

STUDY OF CONTAGIOUS ECTHYMA IN LAMBS OF BASRAH PROVINCE , IRAQ

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ABSTRACT

Contagious ecthyma have been detected and diagnosed in local lamb breeds 3-6 months old of both sexes . The study was conducted to examine 941 local lamb breeds in Basrah, province , Iraq represents eleven (11) flock groups. One hundred (100) local lamb breeds shows different clinical manifestations belong to contagious ecthyma. Twenty five (25) clinically healthy local lamb breeds considered as controls. Diseased lambs show different clinical manifestations such as Anorexia, depression and dullness , Unable to sucking or graze , However, Orf lesions was seen in the form of papules, pustules, vesicles, and scabs which indicated in all diseased animals, Moreover, Orf lesions distributed around mouth commisure and muzzle , Furthermore, lesions was detected at upper and lower part of the lips , additional, Orf lesions was seen on upper and / or lower eyelids . Fissuring lesions were also detected . Moreover, a slight , lesions were also detected on coronets, ears, anus, and vulva in (6%) of diseased animals. Data concerning clinical examinations of diseased lambs show a significant increase in body temperature, respiratory and heart rate of diseased lambs than in controls. The results of hematological examinations of diseased lambs infected with Orf and controls indicated leucocytosis due to lymphocytosis, Moreover, the rate of erythrocyte sedimentation of red blood cells indicated a significant increase in diseased lambs than in controls. On the other hands, The results of the acute phase response also indicated a significant increase in both Haptoglobin values and Fibrinogen time in diseased lambs compared with controls. The results of PCR on gel electrophoresis show that the ORFV virus has 147 base-pair specific PCR amplicons were detected, In addition, Sequence analysis of submitted *Orf virus*

amplified *GFR gene*. The raw nucleotide sequences of all samples were processed by FinchT.V 1-4 version software for trimming the unwanted sequences and bases with low sequence quality (lower than 20% signal intensity). Furthermore, The evolutionary history was inferred using the Neighbor-Joining method, Since, The optimal tree with the sum of branch length = 0.02625234 is shown. (above the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 12 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 79 positions in the final data set. Evolutionary analyses were conducted in MEGA7 . Results of the histopathological examinations show papillomatus hyperplasia of epidermis as well as a numerous immature hair follicles in the proliferation epidermis and proliferation of sebaceous glands, However, an area of apoptosis of structural molecules were also detected , Furthermore, Masses of areas of proliferative sweat glands with large areas of laminated vacuolated structure and deposit tissue like structures in the dermis was determined, However a dark stained blackish deposit hair follicles with some dilated hair follicles and inflammatory cells were also observed, Moreover, scab like formation above the epidermal cells in the upper epidermal layer was seen microscopically. It have been concluded that contagious ecthyma seems to be an endemic viral disease at Basrah providence , Iraq, reflect high morbidity rate which might resulted in a recoded economic losses. However, Secondary bacterial infection or myiasis of affected parts were always follow resulting in more disease complications, Therefore programmed annual vaccination is advised.

INTRODUCTION

Contagious ecthyma, or orf, is a highly contagious ubiquitous disease of sheep, goats, characterized by maculopapular and proliferative lesions affecting the skin around the mouth, nostrils, interdigital regions, teats, and the oral mucosa, The disease caused by the epitheliotropic Orf virus a member of the genus Parapoxvirus. (1,2). Contagious ecthyma also has been reported in other wild and domestic ruminant and in humans (3,4). It's an important disease and common throughout the world wherever small ruminants are raised as farm animals(2).

Orf, is an old English for rough, usually affects the mucocutaneous junctions of the muzzle and lips, although lesions within the mouth affecting the gums, palate, and tongue can occur, especially in lambs and kids. Less frequently, lesions occur on the eyelids, feet, and teats. Lesions of orf progress from papules to pustules and then to thick crusts (5). The infection in sheep and goats is generally known as orf, contagious ecthyma, infectious labial dermatitis, scabby mouth, contagious pustular dermatitis, and sore mouth (6).

Orf virus is a member of parapox viruses belongs to the family poxviridae. This genus also includes pseudocow pox virus and bovine papular stomatitis virus (6,7). The family Poxviridae is characterized by viruses with linear double stranded deoxyribonucleic acid (DNA) molecule of 130 to 300 kilobase pair (kbp) with a hairpin loop at each end (8). Viruses belonging to this family replicate entirely in the cytoplasm because their viruses contain enzymes that synthesize messenger ribonucleic acid (mRNA).

