



RESEARCH ARTICLE

Investigation of Different Serotypes of FMDV in Vaccinated Buffaloes (*Bubalus bubalis*) in Southern Areas of Punjab Province, Pakistan

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ABSTRACT

Foot and mouth disease (FMD) is highly endemic in Pakistan, which induces heavy economic loss to livestock holders in term of decrease milk production, high morbidity and mortality in large and small ruminants. In present study, we observed the clinico-pathological lesions during an outbreak of FMD in vaccinated buffaloes. Overall 31.56% morbidity (119/377), 4.77% mortality (18/377) and 15.12% case fatality (18/119) was recorded during the current outbreak. The morbid animals were lethargic, depressed and exhibited marked lameness, profuse salivation, myositis of tongue, vesicular fluid and epithelial sloughing. Vesicular fluid and epithelial sloughing collected from clinically sick animals were positive for FMDV predominantly O strain and Asia I through ELISA technique. Grossly, mandibular lymph nodes were swollen and hemorrhagic in infected animals. The mucosa of abomasum was severely congested, ulcerated and showed hyperemic edges with centrally yellow necrotic areas. Severe purulent inflammation of jejunum and petechial hemorrhages over base of heart were also observed. Histologically degenerative changes in keratinocytes in stratum spinosum, marked acanthosis and intracellular accumulation of eosinophilic, a cellular, transudate forming characteristic vesicles and bullae were observed in epidermis. The mandibular lymph nodes exhibited prominent capillaries engorged with erythrocytes, necrosis and dendritic cells with engulfed necrotic cells. Hemosiderin accumulation was also observed. Fusion and stunting of intestinal villi along with infiltration of inflammatory cells were characteristic lesions in infected animals. From the findings of our study it can be suggested that clinical signs, necropsy lesions and histopathological changes are valuable and useful tools for the diagnosis of foot and mouth disease in *Bubalus bubalis*. Moreover, strict surveillance, use of good quality vaccines, regular monitoring and geographical distribution of various serotypes of FMDV are valuable tool for establishment of effective control procedures.

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INTRODUCTION

In Pakistan, buffaloes (*Bubalus bubalis*) are the major dairy animal which contributes about 95% of the total milk production in the country (Hussain *et al.*, 2017a).

There are about 30 million heads of buffaloes in Pakistan and still the number is rapidly progressing (Jamil *et al.*, 2015). More than 8.42 million farmers Pakistan are dependent on rearing of animals for their livelihood (Ali *et al.*, 2016; Qayyum *et al.*, 2016). The livestock

production systems are facing various bacterial (Hussain *et al.*, 2017a; Mahmood *et al.*, 2017; Hussain *et al.*, 2018a), viral (Khan *et al.*, 2018), and parasitic infections (Hussain *et al.*, 2017b; Mahmood *et al.*, 2017; Zaman *et al.*, 2017; Hussain *et al.*, 2018b; Khater *et al.*, 2018; Li *et al.*, 2019; Shaikat *et al.*, 2019; Zafar *et al.*, 2019) resulting in huge economic losses. Among the various infectious diseases of livestock, Foot and Mouth disease (FMD) being a transboundary disease is a major and highly transmissible infection of cloven-hoofed animals that causes huge economic losses to the livestock industry worldwide (Rehman *et al.*, 2014). It is a highly contagious disease of domestic animals caused by RNA virus belonging to genus Aphthovirus (Elnekave *et al.*, 2016). Being complex in its genomic sequence, the virus has seven distinct serotypes such as O, A, C, SAT1, SAT2, SAT3 and Asia-1 (Abubakar *et al.*, 2015).

