



Original Research Article

Isolation of Orf virus (ORFV) from Iraqi sheep and study the Pathological changes in mice

Huda, A. M. Al.Sha'alan and Hussein, A.M. Al.Bayati*

Department of Microbiology, College of Vet. Medicine/ University of Baghdad, Iraq

*Corresponding author

A B S T R A C T

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Orf virus (ORFV) is one of the most widespread viral diseases worldwide. This study considered to be the first in Iraq aimed to isolate the virus from infected sheep then characterization of the virus infection and disease in mice. The virus was isolated using of primary chicken embryo fibroblast. The cytopathic effects of isolated virus were rounded and sloughing cell and detachment from monolayer after 48 hours (hr) post inoculation. The isolated virus was characterized by serum neutralization test by using hyper immune serum prepared in Iraqi healthy sheep. A protocol of intradermal inoculation was employed to inoculate 10^9 TCID₅₀/ 0.5ml of isolated ORFV in the skin of ears, back and labial commissures of mice. Seven out of ten of inoculated mice which showed a clinical course characterized by erythema, macules, papules and vesicles or pustules that eventually converted to dried scabs. Local signs started around days 4 post-inoculation (pi) and lasted 6-10 days. Virus was recovered from lesions between days 7 and 14pi. Histological examination of lesions revealed focal proliferative dermatitis with degeneration and vacuolation (ballooning) as well as acanthosis and hyperkeratosis with inflammatory signs of the skin cells. Virus was recovered from lesions of three animals.

Introduction

Contagious ecthyma also known as Orf or contagious pustular dermatitis is a contagious disease, caused by the epitheliotropic virus, a member of the genus Parapoxvirus at the family *Poxviridae*. The disease has a worldwide distribution. The disease affects primarily sheep and goats and rarely dogs, cats (Reid and Rodger, 2007; Jubb *et al.*, 2007).

The disease also has a zoonotic importance, it has occupational hazard to people working with animals.

Clinical features of the infection vary. In some animals, infection may remain subclinical; however, occasionally and especially in young animals, case fatality may reach up to 80% (Reid and Rodger, 2007).

Generally, the lesions are usually localized around the mouth and the nostrils, frequently originating at the commissures of the lips; lesions can also be seen within the buccal cavity and occasionally, in the esophagus or the abomasum.

In ewes, lesions are primarily observed on the teat or the udder skin and less often in the inguinal area and the thigh (Nandi *et al.*, 2011). Lesions follow a well-defined development pattern of local erythema, followed by formation of papules, vesicles, pustules and scabs (Jubb *et al.*, 2007). As lesions resolve, scabs become dry and are shed, with no scar remaining at the lesion site (Hosamani *et al.*, 2009). Infection by the virus causes proliferative skin lesions in which extensive capillary proliferation and dilation are prominent histological features (Groves, 1991).

Materials and Methods

- 1- The disease was clinically diagnosed from different field cases of sheep in different Iraqi provinces suffering from proliferative skin lesions especially at lips and commissures. The skin lesions was recovered from infected area using spatula like tool to get of skin scraping, then preserved and prepared at highly sterilized conditions to use it for viral isolation.
- 2- The cells used for isolation were originated from chicken embryos at age 10-13 days. The embryo was cut to small pieces using sterilized scissors and forceps and removal of bones and viscera. The remaining tissues were washed by phosphate buffer saline to discard the blood. The embryo body was cut in to small parts by sterile scissor as procedure previously described (Hitchner, 1980). The media were used for growth and maintain cell growth which prepared according special formula its origin GIBCO^R (UK).
- 3- The isolated virus was characterized serologically using specific hyper immune serum prepared in the healthy sheep at college field to known isolate (reference isolate) gift from animal research centre.
- 4- Laboratory animals were including healthy mice BALB/c distributed 10 mice (infected group) and 5 mice (control group). These animals were inoculated with isolated virus after titration until reach 10^9 TCID₅₀/0.5ml. These animals were inoculated at different area (back, commissures, ears).
- 5- Skin biopsies were used for histopathological diagnosis recovered from infected mice using sterilized scissors and forceps through the standard techniques. The slides were prepared and examined inside the Pathology Department of veterinary college. Hematoxylin Eosin (HE) colored slides were used to describe the histological features of the skin infection with using an Olympus optical microscope according to Luna and Lee (1968).

