Immune Response, Gene sequence to Contagious Ecthyma Virus (Orfv) Infected Lambs in Diyala Governorate, Iraq

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Abstract. Contagious ecyhyma Known (Orf, contagious pustular dermatitis, sore mouth, scabby mouth) a disease spreads easily and brought by the epitheliotropic. It's endemic around the world and has a serious economic impact. This study aimed to understand the immune response mechanisms of the lambs to defense against Contagious ecthyma virus and molecular detection of the virus with the determining the sequence and mutant of the viral genome to 91 lambs (41 has clinical signs of Orfv and 50 control do not have clinical sign of Orfv) for the period extended from September 2022 to April 2023. Blood samples were collected from all above groups to apply for detection the level of IgM, IgG, IL-10, and IFN-γ of Orfv by ELISA test. Positive Orfv IgM was 75.6% infected lamb and 72% none infected, and positive Orfv IgG was 90.2% infected lamb and 88% none infected. The result was showed the level of IL-10 a significant increasing, in all Lambs positive IgM and IgG compared with control group. IFN-γ level showed non significant differences between all Lambs positive IgM and IgG compared with control group. Molecular detection of Orfv result was confirmed the positivity of all samples positive Orfv IgM. And the sequencing of the Orfv DNA showed a mutant in the some Allel compared with References strain registered at NCBI. Concluded: high prevalence of Orfv among lambs and happened mutants to the viral genome increased the virus's virulence by affecting the immune response.

1 Introduction

Small ruminants have a significant economic role in human society since they are a major source of fiber, hides, wool, calcium, vitamins, protein and other essential nutrient for peoples worldwide, because of viruses constitute an acute illness, lead to reduce animal products. In addition, harmful animal viruses now account for a greater proportion of infectious illnesses that pose a challenge to the animals business [1, 2]. Contagious ecyhyma also known as (Orf , contagious pustular dermatitis ,sore mouth ,scabby mouth) is a disease that spreads easily and is brought on by the epitheliotropic, its endemic around the world and has a serious economic impact [3]. Clinical signs of Orfv include the development of papules, vesicles, pustules, and rapidly growing scabs that are confined to the lips and snouts, medial canthus

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of the left eye, udder, gingiva, tongue, and dental pad and distal prepuce of affected animals [4, 5]. Contagious ecthyma mostly affects sheep and goats, cause severe sores preventing lambs and kids from feeding their mothers' milk , and challenging for sick animals to swallow food , this sickness typically kills lambs and young animals. Although this disease normally has a low mortality rate, it can occasionally result in up to 10% of deaths in lambs, which is a very high degree of morbidity .The incidence rate of secondary infection in lambs can reach up to 93.7% [6]. Orf virus is a member of the family Poxviridae, subfamily Chordopoxvirinae, and genus Parapoxvirus ,the linear double – stranded DNA genome of Orf virus is 135kb length and encodes 132 genes [7, 8]. Numerous immunomodulatory proteins that control the host's innate and pro-inflammatory responses to infection are encoded by the Orf virus .The virus has specific deletions in the genes encoding the immunodulatory proteins include ,chemokine binding protein (CBP), inhibitor of granulocyte-monocyte colony-stimulating factor, IL-2, interleukin 10 homologue (IL-10) [9]. Immune responses that are cell-mediated and humoral are both triggered to stop viral multiplication. Non-neutralizing antibodies are the initial manifestation of the humoral immune response and are often first detected in serum around one month after infection [10]. To assist in limiting viral replication, the humoral immune response, a cell-mediated immune response, is induced. It has been demonstrated that infected macrophages release cytokines that trigger inflammatory reactions [11]. This study aimed to understand the immune response mechanisms of the lambs to defense against Contagious ecthyma virus and molecular detection of the virus with the determining the sequence and mutant of the viral genome.

2 Material and Methods

2.1 Subjects and samples collection

This study comprised of 41 lambs control and 50 lambs with contagious ecthyma virus symptoms. The sample was obtained by draw the blood from jugular vein (The blood collection via the jugular vein in sheep. The collection time of samples including infected lambs and control lambs between September 2022 to April 2023 with age ranged from (3-45) days as well as collection of skin lesion (pustules). These samples obtained from many areas of Diyala governorate, Iraq. The blood were placed in the vacuum gel tube, centrifugation 20 min at the speed 4000 r.p.m. the collected serum was kept in sterile tube and preserved in -20oC for ELISA test (Sun long Biotech, China) to measure the level of Orfv IgM (Cat #:SL00098Sp), Orfv IgG (Cat #:SL00097Sp), sheep IL-10 (Cat #:SL00128Sp, and sheep INF- γ (Cat #:SL00099Sp). The method was done as manufacture instructions.