In sheep and goats, the disease mostly occurs in young animals 3-6 months old, although neonatal lambs and kids aged 10-12 days old can be severely affected as well (9). However, older sheep may also carry the virus without showing lesions and introduce the disease into susceptible flocks (10,2).

Grazing of coarse pastures or stubbles may predispose to infection with scabby mouth as oral abrasions increase the potential for the virus to gain entry (11). Since, The orf virus infects damaged or scarified skin through rough grazing and replicates in regenerating epidermal keratinocytes.

According to World Organization of Animal Health, Orf is a modifiable and zoonotic disease transmitted from animals to humans(12).

Contagious ecthyma was detected and diagnosed in Basrah province , Iraq, therefore, The main aims of the current study are, clinical and hematological studies and explore the main clinical manifestations of the disease showed by diseased sheep with molecular identification of the causative virus via PCR technique, Histopathological study of the orf lesions and Evaluation of acute phase response of diseased animals via evaluation of haptoglobin and fibrinogen .

MATERIALS AND METHODS

Animals and Study design :- The study was conducted to examine 941 local lamb breeds 3-6 months age and from both sexes in Basrah province , Iraq, represents eleven (11) flock groups. One hundred (100) local lambs breeds shows different clinical manifestations belong to contagious ecthyma. Twenty five (25) clinically healthy local lambs bleeds considered as controls. Complete clinical examinations was applied to all animals, However , coprological and blood smear examinations have been done to exclude blood parasitic infection and gastrointestinal parasite infestations use a routine laboratory methods .

Collection of samples and hematology:- Ten milliliters of blood (10 mL) were drained from each animal by jugular vein puncture and from these (2.5) milliliter of blood mixed with EDTA used to determine Total erythrocyte count (TRBc), Hemoglobin concentration (Hb), packed cell volume (PCV), and Total leukocytes count (TLC), (Hematology analyzer, Genex, USA), Moreover differential leukocytes count were done using Giemsa stain blood smears method according to Weiss and Wardrop (13) ,Furthermore erythrocytes sedimentation rate (ESR)were also estimated according to Stevens *et al* (14) .

DNA extraction and Polymerase chain reaction (PCR):-

Viral DNA was extracted from lip scrape tissue samples of clinically infected with lambs with Orf. The samples were collected in sterile containers under aseptic conditions and transported as soon as possible to post graduate laboratory unite at college of Veterinary Medicine ,University of Basrah, Iraq, and stored at -20°C refrigerator until use for viral genomic DNA extraction.

Viral genomic DNA extraction:- Viral genomic DNA was extracted from lip scrape tissue by using (Genomic DNA extraction tissue kit. Geneaid. USA). The PCR technique was used to detect the polymorphisms of the orf gene using forward and reverse primers TCAACTGCGGCTTCTTCAAC) (GCGTTTCGTTTTCGTACTCC) respectively) , The primers were synthesized by(BIONEER, Korea). Amplification reaction was performed using a DNA thermo-cycler, 1.5% agarose gel electrophoresis was stained with ethidium bromide under UV light(15, 16).

Sequence :- The positive PCR products (12 samples) of ORFV were sequenced (BIONEER, Korea).

Estimation of Haptoglobin (Haptoglobin ELISA method)and fibrinogen :-

According to manufacture instructions,(Biotechnology co -china) Serum was used for evaluation of ELISA Haptoglobin. the stop solution changes the color was measured at 450 nm using a spectrophotometer. Moreover, Fibrinogen, was estimated according to manufacture instructions of (Biolabo / France) using, Blood mixed with trisodium citrate using plasma).

Histopathological examinations :-

Histological preparation of the external organs (lips) was done according to Bancroft and Steven,(19). Since, After fixation of specimens by 10% NBF for 5 days, these specimens were washed by tap water for 2-4 hours to remove excess of fixatives and the specimens were transferred into the classical steps of dehydration, clearing, Infiltration and Embedding, cutting and finally staining with Hematoxylin and Eosin.

Statistical analysis:-Data was analyzed and the significant difference between diseased and control group were statistically analyzed using student *t*-test (SPSS) program (18).

RESULTS

Diseased lambs show different clinical manifestations such as Anorexia, depression and dullness (81%), Unable to sucking or graze(77%), However, Orf lesions was seen in the form of papules, pustules, vesicles, and scabs which indicated in all diseased animals, Fig. 1. Orf lesions distributed around mouth commisure and muzzle was found in (76%), Furthermore, Orf lesions was distributed at upper and lower part of the lips (69%), additional, Orf lesions was seen on upper and / or lower eye lids (12%), Fissuring lesions (10%), Fig.2, Moreover, a slight , Orf lesions was also detected on coronets, ears, anus, and vulva in (6%) of diseased animals .Table 1.



