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RESEARCH ARTICLE

Pathological findings and distribution of viral antigen in the FMDV-induced hepatorenalopathy in naturally infected cattle calves

Monalisa Sahoo[1,2*], Rajendra Singh[1], Sagar Patel[1], Jigarji Chaturji Thakor[1], Tareni Das[1], Jyotrimayee Behera[2], Bhabajyoti Dash[2], Prabin Kumar Sahoo[2], Nihar Ranjan Sahoo[2], Ranjan Kumar Mohanty[2] and Rout George Kerry[3]

[1]Division of Pathology, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, [2]ICAR-National Institute on Foot and Mouth Disease (NIFMD), Arugul, Jatni, Bhubaneswar, Odisha, [3]Centre of Biotechnology, Siksha "O" Anusandhan, Ghatikia, Bhubaneswar, Odisha, India

Address for Correspondence

Monalisa Sahoo, ICAR-National Institute on Foot and Mouth Disease (NIFMD), Arugul, Jatni, Bhubaneswar, Odisha, India, E-mail: vety.lisa@gmail.com

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ABSTRACT

Foot and mouth disease (FMD), the "Risk Group 4" animal pathogen, is causing huge economic loss to the livestock owner due to high morbidity and mortality in young calves. Besides vesicular and cardiac lesions, limited studies are available regarding the FMDV induced hepatorenal dysfunction in the naturally infected cattle. Therefore, the present study was undertaken to investigate the hepatorenalopathy in calves naturally infected with FMDV. The serum from the ailing calves (n=12) were assayed for the estimation of hepatorenal function tests and tumour necrosis factor (TNF)-α. The liver and kidneys from the necropsied calves (n=28) were investigated for pathological, immunohistochemical and molecular investigation along with the virus induced apoptotic changes. The affected calves showed clinical signs of high fever, salivation, vesiculo-ulcerative lesion in the buccal mucosa and skin of hoof cleft. The clinically ailing calves showed elevated serum levels of enzymes in the liver and kidneys (alanine transaminase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen, urea, creatinine) and cytokine TNF-α. Post-mortem observations showed the classical lesions of acute necrotizing myocarditis, vesicular/ulceration lesions in the buccal mucosa and clefts of hooves, comparable with the FMD virus infection. In addition to that, variably enlarged livers with rounded borders, centrilobular haemorrhage/necrosis with lobulation, multifocal hepatitis and fibrosis along with congested/hemorrhagic and oedematous kidneys were observed in majority of the calves. Microscopically, the classical lesions of vesicular inflammatory oral lesions, acute necrotizing myocarditis and skeletal muscle necrosis (tongue) were prominent. In addition to that, liver showed prominent multifocal centrilobular necrosis and haemorrhage, surrounded by degenerated fat-laden hepatic cells (like hypoxic changes), multifocal periportal infiltration of mononuclear cells, variably bridging fibrosis and increased activity of Kupffer cells in the sinusoids. The kidneys showed vascular changes such as congestion, oedema and haemorrhages, vasculitis, glomerulitis, interstitial nephritis and tubular degeneration. The presence of viral antigens in the hepatocytes and kidney tubular epithelial cells by immunohistochemistry along with associated elevated serum enzymes support the role of FMDV, causing hepatorenal injury/dysfunction. The TdT-mediated dUTP nick-end labeling (TUNEL) assay confirmed that the death of the hepatocytes and tubular epithelial cells is due to apoptosis. The viral genome in both the liver and kidneys was confirmed to be type A FMDV in multiplex-PCR assay. These results provide insights into novel tissue tropisms in the liver and kidneys of young calves with natural FMDV infections. Therefore, therapeutic intervention should be directed to boost the liver and kidneys for better management of the infected cases.

Keywords: Apoptosis, calves, FMD, hepatorenal dysfunction, immunohistochemistry, MP-PCR, pathology, serotype-A, serum biochemical analyses

INTRODUCTION

Foot and Mouth disease (FMD) is an acute, highly contagious, transboundary viral disease of cloven-hoofed animals. It is caused by FMDV under the genus *Aphthovirus* belonging to family *Picornaviridae*. India is endemic to FMDV like other developing countries and suffers with a huge economic burden, which negatively impacts the livelihood of farmers due to high morbidity in adults, reduced production efficiency and heavy mortality in young and high-yielding crossbred animals[1]. The economic loss due to FMD in India is estimated to be USD 2,768 million for severe, USD 237 million for moderate and USD 133 million for mild outbreaks[2]. The recent report showed the financial loss per infected animal during the disease episode is estimated to be approximately Rs. 10,149.64, excluding other concurrent expenses among the dairy farmers of the highlands in Jammu and Kashmir during the FMD outbreak[3]. Out of 7 serotypes, 3 serotypes (O, A and

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Table 1. Biochemical changes (Mean ± SEM values) in naturally infected calves with FMD virus serotype A and control calves.

Parameters	FMDV infected calves	Control calves
Total protein (g/dl)	6.07 ± 0.23	8.11 ± 0.07
Albumin (g/dl)	3.75 ± 0.06	4.59 ± 0.16
Glucose (mg/dl)	98.4 ± 3.82	47.35 ± 1.65
ALT (U/L)	73.77 ± 2.94	59.19 ± 2.36
AST (U/L)	83.94 ± 1.47	47.28 ± 1.97
ALP (U/L)	119.61 ± 4.20	86.12 ± 2.72
BUN (mg/dl)	20.47 ± 0.49	18.91 ± 0.76
Urea (mg/dl)	51.89 ± 2.16	27.86 ± 1.89
Creatinine (mg/dl)	1.67 ± 0.05	1.53 ± 0.12

