyte (10^3/UL)	4.28±0.25	2.89±0.18***
Eosinophil (10^3/UL)	0.15±0.005	0.13±0.005
Monocyte (10^3/UL)	0.41±0.02	0.39±0.02

*: Significant at P< 0.05; **: Significant at P <0.01; ***: Significant at P < 0.001

Biochemical analysis

The data showed significant increases in cardiac troponin, lipase enzyme, glucose, non-esterified fatty acid, beta hydroxyl butyric acid, AST, ALT activities, and BUN. On the other hand, serum insulin and amylase showed significant decreases in diseased calves compared with the healthy control group (Table 2).

Table 2. Serum biochemical parameters (Mean values± S.E) of healthy control and diseased calves.

Parameters	Control calves (n=20)	Diseased calves (n=20)
Cardiac troponin (µg/L)	0.24±0.03	14.80±0 .62***
Insulin (µLU/ml)	16.42±1.17	12.89±1.06**
Amylase (U/L)	27.26±1.15	21.46±1.20*
Lipase (U/L)	33.58±2.12	47.98±3.85**
Glucose (mg/dl)	70.2±3.63	94.15±4.60***
Non-esterified fatty acid (mmol/L)	0.56±0.05	1.44±0.22***
Beta hydroxyl butyric acid (mmol/L)	1.35±0.30	4.74±0.43***
AST (IU/L)	63.9±2.74	98.7± 3.62***
ALT (IU/L)	31.6±0.65	52.8±2.32***
Blood urea nitrogen (mg/dl)	22.5±0.40	27.3±0.51***
Creatinine (mg/dl)	1.72±0.007	1.74±0.008

*: Significant at P< 0.05; **: Significant at P <0.01; ***: Significant at P < 0.001

Post-mortem examination

The heart was forming white greyish streaks on the myocardial wall separated by congested areas. The gastrointestinal tract mucosa was hyperemic with vesicle formation (Fig. 3A) The pancreas was hyperemic and firm (Fig. 3B). The Liver, and kidneys were enlarged and pale.

Histopathological examination

The heart of calves showed characteristic coagulative necrosis with massive lympho-histiocytic infiltration beside a few neutrophils together with focal edema between cardiac myofibrils (Fig. 3C & D). Endothelial destruction, perivascular and intermus-

cular edema containing fibrin threads and leucocytic cells mainly lymphocytes and neutrophils (Fig. 3E). The pericardium infiltrated with a number of mononuclear inflammatory cells mixed with fibrin threads (Fig. 3F).

The pancreas showed interlobular edema in the septa with necrosis and atrophy of exocrine and endocrine pancreatic glands together with edematous fibrous tissues infiltrated by leucocytes around pancreatic ducts were noticed (Fig. 4A&B). Additionally, the pancreatic ducts showed metaplasia or desquamation to the epithelial lining with degenerated and hyalinization in its smooth muscle fibers besides necrosis of the adjacent pancreatic glands and lymphocytic infiltration (necrotic pancreatitis) (Fig.4C). Some islets of Langerhans showed hypocellularity and hyaline eosinophilic degenerative changes (Fig. 4D).

The liver showed focal hepatic coagulative necrosis and focal infiltration by mild inflammatory cells mainly lymphocytes and dilatation of the sinusoids (Fig. 4E). Th4e portal area showed hyperplasia of the biliary epithelium and the periductal interstitial congestion of the central (Fig. 4F). Later, the survival animals showed interstitial leucocytic aggregations among the hepatocytes.

The kidney showed cloudy swelling, hydropic degeneration, hypercellularity of some glomeruli with the absence of the glomerular spaces of bowman`s capsules, and endothelial hyperplasia (Fig. 4G). In advanced cases, the kidneys showed severe coagulative necrosis in some renal tubules and focal hemorrhage in the renal parenchyma (Fig. 4H). Some glomeruli revealed degenerative changes and shrinkage in some glomerular tufts.

DISCUSSION

In Egypt, despite the government's frequent vaccination of calves with the FMD vaccine, there are numerous FMD outbreaks in Egypt each year. Since FMD is endemic in Egypt and there have been numerous outbreaks involving serotypes O, A, and SAT-2 for a long time (Abd El-Rhman *et al*., 2021). Immunologically, the viral protein 1 (VP1) coding area of FMDV strains is in charge of protective immunity, serotyping specificity, cell virus attachment, and antigenic heterogeneity. The world reference laboratory has tested RT-PCR techniques using primers for FMD for routine FMD viral diagnosis. A potent method for the accurate detection of FMDV is RT-PCR (Paixão *et al*., 2008). When FMDV infects BHK-21 tissue culture, it typically causes significant cytopathology and cell death. (Herrera *et al*., 2008).

