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Comparative Histomorphological Changes of Bovine Foot and Mouth Disease (FMD) at different Clinical Stages in Bangladesh

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A B S T R A C T

The aim of this study was to determine the comparative histomorphological changes of bovine Foot and Mouth Disease (FMD) at different clinical stages in clinically some infected and non-infected diseased cattle. The disease was characterized by fever and vesicular eruption in the mouth, nares, muzzle, foot, teats and other hairless soft areas of the body and morphologically moderate raised values ($p \leq 0.05$) were recorded in rectal temperature, respiratory and pulse rate, where highest values were during 3 to 7 days of post infection which subsequently reduced after passing the days of infection. Microscopic changes were observed at the advanced stages in the tongue epithelium of the infected cattle, which consisted of ballooned epithelial cells of stratum spinosum showing eosinophilic cytoplasm, acantholysis, pyknotic nucleus with granulocyte infiltration and complete necrosis or dissolution of stratified squamous epithelial surface and the filiform papillae rather than the control, primary and recovery stages. These changes were progressed to microvesicle which on coalescence formed grossly visible macrovesicle (bullae). While, loss of striation of the intercalated bundles of striped muscles fibers and necrosis with intense neutrophil infiltration in the interdigital (foot) space epithelium were observed only at the recovery stages. Therefore the comparative relationship of elevated body temperature, respiratory, pulse rate with histological changes at different clinical stages in this research might be helpful for the virologists, cell-biologists, anatomists and veterinarians to plan for the better management, prevention and future control strategies of FMD.

Introduction

Foot and mouth disease (FMD) is highly contagious viral disease of both domestic and wild cloven hoofed animals (Di Nardo *et al.*, 2011). Of the domesticated species, cattle, buffalo, sheep, goats and pigs are most susceptible, also many species of cloven-hoofed wildlife, such as deer, antelope and wild pigs may become infected (FAO, 1984). It can destroy food supplies and farmer`s livelihoods almost overnight of developing

countries where FMD continues to be endemic especially in Bangladesh (Chowdhury *et al.*, 1994b). Although FMD does not cause high mortality in adult animals, the disease has debilitating effects, including weight loss, decrease in milk production, reproductive failures and loss of draught power resulting in reduced productivity. Mortality, however, can be high in young animals up to 100%, where in the virus causes myocardial degeneration, known as Tiger Heart disease (Gleeson *et al.*, 2003). It is estimated that 25% productivity of individual recovered animals are lost due to FMD infection (Russel and Endington, 1985). It causes low production for the affected countries; severe restrictions are placed on international trade of animal and animal products (meat, milk, hide and butter) due to its transboundary nature of transmission (OIE, 2004). Studies showed that the annual losses due to the outbreak of FMD in Bangladesh have been estimated to be US$ 10.92 million per year (Islam, 2011).

The causative agent, FMD virus (FMDV) is a member of the genus *Aphthovirus*, in the family *Picornaviridae*, of which there are 7 immunologically district serotypes; O, A, C, South African Territories (SAT)-1, SAT-2, SAT-3 and Asia-1, and at least 65 subtypes have been identified (OIE, 2004). In Bangladesh, serotypes A, O and Asia-1 are very common, while type C has been identified scarcely. The pathogenicity in case of FMDV type O is always severe than type A, C and Asia-1. However, recently FMD virus types A and Asia-1 are also found as severe as FMD virus type O (Zinnah, 2008).