Highly significant at p<0.001, Statistically significant at p<0.05

Asia 1) are frequently reported to be associated with the disease outbreaks in India[4]. Of which, serotype O is the predominant one, followed by serotype A associated with frequent outbreaks in India. The disease is characterized by high fever, salivation, lameness, vesicular lesions on the mouth, tongue, feet, snout and teats of infected animals. The morbidity rate is very high (upto 100%), but the mortality rate is low in adults (5%), while the high mortality rate (>50%) is observed in young calves due to acute necrotizing myocarditis[5,6]. Sometimes, healed cardiac muscles in heart do not work properly under stress leading to sudden death in adult animals[5]. Generally, the diagnosis of FMD is based on clinical signs followed by confirmation by laboratory tests. Among the various diagnostic assays, multiplex PCR (MP-PCR) is preferred worldwide due to the serotype differentiation with experimental simplicity, greater cost effectiveness besides decreased effort and shorten time[1]. Despite extensive research carried out over the years, the pathogenesis of the disease still remains unexplored. The recent paper from our lab demonstrated the novel pathological findings along with the distribution of viral antigen in various non-target organs such as thyroid, adrenal glands, pancreas, tonsils, lymphoid organs, lungs, trachea and intestine of calves, in addition to target organs[7]. However, pathology and virus distribution in the liver and kidneys remained untouched. The earlier published reports showing the higher levels of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine levels in the serum of naturally infected cattle and buffaloes suggest the FMDV induced hepatorenal dysfunction. Being the epitheliotropic virus, FMDV is expected to replicate in the hepatocytes of the liver and tubular epithelial cells of the kidney, causing hepatorenal injury. The recent published report showed FMDV-induced hepatic lesions in naturally infected buffaloes[8]. FMDV is reported to excrete and persist in the urine of infected animals for 39 days and helps in the transmission of the disease to the naïve population[9]. However, the detailed pathological findings, distribution of FMDV antigens along with the apoptotic changes in the liver and kidneys of cattle calves naturally infected with FMDV have not been investigated. Therefore, the present study was intended to find out the hepatorenal function tests by serum biochemical assays, levels of pro-inflammatory cytokine tumour necrosis factor (TNF)-α, gross and histopathological changes, immunohistochemical detection of viral antigens by immunohistochemistry along with the associated apoptotic changes by TUNEL assay in the liver and kidneys of calves naturally infected with FMDV.

MATERIALS AND METHODS

Animals

In the organized dairy herd of ICAR-Indian Veterinary Research Institute (IVRI) consisting of 590 dairy cattle, a total of 177 (adults-118, calves-59) animals were affected due to the outbreak of FMD. The ailing calves showed the clinical signs of high fever (39.2-41.7^{O}C), increased heart rate (110-121/min) and increased respiratory rate (39-48/min). The animals showed excessive salivation, catarrhal stomatitis, vesicles on lips, cheeks, gums, hard palate, dental pad, rostrum of the dorsum of the tongue and interdigital skin, dyspnoea, panting, mouth breathing and lameness that were suggestive of FMD. However, a few calves (n=4) died acutely without showing any clinical vesicular lesions. The calves (n=59), in particular, were severely stressed and prostrated, of which 28 crossbred (female-16, male-12) aged <6 months succumbed to the disease and were subjected to the postmortem investigations at the Division of Pathology,

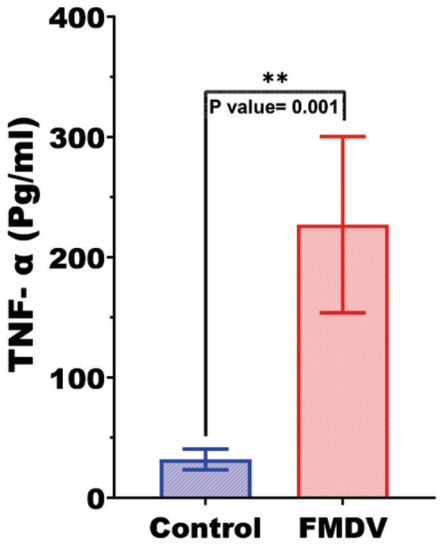

Fig. 1. The level of TNF-α level in the serum is showing significantly higher in FMDV infected calves as compared to control calves. Mean with indicates statistically significant values (P<0.001) as compared to the control calves. Error bars represent ± SEM.

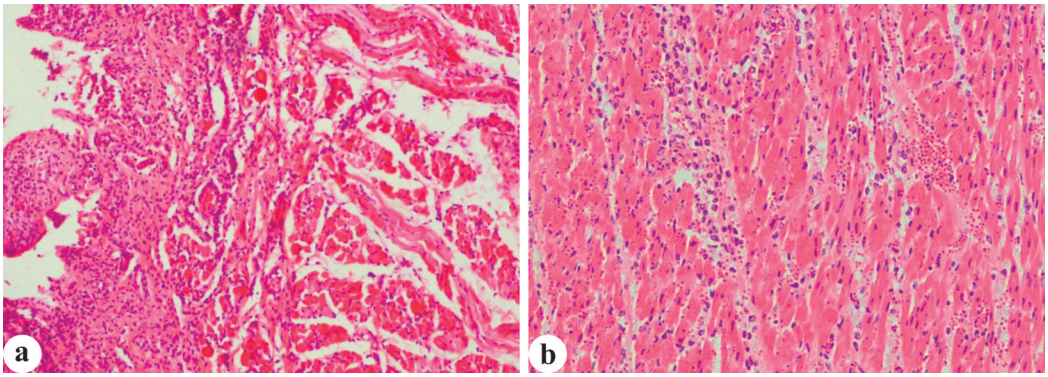

Fig. 2. Histopathological lesions in calves naturally infected with FMDV showing **a.** Marked infiltration of mononuclear cells in the SSE of the tongue. **b.** Acute necrotizing myocarditis with lymphocytic and macrophage infiltration (H&E x100).

ICAR-IVRI, Bareilly, India. The remaining 31 calves were recovered after the treatment by the clinician. The routine vaccination of animals at the farm was being practiced on a 6 monthly basis with the commercially available inactivated FMDV trivalent vaccine (serotypes A, O and Asia 1) (Indian Immunologicals Ltd). All the carcasses were systematically necropsied on their submission and gross lesions were recorded.

The representative tissues (5x10x20 mm) from the tongue, heart, liver and kidneys were collected in 10% neutral-buffered formalin for each case. After 48 hours of fixation, tissue pieces were trimmed for histotechnique. Thin, unfixed tissue pieces from the corresponding sites of organs were collected on ice and later kept at -20^{O}C for molecular detection of FMDV. The liver and kidney pieces without showing any appreciable gross lesions from 2 healthy calves during the routine necropsy unrelated to the present episode were included in the study for comparison purposes.

Serum biochemical analyses

Jugular vein blood was collected into BD Vacutainer rapid serum tubes from FMDV - infected calves (n=12) and FMDV seronegative controls (n=4) [confirmed by virus neutralization test (VNT) assay]. The serum was harvested and then stored at -20^{O}C. The levels of serum concentration of total protein, albumin, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine were determined using commercial kits (Coral Diagnostics, Perkin Elmer, USA) by Celltac-Biochemical analyzer (MEK-6550, Germany).

