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RESEARCH ARTICLE

Hematological and Biochemical Evaluation of Small Ruminants Naturally Infected with ORF Virus in Punjab Province, Pakistan

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ABSTRACT

The ORF virus causes contagious ecthyma in sheep and goats, which is an economically significant viral skin disease. The goal of this research was to observe the influence of ORFV on small ruminants' hematological parameters as well as serum biochemical profiles across various districts of Punjab, Pakistan. In 58/441 animals, ORFV was confirmed by molecular analysis. ORFV lineages were classified into five separate groups based on phylogenetic analysis of the GIF/IL2 gene of Parapoxviruses. ORFV sequences from Pakistan isolates were grouped with viruses from China and India in Cluster I. In ORFV infected animals, hematological tests manifested mild to moderate leukocytosis, granulocytosis, severe neutrophilia and severe lymphopenia. Eosinophils, basophils, monocytes, thrombocytes, red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were not influenced statistically ($P>0.05$). Infected animals had significantly higher ($P<0.05$) alanine transaminase (ALT) and albumin to globulin (A/G) ratios when serum biochemical parameters were assessed. Total protein, albumin, globulin, creatinine and urea all indicated a significant decline ($P<0.05$) in the analysis of data. Bilirubin, aspartate aminotransferase (AST), and alkaline phosphatase (ALP), on the other hand, did not differ significantly ($P>0.05$). On the lateral commissure of the lips, muzzle, and nostrils of ORFV infected animals, diffused papular and pustular lesions as well as scab formation were visible. Microscopic investigations revealed acanthosis, parakeratosis with micro abscess zones, hyperkeratosis, spongiosis, epithelial cell degeneration, necrotic foci with patches of inflammatory cell aggregation and intracytoplasmic eosinophilic inclusion body in keratinocytes. It may be inferred that the ORFV had a considerable impact on sheep and goats hematological and serum biochemical parameters. The findings helped in the comprehension of disease mechanisms and controlling the infection.

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INTRODUCTION

Contagious ecthyma (CE) is a viral cutaneous disease that affects small ruminants and is found all over the world. ORF virus (ORFV), a double-stranded DNA virus of the genus *Parapoxvirus* (PPV) and family *Poxviridae*, is the etiological agent of CE. Bovine papular stomatitis virus (BPSV), pseudo cowpox virus (PCPV) and parapoxvirus of red deer in New Zealand (PVNZ) are all members of this family. ORFV is distinguishable from other *Poxviridae* morphologically by its epitheliotropic properties and tiny oval form (Zhang *et al.*, 2014; Zhou *et al.*, 2022).

Contagious ecthyma is also known as pustular dermatitis, scabby mouth as well as sore mouth (Kazemi Asl *et al.*, 2018). It is identified by vesiculo-proliferative skin lesions that are mainly restricted to the lips, nostrils, mouth, udder and feet of affected animals (Tedla *et al.*,

2018). The ORFV primarily affects sheep and goats but it has also been recorded in other animals such as Japanese serows, musk ox, camels and reindeer (Özmen and Hüseyin, 2018). Contagious ecthyma has a high morbidity rate and a low fatality rate (5-10%), with a 1-2 month incubation period (Venkatesan *et al.*, 2014) The ORFV is difficult to diagnose based on clinical indications as other conditions including foot and mouth disease (FMD), bluetongue and PPR have similar clinical symptoms (Kumar *et al.*, 2014). Conventional polymerase chain reactions (PCR) based on the B2L and GIF/IL-2 genes amplification are frequently employed to identify ORFV in clinical samples (Nandi *et al.*, 2011).

The ORFV is endemic in different geographical locations of Bangladesh, India, China and Iran (Azad *et al.*, 2016; Karki *et al.*, 2019; Zhong *et al.*, 2019). However, there is a dearth of information in Pakistan about ORFV's origins and clinical characteristics. As a result, the current investigation was carried out to determine (i) the molecular characteristics and phylogenetic analysis of the ORFV GIF/IL-2 gene, and (ii) to demonstrate the pathological indices including hematology, serum biochemistry profile and histopathological alterations induced in infected animals. The calculation and assessment of the hematological and biochemical profiles might help in both understanding the pathogenesis and controlling the disease.