Sporadic incidences of the disease have been reported in FMD-free countries with characteristic vesicular features in domestic and wild cloven-hoofed animals with devastating consequences. In Pakistan, FMD is endemic and continuously present in acute or mild forms in dairy animals including cattle and buffaloes (Rehman *et al.*, 2014; Abubakar *et al.*, 2015). Despite of mass vaccination, use of good quality vaccine, strict surveillance and regular monitoring, sporadic outbreaks of the disease occur with high morbidity (Abubakar *et al.*, 2015). Hence, the disease is inflicting heavy economic losses to the livestock sector due to high morbidity and decrease in production in adults and by high mortality in young animals (Abubakar *et al.*, 2015). Due to differences in genetic variability among the multiple outbreaks of FMD, serotype O has been reported since last few years particularly in the Landhi Dairy Colony which is a world largest colony of buffalos in Pakistan (Jamal *et al.*, 2013). Lack of sero-surveillance and due to increased trans-boundary migration of livestock within and from the neighboring countries including Iran, Afghanistan, Bangladesh and China, genomic reshuffling and mutated forms of FMDV are increasing which resulted in disease outbreaks. Earlier studies have reported that viral genomic sequencing and phylogenetic dendrogram of FMD Virus from North-East of Iran has close proximity to that of isolated serotype of FMDV in Pakistan (Rashtibaf *et al.*, 2012). Different studies have indicated the presence of FMD in dairy Cattle in North-East areas of Iran (Rashtibaf *et al.*, 2012), Egypt (El-Moety *et al.*, 2012) and China (Chang *et al.*, 2014) in livestock and wildlife species. However, some serotypes of FMDV have a restricted geographical distribution and there is no cross-protection between these serotypes (Alexandersen *et al.*, 2005). It is reported that sometimes the cross protection is conferred by vaccine even of same serotype (Abubakar *et al.*, 2012; Jamal *et al.*, 2013). The exact monitoring, sero-surveillance, geographical information including climatic conditions, animal movements, information about the interaction among various serotypes of FMDV circulating in the field and their vaccines along with dynamic patterns of the disease in different parts of the country could be a valuable tool for the establishment of effective control procedures. Therefore, in the current study we report the prevalence of different serotypes and clinico-pathological picture of FMD infection in buffaloes in Pakistan.

MATERIALS AND METHODS

The study was conducted to investigate the outbreaks of foot and mouth disease (FMD) in buffaloes at three districts (Bahawalpur, Lodhran and Vehari) of Southern Punjab. On the basis of clinical picture, the infected animals having typical signs of the disease were treated with antibiotics (procaine penicillin and colistin), multivitamins and anti-allergics and were offered palliative diets. Despite of treatment therapy, few of the infected animals (n=18) having severe infection signs were died of the disease.

Necropsy and tissue collection: Post-mortem was conducted immediately after the death of the animal to observe various gross abnormalities. Morbid tissues exhibiting lesions including lymphnodes, sloughed oral mucosa, abomasum, intestine and heart particularly inter-ventricular, atrial and ventricular parts were collected and fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, sections of about 5 µm thickness were prepared and stained with Hematoxylin and Eosin (H&E) stains (Yilmaz *et al.*, 2016). For virus identification, different samples such as saliva, vesicular fluid, epithelial sloughing from freshly ruptured lesions or newly formed vesicles at mouth, feet and udder of the clinically sick animals were collected using sterile cotton swabs and kept in buffered glycerine for further processing (Abubakar *et al.*, 2015). The samples were subjected to antigen detection Indirect Sandwich-Enzyme Linked Immunosorbent Assay (Biological diagnostic services limited, Pirbright, United Kingdom) for serotyping of FMDV (O,A,C, Asia 1).

RESULTS

Clinical ailments: The infected animals were lethargic, depressed and exhibited profuse salivation (drooling). The intensity of clinical signs was aggravated from 2nd to 4th days of infection despite of palliative diet and antibiotic therapy and the milk yield was greatly reduced in morbid animals. The animals with foot lesions exhibited marked lameness, were reluctant to move and showed weight loss. Few animal exhibited ulceration at teats and udder that lead to invasion of secondary pathogens to the udder thus showed subclinical mastitis. An overall 31.56% (119/377) morbidity, 4.77% (18/377) mortality and 15.12% (18/119) case fatality was recorded during the current outbreak. No significant results were recorded in the prevalence of FMDV on the basis of age and sex of animals (Table 1). Mortality was relatively high in calves due to severe hemorrhagic enteritis and off feeding compared to adults. Results showed that serotype (Asia-1) was predominant in morbid animals (Table 2). The sick animals were in arched-back position due to abdominal pain. The margins of the tongue and gingival mucosa were hyperemic at early stage however, the animal in relapsing condition were exhibiting raised healed ulcers. A whitish pseudo-membrane and a few eroded vesicles were also observed at the frenulum linguae and inside the mucosal surface of the digital dental arcade.