Clinically, the disease is characterized by the rapid appearance of high body temperature, respiratory and pulse rate following by the formation of vesicles on the tongue epithelium and skin particularly on inter-digital space epithelium of foot (Sahan, 1962 and Bachrach, 1968). The classical clinical features of FMD in cloven hoofed mammals occur following 2-14 days incubation period as described by Remond *et al.,* 2002. Initial sign includes, pyrexia [up to 106°F (40^{o}C) lasting 1-2 days before other clinical signs], pyrexia is followed by anorexia, agalactia in milking animals and appearance of vesicles (Clavijo *et al.*, 2004). Painful lesions cause profuse drooling, bruxism, foot stamping, and lip smacking with rupture of vesicles occurring within hours to 2 days, leaving erosions and recovery generally occurs within 8-15 days. Characteristic microscopic change was however, observed in keratinized epithelium of tongue and foot (Mohan *et al.,* 2008) which consisted of ballooned epithelial cells of stratum spinosum showing eosinophilic cytoplasm, acantholysis and pyknotic nucleus with granulocyte infiltration. These changes progressed to microvesicle which on coalescence formed grossly visible macrovesicle (bullae). However, there is many relevant studies have been performed throughout the world on emergence and re-emergence of FMD virus, epidemiological studies and risk analysis, targeted surveillance in high risk areas, and effective vaccination both in locally and globally. On the other hand, the effectiveness of treatment at different stages in the cellular level is still unknown that's resulting frequent infection in every year in Bangladesh. Therefore, comparative study on the clinical pictures in associated with histological features of bovine FMD outbreaks under field condition, might also be helpful in order to avail clinicians, researchers and field practitioners for better understanding of the disease process at various clinical stages.

Materials and Methods

Statement of the experiment

The research on "Comparative histomorphological changes of bovine Foot

and Mouth Disease (FMD) at different clinical stages in Bangladesh" was conducted in the laboratory of the Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh during 6 months from January to June 2016, clinically in foot-and-mouth disease cattle and some non-infected cattle (for control group) above age of 1 year were selected to investigate the effective histomorphological changes.

Experimental design

A total of 15 samples of tongue epithelium and tissues of inter-digital lesion from clinically foot-and-mouth disease affected cattle of different outbreak areas of Natore, Mymensingh and Bandarbon districts in Bangladesh.

Animals had suffered from fever, nasal discharge, salivation with buccal lesions, especially on tongue and foot lesions on inter-digital spaces were diagnosed as foot-and-mouth disease (Blood and Radostits, 1989; Samad, 1996). These collected samples were divided into three (3) groups (each group consist of 5 samples). Two pieces of 3/1-cm bovine skin, along with the about 2 cm thick subjacent tissues, were collected from two apparently healthy cows that had been recently slaughtered in the usual manner in a local slaughter house, used as a control group and all necessary data had been recorded from these two healthy cows before slaughter.

Group A (Primary stage): Referred to as primary stage group where animal are affected with FMD disease of 1^{st} to 2^{nd} days of post infections.

Group B (Advanced stage): Referred to as advanced stage group where animal are affected with FMD disease of 3^{rd} to 7^{th} days of post infections.

Group C (Recovery stage): Referred to as recovery stage group where animal are affected with FMD disease of 8^{th} to 14^{th} days of post infections.

Group D (Control group): Group D is control group with normal physiological condition.

Sample collection, preservation and processing

The tongue epithelia and foot tissues were collected from foot-and-mouth disease cattle (Figure 1). The tissues were collected aseptically in separate plastic containers (Falcon® tube 50ml) with special transportation medium and were put into ice box immediately after collection and carried to the Department of Anatomy and Histology, Bangladesh Agricultural University, for the evaluation of effective findings. Then the collected samples were preserved in fixatives (10% formalin) and performed routine Hematoxylin and Eosin (H & E) staining.

Clinical study

The general clinical examination was performed to determine the temperature, respiratory, pulse rate and physical condition of each foot-and-mouth diseased cattle. The method of inspection and palpation were used to examine the mouth and foot. The tongue was checked for injury, ulceration, vesicles, abnormal mobility and consistency. The foot of each animal was examined to detect any lesion on the inter-digital spaces. All clinical findings observed during this study were recorded carefully.

Microscopic photography and illustration

Necessary histological investigation was performed by using high power light microscopy (X4, X10, X40, and X100) for better illustration of the result. The gross

anatomical pictures were taken directly from the organs by using digital camera and the histological pictures were taken through light microscope. The Olympus-BX-51 photographic light microscope was used and necessary illustration was carried out by Adobe Photoshopt® 2007.

Statistical analyses

During the study period the data of daily temperature, respiratory rate, pulse rate, clinical sign and symptom of tongue and inter-digital space (color and shape) were recorded and all the collected data were then analyzed by using IBM SPSS Statistics (version 20) software and revealed the results in necessary forms. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc Duncan's test. Results were expressed as mean ± standard error (S.E). Differences between groups were considered significant at $p \leq 0.01$ and $p \leq 0.05$ level.