MATERIALS AND METHODS

Sample collections: Between July 2020 to July 2021, sheep and goats with probable diagnostic lesions had been chosen for collecting blood as well as cutaneous tissue samples in 14 districts of Punjab province (goats, total no=350; sheep, total no=91) (Table 1). Animals with nodular and diffuse proliferative cutaneous lesions were evaluated for sampling. Skin tissue sections with nodular lesions were collected in a sterile polystyrene vial for PCR and in 10 percent neutral buffered formalin solution for microscopic investigations. All the investigations were permitted by the UVAS Lahore ethical review committee (Permit Number: DR/452 dated 06/10/2020).

Virus genome extraction: The ORFV genome was retrieved following manufacturer's instructions using a commercial DNA extraction kit (GeneJET Genomic DNA Purification Kit having Catalog Number K0722).

Polymerase chain reaction (PCR): ORFV's GIF/IL-2 was successfully amplified by applying forward (5'-GCTCTAGGAAAGATGGCGTG-3') and reverse (5'-GTACTCCTGGCTGAAGAGCG-3') primers as previously described by Kumar *et al.* (2014). The PCR reaction was carried out with a total volume of 40µL of reaction mixture containing 20µL of master mix (PrimeSTAR Max DNA polymerase, Catalog Number: R045A), 2 µL of DNA template, 2µL of each forward and reverse primers and 14µL of PCR grade water. Preliminary denaturation (95°C, 5 min) followed by 35 cycles of denaturation (94°C, 30 sec), annealing (58°C, 30 sec) and extension (72°C, 45 sec) accompanied by a final extension (72°C, 7 min) were reaction settings used in the PCR procedure. Amplicons had been seen on a 1.5 percent agarose gel as well as stained with GelRedTM Nucleic Acid Gel Stain during gel electrophoresis (110V, 230mA, 30 min) before being noted in a gel documentation system (Bio Rad Laboratories, USA).

Sequencing and phylogenetic analysis: Polymerase Chain Reaction Purification Kit (GeneJET) was used to purify the product of PCR (Thermo Fischer Scientific having Catalog Number K0702). For sequencing procedure, the specimens were sent to Comate Bioscience Co., Ltd. in China (Sanger method). Applying NCBI and BLAST, the ORFV sequencing was performed and matched to ORFV sequences obtained from the GenBank Database. The phylogenetic tree had been built by using the Neighbor-Joining method with 1,000 bootstrap replicates on MEGA Software version 6 utilizing inferred nucleotide sequences of parapoxviruses (Kumar *et al.*, 2014).

Hematology and serum biochemistry profile: Hematological parameters (Table 2) were measured through an automatic hematological analyzer (MS4s MELET Compteur Analyser, France). The serum biochemical parameters (Table 3) were evaluated using commercial kits on an auto-analyzer (Microlab 300, semi-automated clinical chemistry analyzer).

Gross lesions and histopathology: Gross lesions and histological examinations were recorded after confirmation of suspected cases through PCR. Cutaneous surfaces of the infected animals including the buccal commissure, lips, muzzles and noses were thoroughly examined for pathological lesions. The skin scrapings were also preserved in a 10 percent neutral buffered formalin (NBF) solution for microscopic examination. The tissue sections were dissected and hydrated overnight in tap water, dehydrated using ethanol by order of increasing concentration, paraffin embedding and subsequently sectioned (4 µm) with a microtome (SLEE, Germany). The tissue sections were stained (with hematoxylin & eosin stain) and studied using an Olympus (Japan-made) light microscope (Anjum *et al.*, 2020).

Statistical analysis: All statistical analyses were carried out using GraphPad Prism® version 8.0. The t-test was conducted to compare means of hematological and serum biochemical parameters. The differences were declared significant when the probabilities of equality and p values were less than 0.05 ($P<0.05$).