Results and Discussion

Virus isolation by cell culture

The eighth passage of virus in chicken embryo fibroblast cells the cytopathic effect CPE shown rounded cells first detected at 24hr after inoculation. The virus increased gradually until 48 hr post infection and show sloughing of infected cells. In the second passage, the CPE appeared 72hr diffuse rounded cells and more sloughing cell, than the first passage which increased gradually and daily monitored until CPE appear in all surface of the tissue culture which completed after 6 days that agreed with Al-Nada, (1983) and Al-Sha'alan, (2003). The isolated virus was titrated until reach the reach 10^9 TCID₅₀/0.5ml at 8th passage. This virus titer was used for mice inoculation. After titration of the isolated virus was doing, the serum neutralization test was performed by using of hyper immune serum against reference strain was prepared in healthy lamb. The neutralization titer was 128 after 28days of injection.

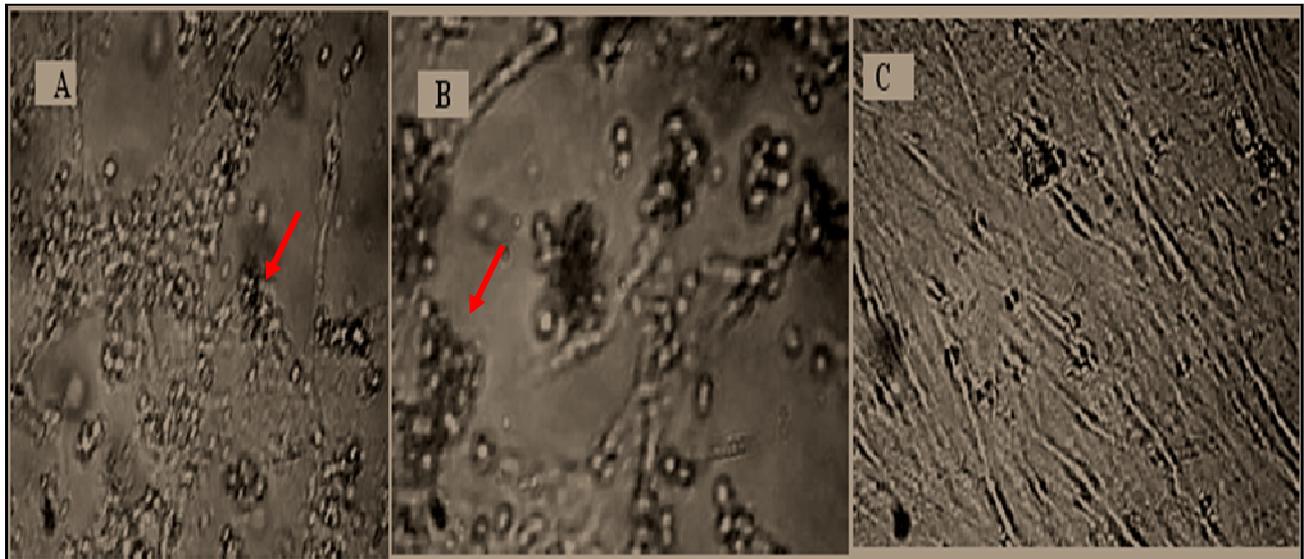


Figure.1 A. Cytopathic effect of Orf virus inoculated in the chicken fibroblast showed rounded cell aggregation and sloughing of the fibroblast cell at 8th passage after 24 hours. B. Cytopathic effect of Orf virus inoculated in the chicken fibroblast showed cell aggregation and sloughing of the fibroblast cell and detachment from cell sheet at 8th passage after 48 hours. C. Un infected cell culture (control) (40X).