Results and Discussion

Clinical pictures at different stage groups

Most of the cattle affected with foot-and-mouth disease showed high rectal temperature (38.4 to 41.9°C), respiratory rate (26.4 to 28.8%) and pulse rate (79.7 to 85.2%), accompanied by severe dejection and anorexia, followed by painful stomatitis at 1 to 14th days of post infection. During primary, advanced and recovery stages the temperature were 38.6 to 41.1°C, 38.1 to 41.9°C and 37.2 to 38.6 °C respectively. The respiratory rate during primary, advanced and recovery stages were 26.3 to 26.8%, 23.7 to 28.8% and 21.2 to 26.3% respectively and the pulse rate of the following respective groups were 78 to 85.2%, 76.6 to 84.7% and 76 to 78.6% respectively (Table 1).Where, the highest temperature 41.9°C and highest respiratory rate 28.2% were observed at 3rd days of post infection that means at the advanced stage but the highest pulse rate 85.2% was observed at 2nd days of post infection at primary stage.

Morphological study at different stage groups

The first clinical sign and symptoms recorded in FMD affected cattle were pyrexia, lassitude and anorexia. The affected animals showed abundant salivation and saliva hanging in long, ropey strings. Vesicles appeared on the oral mucosa, especially on the tongue (Figure 2a) and then inter-digital space of the feet which ruptured to form ulcer. Concurrently with the oral lesions, lesions appeared on the feet particularly on the clefts and on the coronet/inter-digital space (Figure 2b). In uncomplicated cases, the oral vesicles and ulcers usually healed within 10 days either by gradual replacement of the epithelium or after scab formation (Table 2).

In complicated cases, the course of the disease ranged from 10 to 20 days and most of the cases were complicated with secondary bacterial infection. The clinical pictures resembled very closely to those described by Blood and Radostits (1989) and Samad (1996). However, skin lesions, eye and udder lesions with mastitis (Singh *et al.*, 1979), atypical lesions and allergic reactions (Jana and Mailty, 1997) reported in literatures had not been observed in this study.

The age of the lesion in days of infection is displayed in Table 3. Once the vesicles have ruptured, the rate of healing can be influenced by many factors. Therefore it is generally possible to give an approximate estimation of the age of a lesion. Between day 0 to day 7th, it should be possible to date them accurately within a margin of 1st day, but after this period the ability to date them precisely decreases. It is important to bear in mind that ageing of

lesions can only be approximate as other factors such as secondary infection, can alter the rate at which the lesions heal.

Histological changes at different stage groups

In light microscopic examination by H & E staining of tongue epithelium of the control group (Group D) showed that the tongue was composed of intercalary bundles of striped muscle fibers which were embedded in a bedding substance called lamina propria, made up of areolar tissue with containing blood vessels, nerves and glands. The muscle fibers were disposed in three directions: oblique, transverse and longitudinal. The entire tongue was covered by mucous membrane lined by squamous epithelium (Figure 3) and inside we found connective tissue, skeletal muscle organized in packages with different orientations. Along the ventral surface of the mucous membrane was smooth but along the dorsal surface it was thrown into a number of projections called lingual papillae (Onet and Kelleher, 2005; Ibrahim and Hussin, 2018).

On the other hand, the histological changes at the primary stage (Group A) group showed that, the surface of stratified squamous keratinized epithelium started to degeneration and dissolution, the filiform papillae started to damage but lamina propia and striated muscle bundles were still present rather than the control group (Figure 4). Similarly, the advanced stage (Group B) group showed that a complete necrosis and dissolution of the surface of stratified squamous epithelium and the filiform papillae than the control group. There was no evidence of lamina propia but extensive necrosis and hyperplasia with a dense infiltrate of inflammatory cells. The surface epithelium was showing varying sized circular micro-vesicle (Mohan *et al.,* 2008). The micro-vesicles were consisted of ballooned epithelial cells of stratum spinosum with eosinophilic cytoplasm, pyknotic nucleus and granulocytic infiltration. A complete loss of intercellular bridge of muscle fibers term as acantholysis (Mohan *et al.,* 2008) was also revealed clearly (Figure 5). As well as in higher magnification, vacuolar degeneration in the superficial layers of the epithelium (El-Amir *et al.,* 2014) and reticular keratinocyte degeneration within the hyperplastic epidermis were observed.