Fig. 1: Photograph of a sheep showing different proliferative orf lesions with thick scab.



Fig.2: Fissuring of lips.

Table(1): Clinical manifestations of infected lambs with Orf

<i>Clinical signs</i>	<i>Diseased sheep n=100</i>	<i>%</i>
Anorexia,depression and dulness	81	81%
Unable to sucking or graze	77	77 %
Orf lesions in the form of Papules, pustules, vesicles, and scabs	100	100%
Orf lesions distributed around mouth commesure and muzzle	76	76%
Orf lesions distributed at upper and lower part of the lips	69	69%
Orf lesion on upper and / or lower eye lids	12	12%
Fissuring lesions	10	10%
Orf lesions on coronets, ears, anus, and vulva	6	6%

Data concerning clinical examinations of diseased sheep show a significant ($p<0.05$) increase in body temperature, respiratory and heart rate of diseased animals than in controls Table 2.

Table(2): Body temperature, respiratory and heart rate of diseased lambs infected with Orf and controls

Parameters	Controls n=25	Diseased sheep n=100
Body temperature C°	39.4± 0.08	41.3 ± 1.2**
Respiratory rate/ min	25± 0.75	55.6± 8.2**
Heart rate/ min	75.2±0.74	91.8± 10.21**

*Values are mean ± standard error of mean. ** (P<0.05).*

The results of hematological examinations of diseased lambs infected with Orf and controls indicated leucocytosis due to a significant (p<0.05) increase in the number of total leukocyte count and a significant (p<0.05) increase in the absolute number of lymphocytes, (lymphocytosis), Moreover the rate of erythrocyte sedimentation of red blood cells indicated a significant increase (p<0.05) in diseased sheep than in controls. Table 3.

Table (3): Hematological parameters of diseased lambs infected with Orf and controls

Parameters	Controls n=25	Diseased lambs n=100
RBC ×10 ⁶	7.93±1.46	7.97±0.35
Hb g/dl	13.23 ± 1.77	13.6±0.3
PCV %	32.61 ± 4.64	33.93±1.09
Thrombocytes ×10 ³	9704.57±568.34	9604.42±448.23
TLC ×10 ³	11.43±1.54	14.84±0.75**
Neutrophiles ×10 ³	4.39± 0.16	4.45±0.84
Lymphocytes ×10 ³	5.55± 0.42	8.91±0.35**
Monocytes ×10 ³	0.53± 0.07	0.54±0.06
Esinophiles ×10 ³	0.56 ± 0.13	0.61±0.14
Basophiles	0.08±0.04	0.08±0.02
ESR mm/24hr	6.36± 4.72	18.32± 5.42 **

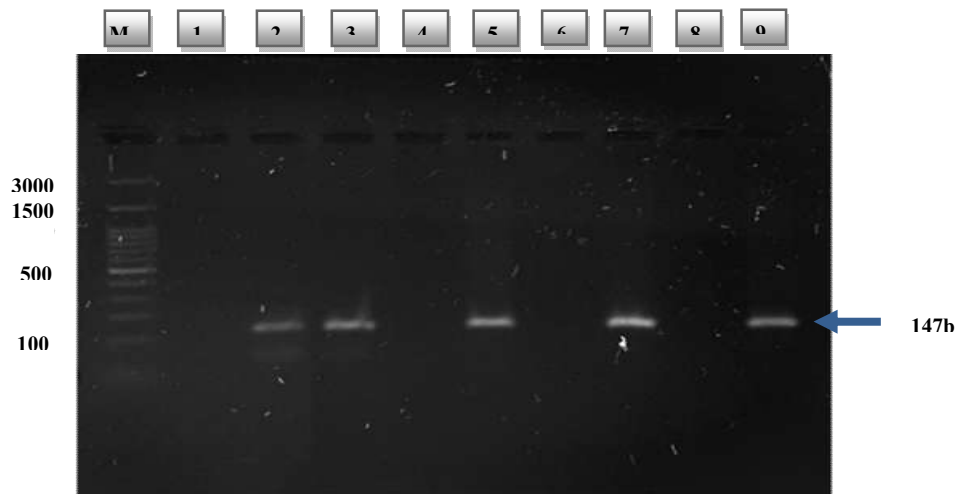
*Values are mean ± standard error of mean. ** (P<0.05).*

The results of the acute phase response also indicated a significant increase (p<0.05) in both Haptoglobin values and Fibrinogen time in diseased lambs compare with controls .Table 4.

