J. Vet. Malaysia (1992) 4(1): 45-49

THE PATHOGENICITY OF A CAPRINE CONTAGIOUS ECTHYMA VIRUS IN SHEEP

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SUMMARY: The lesions that developed following the inoculations of tissue culture adapted caprine contagious ecthyma virus into the damaged skin of lambs were compared to those produced by ovine contagious ecthyma virus. After three inoculations at days 0, 8 and 50, two episodes of histological lesions typical of contagious ecthyma developed. The earliest lesions were observed at 36 hours after the first inoculation and persisted for 7 days and later reappeared at day 16, about 6 days after the second inoculation. Third inoculation failed to reproduce lesions. The gross and histological lesions produced by both the caprine and ovine isolates in lambs were generally similar.

Keywords: contagious ecthyma virus, pathogenicity, sheep

INTRODUCTION

Contagious ecthyma (orf or contagious pustular dermatitis) is a natural viral disease of goats and sheep which is characterised by the formation of vesicular lesions on the lips. The disease is frequently observed in young animals although adults with no immunity may also develop the lesions (Robinson and Balassu, 1981). The lesions usually develop following minor damage to the skin caused by dry and prickly pasture (McKeever *et al.*, 1988). Following the infection, progressive epidermal lesions develop from macule, papule, vesicle, pustule through to scab (Glover, 1928).

Most studies on contagious ecthyma, however, were made on sheep using the virus isolated from the same animal species. Report of experimental infection in sheep using isolates from goats are less common, although considerable heterogeneity based on the restriction endonuclease analysis was observed between contagious ecthyma isolates (Robinson *et al.*, 1982; Rafii and Burger, 1985). This report describes the pathological lesions and compares the pathogenicity of infection by contagious ecthyma virus isolated from goat and sheep in lambs.

MATERIALS AND METHODS

Virus

A tissue culture adapted goat isolate (V1) and a sheep reference strain (ORF II**) were used. The V1 was isolated from the gum of a kid showing severe orf lesions in the buccal cavity. The virus isolate was passaged five times in caprine testicular tissue culture before it was harvested, purified (Nagington *et al.*, 1964) and prepared in 10 per cent suspensions for inoculation (Sinha *et al.*, 1986).

Experimental Animals

Six conventionally raised cross-bred lambs between 2 to 3 months old with no previous history of contagious ecthyma were randomly selected. They were divided into three equal groups and kept in separate rooms.

Experimental Procedure

Lambs in the first group were infected with the V1 virus in the skin of lips and inner thigh using the skin scarification technique (Abdussalam, 1957). In each site, 0.1 ml of the suspension was introduced. Lambs in group 2 were infected with the sheep isolate (ORF II) and group 3 were uninfected control receiving phosphate-buffered saline (PBS) using the same method and at the same sites as those described in group 1.

Eight days after the first inoculation, all groups were re-infected with their respective viral strain using the skin scarification method described earlier followed by the third infection at day 50.

The progress of gross lesion development was recorded daily. Skin biopsies were collected at 4, 8, 12, 16, 24, 36, 48, 60, 72, 96, 120 and 144 hrs as well as at days 7, 8, 12, 16, 20, 26 and 30 after the first infection for the histological and transmission electron microscopic (TEM) examinations.

Sample Processing

All skin specimens were fixed in a modified Bouin solution (McKeever *et al.*, 1988), embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin for histological examination. Skin specimens for TEM were fixed in 2.5% glutaraldehyde in phosphate buffer at 4°C for four hours, stained in osmium tetroxide, dehydrated in a graded alcohol series and embedded in resin. Ultra-thin sections were placed on copper grids and stained with lead citrate and uranyl acetate for transmission electron microscopic examination.

RESULTS

Gross Changes

The challenged sites on lambs in groups 1 and 2 developed scabby lesions typical of contagious ecthyma virus infection. These lesions first appeared on the second day after infection as red erythematous lesions of about 1-2 mm in diameter, became progressively severe and scabby with time of infection and completely healed at about

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** The ORF II strain was kindly supplied by Dr. H.W. Reid, Moredun Research Institute, Scotland.

7 days. The lesions did not develop in uninfected control lambs of group 3 but the induced abrasive skin lesions recovered completely within 72 hours.

Three days after the second infection, both groups developed similar gross lesions which became most severe at day 16 and resolved completely by day 26. None of the lambs orf lesions developed following the third infection.