The inter-digital lesion usually appeared in the recovery stage that means after 7 to 8 days of the post infection. Histological architecture of a fresh skin tissue of animals presenting all normal structure of the skin layers consists of epidermis, dermis, muscles fibers and adipose tissue (Ibrahim and Hussin, 2018). On contrast, the histological changes at the recovery stage (Group C) group showed necrosis of muscle fiber's bundles, loss of striation of intercalated bundles of striped-muscle fibers and dermal necrosis accompanied by an intense neutrophil infiltrate at the recovery stage (Group C) group than other respective groups (Figure 6).

In conclusion, the results of the present study can be concluded that clinical pictures among the FMD affected animals showed significant increase in the mean values of temperature, pulse rate and respiratory rate ($p \leq 0.05$) with severe vesicular erosion in mouth at the advanced stage in comparison with the control group which subsequently reduced after passing the days of infection followed by the recovery stage. As well as, the margins of lesion and red raw appearance were more progressive which followed by extensive fibrin infilling with early granulation and fibrous tissue proliferation that lead to the healing of vesicles at recovery stage.

Table.1 Physio-clinical (Temperature, Respiratory and Pulse rate) examination in the FMD at primary, advanced, recovery and normal control groups

Groups	Primary		Advanced					Recovery							Control
DPI	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Temperature (°C)	38.6	41.1	41.9	41.3	39.5	38.4	38.1	38.6	37.8	37.2	38	37.5	37.5	-	39.5
Respiratory rate (%)	26.3	26.8	28.8	24.7	23.7	26.4	25.9	23.7	24.6	23.4	21.2	22.6	26.3	-	20-25
Pulse rate (%)	78	85.2	84.7	82.7	81.4	79.7	76.6	76	77.2	78.4	77.4	76.8	76.2	-	60-70
Level of Sig.	NS		*					NS							-

Results are Mean ± SE (Standard Error) of 5 cattle in each group. One-way analysis of variance (ANOVA) followed by post hoc Duncan's test was performed as the test of significance. The difference was considered to be significant when ** $p \leq 0.01$, * $p \leq 0.05$ compared to FMD control group.
NS = Not significant

Table.2 The clinical signs and symptoms followed at the different stages

Stages	Clinical signs and symptoms
Primary stage (1-2 days of post infection)	Excessive slobbering and smacking of lips Profuse salivation Depression, dullness and anorexia Nasal discharge Raised temperature (38.6-41.1°C)
Advanced stage (3-7 days of post infection)	Lethargy Shivering and fever Blisters on tongue Reduced production Vesicles and erosion of tongue epithelium Tender and sore feet
Recovery stage (8-13 days of post infection)	Sore and blisters on feet (coronary band) Dyspnea Hoof deformation Un-thriftiness Weight loss Lameness Coronary band lesion first appears blanched Blisters and erosions form between the digits Stamping

Fig.1 Sample collection from tongue and inter-digital space

Fig.2 (a) 3 days-old ruptured vesicle in tongue with sharp margins of lesion and red raw (arrow) appearance; **(b)** 10 days-old ruptured vesicle (arrow) in the inter-digital space.