RESULTS

ORFV isolation and identification: In 58/441 animals, amplification of the partial GIF/IL-2 gene (408 kb) verified the ORFV (51 goats, 7 sheep). The specifics of isolated specimens taken from various districts are shown in (Table 1). Variable nucleotide sequences (total=12) were reported to the NCBI GenBank Database and can be retrieved under the following accession numbers: MZ054280 (Dera Ghazi Khan/7/Goat/2020), MZ054282 (Muzaffargarh/12/Goat/2020), MZ054283 (Muzaffargarh/13/Goat/2020), MZ054284 (Muzaffargarh/15/Goat/2020), MZ054287 (Lahore/24/Goat/2020), MZ054289 (Lodhran/2/Goat/

Table 1: The positive percentage of ORFV cases in different districts of Punjab, Pakistan confirmed positive through PCR

Different Districts	Goats		Sheep	
	Positive animals/Total animals	Positive Percentage	Positive animals /Total animals	Positive Percentage
Bahawalnagar	0/32	0.00	0/5	0.00
Bahawalpur	0/39	0.00	0/5	0.00
Bhakkar	0/26	0.00	0/0	0.00
Dera Ghazi Khan	12/29	41.38	0/9	0.00
Jhang	0/18	0.00	0/6	0.00
Kasur	0/17	0.00	0/2	0.00
Khushab	0/22	0.00	0/7	0.00
Lahore	17/35	48.57	0/4	0.00
Layyah	0/15	0.00	0/3	0.00
Lodhran	14/33	42.42	0/8	0.00
Multan	8/28	28.57	0/8	0.00
Muzaffargarh	0/25	0.00	7/13	53.85
Rahim Yar Khan	0/13	0.00	0/14	0.00
Rajan Pur	0/18	0.00	0/7	0.00
Total	51/350	14.57	7/91	7.71

Table 2: Comparison of hematological values of normal and ORFV infected sheep and goats

Parameters	Goat		P-value (P<0.05)	Sheep		P-value (P<0.05)
	Infected	Normal		Infected	Normal	
WBC	16.40±2.03^{b}	9.21±2.53^{a}	<0.0001	16.69±1.55^{b}	7.64±2.44^{a}	<0.0001
Lymphocyte	36.20±6.25^{a}	61.30±7.86^{b}	<0.0001	38.12±6.24^{a}	62.80±10.19^{b}	<0.0001
Monocyte	3.11±0.67	3.18±0.46	0.5704	3.12±0.69	3.11±0.45	0.9375
Granulocyte	60.70±6.36^{b}	43.10±6.87^{a}	<0.0001	58.70±6.11^{b}	34.94±12.41^{a}	<0.0001
Neutrophil	57.40±6.43^{b}	28.20±9.16^{a}	<0.0001	55.32±6.16^{b}	30.19±9.03^{a}	<0.0001
Eosinophil	2.93±0.75	2.97±0.22	0.8117	2.95±0.74	2.89±0.30	0.6622
Basophil	0.49±0.19	0.59±0.21	0.5024	0.40±0.49	0.47±0.46	0.3367
RBC	11.60±3.16	12.40±3.15	0.2791	12.16±3.26	12.35±1.97	0.7708
MCV	18.80±4.56	20.10±2.56	0.1618	19.98±4.16	20.10±2.71	0.1072
HCT	20.80±3.67^{a}	30.80±5.06^{b}	<0.0001	20.89±3.75^{a}	31.52±3.45^{b}	<0.0001
MCH	6.92±2.06	6.43±0.75	0.2374	6.74±1.94	6.69±1.22	0.8668
MCHC	37.10±7.91	35.50±4.05	0.3156	37.79±7.69	35.31±3.75	0.328
Hb	7.55±1.25^{a}	10.10±1.39^{b}	<0.0001	7.72±1.21^{a}	10.05±0.56^{b}	<0.0001
Thrombocyte	393.23±122.69	406.64±111.32	0.5956	397.11±125.28	441.82±113.45	0.1061

Within a row, values with different superscripts ^{a,b} were different significantly (P<0.05).

