
SEVERE COMPLICATIONS INDUCED BY EXPERIMENTAL BACTERIAL SUPERINFECTION OF ORF LESIONS

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SUMMARY

Twelve goats about 3 months of age were divided into 4 equal groups. Goats in Groups 1 and 2 were infected with orf virus followed by Corynebacterium pyogenes infection of Groups 1 and 3, 3 days after the first appearance of orf lesions. Goats in Group 4 were uninfected controls. Complicated orf lesions which consisted of wet suppurative scabs around the entire lips were observed in goats in Group 1. The lesions persisted for 24 days but were most severe from days 8 to 13. Goats in Group 2 developed lesions typical of orf virus infection that lasted 10 days, while goats in Group 3 developed small nodules of about 1 cm diameter, 48 hours following the introduction of C. pyogenes, which persisted for only 6 days. No lesion was observed in goats in Group 4. Two goats in Group 1 with complicated orf died after 16 and 22 days respectively.

INTRODUCTION

Orf, also known as contagious ecthyma or contagious pustular dermatitis, is a viral disease of sheep and goats that has a worldwide distribution. The disease is characterised by scabs overlying the ulcerated skin of the lips, occasionally with secondary complications involving bacterial and parasitic infections (Robinson and Balassu, 1981). Higher death rates and prolonged infection have been seen mostly in complicated cases. This report describes the progress of orf lesions in goats complicated by infection with Corynebacterium pyogenes as the secondary agent.

MATERIALS AND METHODS

Animals

Twelve conventionally raised goats of about 3 months of age with no history of previous clinical orf lesions were selected for the study. They were divided into 4 equal groups, kept in separate rooms, fed with cut grass and supplemented feed at the rate of 0.25 kg/animal/day. Drinking water was available ad libitum.

Viral and bacterial preparations

Virus from severe orf lesions on the lips and buccal mucosa of a kid was used. The inoculum was prepared by grinding the samples to produce a 10% suspension in phosphate buffered saline (PBS) pH 7.2 followed by treatment with antibiotics (Mazur and Machado, 1989).

Corynebacterium pyogenes was used to induce secondary bacterial infection of the orf lesions. It was grown on blood agar from which the inoculum was prepared at the rate of $3 \times 10^5$ cfu/ml according to the method of Lennette et al. (1974).

Experimental procedure

Goats in Groups 1 and 2 were infected with 1.5 ml of the orf virus inoculum by the scarification technique of Abdussalam (1957) into the skin of the lips. Goats in
Groups 3 and 4 were inoculated with phosphate buffered saline (PBS) using the same inoculation technique.

Seventy-two hours after the first appearance of orf lesions *C. pyogenes* was introduced into goats in Groups 1 and 3 by applying the prepared inoculum at the rate of 1 ml per goat. The bacteria were injected either directly into the orf lesions of Group 1 or into the skin of the lip of Group 3 goats. Other groups received similar amounts of PBS.

**Sample collection and processing**

Skin biopsies were collected at 0 and 24 hours after virus infection followed by further sampling at 5, 24, 48, 72 hours and at 9 and 20 days after the secondary bacterial infection. Skin samples were divided into 3 parts; the first was fixed in a modified Bouin solution (McKeever *et al.*, 1988), embedded in paraffin wax, sectioned at 5 μm thick and stained with haematoxylin and eosin (HE) for histological examination. The second was used for the isolation and quantitation of *C. pyogenes* according to the method of Saleha and Basri (1985) while from the third part, frozen sections of about 4 to 6 μm thick were prepared to demonstrate the orf virus in tissue sections by the fluorescence technique of Chubb and Couch (1985).

**RESULTS**

**Clinical observations**

Skin lesions typical of orf were first observed 48 hours after inoculation of goats in Groups 1 and 2. The lesions consisted of several small, raised and swollen nodules of about 4 mm diameter with dark necrotic tissue at the centre which later increased in size. Goats in the remaining 2 groups had no lesions.

Twenty-four hours after the bacterial infection, the lip lesions in Group 1 became wet and swollen. The number of orf nodules had increased and were markedly swollen and painful on palpation. The scabby nodules affected the whole upper and lower lips at 72 hours which became severely swollen and cracked with pus oozing out from the lesions. The anterior half of the face was swollen.

Six days after the bacterial infection, there were obvious subcutaneous accumulations of pus causing the lips of goats in Group 1 to appear severely swollen. The presence of these lesions affected the appetite causing loss of body weight. At 9 days the entire left and right side of the upper lip were markedly swollen with pale necrotic and granulomatous-like wet suppurative nodules of about 4 cm diameter. The lip swellings were markedly reduced, dry and healing at day 13, leaving fresh new skin underneath. Two goats died on days 16 and 22. Complete recovery was seen in the remaining goat in this Group on day 24.

Goats in Group 2 had several small scabby lesions typical of orf which healed after 10 days. The goats in Group 3 had slightly swollen lips 24 to 48 hours after inoculation with *C. pyogenes*. The swollen lips developed between one to 3 small nodules containing small amounts of pus after 48 hours. The lesions persisted for 6 days. Group 4 did not have any lesions.

**Histopathology**

Lesions observed at 24 hours after orf virus infection in Groups 1 and 2 were typical of orf. There was hydropic degeneration of the epidermis with dead cells forming scabs. The dermis and part of the epidermis were infiltrated by numerous mononuclear leukocytes. Several polymorphonuclear leukocytes were observed among these cells.

Five hours after the bacterial infection in Group 1, there was a marked increase in the number of infiltrating cells, particularly polymorphonuclear leukocytes. Some