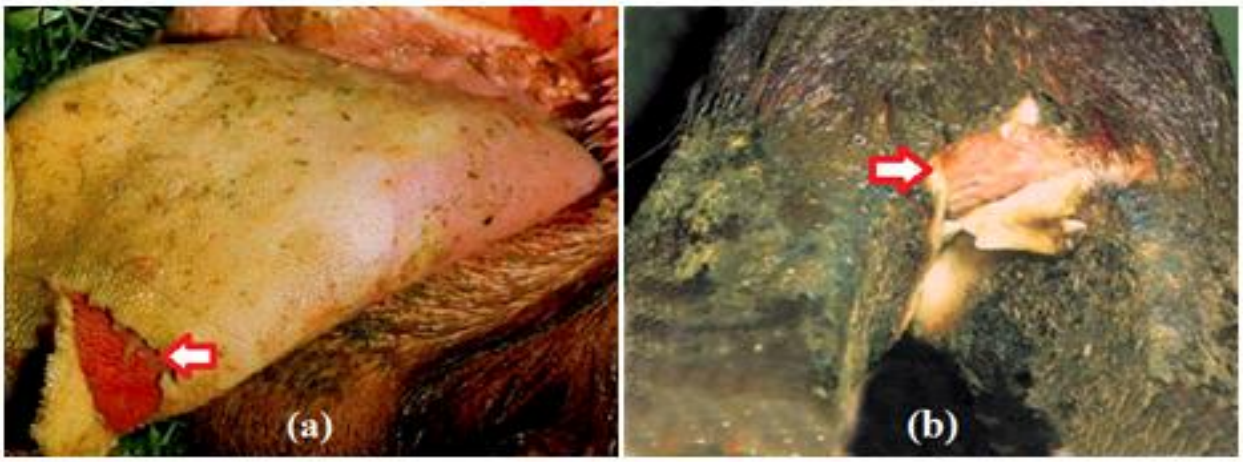


Fig.3 Histological architecture of tongue in control group (Group D) presenting all normal striated muscle bundles (M), lamina propia (LP), filiform papillae (FP), stratified squamous keratinized epithelium (SSKE).

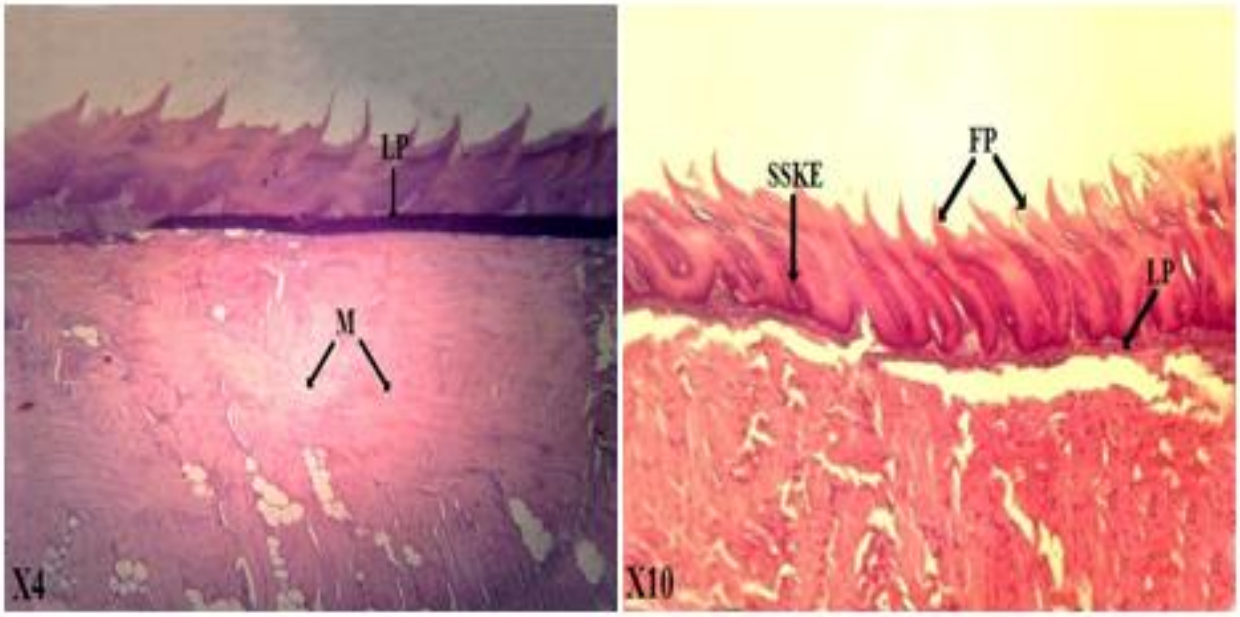


Fig.4 Histological architecture of tongue at primary stage (Group A) group showing damaged and degenerated filiform papillae (FP), stratified squamous keratinized epithelium (SSKE) and lamina propia (LP).