2.2 Molecular detection and sequencing of sheep Orf virus

The DNA –extraction kit from ELK Biotechnology Cat #: (EP007) , Package : 50T was used to extract DNA sheep Orf virus. Master mix kit (Entilink TM PCR Master mix (Blue) from ELK Biotechnology (Cat.#EQ004-01)was used in the PCR method and is ready to contain classical PCR premix solution composed from (Taq DNA plomerase,MgCL2 ,dNTP mixture ,and buffer). The primer that designed to use in this investigation for sheep Orf virus is shown in Table 1 . Skin lesion collected from infected lambs with ecthyma virus skin lesion by used surgical blades then put the skin pustules in phosphate buffer saline and preserved in -20 oC for PCR test to detection the virus in skin scar (pustules). The method was done as manufacture instruction

Table 1. Primer and markers utilized in the current study

Length 508bp	Sequence 5-3	TM	GC%	Self Complementarity	Self 3 Complementarity
Forward	GTCCGTGTCCACCA TCAAGA	59.68	55.00	4.00	2.00
Reverse	GAACTTGCGCACGG ACATG	60.15	57.89	6.00	4.00

2.3 Statically analysis

Data were analyzed by IBM SPSS statistical software, package 20. ANOVA ONE WAY and T test were used for analysis the data and the results were presented as mean ± SD. Significant at P value ≤ 0.05.

3 Results

3.1 Distribution of Contagious Ecthyma Virus antibody in Lambs

The result was showed that distribution of Orfv IgM was 31 from 41 infected lamb (Figure 1) and 36 from 50 none infected (did not have clinical sign for Orfv) as presented in (Table 2). The Infected lambs were given positive Orfv IgG for 37 from 41 and 44 from 50 none infected as presented in (Table 2).

Fig. 1. Clinical signs of Contagious ecthyma virus infected to lambs.

Table 2. Showed distribution of Contagious Ecthyma Virus (Orf) IgM and IgG in Lambs among study groups

Groups	Orf IgM		Total	Orf IgG		Total
	Positive	Negative		Positive	Negative	
Non infected	36 (72%)	14 (28%)	50	44 (88%)	6 (12%)	50
Infected	31 (75.6%)	10 (24.4%)	41	37 (90.2%)	4 (9.8%)	41
Total	67	24	91	81	10	91

3.2 Immunoregulatory effect by Contagious Ecthyma Virus

To investigated effect of Orfv infection on immune response to lamb detection the level of IL-10 and IFN-γ in the infected lambs and related with the level of IgM and IgG. The results of IL-10 showed a significant increasing at (P= 0.000) in all Lambs positive Orfv IgM at

(Mean ± SD):(54.58 ± 38.47) pg /ml compared with negative Orfv IgM Lambs (8.56 ± 6.43) pg/ml as shown in Figure 2.

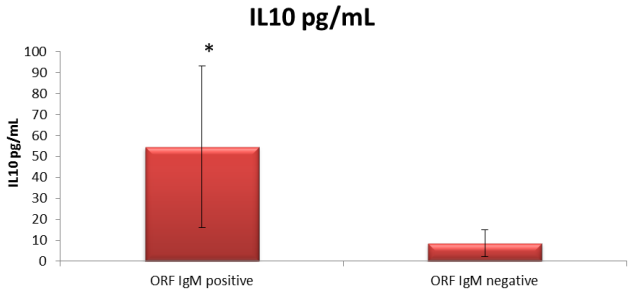

Fig. 2. Showed the level of IL-10 among Contagious Ecthyma Virus (ORF) IgM positive and negative in Lambs. The data was presented as mean ± SD, *Significant at P = 0.000

The result of IFN-γ showed a non significant in all Lambs positive Orfv IgM at (Mean ± SD) : (111.13 ± 71.13) pg /ml compared with level of IFN- γ negative Orfv IgM Lambs at (Mean ± SD):(101 ± 06) pg/ml as shown in Figure 3.