Estimation of TNF-α levels in serum

The levels of TNF-α were estimated in the sera of FMDV-infected calves (n=12) and FMDV seronegative calves (n=4), using a commercially available bovine TNF-α ELISA kit (MBS 2609886, My Biosource, USA) based on the double antibody sandwich technique. The concentrations were expressed in pg/ml.

Histopathology

The NBF-fixed tissues were routinely processed for the preparation of 4-5 µm thick paraffin tissue sections and stained with Haematoxylin and Eosin (H&E) stains[10]. The histopathological tissue sections were evaluated and photographed under the Medicus pro T microscope (Helmut Hund Gmbh, Germany) with the attached ToupTek camera (C-Mount adapter 0.5X). The lesion scoring was done based on the degree of pathological changes in different constituents of the liver compartments (portal triad, hepatic cords, terminal/central vein) and kidney compartments (renal cortex and medulla). The changes were assigned on a scale of 0-3. The histopathological lesion scoring in the liver and kidney was assigned to 0 if there was no damage/normal, a score of 1 if the damage was mild, a score of 2 if the damage was moderate and a score of 3 if the damage was extensive. The maximum score was taken as the

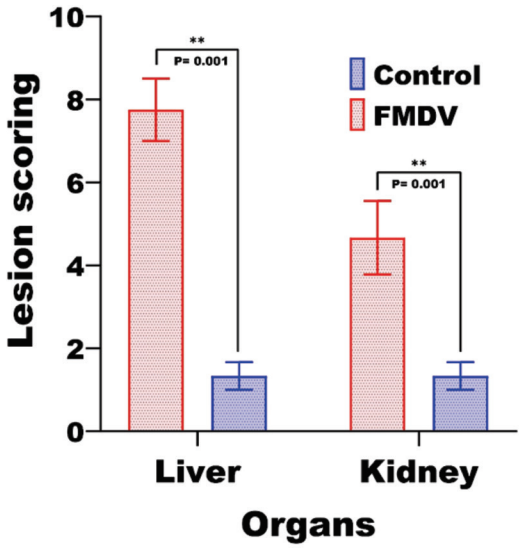

Fig. 3. Histopathological lesion scoring of liver and kidneys of FMDV infected calves showing significantly higher pathological lesions as compared to control calves. Mean with indicates statistically significant values (P<0.001) as compared to the control calves. Error bars represent ± SEM.

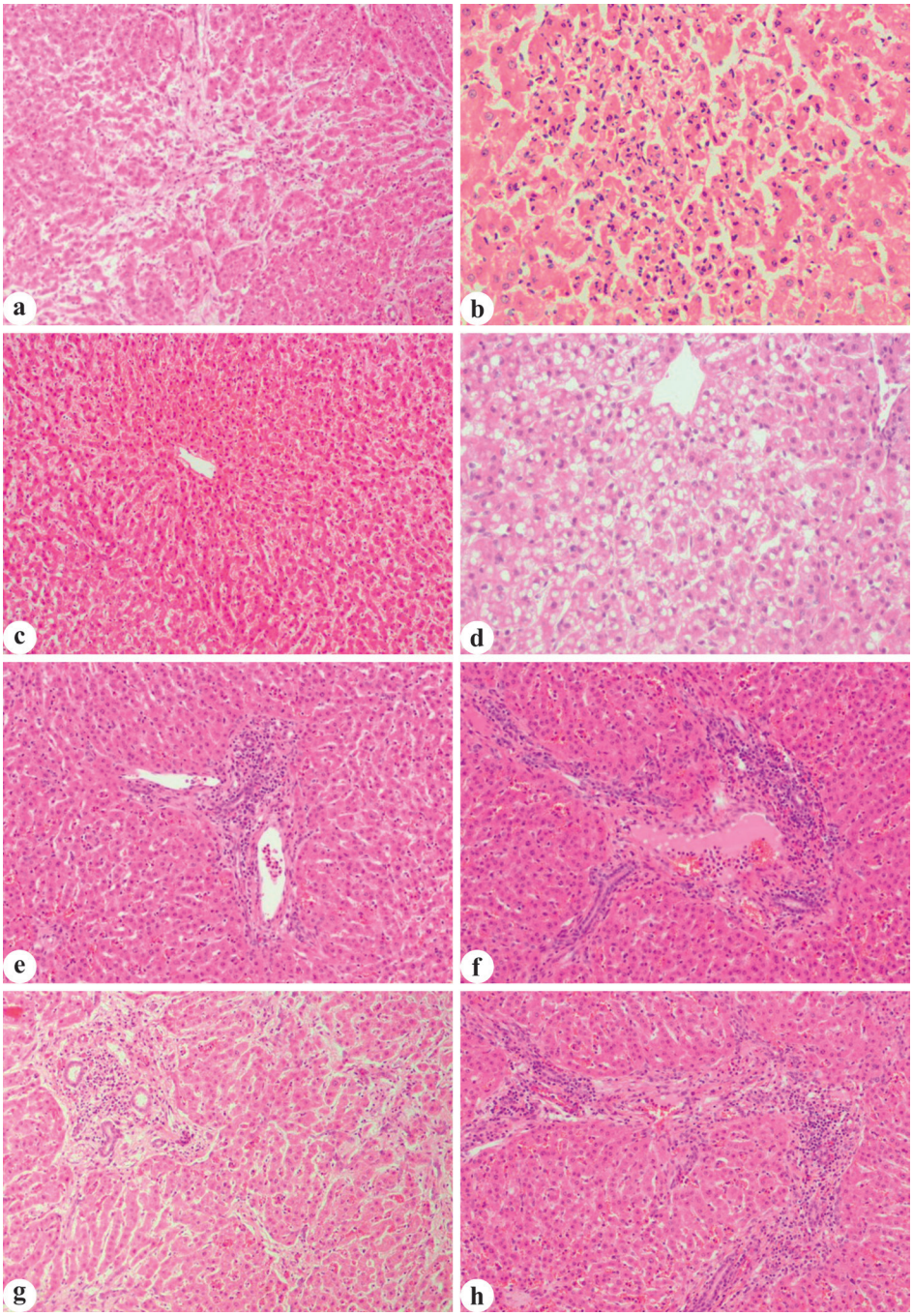

Fig. 4. Histopathological lesions in the liver of calves naturally infected with FMDV showing **a.** Centrilobular necrosis. **b.** Increased Kupffer cells activity. **c.** Passive venous congestion. **d.** Fatty change in zone. **e & f.** Periportal hepatitis with infiltration of mononuclear cells within the portal triad. **g.** Portal fibrosis and infiltration of mononuclear cells. **h.** Bridging portal fibrosis and hepatitis/necrosis (H&E x100).

most severe inflammatory/pathological changes. The lesion scoring of various organs was done in 10 infected and 2 control calves. The lesion scoring was done blind folded by 2 independent pathologists to avoid the bias and reliability of the observations.