Table (4): Haptoglobin values and Fibrinogen time of diseased lambs infected with Orf and controls

Parameters	Controls n = 25	Diseased lambs n = 100
Haptoglobin g/dl	0.024 ± 0.011	0.035± 0.007**
Fibrinogen time / Sec	27.18 ± 8.31	35.65± 7.53**

The results of PCR on gel electrophoresis show that the ORFV virus has 147 base-pair specific PCR amplicons were detected (Fig.3).



PCR product of Orf virus GIF gene (1.5%) agarose gel, M: ladder, lane 2, 3, 5, 7 and 9 positive results ; 1,4,6: negative results; 8, negative control

Sequence analysis of submitted *Orf virus* amplified *GFR gene* was conducted by BLAST alignment tool (Blastn) accessed through <https://blast.ncbi.nlm.nih.gov/Blast.cgi>, the homologues species/isolates were chosen by highest identity percentage with best query cover and lowest E-value. The raw nucleotide sequences of all samples were processed by FinchT.V 1-4 version software for trimming the unwanted sequences and bases with low sequence quality (lower than 20% signal intensity). The identity percentage, accession number and nucleotides variation presented in the Table 5.

Table (5):Sequence analysis :

			country		Locati	Substa	Type	
Orf virus 1	Orf virus strain AH-GY13 GM-CSF/IL-2	100	China	MF770655	-- -- -- -	----	-----	316 to 407
Orf virus 2	Orf virus strain AH1704 GM-CSF/IL-2	100	China	MF489147	-- -- -- -	----	-----	316 to 406
Orf virus 3	Orf virus strain B029,	99	Germany	KF837136	1 1 7 6 3	A-- -T	Transversion	117135 to 117226
Orf virus 4	Orf virus strain F94.848R GM-CSF/IL-2	99	Finland	JF773684	3 4 2	A-- -T	Transversion	315 to 406
Orf virus 5	Orf virus isolate OrfV/KPM/TN/Sheep/12 GM-CSF/IL-2	100	India: Tamil Nadu"	KY077479	-- -- -- -	----	-----	327 to 408
Orf virus 6	Orf virus isolate OrfV/MEC/TN/Goat/13 GM-CSF/IL-2	100	India	KY077478	-- -- -- -	----	-----	327 to 408
Orf virus 7	Orf virus strain AH1701 GM-CSF/IL-2	99	China	MF489146	9 9	N-- --	insertion	315 to 407
Orf virus 8	Orf virus strain F94.848R GM-CSF/IL-2	100	India	KY077479	-- -- -- -	----	-----	328 to 408
Orf virus 9	Orf virus strain AH1402 GM-CSF/IL-2 i	99	China	MF489139	3 6 9	C-- A	Transversion	316 to 407
Orf virus 10	Orf virus strain AH1505 GM-CSF/IL-2	99	China	MF489141	3 6 1	A ...C	Transversion	315 to 406
Orf virus 11	Orf virus isolate ORFV/Hyderabad/25/Sheep/2006 GM-CSF	100	India	MF414634	-- -- -	----	-----	327 to 406
Orf virus 12	Orf virus strain AH1604 GM-CSF/IL-2	100	China	MF489143	-- -- -- --	----	-----	312 to 406

Multiable alignment:

Table 6:Multiable alignment of orf virus GFR gene



Phylogenetic Tree:

The evolutionary history was inferred using the Neighbor-Joining method (19). The optimal tree with the sum of branch length = 0.02625234 is shown. (above the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method (20), and are in the units of the number of base substitutions per site. The analysis involved 12 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 79 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (21).

the epidermal cells in the upper epidermal layer was seen microscopically (Fig.8- 10).

