

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) pH7.2 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 3×10^5 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

sections had oat cells among the numerous dead and living polymorphonuclear leukocytes in the extensive areas of coagulative necrosis in both the dermis and epidermis at 24 hours. The necrotic lesion was not observed in goats in Group 2 infected with orf virus alone. Three days after the bacterial infection, the lesions consisted of clear cellular necrosis and lysis particularly in the epidermis which detached from the severely inflamed dermis. Bacterial colonies were readily observed in both epidermis and dermis in this area. Similar but much less severe lesions were observed in Group 3 infected with *C. pyogenes* alone.

Progressively severe necrotising lesions were observed in Group 1 with the eventual formation of many microabscesses. At day 20, there was evidence of partial recovery with the formation of new epidermis, although numerous mononuclear cells were still present.

Microbiology

Specific fluorescence was detected in the epidermis 24 hours after orf virus infection in Groups 1 and 2, and remained detectable in the necrotic epidermal cells for 20 days after the secondary bacterial infection in Group 1.

At the peak of lesion development, there were obvious differences in the quantity of *C. pyogenes* found in the lesions between goats in Groups 1 and 3. The bacteria were isolated in pure cultures in both groups in numbers ranging from 5×10^3 to 5×10^4 cfu per gram of skin tissue in Group 1 and 1×10^2 to 7×10^2 in Group 3.

DISCUSSION

Complicated orf is an important factor in determining the death rate, the severity of body weight loss and the speed of recovery from orf virus infection (Robinson and Balassu, 1981). This study has shown that complicated orf produced the most severe lesions, in which the orf virus induced initial lesions which became severe following secondary infection. Although the secondary bacterial infection had established in the lesions initiated earlier by orf virus, the virus remained in the necrotic epidermal cells for 20 days. Without the secondary infections, the lesions were mild and healed rapidly. The longer recovery period that was observed in the complicated orf reduced the ability of the animal to eat and drink which eventually lead to chronic starvation and death. Since the recovery is rapid and spontaneous in uncomplicated orf (Robinson and Balassu, 1981), prevention of secondary infection of orf is essential.

ACKNOWLEDGMENTS

We thank Dr Azri Adzhar for advice. The study was supported by IRPA research grant 50332.

Accepted for publication June 1992

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COMPLICATIONS SERIEUSES DUES A UNE SURINFECTION BACTERIENNE EXPERIMENTALE DES LESIONS DUES AU VIRUS ORF

Résumé—Douze chèvres âgées de près de 3 mois ont été réparties entre 4 groupes égaux. Les chèvres des groupes 1 et 2 ont été infectées avec les virus ORF, suivi d'une infection à *Corynebacterium pyogenes* chez les groupes 1 et 3, trois jours après la première apparition des lésions dues au virus ORF. Les chèvres du groupe 4 représentaient le groupe témoin non-infecté.

Des complications dues aux lésions ORF formées de croûtes purulentes et suintantes tout autour des lèvres ont été observées chez les chèvres du groupe 1. Les lésions ont persisté 24 jours, mais ont surtout été graves du 8^e au 13^e jour. Les chèvres du groupe 2 ont développé des lésions typiques de l'infection par virus ORF qui ont persisté pendant 10 jours, tandis que les chèvres du groupe 3 ont développé de petits nodules d'environ 1 cm de diamètre, 48 jours après l'inoculation de *C. pyogenes* qui ont duré 6 jours seulement. Aucune lésion n'a été remarquée chez les chèvres du groupe 4. Deux chèvres du groupe 1 atteintes d'ORF avec complications sont mortes après 16 et 22 jours respectivement.

GRAVES COMPLICACIONES EN LESIONES DE ECTIMA CONTAGIOSO INDUCIDAS MEDIANTE SUPERINFECCION BACTERIAL EXPERIMENTAL

Resumen—Se formaron 4 grupos de cabras de 3 animales cada uno, con una edad aproximada de 3 meses. Las cabras de los grupos 1 y 2 se infectaron con el virus del ectima contagioso. A los 3 días de la aparición de las lesiones de ectima contagioso los grupos 1 y 2 se infectaron con *Corynebacterium pyogenes*. Las cabras del grupo 4 sirvieron de controles no infectados. Los animales del grupo 1 presentaron lesiones complicadas de ectima contagioso que consistían en escaras húmedas y supurativas y que cubrían todos los labios. Las lesiones persistieron 24 días, siendo más graves desde el día 8 al 13. Las cabras en el grupo 2 desarrollaron lesiones típicas de ectima contagioso que duraron 10 días, mientras que las del grupo 3 desarrollaron pequeños nódulos de cerca de 1 cm de diámetro 48 horas después de la infección por *C. pyogenes* que persistieron solo 6 días. No se observaron lesiones en las cabras del grupo 4. Dos cabras del grupo 1 con ectima contagioso murieron a los 16 y 22 días respectivamente.