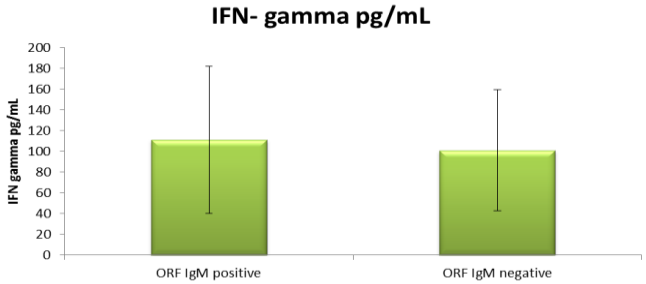

Fig. 3. Showed the level of INF gamma among Contagious Ecthyma Virus (ORF) IgM positive and negative in Lambs. The data was presented as mean ± SD.

The results of IL-10 showed a significant increasing at (P=0.000) in all Lambs positive Orfv IgG at (Mean± SD) : (45.18 ± 39.84) pg/ml compared with level of IL-10 in a negative Orfv IgG Lambs at (Mean ± SD),(20.34 ± 19.95) pg /ml as shown in Figure 4.

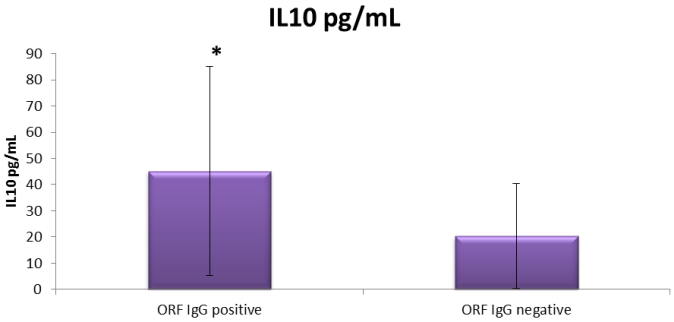

Fig. 4. Showed the level of IL10 among Contagious Ecthyma Virus (ORF) IgG positive and negative in Lambs. The data was presented as mean ± SD, *Significant at P = 0.000

The results of IFN- γ showed a non significant differences in all Lambs positive Orfv IgG at (Mean ±SD) : (110.09 ± 67.88) pg/ml and the level of IFN – γ in a negative Orfv IgG Lambs at (Mean ± SD) : (95.33 ± 69.41) pg/ml as shown in Figure 5.

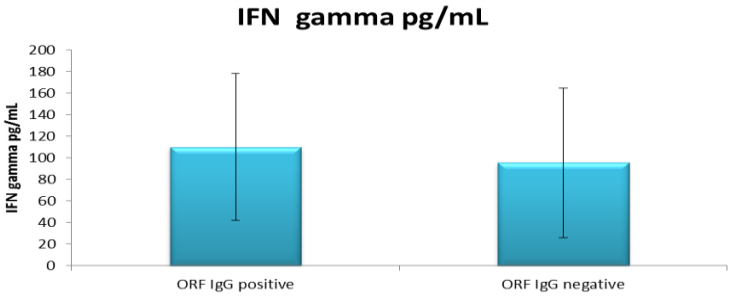

Fig. 5. Showed the level of IFN- gamma among Contagious Ecthyma Virus (ORF) IgG positive and negative in Lambs. The data was presented as mean ± SD.

3.3 Molecular detection and gene sequence of Contagious ecthyma virus

Molecular detection of Contagious ecthyma virus was perform by DNA extraction and applied in PCR method for detection the viral DNA as presented in the Figure 6.

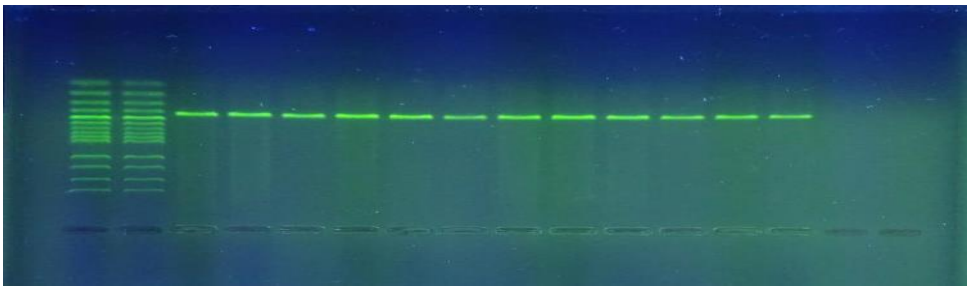

Fig. 6. Contagious ecthyma virus DNA detection by PCR method in the infected lambs

Some of Contagious ecthyma virus DNA was applied for the sequencing to detected the genome sequence for Contagious ecthyma virus infected lambs in Iraq and identification the mutation in the virus genome compared with Contagious ecthyma virus registered in the NCBI library.