Table 1: Overall morbidity of buffaloes on the basis of sex and different age groups

Parameters	No. of examined	Positive		Negative	95% CI	Odd Ratio/P value
		n	%			
Sex						
Male	109	36	33.02	73	24.69-42.26	OR=1.10 [reciprocal=0.91]
Female	268	83	30.97	185	25.65-36.70	
Age groups						
1-2 year	107	39	36.44	68	27.74-45.88	Extended Mantel-Haenszel chi-sq. P=0.344
3-5 year	153	47	30.71	106	23.80-38.37	
>6Years	117	33	28.20	84	20.62-36.86	
Overall	377	119	31.56	258	27.02-36.39	-

Table 2: Area based frequency of different serotypes of foot and mouth disease virus in buffaloes

Study area	Suspected outbreaks	Confirmed outbreaks	Serotypes (%)		
			Asia-I	A	O
Bahawalpur	35	31	9	3	19
Lodhran	43	39	11	2	26
Vehari	41	38	07	5	26
Total	119	110	27(24.54)	10(9.09)	71(64.54)

At about 5th day of clinical disease, the appetite and general condition of these animals was improved. Lesions at muzzle, dental pad and tongue were relatively healed but the affected animals were still reluctant to move and showed lameness when forced to rise or walk. The body temperature of infected animal was significantly high (104°F) during the active phase of the disease. Initially, infected animals revealed small and blanched areas in oral cavity including gums, dental pads and tongue. Later on, the fluid accumulated in these areas was quickly transformed into vesicles that reached to about more than 2 cm in diameter especially on the dorsum of the tongue. At few areas, more than one blister joined and formed the bullae; those occupied more than half of the surface of the tongue in infected animals. Less number of intact vesicles were found on tongue while raw surface fringed with blanched flaps of epithelium was observed over the dorsum and lower gingival surface. Aphthae were also present between the claws of the feet and along with the coronary band of hoof. Feet appeared to be skippered due to separation of horn of heel with eroded underlying corium.

Gross and histo-pathological lesions: At necropsy, severe ulceration at the dorsum and margins of the tongue and dried healed ulcers at the teat and claws were observed. Inguinal and intra-mammary lymph nodes were significantly enlarged and hemorrhagic. Moreover, the mandibular lymph nodes were severely hyperemic and congested in all infected cases (Fig. 1). The mucosa of abomasum was severely congested and exhibited severe ulcerations. Ulcers were observed at the surface of gastric mucosa with hyperemic raised edges and had centrally yellow necrotic areas (Fig. 2). Multiple petechial hemorrhages over the base of the heart, at the coronary fat and myocardium were prominent (Fig. 3). Liver was found normal and gall bladder was full of bile. Purulent inflammation of jejunum was observed and the small intestine was filled with yellowish exudates.

Histologically, lymph nodes were found necrosed having thickened capsule, severe congestion, capillaries were engorged with erythrocytes and depletion of lymphocytes was observed at their germinal centers and peripheral zones (Fig. 4). Due to severe congestion and hemorrhages, hemosiderin accumulation was also observed in hemorrhagic areas in spleen. The duodenum

showed severe congestion in mucosa as well as in submucosal capillaries and infiltration of inflammatory cells particularly neutrophils, lymphocytes and macrophages (Fig. 5). Severe ulceration, congestion of mucosa, fusion and stunting was observed in the villus surface of jejunum. Moreover, myocardium showed multifocal myocardial necrosis with infiltration of chronic inflammatory cells particularly lymphocytes.

