African Swine Fever IV. Demonstration of the Viral Antigen by Means of Immunofluorescence

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SUMMARY

African swine fever immunofluorescent conjugates were prepared in swine and used successfully in the demonstration of viral antigen in frozen tissue sections and in inoculated tissue culture cells. Cross reactivity was observed with the six strains used in the inoculation of swine. The high antibody content of the serum of immune swine did not interfere with demonstration of the antigen in frozen tissue sections of certain of their organs. The localisation and extent of antigen varied with the stage of infection. The virus was demonstrated in spleen and other organs as early as after one day of pyrexia and until after death of the animal. A pool of hog cholera and African swine fever conjugates stained with dyes of different colours was used in the localisation of respective antigens in experimental mixed infection.

RESUME

Des conjugués immunofluorescents anti-peste porcine Africaine préparés avec des sérums de porcs immuns furent employés avec succès pour démontrer l'antigène à virus dans des coupes de tissus congelés et dans des cultures cellulaires. Des réactions croisées furent observées entre les six souches de virus employées dans l'inoculation des porcs. Les taux élevés d'anticorps dans le sérum des porcs résistant à l'infection n'ont pas empêché de démontrer l'antigène dans des coupes de tissu congelé provenant de ces porcs. La distribution et l'étendue de l'antigène ont varié avec le stage de la maladie. Le virus fut démontré dans la rate et autres organes dès le premier jour de pyrexie et jusqu'après la mort de l'animal.

Un mélange de conjugés anti-peste porcine américaine et anti-peste porcine africaine, tint avec des réactifs de couleurs différentes, fut employé pour localiser respectivement ces antigènes dans des tissus infectés avec les deux virus.

Immunofluorescence has proved in this laboratory and many others to be a practical method for the demonstration of certain bacterial, protozoan and viral antigens in tissues from infected animals (1-5). The method seemed particularly promising for the diagnosis of hog cholera (5). This in-infection does not exist in Canada at present but in the last outbreak, eradicated in 1963, the swine population proved highly susceptible. It was in fact so susceptible that, on a few occasions it would have been difficult to differentiate hog cholera (HC) from African swine fever (ASF) on clinical grounds.

The present study was undertaken to determine the specificity and reliability of the fluorescent-antibody technique for the detection of ASF viral antigen directly in frozen swine tissue sections or in tissue culture cells inoculated with suspected material.

Materials and Methods

INFECTIOUS AGENTS

Six ASF virus strains were used in this study. Their origin and source were described in a separate publication (6). Five of the strains — the Spencer, the Gasson, the Portuguese, the Madrid 1 (1960) and the Madrid 2 (1965) — were virulent isolates whereas the sixth, a vaccine strain, was the Portuguese tissue culture modified virus.

Where HC infection was required the Green strain of virus was used.

IMMUNE SERA

Various methods tried in the production of immune sera will be described in a separate publication (6). The method which proved the most useful in swine consisted of vaccination with the 81st. tissue culture passage of the Portuguese attenuated strain, followed at 71 days post-vaccination (dpv), by the feeding of tissues infected with the Portuguese virulent strain. A second challenge, in the form of 3.0 ml. virulent blood was given intramuscularly at 87

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dpv. The majority of conjugates were prepared with serum 117 which had a modified direct complement-fixation end-point titre of 1:80. A limited number of tests were also performed with serum from pig 115 which had a titre of 1:5120. (7).

EXPERIMENTAL ANIMALS

The first part of the experiment was designed to determine the interval between exposure and the appearance of detectable viral antigen in infected tissues. For this purpose a group of 14 swine weighing approximately 50 to 100 pounds, and 3 breeding sows were exposed by feeding tissues infected with the Spencer virus. Temperatures of all animals were recorded daily, and examination for clinical manifestation conducted. The swine were killed at various intervals after exposure following a pyrexia of 1 to 5 days as outlined in Table I. Specimens of tonsil, spleen, liver, lung and kidney were collected from these swine as well as from those which died of the infection.

The second part of the experiment was designed to demonstrate cross-reactivity between various strains of ASF virus. For this experiment, five groups of 2 swine weighing 50 to 100 pounds were exposed by feeding virulent tissue infected respectively, with one of the 5 previously described virulent strains. A sixth group of 3 swine was infected with the Portuguese vaccine strain, given intra-muscularly in the form of 3.0 ml. of infected blood. Swine were observed daily and killed at various intervals after exposure, as listed in Table II. The same organs were collected for examination as listed in the first part of the experiment.

A third part of the experiment was designed to assess the value of immunofluorescence in differentiating HC and ASF viral antigens in swine infected with both agents. For this purpose, swine were infected by feeding tissues containing virulent HC virus, followed 3 days later by similar exposure to ASF virus. Animals were destroyed *in extremis*, and tissues were collected for examination as described previously.

