drome identique aux cas observés en pratique. La thérapeutique implique le repos et les précautions ordinaires pour empêcher le pied de se renverser.

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CONTAGIOUS ECTHYMA OF SHEEP

I. ATTEMPTS TO INFECT OTHER HOSTS

By ANDREW S. GREIG[1]

Contagious ecthyma (ovine pustular dermatitis) is a disease of sheep and goats occurring in parts of Europe, Australia, Britain and North America. In Canada it occurs sporadically in the western sheep-raising areas and has been reported in eastern Ontario. The uncomplicated disease is usually benign and is found primarily in lambs and nursing ewes. The disease is characterized by the formation of lesions on the lips of susceptible animals 2 or 3 days after virus invasion. In ewes lesions may occur on the epithelium of the teats. The lesions commence as papules on the skin at the site of invasion and rapidly progress through the vesicular stage to pustules. Within a few days the pustules rupture and scabs form over the resulting ulcers. The lips become swollen and tender and the animal may stop eating. Although head lesions are usually confined to the lips, they may occur on the mucous membranes of the mouth and pharynx and around the eyes and nostrils. Signs of systemic reaction are usually absent although there may be swelling of the submaxillary glands.

The virus etiology of the disease was first demonstrated by Aynaud (1). The virus is killed by heating to 59°C for 30 minutes, but dried scabs held in the refrigerator (5°C) will remain infective for several months. In this laboratory scabs frozen and held at -15°C have been found infective after 3 years. The virus is poorly filterable. Although the lesions are formed as a result of the action of the virus, secondary invaders play a role in the severity of the disease. In the south-western United States the lesions are frequently invaded by the larvae of the fly *Cochliomia macellaria* (2).

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The natural disease seems to be confined to sheep and goats, although vesicular-pustular lesions on the face and hands of men handling sheep have been attributed to the virus of contagious ecthyma. The disease in humans, known as Orf is usually uncomplicated, the lesions remaining localized but persisting 4 or more weeks (3).

The purpose of this paper is to report attempts to propagate three field strains of contagious ecthyma virus in hosts other than sheep.

MATERIALS AND METHODS

Each of the three strains used in this work was obtained from specimens of sheep scab sent to the laboratory for diagnosis. Two were from Ontario and one from Alberta. Each gave identical reactions when inoculated into the skin of lambs. The strains are designated through this work as strain I, II or III.

Virus inoculum was, in some cases, prepared simply by triturating infective scab in saline by means of a glass tissue grinder to a final dilution of 1:100. Occasionally 10% scab suspensions in saline were treated by differential centrifugation to give inocula relatively free from extraneous material. The saline scab suspensions were first centrifuged at 2000 RPM for 20 minutes. The supernatant portions were again centrifuged under refrigeration at 20,000 RPM for 1 hour. The resulting precipitates were resuspended in one-tenth the original volume of saline and were cleared of particles which would not readily resuspend by a final centrifugation at 1,500 RPM for 10 minutes. Five hundred i.u. of penicillin and 500 micrograms of streptomycin per ml. of inoculum were added for some studies. Although no attempts were made to compare the virus contents of centrifuged preparations with original suspensions, lambs inoculated with the former confirmed the presence of virus in substantial quantities.

Rabbits, guinea-pigs and mice were prepared for inoculation by removing the fur from an area on the flank of each animal by shaving. The area to be inoculated was lightly scarified with a sharp, sterile needle, and virus suspension was applied with a sterile cotton swab-stick.

Suckling mice were used before fur had developed. Embryonated eggs were inoculated on the chorioallantoic membranes (CAM), in the yolk sacs (YS), or in the allantoic cavities (AC) using inocula prepared by centrifugation and with antibiotics added. Twelve day old eggs were used for chorioallantoic inoculations, and 9 day old embryos for the other routes. Eggs were used mostly in groups of 8. Incubation was at 37°C.

Lambs and kids were inoculated on lightly scarified areas of the groin.

RESULTS

Three groups of 3 guinea-pigs each were inoculated with suspensions of each virus strain. None of the animals showed any evidence of infection

over a 20 day period of observation. Virus strains I and II were used to inoculate 2 groups of 10 adult mice but again signs of infection failed to appear. Two families of suckling mice inoculated at 1 day of age likewise did not develop visible lesions with virus strains I and II.

A total of 19 rabbits, including control animals were used in attempts to propagate the virus. In the first attempt with strain, 1. 2 rabbits receiving virus, and 1 control rabbit which was scarified but inoculated only with sterile saline, all developed a thin almost colourless scab on the lines of scarification within 24 hours. There was no erythema or evidence of pustular development. Since the slight reaction was identical on inoculated and control rabbits, it was not considered significant.

In the second experiment, using strain II virus, 4 rabbits were inoculated with virus suspension alone, 2 with virus suspension to which 200 tissue reducing units of hyaluronidase per ml. had been added, 1 rabbit with saline and 1 with saline + hyaluronidase. The hyaluronidase was used in an effort to increase tissue permeability. The rabbits receiving virus and virus plus hyaluronidase developed several round unraised reddened areas 1 to 2 mm. in diameter over the sites of inoculation but not necssarily on the lines of scarification. These lesions usually appeared 4 to 5 days after inoculation and persisted 3 or 4 days after which they disappeared completely. No such lesions were found on the control rabbits and the presence of hyaluronidase did not influence the degree of reaction.