Molecular detection of FMDV

The total tissue RNA was extracted from the frozen tissues of the tongue, heart, liver and kidney of 28 calves using the RNeasy kit (Qiagen, Germany) according to the manufacturer's instructions. The cDNA synthesis was performed using 100 units of M-MuLV reverse transcriptase enzyme (Promega, USA) and 1 µg of oligo (dT) primer (Thermo Scientific) per reaction. The identification of the specific serotype involved was done by MP-PCR using three serotype-specific forward primers consisting of DHP 13, DHP 15 and DHP 9 targeting the serotypes O, A and Asia 1, respectively,

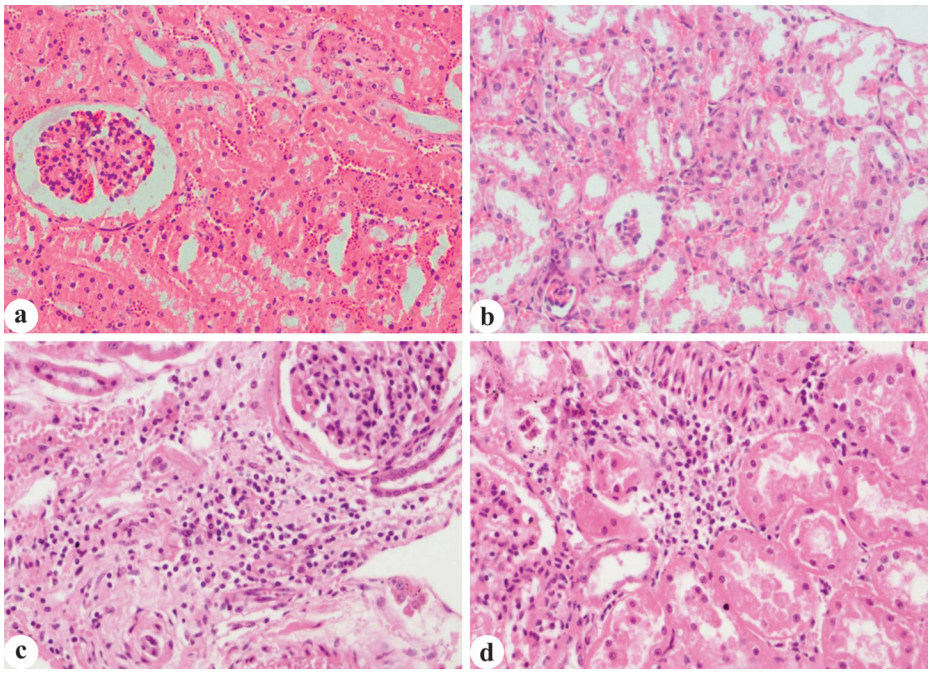

Fig. 5. Histopathological lesions in kidneys of naturally infected calves showing **a.** Vascular congestion in glomerulus and tubules (H&E X100). **b.** Necrosed glomerular tuft with degenerative changes in the tubules (H&E X100). **c.** Peri-glomerulitis (H&E X200). **d.** Interstitial nephritis (H&E X200).

and the universal FMDV-specific NK-61 primer (5'-GACATGTCCTCCTGCATCTG-3') as mentioned in the previous report[11]. Further, cDNAs were tested to rule out the intercurrent viral pathogens such as Bovine Viral Diarrhoea virus (BVDV)[12], Malignant Catarrhal Fever (MCF)[13], Bluetongue Virus (BTV)[14] and Vesicular Stomatitis Virus[15].

Distribution of FMDV antigen

The paraffin tissue sections of the liver and kidneys that were positive to FMDV by MP-PCR assay were mounted on poly-L-lysine-coated slides from FMDV affected calves (n=10) and processed by indirect immunoperoxidase technique (IPT) for the distribution of viral antigen in the cells of different compartments. After deparaffinization and rehydration, endogenous peroxidase activity was quenched with BLOXALL (Vector labs, USA) for 30 min, followed by washing in phosphate-buffered saline-Tween (PBST, 0.05 M, pH 7.6) thrice for 5 min each. The antigen retrieval was done by heating the slides in a microwave oven with the citrate-based antigen unmasking solution (Vector labs, USA) for 25 min. The sections were thoroughly washed in PBST followed by incubation in a humidifier chamber with prediluted 2.5% normal horse serum blocking solution (Vector labs, USA) at 37^{O}C for 1 h to block non-specific sites. Afterwards the sections were incubated with rabbit polyclonal anti-FMDV Polyprotein (3D polymerase) primary antibody (bs-4524R, Bioss antibodies 1:100 optimal working dilution) and incubated overnight at 4^{O}C. Sections were thoroughly washed in PBST three times to remove unbound antibody. Afterwards the sections were incubated with prediluted ImmPRESS® HRP universal (Horse Anti-Mouse/Rabbit IgG) antibody polymer agent (Vector labs, USA) at 37^{O}C for 1 h. Then the sections were washed thrice with 5 min each wash with PBST. The sections were treated with a freshly prepared solution of 1 drop of ImmPACT DAB Reagent with 1 ml ImmPACTDAB 3-3'-diaminobenzidine tetrahydrochloride (DAB) diluent (ImmPACT® DAB Substrate kit, Vector labs, USA) for the 30s for the development of brown colour. Afterwards the slides were counterstained with Mayer's haematoxylin for the 30s. The sections were washed and mounted with CC/Mount™ (Sigma-Aldrich, USA). For the negative controls, the primary antibody was substituted with rabbit IgG isotype control (Novus Biologicals, USA) and with PBS with 1% BSA to rule out false positive staining.

***In-situ* detection of apoptosis**

The labeling of apoptotic cells in tissue sections of the liver and kidneys of FMDV-infected calves (n=10) was performed with TUNEL (transferase-mediated dUTP nick end labeling) assay kit using the manufacturer's instructions.