The result of ten genome for Contagious ecthyma virus DNA was appeared there is a mutant in the some Contagious ecthyma virus (Figure 7) compared with reference and consensus. And the frequency of mutant Allel and genotype was presented in the Table 3 and 4.

Table 3. Mutant percentage and frequency for Allel type in Contagious ecthyma virus gene sequence

Allel	Frequency	Percentage
A	25	42.37 %
T	8	13.56 %
G	4	6.78 %
C	22	37.29 %
Total	59	100

Table 4. Mutant percentage and frequency for genotype in Contagious ecthyma virus gene sequence

Genotype	Frequency	Percentage
TC	20	51.28 %
AT	2	5.13 %
AG	5	12.81 %
CG	4	10.26 %
GG	2	5.13 %
AC	2	5.13 %
GC	2	5.13 %
GT	2	5.13 %
Total	39	100

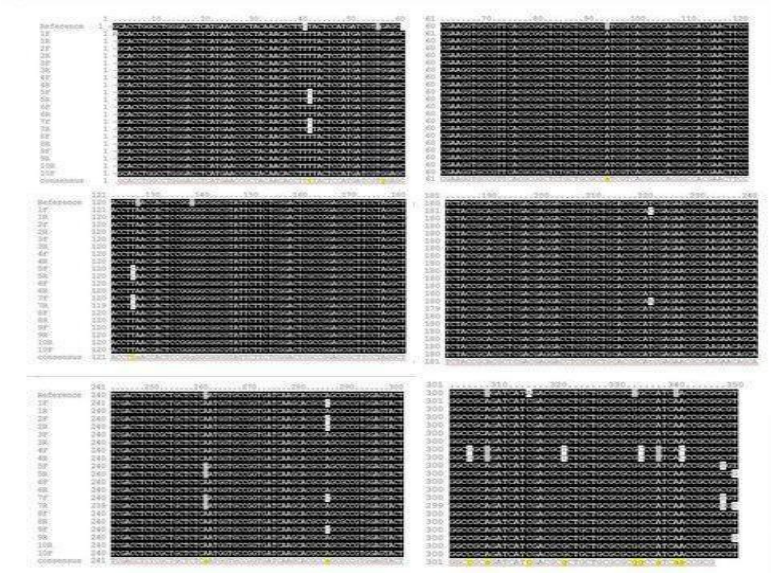

Fig. 7. Sequencing of Contagious ecthyma virus gene compared with reference sequen obtained from gene data bank of NCBI.

4 Discussion

Due of the disease's high morbidity incidence but low fatality rate, owners have paid less attention to it. Animal's lips and nose frequently become infected with the Orf virus. This is because the animals are kept in close quarters and have a grazing behavior that leads to the development of tiny abrasions on the mouth and lips of the animal when feeding. In nursing animals, where the infected lambs may be the source of infection during suckling, a very low prevalence of the virus was also seen on the animal's udder and teat [12].

Sheep were more susceptible to the illness than goats, with an incidence of 15.5% in sheep and 8.5% in goats. Although sheep have a higher incidence than goats, the severity of the infection is the opposite [13]. Compared to sheep, goats were more susceptible to the infection, which was marked by proliferative lesions in the first two to three months. Because both sex groups in the region were maintained under the same management regime, there was no correlation between the animal's sex and the occurrence of the illness the effectiveness of the serological test used is shown to be a good and suggested tool for future applications. The seroprevalence of the Orf virus in Basrah province was evaluated to determine the level of virus exposure in the sheep population.

The prevalence rate in sheep aged 1-6 months was significantly higher than that of the other age groups. Lambs have a higher probability of infection than adults because lambs' immune system is still underdeveloped and depends mostly on innate immunity and maternal antibodies to protect against the Orf virus [14]. The result of this study was detected the high sero-prevalence of Orf virus IgM (73.63%) and IgG (89%) among the lamb with or without clinical signs of Orf virus infection.