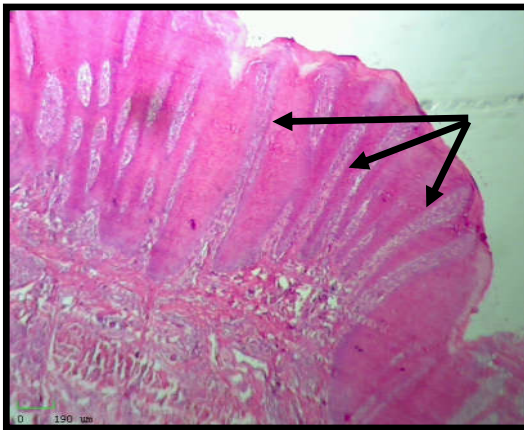


Fig. 5: Panillomata's hyperplasia of epidermis 4X



Fig. 6: Numerous immature hair follicles. 10 X

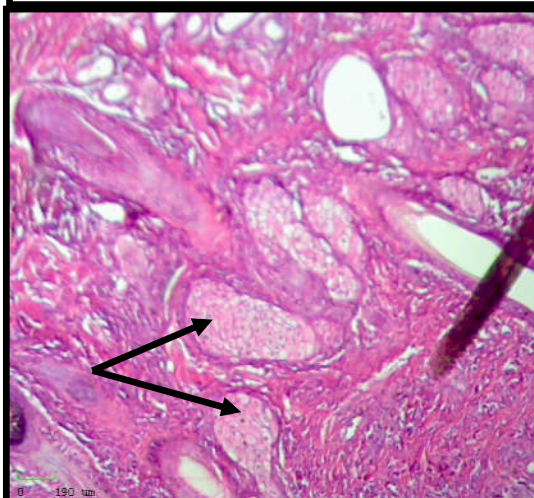


Fig. 7: proliferation of sebaceous glands . 10X



Fig.8: Areas of apoptosis formed of structural molecules(non cellular). 10X



Fig. 9: Masses of areas of proliferating sweat glands .

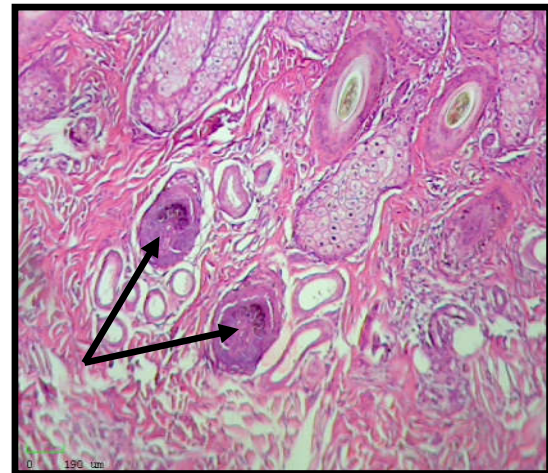


Fig.10: Hair follicles with dark stained blackish deposit. 10X

Contagious ecthyma is a highly contagious, viral skin disease that affects sheep, goats and some other domesticated and wild ruminants, although a number of proprietary treatments as well as homeopathic preparations are available, (22). Since infection occurs by direct contact with the virus which is highly resistant and can survive in the environment for more than one year, In addition the disease occurs most commonly in young ages, However, occasionally seen and registered also in older sheep whom mostly grazing of rough pastures those had a cut stalk of cereal plants that may predispose to infection with scabby mouth as oral abrasions increase the potential for the virus to gain entry (2). In this study, we presented the first clinical report of Orf virus in small ruminants based on molecular identification and histopathological diagnosis at Basrah province , Iraq .

The disease has a high morbidity rate whilst, the mortality rate is low, as a result, less attention has been given by the owners. However, the loss of condition of the diseased animals due to the disease significantly might expose to danger the market value (11). Over the study period, we have recognized the lack of adequate diagnosis and effective treatment of clinical cases which is the main contributing factor for the occurrence of disease outbreaks at any time points.

Diseased lambs show different clinical manifestations which are mentioned by (2,11,12,23), As, Orf infection can be clinically manifested as simple lesions around the commensure of the mouth, These lesions usually begin as erythema, followed by papules, pustules, which develop into brownish dry scabs. The time span of these stages is usually four to six weeks, more or

less. Nevertheless, under certain circumstances as in young malnourished lambs the disease may take a chronic form, which might take more than 4-6 months to heal completely, in such chronic cases, the lesions might spread to other parts of the skin regions (24).

It had been shown previously, that contagious ecthyma is endemic all over the world, but its less reported in the literature because of its low morbidity and minimal economic consequences (25). In Basrah province, more cases of contagious ecthyma in sheep and goat populations have been observed over the past several years, but no specific vaccination or control programs has been applied to control and eradicated this disease.

Contagious ecthyma may become a serious problem in young, stressed, immuno-suppressed or overcrowded animals (26). The differential diagnosis of the diseases causing crusts in small ruminants like contagious ecthyma, pox and PPR which have, in some cases, similar clinical symptoms may be a problem, with the development of molecular biology, the PCR technique has met the demands for specific and sensitive diagnosis of orf virus infection in the field specimens from affected animals (25,27). The present study has demonstrated for the first time the characteristics and specifications of lambs contagious ecthyma in Basrah province , Iraq.