Statistical analyses

The data were expressed as means ± standard error of mean and subjected to statistical analyses using independent t-test for all the parameters in GraphPad Prism 8.0.2 except for histopathological lesions scoring. The histopathological lesion scoring of the livers and

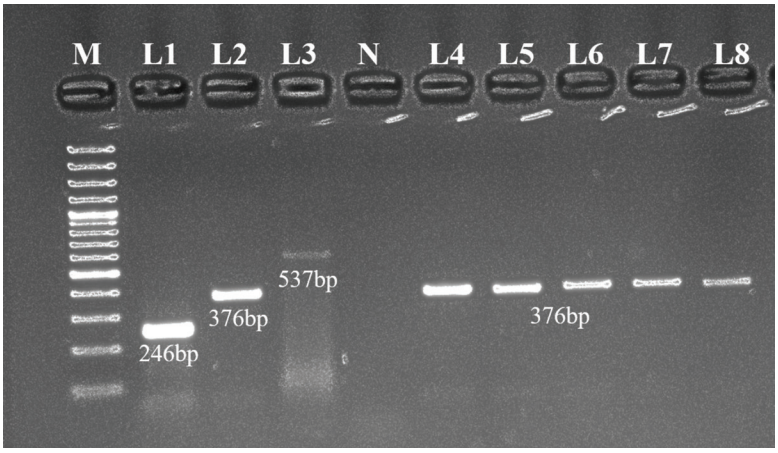

Fig. 6. Molecular detection of FMDV. M: Marker (100 bp), L1: Type O positive control (246 bp), L2: Type A positive control (376 bp), L3: Asia 1 positive control (537 bp), N: Negative control, L4: Tongue epithelium, L5: Heart, L6: Liver, L7: Liver, L8: Kidney.

kidneys of FMDV-infected and control calves was analyzed using the Mann-Whitney U test. Statistical significance was set at $p<0.05$ for significant and $p<0.001$ for highly significant.

RESULTS

Serum biochemical investigation

The FMDV-infected calves showed significantly reduced values of total protein concentrations and albumin levels ($p < 0.001$) and significantly higher ($p < 0.001$) glucose concentrations as compared to controls. The FMDV-infected calves showed significantly higher levels of ALT, AST and ALP liver enzymes ($p < 0.05$) as compared to controls. Similarly, the FMDV-infected calves showed significantly higher serum levels of BUN and urea ($p < 0.05$), whereas creatinine levels, although increased failed to show any significant change as compared to control calves.

Estimation of TNF-α level

The serum of FMDV - infected calves showed significantly higher levels of TNF-α ($p < 0.001$) as compared to controls (Fig. 1).

Gross and histopathological lesions

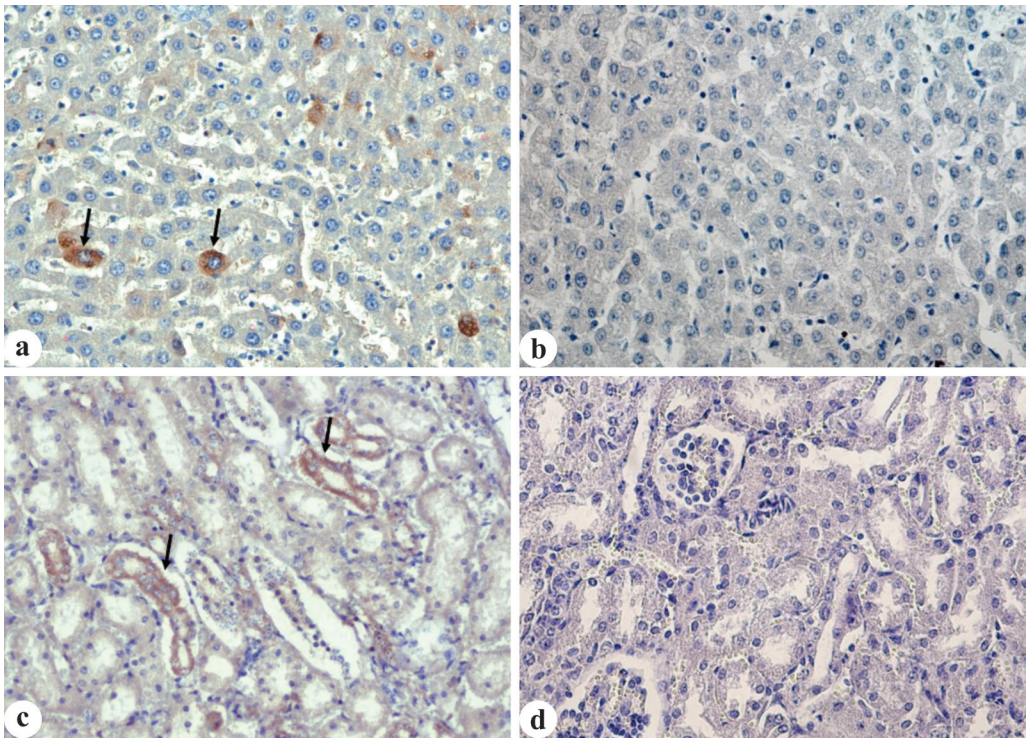

Fig. 7. Immunohistochemical detection of FMDV antigens. **a.** Hepatocytes (arrow). **b.** Absence of any immunoreactivity, negative control, liver. **c.** Renal tubules (arrow). **d.** Absence of immunoreactivity in the negative control section, kidney (IHC x100).

There were consistent vesicular lesions in the buccal mucosa, the interdigital skin and in addition, the necrotizing myocarditis (tigroid heart) in the majority of the cases. The buccal mucosa of 24 calves showed few to many vesicles (intact/ruptured) of varying sizes over the tip, lateral border and dorsum of the tongue, cheeks, gums, dental pad, lower lip and muco-cutaneous junction of the exterior nares. The heart in all the cases (n=28) showed grey to pale foci/stripes of necrosis of varying sizes alternating with red myocardium, resembling tiger stripes (Tigroid heart appearance). Microscopically, the stratified squamous epithelium (SSE) of the tongue showed marked infiltration with mononuclear inflammatory cells (Fig. 2a). Below the epithelium layer, the connective tissue, striated muscles, adipose tissue and salivary glands showed vascular congestion with perivascular infiltration of mononuclear cells. The affected heart showed acute necrotizing interstitial lymphocytic myocarditis (Fig. 2b).