TISSUE CULTURE METHODS

Methods used in the tissue culture studies are described in detail in another publication (8). Kidneys from secondary specific pathogen-free pigs between three weeks and three months of age were treated by standard trypsinization procedures to obtain a suspension consisting primarily of tubular epithelial cells. The cells, packed by centrifugation for two minutes at 1000 RPM, were resuspended in growth medium to an approximate concentration of 0.5 per cent (VV). The suspended cells were then seeded in 5.0 ml. amounts into 28 x 110 mm. Leighton tubes in which 18 x 75 mm cover slips had been placed.

The growth medium contained 0.5 per cent lactalbumin hydrolysate, 0.1 per cent proteose peptone No. 3, 0.001 per cent cysteine HCl (W/V) and 10 per cent (V/V) calf serum in Hank's balanced salt solution. Penicillin (200 iu/ml.) and streptomycin (200 ug/ml.) were incorporated in the medium. Monolayer sheets were complete after 4 or 5 days' incubation at 37°C, and the growth medium proved capable of holding the cultures without necessity of a change until they were inoculated. This was usually done on the 4th day.

At the time of inoculation the growth medium was changed to a maintenance medium in which the specimen was suspended. Inocula consisted of either spleen suspensions from pigs or tissue culture fluids containing ASF virus serially passaged in primary pig kidney cells. Mixed HC and ASF infections were also produced by inoculating the tissue cultures with HC virus followed immediately by the inoculation of ASF virus. In the case of spleen, one gram of tissue, either fresh or frozen, was suspended in 5.0 ml. of medium by grinding with a glass tissue grinder. This suspension was centrifuged at 2000 RPM for 15 minutes, and the supernatant was further diluted 1:10 in maintenance medium. This latter suspension was used to replace the growth medium in equal volume in one or more Leighton cover-slip cultures. Tissue culture-passaged virus was diluted 1:10 or 1:100 in maintenance medium for further passaging.

The maintenance medium used was a modified Lepine's medium (9) consisting of 0.05 per cent casein hydrolysate, 0.015 per cent glutamine, 0.001 per cent cysteine HCl (W/V), 1 per cent Eagle's basal vitamin mixture and 5 per cent lamb serum (V/V) in Earle's saline. Antibiotics, unless otherwise stated, were used at the same concentrations as in the growth medium.

SECTIONING OF TISSUE

The tissues were mounted on tissue hold-

ers using a drop of Lab-Tek O.C.T. compound zone I and quickly frozen in a container of crushed dry ice. Frozen blocks were placed on the freezer bar of the cryostat to adjust to the cutting temperature of –20°C. A Lab-Tek cryostat with the microtome set at two microns was used to cut the tissues. Sections were lifted on the small wire pickup device, placed on cold microscope slides and thawed in place by the warmth of a finger held against the back of the slide. Preparations were then kept frozen until ready for fixation and staining.

FLUORESCENT ANTIBODY TECHNIQUE

Precipitation of globulins — Serum globulins from normal and immune swine were fractionated separately by dialysis for 24 hours at 9°C against nine volumes of 1.88 M ammonium sulphate solution. The precipitates, obtained by centrifugation, were resuspended in distilled water to approximately one tenth the original serum volumes. Ammonium sulphate was removed by dialysis against physiological saline for four days, changing the saline twice daily. Globulin solutions were clarified by centrifugation in the cold, dispensed in small vials, and stored frozen.

Labelling of globulins — The protein content of concentrated globulin solutions was determined by a micro-Kjeldahl or by a Biuret method and the globulins diluted so that each 1.0 ml. contained 10.0 mg. of protein. A solution containing 10.0 ml. of physiological saline, 3.0 ml. of carbonate-bicarbonate buffer (0.5 M, pH 9.0) and 2.0 ml. of acetone was cooled slowly in a dry ice-alcohol bath until ice crystals formed. With stirring, 10.0 ml. of the properly diluted globulin solution (1.0 ml. = 10.0 mg.) was added and the mixture kept cool. Then 1.5 ml. of acetone containing 0.05 mg. of fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate[1] per mg. of protein was added and shaken overnight at 9°C. The mixture was dialysed for 24 hours at 9°C against a large volume of 0.01 M phosphate buffered saline (PBS) pH 7.2, containing 20.0 gm. Dowex[2] AG2-x4 anion exchanger (chloride form), and then for a further 24 hours against the buffer alone. The conjugate was dispensed in small vials and stored frozen.

[1]Baltimore Biological Laboratories, Baltimore, Md.
[2]Bio-Rad, Distributed by Calbiochem. P.O. Box 54282, Los Angeles 54, Cal.