In this current study, unruptured or recently ruptured vesicles from the tongue of clinically suspicious calves were used as trials for isolating FMDV from epithelial tissue. Samples were injected into a confluent BHK-21 cell culture and developed of CPE. By 12 hours after infection, characteristic CPE had begun to manifest,

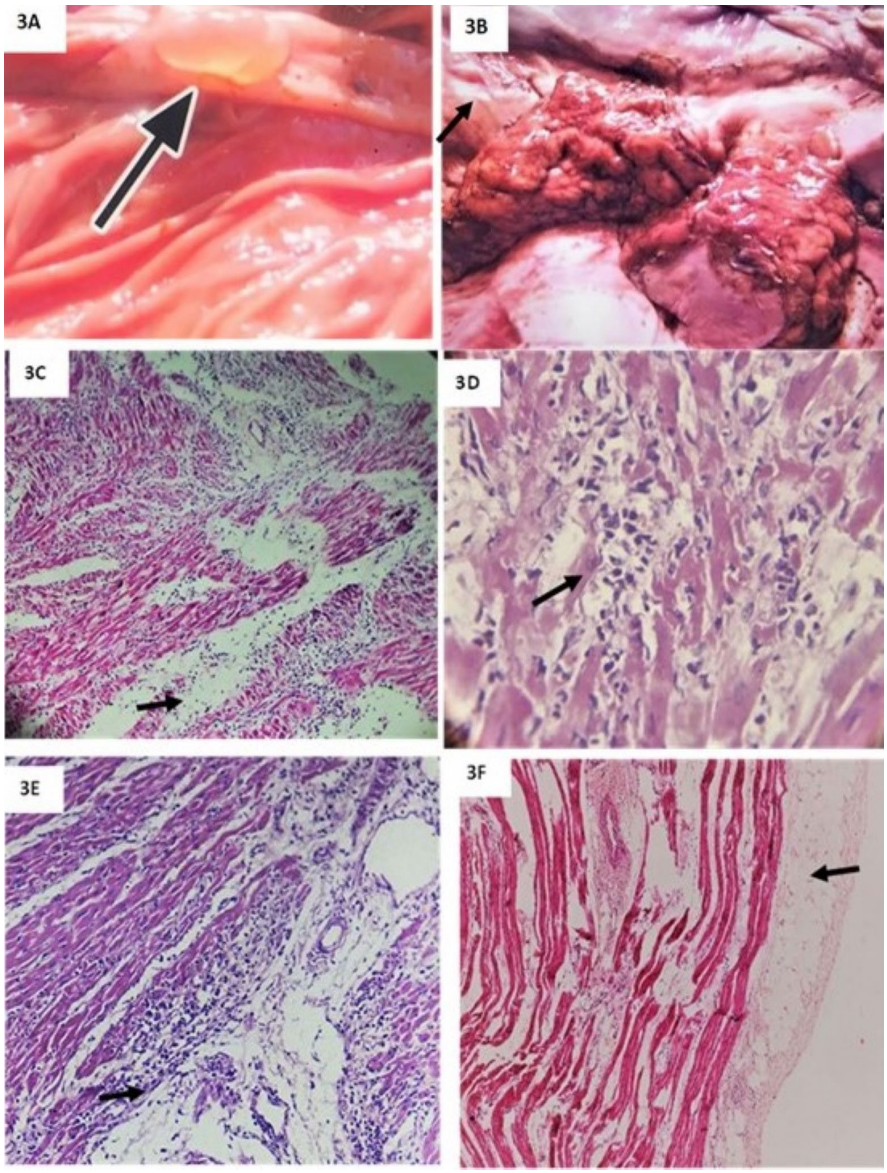

Fig. 3. (A) Vesicle formation on the reticulum surface. (B) The pancreas was hyperemic and firm. (C & D) Heart showing severe coagulative necrosis of the cardiac muscle fibers with massive lympho-histiocytic infiltration replaced the myocardial muscles and edema. (E)Heart showing Perivascular edema containing fibrin threads and leucocytic cells. (F) The pericardium infiltrated with many mononuclear inflammatory cells mixed with fibrin threads (arrows) (H&E X 100,200&400).

which comprised flattening and rounding cells, the production of multinucleated cells, the dissolution of intracellular bridges. Within 48 hours, more than 60% of cells had died, and by 72 hours, the cell monolayer had separated from the surface of the culture vessels.BHK-21 cell isolation of FMDV is a highly sensitive technique.