Out of 28 cases, 16 calves showed gross liver lesions. The livers were swollen, mottled with distended gall bladder, of which, 12 livers were mild to moderately congested and 6 had whitish necrotic areas on its surface. Further, out of 28 cases, 19 cases showed moderately congested cortex and medulla of the kidneys. Microscopically, the liver and kidneys of the FMDV-infected calves showed significant ($p < 0.001$) pathological alterations as compared to control (Fig. 3). The liver showed centrilobular necrosis of hepatic cells with infiltration of mononuclear cells in 19 cases (Fig. 4a), vasculitis, swollen endothelial lining cells of blood vessels with hyperplasia of Kupffer cells in 14 cases (Fig. 4b). The liver of 16 calves showed passive venous congestion (Fig. 4c), oedema and infiltration of inflammatory cells within the hepatic sinusoids. The liver of the infected calves showed multifocal areas of periportal hepatic necrosis accompanied with the infiltration of inflammatory cells. A total of 12 cases showed fatty change around the central vein (Fig. 4d) characterized by the presence of variable sized fatty vacuoles in the hepatocytes. The liver showed non-suppurative hepatitis characterised by the infiltration of mononuclear cells around the portal triad and the central/terminal vein in 11 cases (Fig. 4e & f). Portal-central bridging hepatitis (Fig. 4g) and portal fibrosis (Fig. 4h) were observed in 8 and 5 cases, respectively. The kidneys showed severe vascular changes characterized by congestion and oedema in the capillary plexuses of the glomerulus and tubules of 12 cases (Fig. 5a). The glomerular necrosis (Fig. 5b) was observed in 8 cases, and 6 cases showed mesangial cell proliferation. The tubules showed degenerative changes with presence of proteinaceous exudates within the lumen (Fig. 5b). The periglomerulitis (Fig. 5c) was observed in 7 cases. The interstitial nephritis (Fig. 5d) characterized by infiltration of mononuclear cells was observed in the interstitial space of 5 cases.

Molecular detection of FMDV

In MP-PCR assay, the heart and tongue of all the

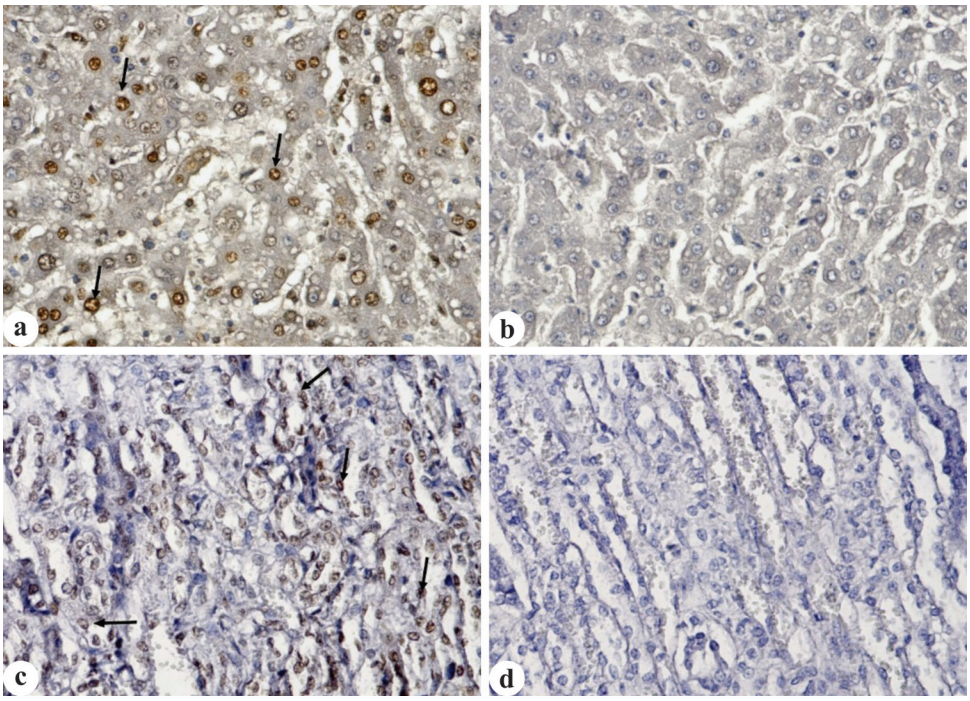

Fig. 8. Detection of apoptosis in calves naturally infected with FMDV. **a.** Apoptotic nuclei (arrow), hepatocytes, liver. **b.** Absence of immunoreactivity, negative control, liver. **c.** Apoptotic nuclei (arrow) in renal tubular cells of renal medulla. **d.** Absence of immunoreactivity in negative control, kidney (TUNEL x200).

cases showed the presence of serotype A by amplifying 376 bp. Out of 28 calves tested, the presence of viral genome was detected in 19 and 13 cases of liver and kidneys, respectively (Fig. 6). The heart, tongue, liver and kidneys failed to show the amplification for other viral differentials such as MCF, BTV, BVDV and VSV.

Distribution of viral antigen

The liver (n=19) and kidneys (n=13) of MP-PCR positive cases showed the presence of viral antigen in the hepatocytes and tubules of 11 and 8 cases, respectively (Fig. 7).

***In-situ* detection of apoptosis**

In TUNEL assay, significantly increased number of apoptotic cells were observed in the hepatic lobules of the FMDV-infected calves (Fig. 8a). The inflammatory cellular exudates within the central vein also showed apoptosis. The negative control section failed to show any immunoreactivity for apoptotic nuclei (Fig. 8b). The medullary tubules of the kidney showed the increased number of apoptotic nuclei (Fig. 8c), whereas negative control sections failed to show any immunoreactivity (Fig. 8d).

DISCUSSION

The FMDV-infected calves had higher physiological values as compared to control calves, which are in agreement with the earlier observations[8,16]. The vesicular and necrotizing myocarditis lesions were documented as reported in earlier reports[7,17-19]. The FMDV-induced necrotizing myocarditis is the primary reason for the death and high mortality in calves. The reduction in serum total protein and albumin levels that might be attributed to the starvation due to oral lesions, hepatic and renal damage due to virus replication and passive congestion due to the myocarditis as reported in earlier studies[16,19-22]. The liver damage and protein-losing enteropathy due to FMDV might be the reason for hypo-proteinemia and hypoalbunemia[20]. The higher blood glucose level in FMD infected calves might be due to lower insulin level resulting due to replication of FMDV in the beta Langerhans cells of pancreas. The similar findings are in congruent with the earlier observations[7,23]. The increased level of glucose might be another reason for the decrease in protein concentrations observed in this study[21,24]. The elevated levels of the ALT, AST and ALP liver enzymes suggest the hepatic cells damage leading to the subsequent release of hepatic enzymes into the circulation. Similar results are reported in the FMD cases in various reports[16,20,25]. The high rise of AST level is frequently regarded as a sensitive indicator of hepatocyte damage, even in cases of subclinical infection[25]. Moreover, high rise of AST levels can be due to myocardial damaged cause by FMDV resulting from acute necrotizing myocarditis. The high rise of BUN, urea and creatinine levels in the serum of FMDV infected calves suggest the renal impairment. This might lead to development of prerenal azotemia due to reduced rate of glomerular filtration rate leading to high levels of creatinine in the serum. The high rise of BUN, urea and creatinine levels in case of FMDV-infected cattle and buffaloes were reported in earlier studies[16,20]. The higher levels of TNF-α in the sera of FMDV infected calves might suggest the continued stimulation of the immune cells producing this inflammatory cytokine due to potential consequence of virus replication causing systemic inflammatory reaction[26,27]. The presence of TNF-α might act as host defense mechanism against FMD infection[28].