For the study of mixed HC and ASF infections, HC immune globulins were conjugated with fluorescein isothiocyanate (green fluorescence) whereas the ASF immune globulins were conjugated with tetramethylrhodamine isothiocyanate (brick-red fluorescence). These conjugates were used singly or as a pool after their optimum reactivity had been determined in preliminary trials.

Absorption of conjugate with tissue powder. To reduce non-specific fluorescence, the conjugates were absorbed with swine kidney and spleen tissue powders prepared by extraction with acetone and ether. Sufficient conjugate to fill requirements for two weeks was absorbed at one time. For absorption 100.0 mg. of tissue powder per 1.0 ml. of conjugate to be absorbed was moistened with PBS and the excess liquid was removed after centrifugation, to minimize dilution of the conjugate. The moistened powder and conjugate were mixed and placed on a mechanical shaker for one hour at room temperature. The absorbed conjugate was recovered by centrifugation, and stored frozen in small quantities. Before use preliminary titrations were made of immune conjugates to determine the end-point dilution giving optimal fluorescence.

Fixation and staining techniques — The cover slips of Leighton tubes inoculated with the specimens, after incubation for 2 to 5 days, were removed, washed with PBS and dried quickly before a fan. When dry they were fixed for 10 minutes in acetone in a dry ice-alcohol bath. After fixation, cover slip tissue cultures were dipped briefly in PBS and dried as before. The same procedure was used for fixation of tissue sections except that the washings with PBS were omitted.

The staining procedure was the same for both tissue sections and tissue cultures. Acetone-fixed preparations were flooded with diluted immune conjugate, incubated for one hour in a moist chamber at 37°C, rinsed with PBS and air dried in the dark. The reverse side of each preparation was cleaned with moistened soft tissue paper before mounting on microscope slides or cover slips with 10 per cent glycerol in PBS. A minimum of mounting medium was used, and any excess was squeezed from between the preparation and the covering slide by gentle pressure.

As controls on the staining procedure, known infected and non-infected preparations were stained with immune and normal conjugates for each of the unknown specimens.

Microscope — Examination of the preparations were made the same day as mounted by means of a Zeiss Standard Universal microscope equipped with an OSRAM high pressure mercury lamp HBO 200 W and dark field condenser. Exciter filters 38/2.5 (a fixed filter) and BG 12/4 and barrier filter 44 (440 millimicrons transmittance) were employed. Examinations were made using Neofluar 16 x or 40 x objectives and 8 x oculars. Photographs were taken on Kodachrome II films with dark-field condenser giving an exposure of 4 minutes with the above two filters, then a further exposure of 3 seconds using only barrier filter 44.

Results

Reactivity of the immune conjugates

When pig 117 was destroyed, its serum had a modified direct complement-fixation titre of 1:80, and was usable as a conjugate in 1:20 dilution. However, fluorescence was still visible when this conjugate was used in 1:64 dilution. The modified direct complement-fixation titre of the serum from pig 115 was 1:5120 when this animal was destroyed. We have not encountered such a high serum titre in any of the virus diseases studied previously. The reactivity of the latter serum was also remarkable in immunofluorescence. Fluorescence was still discernible when this conjugate was used in a 1:256 dilution on infected frozen tissue sections, and in 1:1024 dilution when it was used on infected tissue culture cells. In routine examination this conjugate was usable in 1:64 dilution with maximum fluorescence.

Coexistence of viral antigen and antibodies in organs of immune swine

Pig No. 117 — Three weeks after vaccination with the Portuguese attenuated virus the serum of this animal had a modified direct complement-fixation titre of 1:40. This titre fluctuated between 1:40 and 1:80 until the 62nd post-vaccination day. On the 98th day, that is 11 days after the second exposure of this pig to the virulent Portuguese virus, the titre of its serum was 1:80. This animal was destroyed on this day, but as reported previously (7, 10) we were unable to demonstrate the virus in its tissue by the complement-fixation test or the agar double diffusion precipitation test. However, small numbers of fluorescent cells typical of ASF virus infection were observed in the lung, spleen and lymph node from this animal.

Pig No. 115 — The serum antibody titre of this animal in the modified direct complement-fixation test fluctuated from 1:10 to 1:20 from the 21st to the 62nd post-vaccination day. By the 98th day, that is 11 days after the second challenge with the virulent Portuguese strain, the antibody titre had risen to 1:80. Thereafter, the temperature of this animal fluctuated, showing a peak of 105.2°F on the 123rd day and another of 106.4°F on the 136th day. Meanwhile the complement-fixation titre of the serum increased to 1:320 on the 120th day and to 1:5120 by the 136th day. However, viral antigen could not be demonstrated by the modified direct complement-fixation test or the agar double diffusion precipitation test in the tissues of this animal obtained at necropsy (7, 10). The liver and spleen from this animal, instead of reacting as an antigen as would be expected normally, reacted with known position antigens as if the suspected tissue contained antibodies. They did not react with known negative control tissue extracts. The tonsil, spleen, liver and kidney of this animal were devoid of any evidence of virus infection when examined by immunofluorescence, but in lung tissue section innumerable fluorescent cells typical of ASF virus infection were seen.