In general contagious ecthyma will commonly affects animal lips, However mouth and surrounding skin were also targeted and harmed, Furthermore the disease can also affect the face, feet and even the udder skin of lactating ewes, As the causative virus will cause superficial sores, which then will crusted and finally scabbing over and then falling down. Moreover, the underlying skin heals without scarring, where, this cycle takes approximately 4-5weeks or may be less, Thereby, lambs will lose its condition as they are reluctant to eat and it is too painful for ewes to feed suckling lambs (2) . On the other hand, Hosamani, *et al* (6), Emphasized that, Orf lesions promotes through the stages of erythema papule, vesicle, pustule, scab formation, and finally resolution, Since, Orf pustules will develop within a few days, However, when it will ruptured ulcers and a thick scab will formed which shed within 3-4 weeks, leaving no scar tissue, Although the immunity is solid but could only last for eight months, In addition, there was an antibody

response to the virus, Furthermore, these sores may become infected by opportunistic bacteria, causing further infection (11).

In this study, it was indicated that the infection of Orf virus is common around mouth and lips of the diseased animals. This is due to the close confinement and grazing habit of the animals which causes the formation of minor abrasions on the mouth and lips of the animal during feeding. A very low incidence of the virus was also observed at other parts like on the teat and udder of the animal particularly in nursing animals in which the infected lambs could possibly be the source of infection during suckling (2,6).

The diagnosis of contagious ecthyma is based upon the finding of large proliferative lesions in the animal body, In the current study the clinical diagnosis of ORF in sheep show wart-like lesions which were distributed in the skin of the lips, gums and muzzle . This agreed with the study which stated that the clinical signs of ORF include multifocal to coalescing papillary, proliferative and ulcerated lesions in the epidermis of the muzzle and lips moreover, In some cases the lesions appear on and in the nostrils, around the eyes, on the thigh, coronet, vulva, udder and axilla (28,29).

The traditional methods of diagnosis which depends on the characteristic clinical signs could be inaccurate, but virus isolation and culturing is thought to be a gold standard method of confirmation, although it is time-consuming (8). With the development of molecular biology, PCR technique has become widely used to amplify the desired genomic fragments of tissue specimens, and it has become a powerful tool in molecular diagnosis. This method is recommended for proper identification of the pathogen through gene amplification using specific forward and reverse primers (10). Workers in various different parts of the world have reported contagious ecthyma as a common outbreak of sheep and goats and indicated that PCR is a quick confirmatory test for Orf virus (21).

Results of the current study indicated leukocytosis due to significant increase in lymphocytes number (Lymphocytosis) this agreed with (13) , whom mentioned that leukocytosis can be indicated as a reaction to different

infectious, inflammatory, However in those conditions, This reaction could be mediated by several molecules, which are released in response to stimulatory events that include growth or survival factors such as granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, c-kit ligand, or adhesion molecules and various cytokines, Moreover, because the demands on the leukocyte producing tissues in the bone marrow have increased to the point at which there is an insufficient number of mature cells for delivery into the circulation, as the infection subsides, the number of younger forms and the total white cell count decrease and ultimately return to normal , during the period of repair following an inflammatory reaction, the monocytes may increase in number, and subsequently the lymphocytes will become more numerous (30), on the other hand , Certain types of infection specially viral types are characterized from the beginning by an increase in the number of small lymphocytes unaccompanied by increases in monocytes or granulocytes, and such lymphocytosis is usually of viral origin, However , moderate degrees of lymphocytosis are encountered in certain early acute and chronic infections , (31), Furthermore , (32) added that some infectious diseases, caused by some viral infection were associated with the appearance of unusually large lymphocytes (atypical lymphocytes).

In the current study the diagnosis with histopathological features reveal, papillomatus hyperplasia of epidermis, as well as a numerous immature hair follicles in the proliferation epidermis and proliferation of sebaceous glands, However, an area of apoptosis of structural molecules were also detected , Furthermore, Masses of areas of proliferative sweat glands with large areas of laminated vacuolated structure and deposit tissue like structures in the dermis was detected, Same results are also indicated by (33,).Whom describe the histopathological lesions, As, they mention that, the crusts around nose and lips. These lesions are the late stage of the disease, are formed after rupture of vesicles and pustules, and are responsible for the common name scabby mouth, Moreover, Blood may be incorporated into crusts following severe exudation and inflammation that can damage vessel walls secondarily, However, The blood may contribute to the darkly colored crusts , In addition, The epidermal hyperplasia (acanthosis), ballooning degeneration, vesicle and neutrophils accumulating in the vesicle, which subsequently

results in the formation of a pustule. Free red blood cells are present in the epidermis to the left of the vesicle. Epidermal hyperplasia, upward movement of the pustule, and rupture of the vesicle or pustule contribute to crust formation. Furthermore, (34) added that, hyperplasia of the epidermis and follicular infundibula results in a papillary appearance of the surface that is further accentuated by stacks of exudative crust overlying the congested and inflamed dermal papillae. However, Segment of ballooning degeneration of keratinocytes in the stratum granulosum near the edge of a lesion were also detected, As well, Affected keratinocytes are swollen with pale eosinophilic cytoplasm, The keratohyalin granules are peripheralized, but the nucleus remains in the center of the keratinocyte. One or more large, acidophilic cytoplasmic viral inclusion bodies typical of parapoxvirus infection.