The third and fourth experiments repeated the second, except that virus strain III was used. In one case a crude suspension of scab was used for inoculation and in the other the inoculum was clarified by means of differential centrifugation. In each case the reaction was the same as in experiment 2. All six rabbits receiving virus developed small erythematous spots not found on the control rabbits.

Four trials were made to propagate the virus in embryonated eggs. In the first trial, using strain I, one group each of YS, AC, and CAM inoculated eggs were examined daily but failed to reveal any embryo deaths or abnormalities. Opening and examination of the eggs in 5 days revealed no lesions.

The second trial utilized strain I virus again but was confined to CAM inoculation. A larger group of eggs (20) were inoculated and groups of 4 opened at 24 hour intervals. From 48 hours on, 1 or 2 eggs of each group showed slight opacities and thickened areas on the membranes. Control eggs inoculated in a similar manner with saline revealed the occasional thickened areas, but no opacities. A second passage in eggs was made using a suspension of primary passage membrane harvested 72 hours after inoculation. Examination of the second passage eggs after 3 days incubation revealed some thickened membranes but no opaque areas. A third passage was made, and portions

of thickened chorioallantoic membrane used to prepare a suspension for the inoculation of a lamb. When within 3 weeks the lamb failed to show any evidence of infection, it was subsequently challenged and found fully susceptible to the virus.

The third and fourth trials in eggs utilized strains II and III. Strain II was inoculated only on the CAM of 1 group of eggs, and on examination 3 days later slight opacities and thickened areas of tissue were found on the membranes of about half the inoculated eggs. Portions of the affected membranes were harvested, a suspension prepared in saline and used to inoculate a lamb. No evidence of infection was found. Similar results were obtained with strain III virus inoculated by the CAM route. In addition, strain III virus suspension was inoculated into groups of eggs by the YS and AC routes. Yolk sac and AC fluids were harvested after 3 days incubation and used to inoculate 2 lambs. There was no evidence of infection in either lamb.

Three kids, each inoculated with a different virus strain, all showed evidence of typical infection. Within 48 hours the site of inoculation in each case was inflamed and slightly swollen. In another 48 hours pustules developed over the scarifications, and these were followed in a few days by scab formation. The scabs persisted for about 2 weeks after which the inoculation sites returned to normal. Each kid was reinoculated 2 months later with a different virus strain than had been used initially, but all three animals proved to be resistant to re-infection.

DISCUSSION

The purpose behind the search for a host other than the sheep is, of course, to facilitate laboratory studies. Then too, although the disease is easily prevented by the use of a vaccine prepared from infectious scab material from sheep, the risk of disseminating other more serious disease agents of sheep in such a product must not be overlooked.

Within the limited range of animals tried in this study, only lambs and kids showed any outstanding degree of susceptibility to infection with the virus of contagious ecthyma. The very slight reaction in the rabbit perhaps indicates that the strain of rabbit used was slightly susceptible, but insufficient to be useful for diagnostic or laboratory purpose. Bennet, Horgan and Haseeb (4) on the other hand were successful in transmitting infection to rabbits to a much greater extent. In their work they describe skin eruptions following inoculation consisting of coarse, yellowish, pustules with appreciable erythema around the peripheries of the lesions. They also found hemorrhagic spots about 3 mm. in diameter scattered throughout the area of inoculation. Likewise rabbits inoculated by Blanc (5) developed severe pustular lesions which later became crusted and dry. Between the lines of scarification red macules appeared about the third day. He was able to transmit infection from rabbit to rabbit.

Glover (6) working in England found that the rabbit as well as the guinea-pig, mouse, fowl and pigeon were refractory to the virus by any route of inoculation tried. Similarly in the United States Broughton and Hardy (2) were not successful in their attempts to infect rabbits, guinea-pigs, a calf and a dog. It is possible that differences in the strain of virus or strain of rabbits used might account for the diversity of results obtained by the different workers.

Although there appeared to be slight opacities formed on the membranes of embryonated eggs inoculated with contagious ecthyma virus they are apparently not the result of virus propagation. Pask (3) and his workers mention similar lesions on eggs inoculated by them but were not able to recover virus from inoculated eggs.

SUMMARY

Three strains of contagious ecthyma virus were used in attempts to infect mice, guinea-pigs, rabbits, kids and embryonated eggs. Mice, guinea-pigs and embryonated eggs proved refractory. In rabbits small reddened areas developed around the sites of inoculation, but other signs of infection did not appear. Kids proved to be as susceptible as lambs to the viruses employed.

RESUME

Trois souches du virus de l'ecthyma contagieux ont été utilisées dans des tentatives d'infection chez la souris, le cobaye, le lapin, le chevreau et sur l'oeuf embryonné. La souris, le cobaye et l'oeuf embryonné se sont avérés réfractaires. Chez le lapin, de petites aires rougeâtres se sont développées au pourtour du point d'inoculation et il n'y eut pas d'autres signes d'infection. Dans l'infection expérimentale, le chevreau paraît présenter la même susceptibilité aux virus utilisés que l'agneau.

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