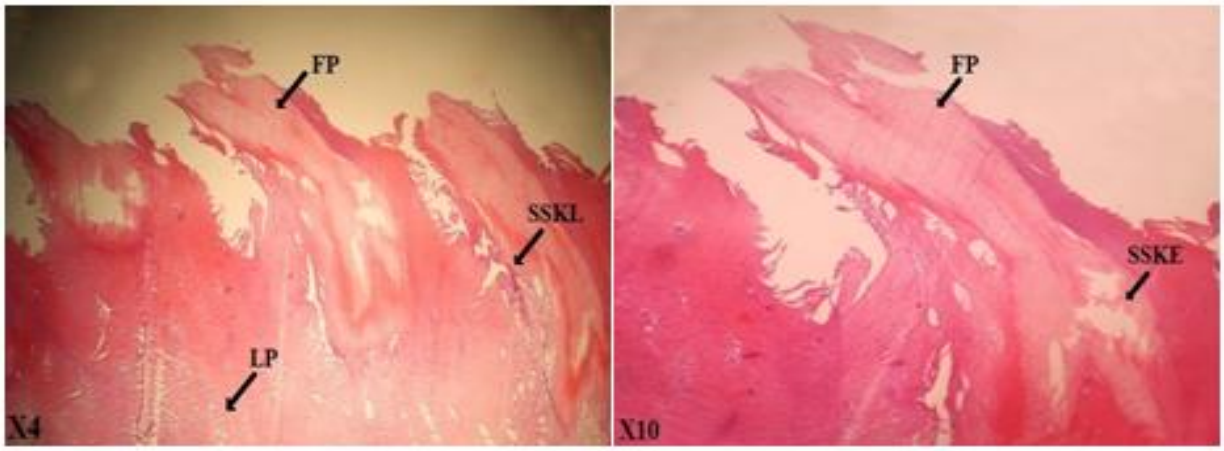


Table.3 Clinical appearances of lesion with successive days of infection

Day of clinical disease	Appearance of lesion
Day 1	Blanching of epithelium followed by formation of fluid filled vesicle
Day 2	Freshly ruptured vesicles characterised by raw epithelium, a clear edge to the lesion and no deposition of fibrin
Day 3	Lesions started to lose their sharp demarcation and bright red colour. Deposition of fibrin was started to occur.
Day 4	Considerable fibrin deposition had occurred and regrowth of epithelium was evidenced at the periphery of the lesion.
Day 7	Extensive scar tissue formation and healing had occurred. Some fibrin deposition was usually still present.
Day 8-14	Animals recovered with in 2 weeks.

Fig.5 Histological architecture of tongue at advanced stage (Group B) group showing dissolution of surface keratin layer (KL), complete necrosis in the stratified squamous epithelium (N); complete loss of filiform papillae (FP) with varying sized circular micro-vesicle (MV); which consisting of ballooned epithelial cells with eosinophilic cytoplasm (BE); vacuolar degeneration (VD); acantholysis condition (A); Pyknotic nuclei and granulocytic infiltration (PN).

