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ABSTRACT

Study of Some Immunological Parameters in Lambs Infected with *Contagious Ecthyma* Virus in Diyala Governorate

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Contagious ecthyma is additionally referred to as (Orf) and it is known in many names; scabby mouth, sore mouth, and contagious pustular dermitis. It is a disease that spreads easily and is brought on by the epitheliotropic, is endemic around the world and has a serious economic impact. Present 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 of the gene and mutant in the gene sequence of the virus in 91 lambs (41 lambs have clinical signs of Orfv and 50 lambs control do not have clinical sign of Orfv) for the period extended from September 2022 to April 2023 by using two methods in diagnosis Enzyme.-linked immune sorbent assay(ELISA) and molecular identification Polymerase chain reaction (PCR).

Blood samples were collected from all the mentioned groups to apply for detection the level of IgM, IgG, IL-10, IFN- gamma (IFN- γ) of Orfv using the ELISA test. Subsequently a diagnosis of the positive and negative Orfv IgM and IgG in serum blood samples.

The distribution Orfv IgM in infected lambs showed positive result in 75.6% (31out of 41of the infected lamb), while in the non-infected group, it was 72% (36 out of 50) of the lambs. Regarding the distribution of Orfv IgG in

Lambs was found that among the infected lambs at 90.2% (37 out of 41) tested positive for Orfv IgG. Similarly among the non-infected lambs showed positive Orfv IgG results 88% (44 out of 50).

Furthermore this study aimed to investigate the effect of Orfv infection on the immune response by examine the level of IL-10 and IFN- γ in the infected lambs and their correlation with IgM and IgG level . The results showed a significant increasing in IL-10 levels in all Lambs with positive Orfv IgM at (54.58 ± 38.47) pg /ml compared with negative Orfv IgM Lambs (8.56 ± 6.43) pg/ml. However the level of IFN- γ did not show significance difference between lambs with positive Orfv IgM at (111.13 ± 71.13) pg /ml and lambs with negative Orfv at (101 ± 06) pg/ml . Regarding for to related Orfv IgG, the level of IL-10 showed a significant increasing in all Lambs positive Orfv IgG at (45.18 ± 39.84) pg/ml compared with level of IL-10 in a negative Orfv IgG Lambs at (20.34 ± 19.95) pg /ml. and the level of IFN- γ showed a non-significant differences in all Lambs positive Orfv IgG at (110.09 ± 67.88) pg/ml and the level of IFN – γ in a negative Orfv IgG Lambs at (95.33 ± 69.41) pg/ml.

Molecular detection of *Contagious ecthyma* virus was perform by DNA extraction and applied in PCR method for the detection of the viral DNA. The molecular detection Orf virus result in this study was confirmed the positivity of all samples and gave positive Orfv IgM. And the sequencing of the Orf virus DNA showed a mutant in the some allele compared with references strain registered at NCBI.

Based on the findings of this study, it can be concluded that there is a high prevalence of *Contagious ecthyma* Virus among lambs. Furthermore, the presence of mutants in the viral genome contributes to increased virulence of the virus which subsequently impacts the immune response.

Chapter One

Introduction

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 viruses constitute an acute illness, it is thought that the economic losses they produce are not accurately reported by reducing the quantity and quality of milk. In addition harmful animal viruses now account for a greater proportion of infectious illnesses that pose a challenge to the animals business (Chakraborty *et al.*, 2014; Yan *et al.*, 2020).

Contagious ecthyma (Orf) is known in many names; scabby mouth, sore mouth, and contagious pustular dermatitis. It is a disease that spreads easily and is brought on by the epitheliotropic, the illness is endemic around the world and has a serious economic impact (Spyrou and Valiakos, 2015). Papules, vesicles, pustules, and quickly expanding scabs that are limited to the lips and snouts as well as the medial canthus of the left Eye are among the clinical indications of Orf sickness udder ,gingiva, tongue, dental pad and distal prepuce of affected animals (Cargnelutti *et al.*, 2011; Kinley *et al.*, 2013).

Contagious ecthyma, which mostly affects sheep and goats. As result to severe sores preventing lambs and kids from feeding on 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 %(Chi *et al.*, 2017).

The classification of the family Poxviridae include two subfamily Chordopoxvirinae and Entomopoxvirinae, while Orf virus (Orfv) is one of the poxviridae family, chordopoxvirinae subfamily and parapoxvirus genens. The linear double-stranded DNA genome of Orf virus is 135kb length and encodes 132 genes, (Lacasta *et al.*, 2021; Coradduzza *et al.*, 2022).

The Orf virus encodes a large number for proteins act as immunomodulators and regulator for immune response and inflammation. Immunomodulatory proteins such as IL2, IL10, granulocyte, monocyte colony stimulator factor, and chemokine binding protein have particular deletions in the virus. (Martins *et al.*, 2021).

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 (Reina *et al.*, 2013). To assist in limiting viral replication, immune response must be enhanced as cell mediated and humoral response and it has been demonstrated that infected macrophages release cytokines that trigger inflammatory reactions (Jarczak *et al.*, 2016) .

Aim of the study:

The primary objective of the current study was to investigate the immune response mechanisms of the lambs to defense against *Contagious ecthyma* virus and molecularly detection of the virus by determining the sequence and identifying mutation in viral genome.

The objectives to achieve the aim of the study are:

1. Detection of *Contagious ecthyma* virus DNA from skin lesion pustules of infected lambs and subsequent application of PCR techniques for detection of the virus.
2. Detection the *Contagious ecthyma* virus- a specific IgM, IgG, IL-10 , IFN- γ in serum of both infected lambs and control lambs (those without clinical sign to infects).
3. Application the CEV DNA for gene sequence to identify any mutation present in the gene.