Swinepox. Virus Isolation, Experimental Infections and the Differentiation from Vaccinia Virus Infections

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With 13 Figures
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Summary

The isolation of swinepox virus in primary pig kidney cell cultures is reported. The differentiation from vaccinia virus was possible with challenge infections of convalescent pigs and the use of the agar gel diffusion precipitation (AGDP) test and immuno-electroosmophoresis (IEOP). Using both immune precipitation tests reactions of identity were obtained between the heterologous antigens of swinepox and vaccinia viruses. A total of 829 pig sera from the field were tested for precipitating antibodies with the IEOP. Antibodies were detected in 65 (=7.8 per cent) of these serum samples.

Introduction

Swinepox virus has been found to have a very restricted host range. The natural host is the pig. Rabbits can be infected by intradermal inoculation, but serial transmission was unsuccessful [DATT (4)]. Swinepox virus infections are considered to have a worldwide distribution. The number of publications on swinepox is small and most reports describe experimental infections in the natural host with accompanying descriptions of the pox lesions. Several attempts to isolate the virus in various tissue culture systems were unsuccessful [MAYR (10)]. Two reports from Ohio and Iowa, however, described the isolation and propagation of the virus in primary cell cultures of porcine origin [KASZA et al. (8); CHEVILLE (2)]. From Illinois MEYER and CONROY (11) reported an additional successful virus isolation in diploid swine kidney cell cultures with the appearance of cytopathic lesions after four blind passages. This paper describes the isolation of swinepox virus in primary pig kidney cell cultures. The differentiation from vaccinia was possible with challenge infections of convalescent pigs and the use of the agar gel diffusion precipitation (AGDP) test and immuno-electroosmophoresis (IEOP). The latter test was used for a field survey. Of 829 pig sera from the field 65 were positive.

An accompanying report describes the electron microscopy of ultrathin sections of swinepox lesions in an experimentally infected pig (14).
Materials and Methods

Origin of the Virus

In September 1971 a group of a hundred 6 to 12 month-old pigs were brought together for further transportation from different sources in The Netherlands. Nearly all animals showed evidence of the pox infection within 5 days after the first appearance of the lesions in the herd. The lesions were mainly observed on and around the ears. The cutaneous regions of the trunk and the legs were also affected, but to a minor degree. The early lesions consisted of hard erythematous papules of 3 to 7 mm in diameter. They developed into typical pox lesions with a central crust and epithelial proliferation but without visible vesicle formation. Virus isolations and experimental infections were performed with 10 per cent suspensions of mixtures of encrusted cutaneous material and biopsied skin lesions in phosphate buffered saline (pH 7.2). Material was obtained from a number of the animals.

Cell Cultures

Cell cultures prepared by trypsinization of the kidneys of 3 to 5 week-old piglets were used throughout the experiments. The isolation of the virus was performed in primary cultures and starting with the second virus passage secondary pig kidney cell cultures were used. The growth medium consisted of Eagles’ MEM (Hanks’ BSS) supplemented with inactivated calf serum (10 per cent) and antibiotics. The maintenance medium was Earle’s BSS supplemented with lactalbumin hydrolysate 0.5 per cent, glucose 0.1 per cent, yeast extract 0.1 per cent and bovine albumin 0.1 per cent (1). Penicillin, streptomycin and mycostatin were added in concentrations of 100 I.U., 100 µg and 50 I.U. per ml of medium, respectively.

Neutralization Tests

Neutralization tests were carried out against 100 TCID₅₀ of the swinepox virus employing a vaccinia hyperimmune serum of bovine origin (kindly provided by Dr. G. van Steenis, National Institute of Public Health, Bilthoven, The Netherlands) and convalescent sera of vaccinia virus- and swinepox virus-scarified piglets. The serum-virus mixtures were incubated at 37°C for 1 hour and then inoculated into secondary pig kidney cell cultures.

Immuno-Electroosmophoresis and Agar Gel Diffusion Precipitation Test

Antigens for the immuno-electroosmophoresis (IEOP) and agar gel diffusion precipitation (AGDP) tests consisted of suspensions of pox lesions and packed cells or 60 to 100× concentrated supernatants of virus-infected pig kidney cell cultures. The concentration of tissue culture fluid was performed by ultrafiltration (Amicon diaflo® systems) or by 7 per cent polyethylene glycol (6 m) precipitation. The AGDP technique was carried out on microscope slides (76 × 26 mm), using 3 ml of 1 per cent agarose (Indubiose® A37, l’Industrie Biologique Francaise S.A., 92-Gennevilliers, France) in PBS of pH 7.2. Hexagon diffusion patterns were used. The diameter of the wells was 4 mm and the distance between the peripheral wells and the edge of the central well was 6 mm. The central well was filled to the rim with antigen, taking care that the fluid came into contact with the entire edge. Undiluted serum was added to the peripheral wells and these were refilled two times within the first half hour. The slides were incubated overnight at room temperature in a moist chamber. Antigen was removed by suction the following day. The slides were then washed in PBS for 24 hours followed by a change in distilled water for at least 48 hours, dried and stained with amido black 10B.

The IEOP test is essentially a combination of the techniques described by PAN et al. (12) and WALLIS and MEHNICK (15). The electrophoresis chambers of the Shandon Vokam electrophoresis apparatus were filled with veronal buffer (diethylbarbituric acid, 1.84 g; sodium diethylbarbiturate, 10.3 g; distilled water to make a 1 liter volume) adjusted to pH 8.6 with hydrochloric acid. The gel used was 1 per cent agarose in the same veronal buffer with 20 per cent lowered concentrations of diethylbarbituric acid.