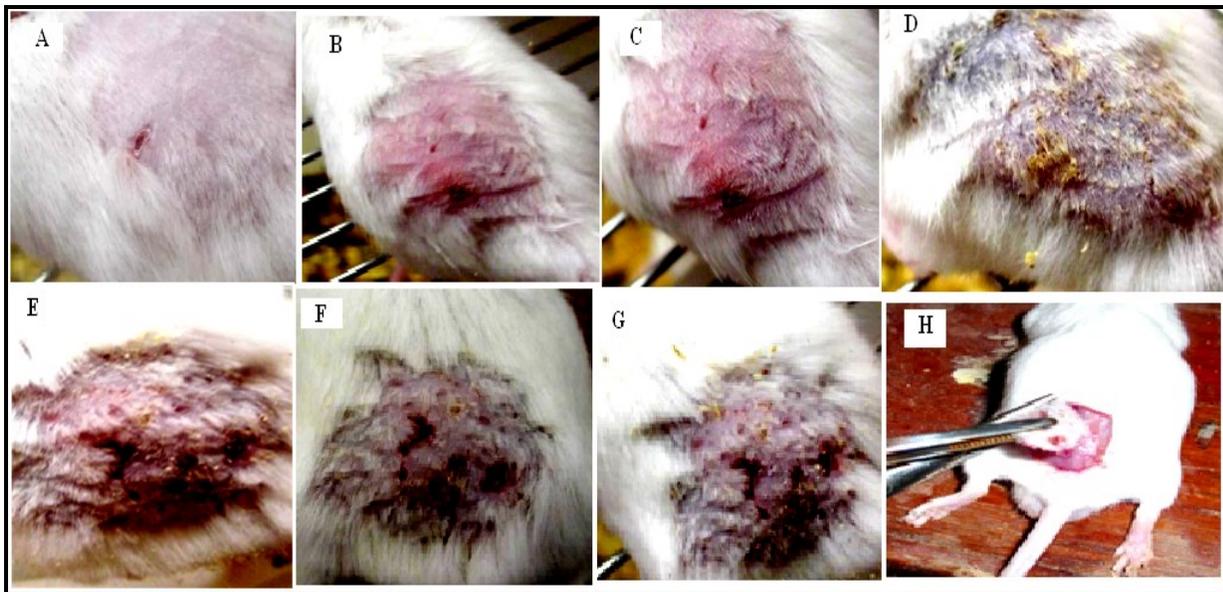


Figure.2 showed the result of gross lesions of virus inoculation in the skin of the mice back. A. normal skin appearance of control animal. B, C. Showed moderate skin lesions of infected group; 72 hours after infection. D, E, F, G. Showed sever skin infection and appearance of scabs and different stages of infection; 72 hours after infection. H. Excision of infected area using sterilized scissor and forceps for sectioning.