Table 3: Comparison of serum biochemistry values of normal and ORFV infected sheep and goats

Parameters	Goat		P-value	Sheep		P-value (P<0.05)
	Infected	Normal	(P<0.05)	Infected	Normal	
Bilirubin	0.075±0.040	0.078±0.030	0.3528	0.072±0.015	0.074±0.018	0.4526
ALT	27.54±4.48^{b}	11.76±3.44^{a}	<0.0001	38.48±3.01^{b}	29.95±2.14^{a}	<0.0001
AST	345.70±40.85	338.90±109.21	0.4688	354.48±42.79	347.20±95.51	0.5856
ALP	256.66±141.46	248.98±68.88	0.5236	235.27±80.19	228.31±80.57	0.6813
T.P	5.29±0.19^{a}	6.77±0.17^{b}	<0.0001	5.12±0.37^{a}	6.80±0.56^{b}	<0.0001
ALB	3.16±0.28^{a}	3.40±0.35^{b}	<0.0001	3.03±0.19^{a}	3.42±0.14^{b}	<0.0001
GLB	2.13±0.19^{a}	3.46±0.33^{b}	<0.0001	2.08±0.30^{a}	4.65±0.67^{b}	<0.0001
A/G	1.50±0.26^{b}	1.28±0.39^{a}	0.0001	1.50±0.34^{b}	1.30±0.37^{a}	0.0097
Creatinine	0.73±0.13^{a}	1.35±0.22^{b}	<0.0001	0.75±0.12^{a}	1.59±0.22^{b}	<0.0001
Urea	12.16±1.29^{a}	15.56±2.82^{b}	<0.0001	12.06±1.29^{a}	14.57±3.72^{b}	<0.0001

Within a row, values with different superscripts ^{a,b} were different significantly (P<0.05).

2020), MZ054290 (Lodhran/3/Goat/2020), MZ054294 (Muzaffargarh/11/Goat/2020), MZ054295 (Muzaffargarh/ 14/Goat/ 2020), MZ054296 (Muzaffargarh/16/Goat/2020), MZ054298 (Multan/21/Goat/2020), MZ054300 (Lahore/25/Goat/2020).

Phylogenetic analysis: ORFV lineages had been generally categorized into five separate groups based on phylogenetic studies of GIF/IL2 gene sequences from 54 Parapoxviruses (PPVs) (Fig. 1). ORFV sequences recovered from goats across Pakistan, China, as well as India comprised Cluster I. ORFV sequences found throughout sheep through China, India, but also Iraq were grouped into Clusters II, III, and V, respectively. ORFV isolates from musk oxen as well as mountain goats had been assigned to Cluster IV.

Gross lesions: ORFV infected animals had extensive, diffusive and excretive papular lesions on lips, muzzle and nostrils (Fig. 2a, 2b). Also, pustular lesions with fibrin deposits were evident at the mouth commeasure and the upper and lower lips of infected goats (Fig. 2c). In addition, scab formation was observed on lateral commissure of lips and nostrils in goats (Fig. 2d, 2e). Furthermore, one animal exhibited papule formation on the scrotum (Fig. 2f).

Hematological examinations: Hematological parameters of ORFV infected goats and sheep are given in Table 2. The WBCs ranged to 16.40±2.03 in goats with P<0.01. The neutrophils count was significantly increased with P<0.001 in infected goats having values 57.40±6.43. The lymphocytes count decreased significantly with values of 36.20±6.25 with P<0.0001). The values of hemoglobin concentration (Hb) were (7.55±1.25 P<0.0001) and hematocrit percentage (HCT) (20.80±3.67; P<0.0001) were significantly decreased than normal value in ORFV infected goats. Meanwhile, the monocytes, eosinophils, basophils, RBCs, MCV, MCH, MCHC and thrombocytes were found normal in ORFV infected goats.