The tongue lesions of coagulative necrosis involving the epithelial and subepithelial structures and vesicle formation containing fluid with infiltration of inflammatory cells are consistent with the earlier observations[7,17,18]. The heart showing acute necrotizing interstitial lymphocytic myocarditis is in agreement with previous studies[5,6,29]. The liver showing multifocal hepatic necrosis and congestion of central and portal vein are in congruent with the earlier observations[8,18]. The fatty change in the affected liver might be due to disruption of the liver's ability to properly metabolize fats, leading to the accumulation of triglycerides in the liver cells. The marked infiltration of mononuclear cells around the portal triad might be due to the entry of viral toxin through the portal tract causing the hepatic damage. The passive venous congestion in liver might be linked with the myocarditis due to impaired venous drainage[8]. The portal fibrosis developed in the present case might be due to continued periportal necroinflammation along with ischemic changes induced by myocarditis lesions might initiate the fibrogenesis destroying the periportal parenchyma in liver. The portal fibrosis in case of FMDV-infected calves has been documented in the earlier report[8]. The kidney showing vascular and inflammatory changes in the glomerulus and tubules suggest the FMDV induced renal dysfunction. The degenerative changes in the tubules might be due to impaired end organ perfusion due to myocarditis lesions. Further, the increased serum levels of BUN, urea and creatinine in the present study support our findings[16,20]. The presence of viral antigen in the tubules suggest FMDV induced renal damage due to replication of virus in the tubules. The earlier report showing the replication of FMDV in continuous bovine kidney cell line supports our findings[30]. The excretion of the virus in the urine of the infected animals might be due to the replication of the virus in the tubular epithelial cells. Further, in MP-PCR assay, the detection of viral nucleic acid in the liver and kidney confirm the association of FMDV with histopathological alterations in liver and kidney. The detection of apoptosis in the hepatocytes and renal tubules suggest the FMDV induced

hepatorenal damage mediated through apoptosis in liver and kidney respectively. Apoptosis is a tightly regulated physiologic and pathologic process that plays a crucial role in the disposal of unwanted cells as well as in maintaining the homeostasis of the immune system[31]. FMDV has been reported to induce apoptosis in tongue and hoof of naturally infected swine[28] and heart showing myocarditis lesions in lamb[32]. The hepatorenal damage in the present study might have association with the FMDV induced myocarditis in calves that restricts the flow of the blood to the liver and kidney causing degenerative changes. The higher apoptotic cells in the liver and kidney of calves, suggesting the key role of apoptosis in FMDV-induced hepatorenal dysfunction. The different viral proteins of FMDV such 2C protein[33], 3C protease[34] and VP1[35] of FMDV are reported to induce apoptosis. The higher level of TNF-α might be responsible for induction of high rate of apoptosis. The association of TNF-α with apoptosis in the tongue of pigs infected with FMD has been reported in previous report[28].

The present study documents the FMDV-induced hepatorenalopathy besides the classical lesions of the FMD. This was accomplished with the higher serum levels of ALT, AST, ALP, BUN, urea and creatinine in the ailing calves, histopathological alterations with the presence of viral antigen and molecular detection of viral genome in the liver and kidney. It remains unresolved whether the findings described herein are specific to the FMDV-type A or irrespective of any serotypes that need to be investigated. Understanding these diverse pathological spectrum in other organs is essential for the accurate diagnosis and effective management of hepatorenalopathy associated with FMDV.

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Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Aslam M and Alkheraije KA. 2023. The prevalence of foot and mouth disease in Asia. *Front Vet Sci* **10:** 1201578.
2. Govindaraj G, Krishnamohan A, Hegde R, Kumar N, Prabhakaran K, Wadhwan VM, Kakker N, Lokhande T, Sharma K, Kanani A and Natchimuthu K. 2021. Foot and Mouth Disease (FMD) incidence in cattle and buffaloes and its associated farm-level economic costs in endemic India. *Prev Vet Med* **190:** 105318.
3. Dilawar A, Hassan N, Farooq S, Bashir S, Muhee A and Nazir T. Economic impact of Foot and Mouth Disease (FMD) on dairy farmers of the highlands. *J Livest Sci* **16:** 322-7.
4. Subramaniam S, Pattnaik B, Sanyal A, Mohapatra JK, Pawar SS, Sharma GK, Das B and Dash BB. 2013. Status of Foot and Mouth Disease in India. *Transbound Emerg Dis* **60:** 197-203.
5. Aktas MS, Ozkanlar Y, Oruc E, Sozdutmaz I and Kirbas A. 2015. Myocarditis associated with foot and mouth disease in suckling calves. *Vet Arh* **85:** 273-282.
6. Stenfeldt C, Diaz-San Segundo F, De Los Santos T, Rodriguez LL and Arzt J. 2016. The pathogenesis of foot and mouth disease in pigs. *Front Vet Sci* **3:** 41.
7. Sahoo M, Singh R, Kumar P, Kumar Mariappan A, Munnuswamy P, Singh K, Mani S, Dhama K, Kondabattula G, Das T and Thakor JC. 2023. Novel pathologic findings and viral antigen distribution in cattle and buffalo calves naturally infected with Foot and Mouth disease virus. *Vet Q* **43:** 1-3.
8. Khafaji MAAL. 2025. Physical and pathological assessment of cardio-hepatobiliary system with salivary gland in buffalo infected with FMD. *Int J Vet Sci Anim Husb* **10:** 107-113.
9. Alexandersen S, Zhang Z, Donaldson AI and Garland AJ. 2003. The pathogenesis and diagnosis of foot and mouth disease. *J Comp Pathol* **129:** 1-36.
10. Luna LG. 1972. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology (3rd Ed.) McGraw-Hill Book Company, New York.
11. Giridharan P, Hemadri D, Tosh C, Sanyal A and Bandyopadhyay SK. 2005. Development and evaluation of a multiplex PCR for differentiation of foot and mouth disease virus strains native to India. *J Virol Methods* **126:** 1-1.
12. Uruno K, Shibata I and Nakane T. 1998. Detection of bovine viral diarrhea virus (BVDV) using reverse transcription polymerase chain reaction assay. *J Vet Med Sci* **60:** 867-70.
13. Sood R, Khandia R, Bhatia S, Hemadri D, Kumar M, Patil SS, Pateriya AK, Siddiqui A, Kumar MS, Venkatesha MD and Kulkarni DD. 2014. Detection and molecular characterization of naturally transmitted sheep associated malignant catarrhal fever in cattle in India. *Trop Anim Health Prod* **46:** 1037-43.
14. Tiwari AK, Kataria RS, Desai G, Butchaiah G and Bandyopadhyay SK. 2000. Characterization of an Indian bluetongue virus isolate by RT-PCR and restriction enzyme analysis of the VP-7 gene sequence. *Vet Res Commun* **24:** 401-9.
15. Nunez JI, Blanco E, Hernandez T, Gomez-Tejedor C, Martín MJ, Dopazo J and Sobrino F. 1998. A RT-PCR assay for the differential diagnosis of vesicular viral diseases of swine. *J Virol Methods* **72:** 227-35.
16. Ghanem MM and Abdel-Hamid OM. 2010. Clinical, haematological and biochemical alterations in heat intolerance (panting) syndrome in Egyptian cattle following natural foot and mouth disease (FMD). *Trop Anim Health Prod* **42:** 1167-73.
17. Mahmoud EA and Neamat-Allah AN. 2016. Hemato-Biochemical Studies on Egyptian Buffaloes and Calves Naturally Infected with Foot and Mouth Disease Virus Serotype SAT2. *Bull Univ Agric Sci Vet Med Cluj-Napoca, Vet Med* **73**.
18. Ali AA, Hafez MH, Ahmed B, Hasanin SA, Algabri N and Sheire HA. 2016. Pathological and molecular investigations on Foot and mouth virus outbreaks among cattle herds in Dakahlia Governorate, Egypt. *Zagazig Vet J* **44:** 128-37.
19. El-Ansary RE, Sofy AR, El-Tabakh MAM, Afify AF, El-Gaffary M, Oraby MI and Elkhiat MA. 2025. Biochemical, hematologic and oxidative stress biomarkers investigation in infected native breed calves with foot and mouth disease virus serotype a. *Adv Anim Vet Sci* **13:** 253-261.