Others (35) were also added that, The histopathological examinations of the proliferative verrucous lesions of the affected animals revealed severe epidermal hyperkeratosis and hyperplasia. There were degenerative changes within the stratum spinosum, with numerous swollen, Vacuolated cells having pyknotic nuclei . Intraepidermal aggregates of inflammatory cells were present, and the formation of intracytoplasmic eosinophilic inclusion bodies was present in vacuolated necrotic prickle.

It had been shown that, lesions induced by viral challenge of mildly abraded skin, indicated that the virus does not establish in the damaged epidermis, but replicates in the cells of an underlying replacement epidermal layer derived from the walls of the wool follicles. The skin reaction consists of a cellular response with necrosis and sloughing of the affected epidermis and underlying stratum papill are of the dermis. Healing is then completed with the formation of a third epidermis derived from the deeper portions of the wool follicles. The previous cutaneous infection did not prevent re-introduction of the disease, even in the same area of the skin, although the lesions were less severe and persisted for a shorter period (2,11).

Results of this study also show a significant increase of acute phase response, Represented by a significant increase of both haptoglobin and fibrinogen . The acute phase response refers to the non-specific and complex innate reaction that occurs shortly after tissue injury, Since , Proinflammatory cytokines are released initially at the site of an insult and are responsible for the induction of local and systemic defenses (36). Of the inflammatory cytokines, the interleukin-6, Tumor necrosis

factor-alpha (TNF- α), and interleukin-1-beta are the major mediators of acute phase protein (APP) synthesis in the liver, which is the main site of APP synthesis, although non hepatic sites have also been recognized(37). Serum concentrations of positive APPs increase by over 25-30% during an acute phase response. However, Some APPs decrease in concentration such as albumin and transferrin, and are referred to as negative APPs. Acute phase proteins function to further activate the immune system, enhance phagocytosis, and clear the products of inflammation. Moreover, APPs may be more sensitive than leukocyte counts as markers of inflammation, are more stable than cellular components, and the assays can be performed on previously frozen and stored serum or plasma. As , it were thought that APPs also have a faster response than changes in WBC counts in situations where new WBCs must be generated by the bone marrow. (38).

Sequence analyses used based on the GFR gene used to determine the ORFV virus. Genomic sequences of ORFV were assembled into a contiguous sequence . The identities of the ORFV strain with other ORFV strains were 99%–100% at the nucleotide level, presences transversion A-C nucleotide (NCBI). (5).

Although the disease is endemic in most parts of the world, there are few descriptions of Orf virus strains and comparisons of these strains between them. Details of 31 Orf virus strains, whose sequence of the envelope gene (B2L) has been reported before.(39).

It had been shown that, Full-length B2L gene encoding for immunogenic major envelope protein from most ORFV isolates was amplified by PCR and the amplicons (1206 bp) were cloned and sequenced. Since , Comparative sequence analysis revealed an open reading frame of 1137 nucleotides (nt) encoding a polypeptide of 378 amino acids (aa). Indian isolates were highly related amongst themselves with sequence identity of over 97% at the nt and aa level. Further, they showed 97-98% sequence identity with sequences of other ORFV isolates from around the world, whereas, 94-95 and 82.7-83.8% sequence identity was observed, respectively, with pseudocowpox and bovine papular stomatitis viruses, the other members of the genus, Phylogenetic analysis also showed that these Parapoxviruses from sheep and goats are closely related to other orf viruses reported worldwide (6). Moreover, The ORFV strain from the Thi-Qar Province showed a close relationship with other strain in Asia . Among strains originating in sheep and goat was closer to strain in India, Germany and Finland Analysis also showed that ten ORFVs were more closely related to the

other region from Iraq (NCBI). The phylogenetic tree based on the GFR gene showed the nine sheep ORFVs and three sheep ORFVs formed distinctly separate branches. Analysis of the phylogenetic trees based on nucleotide sequences of each gene of ORFV. Multiple alignment of the nucleic acid sequence showed that gene presences transversion A-C.(40).The availability of genomic sequences of three sheep ORFVs aids in understanding of the diversity of orf virus isolates in this region and can assist in distinguishing between orf strains that originate in sheep .

دراسة مرض الحمى القلاعية الساري في حملان محافظة البصرة، العراق

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فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة البصرة، البصرة، العراق.