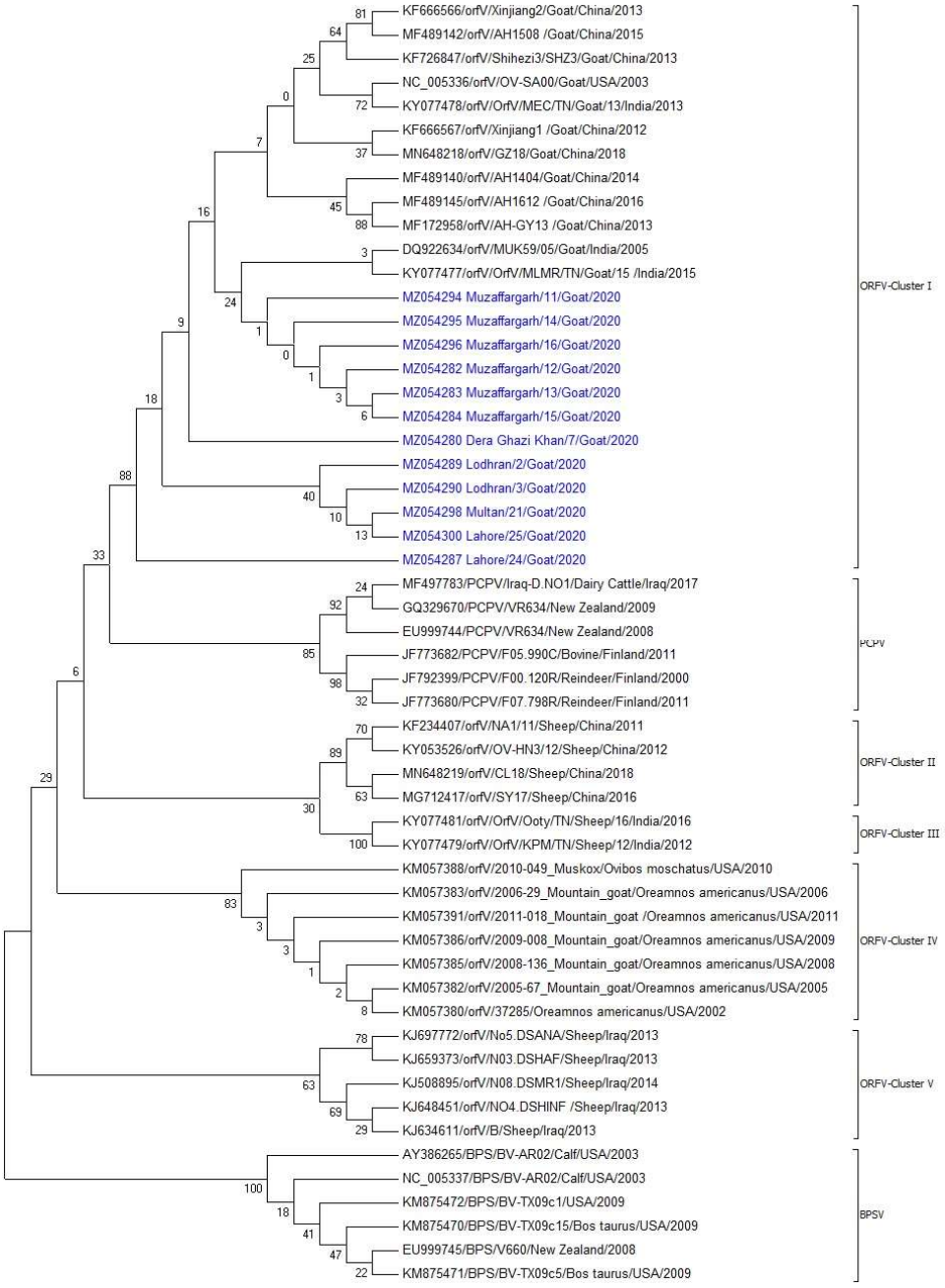

Fig. 1: Phylogenetic analysis of different parapoxviruses based on nucleotide sequences of the GIF/IL-2 gene

The total numbers of WBCs in sheep were significantly increased with the values of 16.69±1.55; P<0.01 while neutrophils count was also increased (30.19 ±9.03; P<0.001) in ORF affected sheep. In comparison neutrophils count in affected goats was slightly higher than contagious ecthyma infected sheep. Meanwhile, the lymphocytes count (38.12±6.24; P<0.0001) was lowered in affected sheep. HCT (20.89±3.75; P<0.0001) was lowered in affected sheep along with the significantly lowered values of Hb levels as compared to non-affected sheep (7.72±1.21; P<0.0001). However, the monocytes, esinophils, basophils, RBCs, MCV, MCH, MCHC and thrombocytes were found normal in ORFV infected sheep.