DISCUSSION

In recent time, the control and management of emerging and re-emerging infectious diseases in livestock mainly depends upon rapid reporting and early diagnosis of the infectious agents, surveillance and determination of risk factors, distribution and understanding of pathogenesis, development of suitable and adjuvant vaccines and epidemiological models (Chand *et al.*, 2015). The monitoring and investigation of such infectious problems in livestock is crucial to uplift the economy of the farmer through production of disease-free and good quality and quantity dairy animals (Chand *et al.*, 2015). Foot and mouth disease being a highly contagious and rapidly transmissible trans-boundary disease is characterized by huge economic losses in terms of poor milk production, high morbidity and mortality as well as causing adverse effects on international trade of livestock products (Kuldeep *et al.*, 2014). It significantly reduces the milk yield that has also been previously reported due to this disease in cattle and buffaloes of different dairy herds of Pakistan (Ferrari *et al.*, 2014). Oral lesions observed in the present study has also been observed in black buck particularly on tongue, inner side of the cheeks and on hard palate and foul smelling with brittle affected epithelium (Chakraborty *et al.*, 2014). Moreover, this disease also caused erosions at the supernumerary digits of wild suids and kudu and lesions at the kneeling pads of warthogs and bush pigs. Although lesions at udder have been observed in different domestic and wild ruminants in variable intensities (Lyons *et al.*, 2015) but such abrasions of the udder or teat have not been observed in the present study. Different clinical ailments like excessive salivation, erosions and vesicles formation in mouth and in inter-digital spaces along with ulceration of coronary band in cattle calves infected with FMD with increased rectal temperature have also been reported (Aktas *et al.*, 2015).

Previously, no report is available about the necropsy signs in buffaloes died of FMDV infection in Pakistan. However, studies have indicated various lesions including petechial hemorrhage and yellowish or grayish necrotic foci in heart of lambs (Gulbahar *et al.*, 2007), myocarditis in cattle calf (Karapinar *et al.*, 2010) and myocarditis in infected pronghorn (Rhyen *et al.*, 2016) and necrosis of



Fig. 1: Mandibular lymph nodes showing severe congestion.



Fig. 2: Gastric mucosa with hyperemic, raised edges and had centrally yellow necrotic areas.

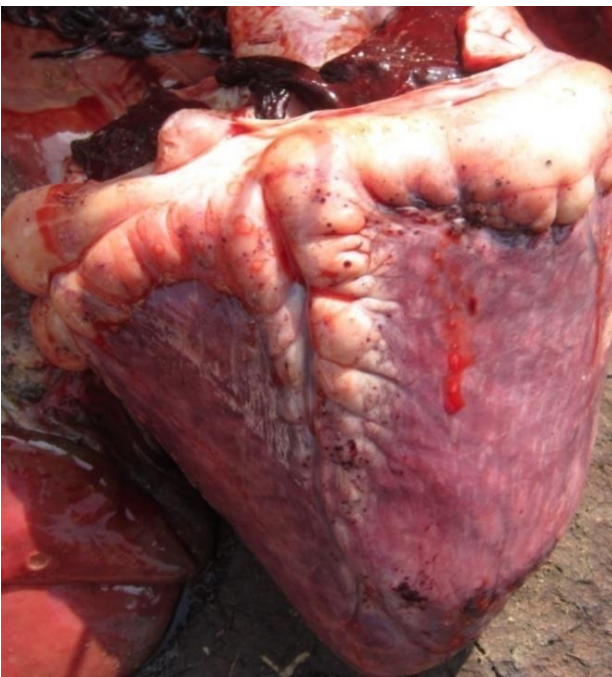


Fig. 3: Multiple petechial hemorrhages over the base of heart, on the coronary fat and myocardium.