To provide further confirmation, Molecular (RT-PCR) diagnosis was used as quick detection and confirmatory test according to OIE (2012), RT-PCR was carried out by choosing viral gene VP1-based primers as a conserved region. The RNA was extracted from the samples The RT-PCR was conducted utilizing certain primer sets that resulted in an amplified fragment of 326 bp, which is equivalent to the FMDV amplicon size expected. The size of the attachment protein gene fragment was the same in the FMDV reference strain and the epithelial tissue test samples. Consequently, it was determined that 10 examined samples were FMD-positive.

Diseased animals in the current investigation had a variety of clinical symptoms previously documented by Alexendersen *et al*. (2003) and Alsaad *et al*., (2020). However, FMD symptoms and severity may differ between animal breeds, and even within breeds, most likely attributable to the virus's strain and serotype, the animals' genetic factors, or their immune systems (Alsaad *et al*., 2020). Clinical findings of fever raised heart and respiratory rates and murmur have conjointly been reportable in myocarditis because of FMD, as seen previously (Karapinar *et al*., 2010). These results therefore illustrate the clinical features of myocarditis-related circulatory insufficiency. The body temperature, pulse rate, respiration rate, and abnormal heart rhythm in auscultation without FMD vesicular lesions have all been described in suckling calves by Karapinar *et al*. (2010) as the major clinical changes in FMD-infected calves. There may or may not be concomitant vesicular lesions when FMDV myotropism develops. According to Arzt *et al*., (2011) the existence of myotropism and vesicles is referred to as enlarged multi-tropism (the muscle and the epithelium).

Hematological analysis of FMD-infected animals revealed a significant reduction in RBCs count, hemoglobin concentration, and PCV%. Similar findings were recorded by Ghanem and Abdel-Hamid (2010) and Gattani *et al*. (2011). Therefore, endocrinopathy that develops as a result of FMD infection may be to blame for the predominance of anemia (Gokce *et al*., 2004), or as a result of insufficient consumption of vitamin B12 and folic acid in the food, liver metabolism issues, and reduced absorption owing to intestinal damage (Nasr El-Deen *et al*., 2017). However, from our histopathological study, the liver and kidneys of infected animals suffered from degenerations and focal necrosis, as erythropoietin is a vital substance produced by the kidneys and responsible for the process of erythropoiesis, one may make a case for the main reason for anemia in FMD infected animals