Serum biochemistry profile: Overall the ALT level was significantly higher in infected goats (27.54±4.48; P<0.001) and sheep (38.48±3.01; P<0.0001), as shown in Table 2. The representative values of total protein, albumin, globulin level, serum creatinine, and urea level were found significantly lower respectively (5.29±0.19, 3.16±0.28, 2.13±0.19, 0.73±0.13, 12.16±1.29; P<0.0001). Similarly, in sheep infected with ORF, the values of total protein, albumin, globulin level, serum creatinine, and urea level were also found significantly lower respectively (5.12±0.37, 3.03±0.19, 2.08±0.30, 1.59±0.22, 14.57±3.72; P<0.0001). The serum bilirubin, AST and ALP were in normal range in ORFV positive goats and sheep.

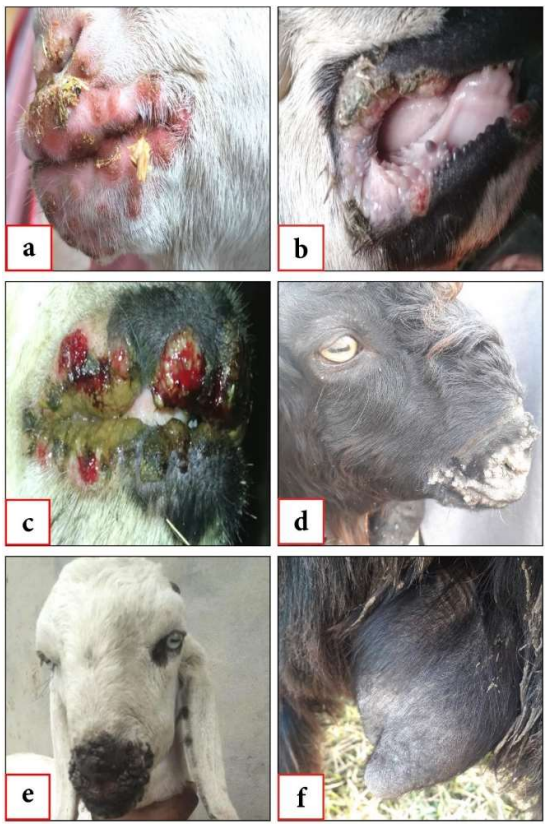

Fig. 2: Gross lesions of ORFV infected animals. a & b): Papular lesions on lips, muzzle and nostrils of goats. c): Pustular lesions with fibrin deposits at the mouth commeasure and the upper and lower lips of infected goats. d & e): Scab formation on lateral commissure of lips and nostrils in goats. f): Papule formation on the scrotum of buck.