The serodetection for disease was important to give the real, more accurate and fast result for the prevalence of the Orf virus. Previous studies were reported the prevalence of Orf virus among sheep and lamb in different countries. The overall prevalence of Orf determined in Basarh in sheep was 25.7%. This prevalence rate was higher compared to that of 12.2% and 16.8% as reported in sheep in Malaysia [15] and also in England, which was 19.51% [16]. On the other hand, it was relatively lower compared to 52.81% and 60% as reported in lambs in Turkey [17] and Saudi Arabia [18]. Animal body condition and viral prevalence were substantially associated, with poor body condition animals showing a greater prevalence than excellent and medium body condition of animals. This is related to the development of a robust immune system in well-nourished animals as opposed to animals that receive less nutrition. The immune system safeguards the health and welfare of animals. In many cases, nutrition can regulate immune function and strike a balance between health and sickness [19]. Sheep that have previously been exposed to Orfv can be infected again, however the severity of the lesions and duration to resolution decrease with each second infection. This suggests that the host immune response can limit illness severity but not prevent reinfection . The presence of immunomodulatory proteins produced by Orfv, which decrease components of the host immunological and inflammatory response, contributes to this. These immunomodulatory proteins have a range of actions, including protecting the virus from the antiviral effects of interferons, permitting viral replication, or limiting the biological activity of key immune system cytokines and decreasing inflammation [20].

The main immunomodulatory genes encoded by Orfv are chemokine binding protein , interleukin-10 , vascular endothelial growth factor, GM-CSF inhibitory factor (GIF), and interferon-resistance gene , which prevents an enzyme dsRNA-dependent kinase from catalyzing protein synthesis [21]. The identification of Orfv-secreted immunomodulatory genes and proteins may provide an explanation for how the virus manages to evade host protection [22]. IL-10 a cytokine with strong anti-inflammatory qualities known as interleukin 10 (IL-10) is essential for controlling the host immune system's response to infections, minimizing host injury, and preserving healthy tissue homeostasis. Increased immunopathology in response to infection and a higher risk of developing a number of autoimmune illnesses are both correlated with IL-10 dysregulation. One of the earliest genes, Orfv-IL-10 is produced by the virus and is found near the terminal right end of the viral genomic DNA.

This study was showed the level of Orfv-IL-10 in all Lambs positive IgM was elevated compared with the Lambs negative IgM. Interferon gamma (IFN-γ) is a cytokine critical to both innate and adaptive immunity, and functions as the primary activator of macrophages, in addition to stimulating natural killer cells and neutrophils. T cells, natural killer (NK) cells, and macrophages are the primary secretors of gamma interferon [23]. IFN-γ, also known as type II interferon, is a cytokine that is essential for innate and adaptive immunity against viral, bacterial, and protozoan infections. IFN-γ is a key macrophage activator and inducer of major histocompatibility complex class II molecule production. A variety of autoinflammatory and autoimmune disorders are linked to abnormal IFN -γ γ expression. IFN-γ is crucial in the immune system because of its potential to directly suppress virus replication and, more critically, because of its immunostimulatory and immunomodulatory activities. As part of the innate immune response [24]. This study was showed elevated of ORFV-IFN –γ in Lambs positive IgM compared with IFN- γ negative IgM Lambs. This result

was gave evidence that the Orf virus effect on the modulation immune response through increase the level of IL-10 in the infected lamb, while caused decreased in immune response and inflammation process by decreasing or balanced the level of IFN- γ in the infected lamb so that enhanced the virus from distribution and be more virulence. The cytokine IL-10 is a crucial facilitator of anti-inflammatory processes that protects a host against exaggerated reactions to infections and microbiota .It also plays crucial roles in a variety of other contexts ,including sterile wound healing ,autoimmune ,cancer ,and homeostasis [25].

Apoptosis a process of programmed cell death ,a type of cell death different from necrosis, is important in processes such as homeostasis and the elimination of damaged cells and may be initiated by cytokines and immune effector cells [26]. Contagious ecthyma virus is a potent genetic carrier capable of controlling apoptosis in infected skin cells, a strategy that serves to evade the host's immunological response. It has been claimed that the virus may persist in the skin and produce recurring infections in the same flock. Mammalian interleukin 10 (IL10) is a cytokine that inhibits immunological and inflammatory responses, and it was discovered that ORFV could also make a related anti-inflammatory virokine (Orfv-IL-10) that severely weakened the virus when this gene was taken out [27].

5 Conclusion

This study was concluded high prevalence of Orfv among lambs and happened mutants to the viral genome increased the virus's virulence by affecting the immune response.

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