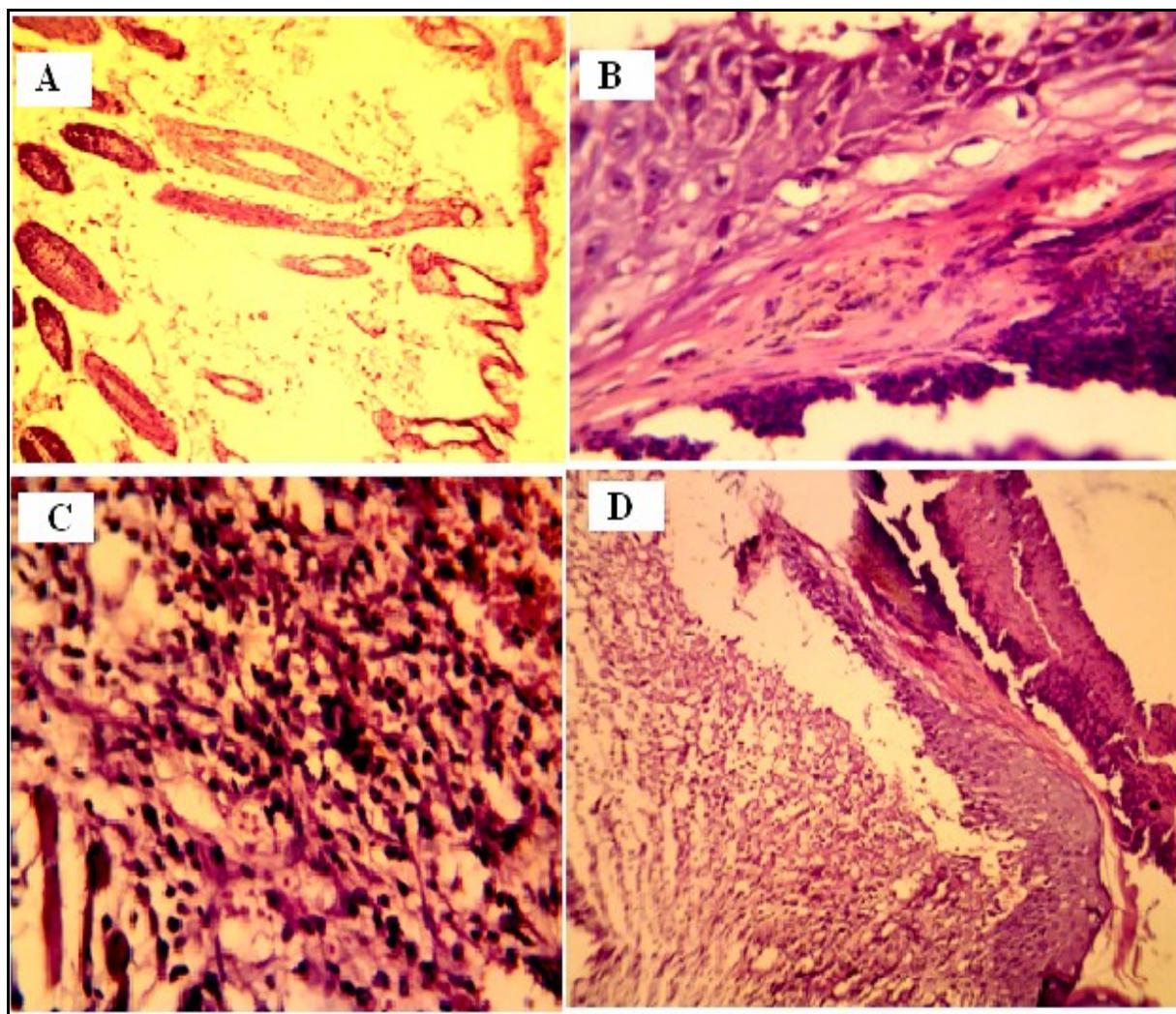


Figure.3 Histopathological sections of mice skin which showed the followings: A. Skin of control group with normal skin structure. (H and E 10X).

B. Skin of infected group showed the hydropic degeneration of prickle cell layer, arrows. C. The sub cutis layer of infected skin was infiltrated with neutrophils with mild hemorrhage and fibroplasias, arrows. D. Skin of infected group showed of acanthosis and hyper keratosis and large number of dead neutrophils on the surface with infiltration of mononuclear cells in the dermis, arrows. (H and E 40X).

Viral inoculation using experimental mice

The inoculated mice were showed their response to virus infection has varied in intensity. The main skin manifestations were thickening and redness at the injection site after 24hr. after three days of infection, were showed formation of blisters and crusts to seven mice of infected group. The lesion was

showed the layer of thick brown-gray crust that may be elevated 0.5-1 mm above the skin surface; were typical for poxvirus infections but more proliferative due to continues epidermal proliferation. This result was in line with Pompei *et al.*, (2010) but it was using of lambs and Al-Sha'alan, (2003) when using of guinea pig and rabbits as animal model; this result may be due to

immune variation of the infected animals, but this result disagreeing with Al-Nada, (1983) that unable to infect laboratory animals this may be due to strain virulence or strain subtype.

Histopathological sections of inoculated skin

The skin section of control group was characterized by normal histological structure Figure (3A).

The skin sections of infected group were characterized by acanthosis and hyperkeratosis. In addition to that showed hydropic degeneration of prickle cell layer, their epithelial cells contains large vacuoles pushed the nuclei aside and viral inclusion bodies like structure were seen Figure (3 B). Also the sub cutis was infiltrated with neutrophils and undergoes mild hemorrhage and fibroplasia Figure (3C).

The skin surface was covered with a thick layer consisting of necrotic debris and neutrophils may be due to secondary bacterial infection. The dermis was infiltrated with mononuclear cells (Figure 3D). Also there was severe destruction in both epidermal and dermal tissue. These results were supported with Pompei *et al.*, (2010); Tizard (2013) and Al-Obidi, (2014) were showed same results when used different animal model, this may be due to skin response to this type of viruses (epitheliotropic virus).

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