Histopathology

The earliest histological lesions were observed at 36 hours after infection of lambs in both groups 1 and 2. The progress and severity of the lesions were generally similar for both groups. There were focal accumulations of necrotic epidermal and inflammatory cells on the epidermis, forming scabs. Several superficial cells in the remaining Malpighian epidermal layer beneath these lesions showed necrosis and hydropic degeneration. The cytoplasm contained vacuoles while the nuclei appeared swollen, making the epidermis appeared focally thickened. Nuclei of several superficial cells were pyknotic. Numerous mononuclear and neutrophilic leucocytes were found in the dermis.

Sixty hours after infection, the lesions were less severe than those seen at 36 hours. There were remnants of the scabs on the epidermis which appeared almost normal. There were still marked infiltrations of mononuclear leucocytes in the dermis. Similar lesions were observed at 5 days after infection.

The lesions were very mild and appeared almost normal at days 6, 7 and 8. There were no evidence of scabs on the epidermis and no hydropic degeneration in the epidermal cells while few lymphocytes were found scattered in the dermis. At day 12, several epidermal cells appeared swollen with vacuoles and there were focal accumulations of lymphocytes, plasma cells and few neutrophils in the dermis, particularly around the sweat glands. The blood vessels in the muscular layer were congested with numerous neutrophilic leucocytes.

The histological lesions seemed to be markedly reactivated at day 16. There were numerous epidermal cells showing severe hydropic degeneration while many superficial cells were necrotic. Severe leucocytic infiltrations were observed throughout the dermis, and the same cells formed small pockets in the epidermis. There were marked proliferation of the fibroblast in the dermis. At day 20, there were marked necrosis of epidermal cells that eventually sloughed off to form scabs following leucocytic infiltrations in between the dermis and the epidermis, separating the two layers. The lesions reduced markedly by day 26.

In the uninfected control group, mild infiltration by the inflammatory cells in the dermis at 12 and 24 hours after infection was the only lesion observed. The epidermis appeared unaffected.

Electron Microscopy

Numerous virions that were mostly in the early stage of maturity were observed in the infected epidermal cells, particularly in stratum spongiosum of animals in groups 1 and 2 at 36 hours. These virions, mostly with incomplete outer membranes, were found in the cytoplasm of the cells until 60 hours after infection. The viral particles were not present after 72 hours of infection.

Few viral particles similar to those observed at 36 hours were observed again in the epidermal cells 12 days after the first infection. Much more virions were observed at 16 and 20 days but disappeared again at 26 days after infection.

DISCUSSION

The results of this study reveals that both caprine and ovine contagious ecthyma virus isolates are able to infect lambs, producing similar gross and histological lesions to those reported earlier in sheep (Glover, 1928; Abdussalam, 1957; McKeever *et al.*, 1988). The major lesions consist of scab, formed by the accumulation of dead epidermal cells and inflammatory leucocytes, hydropic degeneration and necrosis of the epidermis, and the infiltration of mononuclear and neutrophilic leucocytes in the dermis.

The two different isolates used in this study seemed unable to produce different type and severity of gross and histological lesions in lambs, although early analysis showed several differences between the two isolates. This is probably due to the ability of lambs to resist development of different lesions using different viral isolate.

ACKNOWLEDGEMENTS

Thanks to Bohari, O.K. Ho, S. Apparau, K. Kumar and Fauzi Che Yusuf for their assistance. The study was financially supported by IRPA grant 50332.

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RINGKASAN

KEPATOGENAN VIRUS EKTIMA MENULAR KAPRIN PADA BEBIRI

Lesi yang terhasil selepas penginokulatan virus ektima menular kaprin tersuai kultur tisu ke dalam kulit rosak anak bebiri telah dibandingkan dengan lesi yang dihasilkan oleh virus ektima menular ovin. Selepas tiga penginokulatan pada hari 0, 8 dan 50, dua episod lesi histopatologi yang tipikal untuk ektima menular telah terkembang. Lesi terawal dapat dilihat pada 36 jam berikutan penginokulatan pertama dan kekal selama 7 hari sebelum menjelma semula pada hari 16, iaitu 6 hari selepas penginokulatan kedua. Penginokulatan ketiga gagal untuk menghasilkan sebarang lesi. Lesi kasar dan histologi yang dihasilkan oleh kedua-dua isolat virus kaprin dan ovin dalam anak bebiri tersebut umumnya serupa.