
DIAGNOSIS OF AFRICAN SWINE FEVER BY IMMUNOFLUORESCENCE

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SUMMARY

An immunofluorescent method for the diagnosis of African swine fever (ASF) is described. Diagnostic samples inoculated onto PK15 swine kidney cell line monolayers on coverslips, are acetone-fixed and stained with fluorescein isothiocyanate-conjugated ASF convalescent serum globulin at 24, 48 and 72 hours after inoculation. Specific fluorescence is detected in ASF infected cells as granular or globular inclusions. The method detected ASF virus in materials from some infected pigs which produced no haemadsorption at all, or only after two to three serial passages in swine buffy coat cultures. This test may provide a sensitive and rapid method for diagnosing ASF in chronic infection.

INTRODUCTION

Since its development by Malmquist and Hay (1960), the haemadsorption test has been the principal in vitro method for diagnosing African swine fever (ASF). Although reliable for diagnosing acute ASF, the haemadsorption test has been less satisfactory for diagnosing chronic ASF, possibly because of the presence of haemadsorption-inhibiting antibodies in tissue used to inoculate swine buffy coat cultures. Tissues from chronically infected pigs have often required three serial passages at 7 to 10 day intervals before haemadsorption could be detected in the final passage (Botija, Ordas, Garcia, 1971). Sometimes haemadsorption was not obtained and diagnosis was possible only by inoculating susceptible swine producing acute disease and haemadsorption with their tissues (Botija, 1970). Coggins (1968) succeeded in segregating a non-haemadsorbing ASF virus in tissue culture which produced CPE but not haemadsorption in infected buffy coat cultures. A more rapid and sensitive method was therefore needed in diagnosing subacute and chronic ASF.

The value of the fluorescent antibody method for the study and diagnosis of ASF has been well documented (Boulanger, et al. 1967; Carnero, et al, 1968; Colgrove, 1968; Colgrove, et al., 1969; Colgrove, 1969; Heuschele, et al., 1966; Titoli, et al., 1969; Botija, 1970; Botija, et al., 1971; However leucocyte (buffy coat) smears, blood, and tissues from infected animals occasionally cause difficulty in diagnostic interpretation because of considerable non-specific fluorescence. The immunofluorescence method herein described provides a rapid sensitive in vitro diagnostic test for ASF, particularly for cases which fail to produce haemadsorption on first passage, and which might require three to five weeks to definitively diagnose by the haemadsorption test.

MATERIALS AND METHODS

Cell Cultures. Monolayers of the PK15 swine kidney cell line were grown on coverslips in Leighton tubes using either Eagle's minimum essential medium (MEM) with 10 per cent bovine serum or Earle's balanced salt solution with 0.5 per cent lactalbumin hydrolysate and

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0.1 per cent yeast extract (LEY) and 10 per cent bovine serum plus antibiotics as growth medium, and using MEM or LEY with 5 per cent bovine serum as maintenance medium.

Swine buffy coat (BC) cultures were prepared with and without coverslips as previously described by Hess and DeTray (1960).

Virus Inoculations. Growth medium was removed from PK15 cultures and 0.2 ml of inoculum was placed on the monolayer and allowed to absorb for 30 minutes at 37°C before adding maintenance medium. Buffy coat cultures were inoculated with 0.2 ml without removal of medium. Cultures were incubated at 37°C. Several isolates of ASF virus from various sources were tested (Table I). Hog cholera virus (HCV), bovine ephemeral fever virus (EFV) and bovine epizootic fever virus (Japan) (BEFVJ) were inoculated into some cultures as heterologous virus controls. Buffy coat cultures were examined daily for 6 days for haemadsorption and CPE.

Immunofluorescent Methods. The globulin fraction of a convalescent serum (No. 1412) obtained from a pig inoculated with attenuated Tengani ASF virus and surviving challenge exposure to virulent Tengani and Lisbon 60 ASF virus isolates, was used to prepare ASF fluorescent antibody conjugate with fluorescein isothiocyanate as described by Heuschele et al. (1966). Before use, portions of the conjugated ASF antibody were absorbed by three different methods to remove non-specific reactants: (1) absorption with dried rabbit liver powder, (2) absorption with freshly homogenated normal pig spleen, liver and lymph node tissues, and (3) absorption with acetone-dried normal pig tissue powder (spleen, liver, lymph node). Preliminary trials with these conjugates indicated that those absorbed with pig tissues (either as dried tissue powder or as fresh tissue homogenate) had the least amount of background non-specific fluorescence. A 1:40 dilution of conjugates in phosphate-buffered saline (PBS), pH 7.4, 0.1 M was used in the test.

Coverslips were removed from culture tubes 24, 48 and 72 hours postinoculation (hpi) and washed 5 minutes in PBS and air-dried. They were then fixed in acetone at room temperature for 15 minutes and allowed to dry by evaporation. They were again washed 5 minutes in PBS and covered with ASF conjugate. Duplicate cultures were also covered with a hog cholera fluorescent antibody preparation as a control. The reaction of conjugate with coverslip preparations was allowed to proceed for 30 minutes at room temperature. Coverslips were then washed in three changes of PBS for 15 minutes, mounted with buffered glycerol, and examined by fluorescence microscopy.

The specificity of fluorescence was controlled by: (1) absence of similar fluorescence in unoinoculated cultures or cultures inoculated with heterologous viruses (HCV, EFV, BEFVJ), (2) quenching of fluorescence in ASF-infected cultures to which unconjugated ASF convalescent serum 1412 had been applied before the fluorescein-conjugated ASF antibody.

RESULTS

The PK15 and BC coverslip cultures inoculated with 20 ASF virus specimens had cells containing specific fluorescence when stained with ASF conjugate (Table I). Uninoculated cultures and cultures inoculated with HCV, EFV or BEFVJ did not have similar fluorescence when stained with ASF conjugate. Culture preparations inoculated with HCV had specific fluorescence when stained with hog cholera fluorescent antibody conjugate; however, this HC conjugate did not produce similar fluorescence in cultures inoculated with EFV, BEFVJ or ASF virus or in uninoculated cultures.

Only a few PK cells containing globular or fine granular fluorescence in the cytoplasm were seen in ASF-infected cultures examined at 24 hpi. The numbers of fluorescing cells progressively increased at 48 and 72 hpi and, at the later time, often involved clusters of contiguous cells (Fig. 1). None of the preparations had the extensive involvement of most cells in the monolayer usually seen when ASF virus, which has adapted to PK cells is used for fluorescent studies (Heuschele et al., 1966).