20. Gokçe G, Gokce Hİ, Gunes V, Erdogan HM and Çitil M. 2004. Alterations in some haematological and biochemical parameters in cattle suffering from foot and mouth disease. *Turkish J VetAnim Sci* **28:** 723-7.
21. Kar J, Ahad A, Nath SK, Islam Z and Sarker MS. 2015. Haematobiochemical aspects of Foot and Mouth disease in cattle in Chittagong, Bangladesh. *J Inf Mol Biol* **3:** 62-65.
22. Khan D, Sheikh IS, Ullah A, Kasi KK, Mustafa MZ, Din ZU, Anwar I, Kakar N and Waheed A. 2024. Circulation of foot and mouth disease serotypes, risk factors and their effect on hematological and biochemical profiles among cattle and buffalo in Quetta, Balochistan, Pakistan. *Vet World* **17:** 329.
23. Kamal E and Abo Heakal N. 2018. Alterations in some biochemical parameters in cattle affected with foot and mouth disease in Dakahlia Governorate, Egypt. *Mansoura Vet Med J* **19:** 49-59.
24. Barboni E, Mannocchio I and Asdrubali G. 1966. The development of diabetes mellitus in cattle experimentally infected with virus of foot and mouth disease. *Vet Ital* **17:** 339-368.
25. Eldeen NAN, Neamat-Allah AN, Rizk LG and Fareed RSG. 2017. Serological, haematological, biochemical and oxidative markers during foot and mouth disease serotype 'O' infection, Egypt. *Bull Univ Agric Sci Vet Med Cluj-Napoca, Vet Med* **74:** 218-226.
26. Zhang Z, Bashiruddin JB, Doel C, Horsington J, Durand S and Alexandersen S. 2006. Cytokine and Toll-like receptor mRNAs in the nasal-associated lymphoid tissues of cattle during foot and mouth disease virus infection. *J Comp Pathol* **134:** 56-62.
27. Zhang Z, Ahmed R, Paton D and Bashiruddin JB. 2009. Cytokine mRNA responses in bovine epithelia during foot and mouth disease virus infection. *Vet J* **179:** 85-91.
28. Ku BK, Kim SB, Moon OK, Lee SJ, Lee JH, Lyoo YS, Kim HJ and Sur JH. 2005. Role of apoptosis in the pathogenesis of Asian and South American foot and mouth disease viruses in swine. *J Vet Med Sci* **67:** 1081-8.
29. Ryan E, Horsington J, Durand S, Brooks H, Alexandersen S, Brownlie J and Zhang Z. 2008. Foot and mouth disease virus infection in young lambs: pathogenesis and tissue tropism. *Vet Microbiol* **127:** 258-74.
30. LaRocco M, Krug PW, Kramer E, Ahmed Z, Pacheco JM, Duque H, Baxt B and Rodriguez LL. 2013. A continuous bovine kidney cell line constitutively expressing bovine $\alpha v\beta 6$ integrin has increased susceptibility to foot and mouth disease virus. *J Clin Microbiol* **51:** 1714-20.
31. Clarke P and Tyler KL. 2009. Apoptosis in animal models of virus-induced disease. *Nat Rev Microbiol* **7:** 144-55.
32. Gulbahar MY, Davis WC, Guvenc T, Yarim M, Parlak U and Kabak YB. 2007. Myocarditis associated with foot and mouth disease virus type O in lambs. *Vet Pathol* **44:** 589-99.
33. Wang J, Wang Y, Liu J, Ding L, Zhang Q, Li X, Cao H, Tang J and Zheng SJ. 2012. A critical role of N-myc and STAT interactor (Nmi) in foot and mouth disease virus (FMDV) 2C-induced apoptosis. *Virus Res* **170:** 59-65.
34. Yi J, Peng J, Ren J, Zhu G, Ru Y, Tian H, Li D and Zheng H. 2021. Degradation of host proteins and apoptosis induced by foot and mouth disease virus 3C protease. *Pathogens* **10:** 1566.
35. Peng JM, Liang SM and Liang CM. 2004. VP1 of foot and mouth disease virus induces apoptosis via the Akt signaling pathway. *J Biol Chem* **279:** 52168-74.