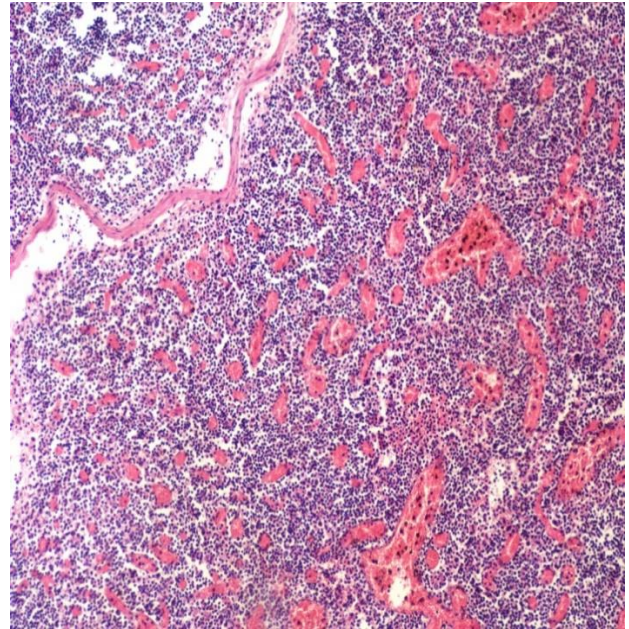


Fig. 4: Photomicrograph of lymph nodes showing thick capsule, severe congestion, engorged capillaries with erythrocytes, necrosis and depletion of lymphocytes at the germinal and peripheral zones.

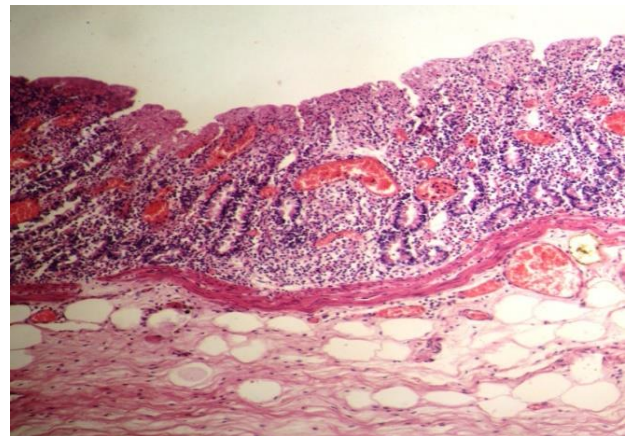


Fig. 5: Photomicrograph of intestine showing congestion of mucosa, submucosal capillaries, infiltration of inflammatory cells particularly neutrophils, lymphocytes and macrophages in infected buffaloes.

myocardium in FMDV infected buffaloes (El-Amir *et al.*, 2014). Previously, intestinal lesions like edema, congestion, necrosis of intestinal glands, hypertrophy and thickening of villi of intestine while atrophy and degenerative changes in lymphoid nodules of cattle calves due to FMDV have been reported. In the present study, different histological lesions in the heart of infected buffaloes including multifocal myocardial necrosis with infiltration of chronic inflammatory cells particularly lymphocytes were observed. Similarly, few studies have shown necrosis of myofibers along with infiltration of lympho-plasmacytic cells between myofibers in pronghorn (Rhyan *et al.*, 2016) and necrosis of myocardial fibers and interstitial myocarditis in buffaloes (El-Amir *et al.*, 2014) infected with the disease.

Furthermore, it has been investigated that FMDV enter into the cells of infected animals through binding of the RGD loop of VP1 located on viral capsid and involving different receptors ($\alpha\beta 1$, $\alpha\beta 3$, $\alpha\beta 5$, or $\alpha\beta 6$) of the target cells leading to development of clinical

lesions. Similar clinical ailments due to FMDV infection in pronghorn have been also reported (Rhyan *et al.*, 2016). Vesicular fluid and different tissues collected from morbid and dead animals were predominantly positive for FMDV O serotype followed by Asia-1. Previous studies have reported similar distinct O serotype in India (Mohapatra *et al.*, 2014), Bangladesh (Nandi *et al.*, 2015), Iran (Rashtibaf *et al.*, 2012); China (Chang *et al.*, 2014) and Pakistan (Abubakar *et al.*, 2012; Rehman *et al.*, 2014; Abubakar *et al.*, 2015). Therefore, the findings of this study suggests that identification of various serotypes of this disease causing virus is of important and crucial for control strategies and handling of disease outbreaks in tropical and subtropical areas.

Therefore, large scale and regular studies are recommended to know the geographical distribution of different serotypes of FMDV in field and monitoring of vaccines along with dynamic patterns of disease in different parts of the country.

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