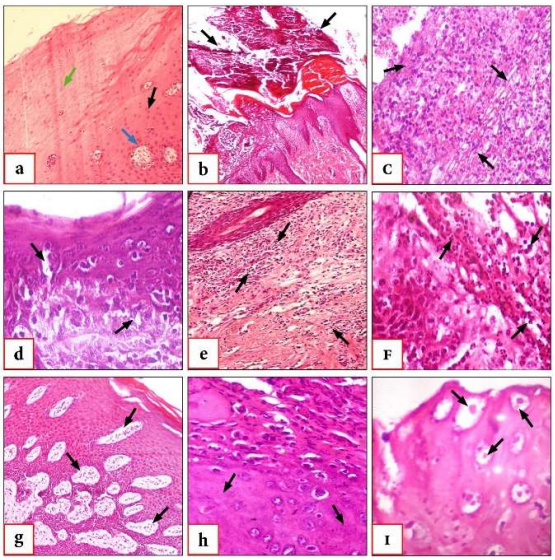

Fig. 3: Photomicrographs of skin lesions of ORFV infected animals. a): Epidermal hyperplasia/ acanthosis (black arrow) and parakeratosis (green arrow) along with 4-5 zones of microabscesses (blue arrow) (H & E stain, 10X). b): Scab formation (H & E stain, 4X). c): Hyperkeratotic epidermis along with threads of fibrin (H & E stain, 10X). d): Epithelium degeneration and ballooning with prominent spongiosis (H & E stain, 40X). e): Inflammatory cells infiltration around the adnexa (H & E stain, 10X). f): Inflammatory cells infiltration (H & E stain, 40X). g): Necrotic foci (black arrows) (H & E stain, 10X). h): Intercellular edema (H & E stain, 40X). i): Intracytoplasmic eosinophilic inclusion body along with the division of keratinocytes (H & E stain, 40X).

Microscopic lesions: Histopathological investigations disclosed epidermal hyperplasia (acanthosis) in addition to parakeratosis as well as 4-5 zones of microabscesses (Fig. 3a). On the upper surface of the hyperkeratotic epidermis, a thick scab with threads of fibrin was formed (Figs. 3b, 3c). In some areas, epithelium degeneration and ballooning with prominent intracellular clear spaces (spongiosis) were evident (Fig. 3d). The dermal portion was markedly infiltrated by inflammatory cells around the adnexa (Figs. 3e, 3f). Typical necrotic foci (Fig. 3g), intercellular edema (Fig. 3h) and intracytoplasmic eosinophilic inclusion body along with the division of keratinocytes were seen (Fig. 3i).

DISCUSSION

Contagious ecthyma is an epitheliotropic viral illness that produces papule, pustule, vesicle and scab formation in sheep, goats, in addition to wild ruminants. The disease is included in the World Organization for Animal Health (OIE) list of notifiable terrestrial animal diseases. The GM CSF/IL2 protein is encoded by the GIF gene (Karki *et al.*, 2019) and the ORFV was detected utilizing molecular techniques by using the GIF gene. The main goal of this study was to learn more about the pathological findings of ORFV prevalent in Punjab, Pakistan. ORFV is generally believed to be a non-systemic infection; however, the current investigation detected considerable variation in hematological and biochemical variables. Twelve isolates of ORFV were effectively isolated and identified from clinically infected animals in 14 Punjab districts in the current investigation.

The parapoxviruses were classified into three categories focusing on gene sequences of GIF/IL2: ORFV, PCPV as well as BPSV demonstrating a tight evolutionary relationship between ORFV and PCPV contrasted to BPSV. In addition, ORFV lineages had been divided into five different clusters. The findings of current research are in accordance with previous studies (Chan *et al.*, 2007; Oem *et al.*, 2009; Zhao *et al.*, 2010; Friederichs *et al.*, 2014; Khalafalla *et al.*, 2020) Identified isolates were grouped in a monophyletic group (Cluster I) of goat originating Asian ORF Virus strains after molecular analysis of the GIF gene. Pakistani isolates were also found in cluster I with Chinese (AH1404, Xinjiang1, AH1612, AH-GY13, Shihezi3, Xinjiang2, AH1508, GZ18) as well as Indian (MUK59/05, MLMR/TN, MEC/TN) isolates. Clusters II, III, and V, on the other hand, contain sheep-originated ORFV isolates from China, India and Iraq respectively (Zhao *et al.*, 2010; Li *et al.*, 2013; Kumar *et al.*, 2014).

The ORFV significantly affected the hematological parameters of infected sheep and goats in current study. WBCs and neutrophils were at higher level than normal value, which indicated severe ORFV infection (Kumar *et al.*, 2015). However, lymphocytes, HCT and Hb level were lower in both sheep and goats in current study. Our hematological results were comparable with the finding of previous studies (Kazemi Asl *et al.*, 2018), where they found that hematological parameters were seriously affected in ORFV-affected small ruminants. One possible reason for hematological changes in infected animals could be inflammatory response by animals to the ORFV

or secondary bacterial infection (Tryland *et al.*, 2018). Tedla *et al.* (2018) observed that ORFV affected animals were in stress condition due to painful lesions on lips and muzzle. Arvind *et al.* (2010) also reported similar findings in tick borne infected animals, which is affected by other parapoxvirus.