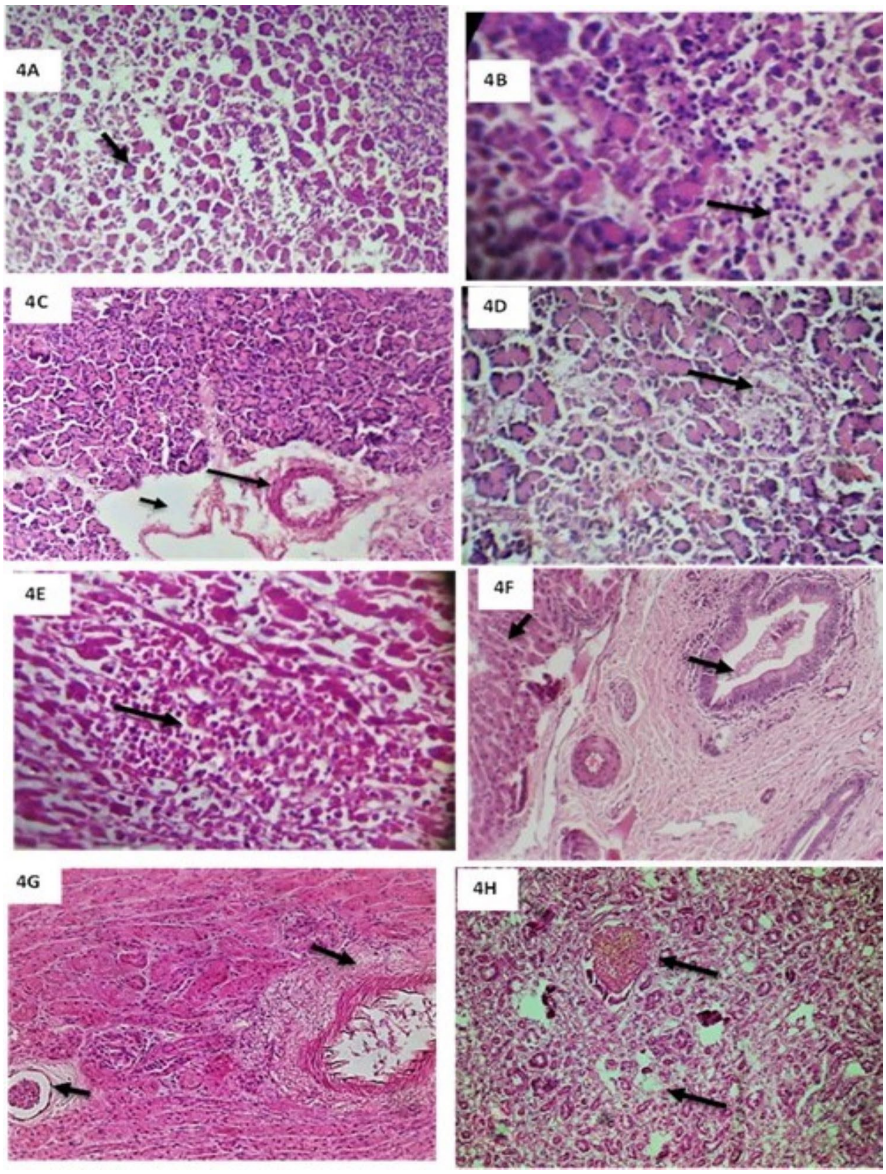

Fig. 4. (A & B) Pancreas showing interlobular edema in the septa with necrosis and atrophy of exocrine glands. (C) Interstitial edema, metaplasia of the epithelial lining with degenerated and hyalinization in its smooth muscle fibers of the pancreas. (D) Islets of Langerhans showing hypocellularity, necrosis of exocrine glands, and edema. (E) liver showing focal infiltration mononuclear cells mainly lymphocytes among the necrotic hepatocytes. (F) liver showing hyperplasia of the epithelial cells lining bile ducts and hepatocellular degenerative changes. (G) Kidney showing cloudy swelling, hydropic degeneration, and endothelial hyperplasia. (H) Kidney showing severe coagulative necrosis of the renal tubules, interstitial edema, and focal hemorrhage (H&E X100,200 and 400)

(Jubb *et al*., 1991).

Leucopenia caused by lymphopenia was found in FMDV infection (gp.2) cases. Such a drop in lymphocytic count may be caused by transitory immunosuppression brought on by FMDV infection of T and B cells during a brief period when infection hits the peak of viremia (Diaz and Sevilla, 2006). This transient immunosuppression promotes widespread viral dispersal and shedding into the environment (Parida *et al*., 2006). Moreover, Olabode *et al*. (2013) recorded a significant drop in total leukocyte count in blood samples taken within three days post foot and mouth disease infection.

Neutrophil, monocytic, and eosinophilic counts of sick groups showed no significant alterations. Similar results were additionally recorded by Krupakaran *et al*. (2009) and Ghanem and Abdel-Hamid (2010).

The earliest biochemical signs of heart muscle injury are serum cardiac troponins (Boccara *et al*., 2000). Because cTnI is 100% tissue-specific for the heart, it is a great tool for use as a biochemical marker for identifying damage to the heart muscle (Bodor *et al*., 1995).

According to Lim *et al*. (2005), the histologic finding of inflammation was a later sign of myocardial harm following viral infection than the blood cardiac troponin levels. The amount of inflammatory and degenerative alterations in the myocardium are likely reflected by the increase in serum cTnI, which is inversely correlated with disease severity.

The cardiac cell damage and degeneration are shown by the significantly substantial increases in cTnI levels in FMD-infected calves compared to control calves. This finding is supported by the work of Sobhy *et al*. (2018) and Aly *et al*. (2020) and is connected to FMDV's myotropism action, which causes cardiac insufficiency and myocardial damage, particularly in heart tissue (Sharma *et al*., 2004).