الخلاصة

شخص مرض الحمى القلاعية الساري في الحملان المحلية ومن كلا الجنسين بعمر 3-6 اشهر. أذ شملت الدراسة فحص 941 من الضأن المحلية مثلت احد عشر من قطاعان ضأن محافظة البصرة ، العراق . عشوائياً تم اختيار 100 من الحملان المحلية والتي اظهرت علامات سريرية متعلقة بالمرض كما اختير 25 من الحملان المحلية السوية سريرياً عدو كمجموعة سيطرة . اظهرت الحملان المصابة علامات سريرية مثل انعدام الشهية والاكئاب والبلادة، عدم القدرة على الرضاعة أو الرعي، فضلاً عن ذلك فقد لوحظت الافات المرضية بشكل حطاطات، بثرات ، حويصلات وندب في جميع الحملان المصابة .لوحظت الافات المرضية حول الفم وفي الشفاه العليا والسفلى، وفي الجفون العليا والسفلى، كما لوحظت افات التنشق ايضاً في الحملان المصابة وتواجدت افات مرضية بشكل قليل جدا حول الاكليل والأذنين والشرج والفرج (6%). كما عانت الحيوانات المريضة من ارتفاع معنوي في معدلات درجات حرارة الجسم وضربات القلب وتردد التنفس. أوضحت نتائج الفحوصات الدموية ارتفاع معنوي في العدد الكلي لخلايا الدم البيض بسبب الارتفاع المعنوي للعدد المطلق للخلايا اللمفية فضلاً عن حدوث ارتفاع المعنوي في سرعة تثفل كريات الدم الحمر في الحملان المصابة بالمرض بالمقارنة مع مجموعة السيطرة ومن ناحية أخرى فقد بينت نتائج استجابة الطور الحاد وجود ارتفاع معنوي في معدلات الهابتوكلوبين ووقت منشيء اللبغين في الحملان المريضة بالمقارنة مع حملان مجموعة السيطرة . بينت نتائج فحص تفاعل البلمرة المتسلسل في الهلام الكهربائي ان الفيروس المتسبب عن الحمى القلاعية الساري له 147 من القواعد الزوجية . تم اختيار الأنواع المتماثلة / العزلات حسب أعلى نسبة هوية مع تغطية أفضل للاستعلام وأدنى قيمة E. كما تمت معالجة متواليات النيوكليوتيدات الخام لجميع العينات بواسطة برنامج إصدار FinchT.V 1-4 لتقليص المتواليات والقواعد غير المرغوب فيها بجودة تسلسل منخفضة (أقل من كثافة الإشارة بنسبة 20%). فضلاً عن

استنتاج التاريخ التطوري باستخدام طريقة الجار - الربط ، حيث يتم عرض الشجرة المثلى مع مجموع طول الفرع = ٠.٠٢٦٢٥٢٣٤. (فوق الفروع). حساب المسافات التطورية باستخدام طريقة أقصى احتمال مركب وهي بوحدات عدد البدائل الأساسية لكل موقع أذ تضمن التحليل ١٢ سلسلة من النيوكليوتيدات وتضمنت اتجاهات الكودون الاول+ الثاني + الثالث + غير المرز . تمت ازالة جميع المواقع الغامضة لكل زوج متسلسل حيث أن هناك مامجموعه ٧٩ وظيفة من مجموع البيانات النهائية. واجريت التحليلات التطورية في MEGA7.

بينت نتائج الفحوصات النسجية المرضية تضخم الورم الحليمي للبشرة وكذلك العديد من بصيلات الشعر غير الناضجة في انتشار البشرة وانتشار الغدد الدهنية ، ومع ذلك ، تم اكتشاف منطقة من موت الخلايا المبرمج للجزيئات الهيكلية. فضلا عن تحديد كتل مناطق الغدد العرقية التكاثري مع مساحات كبيرة من هياكل مغلفة وأنسجة رواسب مثل الهياكل في الأدمة، ورواسب داكنة اللون مع بعض بصيلات الشعر المتوسعة والخلايا الالتهابية. وشوهدت ماثلات الندب فوق خلايا البشرة في الطبقة العليا من الجلد . استنتج من هذه الدراسة أن مرض الحميقاء الساري قد يكون متوطناً في محافظة البصرة وقد يعكس اصابات مرضية مرتفعة قد تؤدي الى هلاكات فضلا عن اصابة الحيوانات المريضة بالنغف أو الإصابات الجرثومية الثانوية والذي قد يعقد عملية الشفاء عليه ينصح بتباع برامج سنوية ممنهجة للسيطرة على المرض.

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