In the current study, the ALT was at significantly higher level, while serum creatinine, total protein and globulin level was lower than normal level in ORFV infected goats and sheep. Narnaware *et al.* (2015) obtained similar results revealing substantially higher ($p<0.05$) ALT readings in CE infected camels in comparison to unaffected affected camels. Kumar *et al.* (2015) reported that AST level has increased in ORFV affected camels which contradict our findings. The liver contains the maximum level of ALT but AST could also be detected in the muscle, heart, brain, kidney, pancreas and lungs (Agrawal *et al.*, 2016). Most likely, starvation during the illness was the cause of the decreased values of creatinine and total protein in this study. The pretentious animals experience trouble feeding due to the excruciating pain induced by lesions in oral cavity and lips (Maganga *et al.*, 2016).

Concerning the gross pathology, the infected animals had the typical multifocal to coalescing ulcerated lesions on the epidermis of gums, lips, mouth commeasure, muzzles, nose and udder. Also, infected animals had severe proliferative papules, pustules, and crust formation. Affected animals frequently developed severe progressive papules, pustules, as well as crusts. Certain animals on the other hand exhibited the progressive stage in the form of thicker, brownish, rapidly accumulative scabs along with granulation. The gross lesions identified in the current study were similar to those seen in contagious ecthyma virus infected sheep and goats in past research (Kinley *et al.*, 2013; Al Saad *et al.*, 2017; Aneed and Alsaad, 2019; Milovanović *et al.*, 2019).

ORF lesions are reported to undergo the stages of macule, papule followed by vesicle, pustule, scab formation as well as final resolution. ORF pustules emerge during a few days, preceded by ulcers in addition to a hard scab development (3 to 4 weeks), without any scar formation (De La Concha-Bermejillo *et al.*, 2003; Abbas and Mughal, 2014).

Acanthosis was diagnosed by histopathological investigations, which demonstrated epidermal hyperplasia accompanied hyperkeratosis and parakeratosis, as well as considerable epidermal thickness. The disintegration of epithelium is substituted with multifocal to coalescing necrotizing sero-cellular scab in epidermal degeneration. Degenerative alterations in spinous cells of the stratum spinosum included spongiosis as well as vacuolation along with pyknotic and karyorrhectic nuclei. The creation of a scab and a micro abscess in the epidermis was also evident. Keratinocytes also had typical intraepithelial ballooning degeneration as well as eosinophilic intracytoplasmic inclusion bodies. In addition, infiltration by inflammatory cells and intracellular edema extended the superficial dermis. Microscopic lesions of the vesicular stage include significant epidermal hyperplasia, ballooning degeneration as well as eosinophilic intracytoplasmic inclusion bodies inside keratinocytes (Sanchez *et al.*, 1985; Barraviera, 2005; Tahir *et al.*, 2014; Turan *et al.*, 2013; Vellucci *et al.*, 2020).

Conclusions: The findings of this study indicated that ORFV altered the hematological and serum biochemical parameters of infected sheep and goats in addition to producing the histological lesions all over lips, muzzle and nostrils. Weight loss, malnutrition, induced disease distress and pathological abnormalities (inflammation and secondary bacterial infection) may be associated with alterations affecting hematological and biochemical indices in ORFV infection. The ORFV strains in Pakistan are genetically diverse, yet these are genetically comparable to ORFV strains found in adjacent nations (China and India). This phylogenetic analysis in this study illustrated twelve ORFV strains exist in Pakistan, however further exploration in this regard is highly recommended.

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Authors contribution: The study was planned and designed by MUR, IH, AA, MUR and AA. The experiments were carried out by AA and IH. The manuscript was written by AA, IH and MUR. The manuscript was reviewed by IH, AA, and MUR. The study was evaluated as well as supervised by MUR. The paper has been read and endorsed by all authors.

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