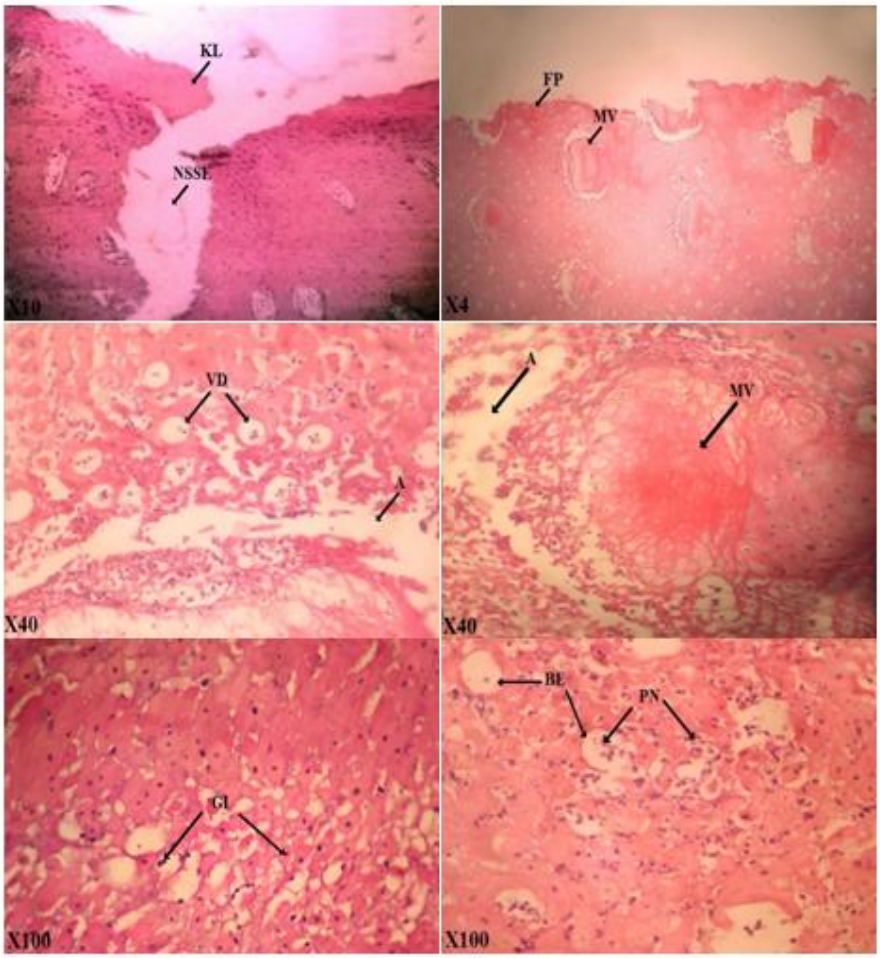
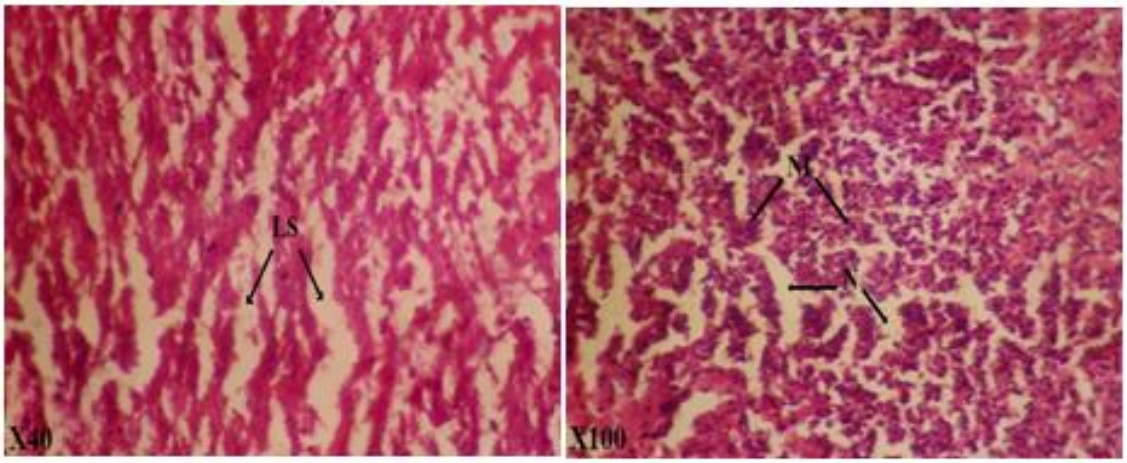


Fig.6 Histological architecture of inter-digital (skin) space at recovery stage (Group D) group showing necrosis of muscle's bundles (N) and loss of striation of intercalated bundles of striped-muscle fibers (LS); Excessive neutrophil infiltration (NI).



Histologically at advanced stage in tongue, ballooned epithelial cells of stratum spinosum were showing eosinophilic cytoplasm, acantholysis, pyknotic nucleus with granulocyte infiltration which coalescence to formed grossly visible macrovesicle (bullae). Furthermore, vacuolar and reticular keratinocyte degeneration within the hyperplastic epidermis in the superficial layers of the epithelium in higher magnification was more conspicuous. But the dermal (inter-digital space) lesions microscopically showed necrosis of muscle fiber's bundles and loss of striation of intercalated bundles of striped-muscle fibers with intense neutrophil infiltrate only at the recovery stage.

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