These results were supported by necrosis of the myocardium in the histopathologic section. The characteristic lesion of myocardial infarction on the heart has been typically observed in FMD virus infection mainly in young calves which could be the cause of sudden death and high mortalities. The Cardiac muscles of calves revealed myocardial necrosis and multifocal lymphohistiocytic infiltration beside a few neutrophils. These results have an agreement with Ali, *et al*. (2016) and Gab-Allah *et al*. (2018) who recorded multifocal lymph histiocytic myocarditis. In our study serum lipase enzyme levels, non-esterified fatty acids (NEFA), and beta hydroxyl butyric acid (BHBA) were considerably increased in diseased calves compared with the control group, our results were approved by González, *et al*. (2011) and Eman *et al*. (2018), the rise in (NEFA) could also because of increasing its mobilization from adipose tissues to blood (Baird, 1982), and later on, increase the level of lipase enzyme in infected calves. The rise in (BHBA) is due to excessive fat mobilization and accumulation of acetyl-coenzyme A which changed into acetoacetate and later on reduced to BHBA by BHBA dehydrogenase (Baird, 1982). In our study, there is a significant increase in the level of glucose and a significant decrease in insulin and amylase levels in the infected calves, our result is compatible with Nasr El-Deen *et al*. (2017) and Saadoon *et al*. (2019). High BHBA concentrations were accompanied by higher insulin resistance and higher NEFAs concentrations were related to a lower insulin secretion considerably (Kerestes *et al*., 2009). That would additionally explain the observed hypoinsulinemia in our present study. Hyperglycemia is because of the direct destruction of pancreatic -cells by FMD virus as a result of replication of the FMD virus within the pancreas which resulted in the destruction of insulin-producing cells (beta cells in the pancreas) and subsequently lead to hypoinsulinemia. These results were supported by necrosis of pancreatic cells as shown in the histopathology. The reduction in serum amylase enzyme level in the present study might be attributed to acute pancreatitis (Krehbiel *et al*, 1995) or as a result of hypoinsulinemia as stated by Patel *et al*. (2006).

According to the results of the current investigation, the serum biochemical examination of calves that had been clinically infected with FMD revealed a considerable rise in AST and ALT activities that might be caused by the virus's damaging effects on the myocardial muscles and hepatocytes, these findings agree with previous studies (Nath *et al*., 2014; Nasr El Deen *et al*., 2017). However, increased creatinine and BUN levels in diseased calves may be brought on by starvation, dehydration, fever, an increase in catabolism, and tissue injury, which all lead to pre-renal azotemia (Kaneko *et al*., 1997). Similar results were reported by Saravanan *et al*. (2020).

The microscopic examination of the pancreas showed necrotic pancreatitis, interlobular edema among the septa with necrosis, and atrophy of exocrine and endocrine pancreatic glands infiltrated by round cells (mainly lymphocytes) around pancreatic ducts. Some islets of Langerhans showed hyaline eosinophilic degeneration and hypocellular. The authors opinion considers these lesions in the pancreas need further studies to explore if it is a primary target organ for viral infection and replication. Arzt *et al*. (2011) recorded pancreatic atrophy in both exocrine and endocrine portions with edema and necrosis besides the absence of some acinar lumen. Also, the previous lesions were partially agreed with those obtained by Thomas (2002) who recorded pancreatic islets and acini exhibit degeneration in chronically infected cattle.

Examination of the liver revealed multi-focal hepatic coagulative necrosis and focal infiltration by mild inflammatory cells mainly lymphocytes and dilatation of the sinusoids. These reports have great accordance with the results of Brown *et al*. (1996) and Ali *et al*. (2016) who recorded centrilobular coagulative necrosis, fatty changes, and some portal area infiltrated by few lymphocytes. Moreover, the lesion in the hepatic capsule and parenchyma (fibrosis) is due to concomitant infection by other pathogens.

The kidneys in this study revealed severe coagulative necrosis in the renal tubules and focal hemorrhage in the renal parenchyma. Some glomeruli were degenerative changes and shrinkage of some glomerular tufts Gab-Allah *et al*. (2018) found severely congested blood vessels, necrosis of renal tubular, hyaline degeneration, and necrotic glomerular tufts. Also, Lubroth (2002) explained that the lesions in liver and kidneys are due to infarction of heart muscle causing focal necrosis and the presence of clostridial bacilli causing a secondary bacterial infection.

CONCLUSION

In conclusion, the findings of this study indicate that the cardiac form of FMD in young animals does not manifest vesicular lesions or pathognomic clinical symptoms. A highly sensitive approach for detecting myocardial cell injury in the early stages of the illness is the measurement of blood cardiac troponin. Necrotic pancreatitis, atrophy of pancreatic glands and islets of Langerhans were obviously need more investigation determined if these lesions might be one of the causes of mortalities due to hypoinsulinaemia. Myocarditis caused by FMDV is closely correlated with age and is more common in younger calves.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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