Appearance of viral antigen in tissue sections and tissue culture

The fluorescence in ASF-infected cells was cytoplasmic and very granular in contrast to hog cholera viral antigen which appeared as non-particulate diffuse luminescence within the cytoplasm.

The distribution of fluorescence in tissue sections of various organs (Fig. I) was also very different from that seen in HC. In the *tonsil*, the predilection sites for HC virus multiplication were the epithelial lining of the tonsillar crypts and in the germinal centers, whereas in ASF infection these structures were devoid of fluorescence. Instead, the fluorescence in ASF was located in the lymphatic tissue surrounding these structures. In the *spleen*, the

Malpighian corpuscles which show fluorescence in HC were devoid of activity in ASF. The same observation applied also to the germinal centers of the *lymph nodes*. In the *kidney* the greater part of ASF fluorescence appeared as numerous isolated granules in the glomeruli and was only rarely seen in the intertubular spaces. The reverse was the case in HC infection. In the late stages of HC infection, marked fluorescence was present in epithelial cells of the tubules but this was not seen in ASF.

In ASF, cells showing typical fluorescence in the various organs appeared to have a similar structure, and did not seem to form part of the parenchyma. The cells most commonly seen with specific fluorescence were large, round, irregular cells, approximately five times the dimension of granulocytes, without apparent nucleus. The fluorescence was granular, and often the cells contained a globule of dense solid fluorescence. These cells could possibly be macrophages. A second type of cells, about the size of a granulocyte, also often showed specific fluorescence. These cells were nucleated, and usually resembled small lymphocytes, but some exhibited eccentric nuclei suggestive of plasma cells. Their nuclei were not stained; the fluorescence was cytoplasmic and granular. Very often at the edge of the nucleus, in the cytoplasm, a globule of dense fluorescence was present. In advanced infection some of the above cells appeared "exploded", leaving numerous fluorescent granules and some globules dispersed in the tissue spaces.

In ASF the specific fluorescence as affected by fluorescein isothiocyanate conjugate was very pronounced and had a distinct yellowish green tint easily differentiated from the non-specific yellowish coloration of the granulocytes. With tetramethylrhodamine isothiocyanate conjugate this specific fluorescence took an intense brick red coloration. The ASF rhodamine conjugate, as a pool with the HC fluorescein conjugate, was useful in differentiating and localising both HC and ASF viral antigens in tissue culture or in tissue section of organs from swine infected with both viruses. (Fig. I).

The distribution of ASF antigen and the appearance of fluorescence in inoculated tissue culture cells varied with the interval of incubation between inoculation and examination of the cultures (Fig. I). It was also influenced by the normal degeneration rate of the tissue culture. When examination was performed 1 or 2 days after inoculation, the fluorescent antigen was usually located in single cells which were elongated. The fluorescence appeared as a film covering the entire cells. The intensity was more pronounced at the periphery of the cytoplasm. It was usually difficult to locate the nucleus in single infected cells. The fluorescence was more granulous than in hog cholera but was not as granular as after longer incubation period. In the cytoplasm of a few cells, globules were present which stained more opaque green.

As incubation progressed, by the 3rd or 4th day groups of 2 to 6 cells were showing fluorescence. More than half of them had a rounded appearance. In many cells the nuclei were faintly stained and visible as a dark area covered by a faint greenish film of fluorescence. Depending on the progress of infection, and possibly also on the keeping quality of the cells, minute to moderately large globules might be present in the cytoplasm of many cells. Some of these inclusion-like globules were alone outside of the cells. It was impossible to determine the role played by these globules in the start of new infected areas. Often the globules were seen at their maximum only after 5 or 6 days of incubation. Usually at that time some cells were undergoing degeneration and fluorescent filaments or projections were visible in between these cells. With longer incubation, as the degeneration of the cells progressed, it was not rare to see only isolated rounded cell masses showing fluorescence.

Influence of exposure interval on detection of virus in tissue

As shown in Table I, the virus was detected by immunofluorescence in tissues harvested from the first day of temperature rise until after the animals died of infection. In every case, positive results were obtained also by immunofluorescence applied to tissue cultures inoculated with spleen from the above animals. The amount of fluorescence observed in inoculated tissue cultures did not vary appreciably when examined 3 or 6 days after inoculation.

However, the extent of fluorescence observed in frozen tissue sections of the various organs of an animal varied with the duration of pyrexia and the stage of the disease at the time of collection of specimens. On the first day of temperature the

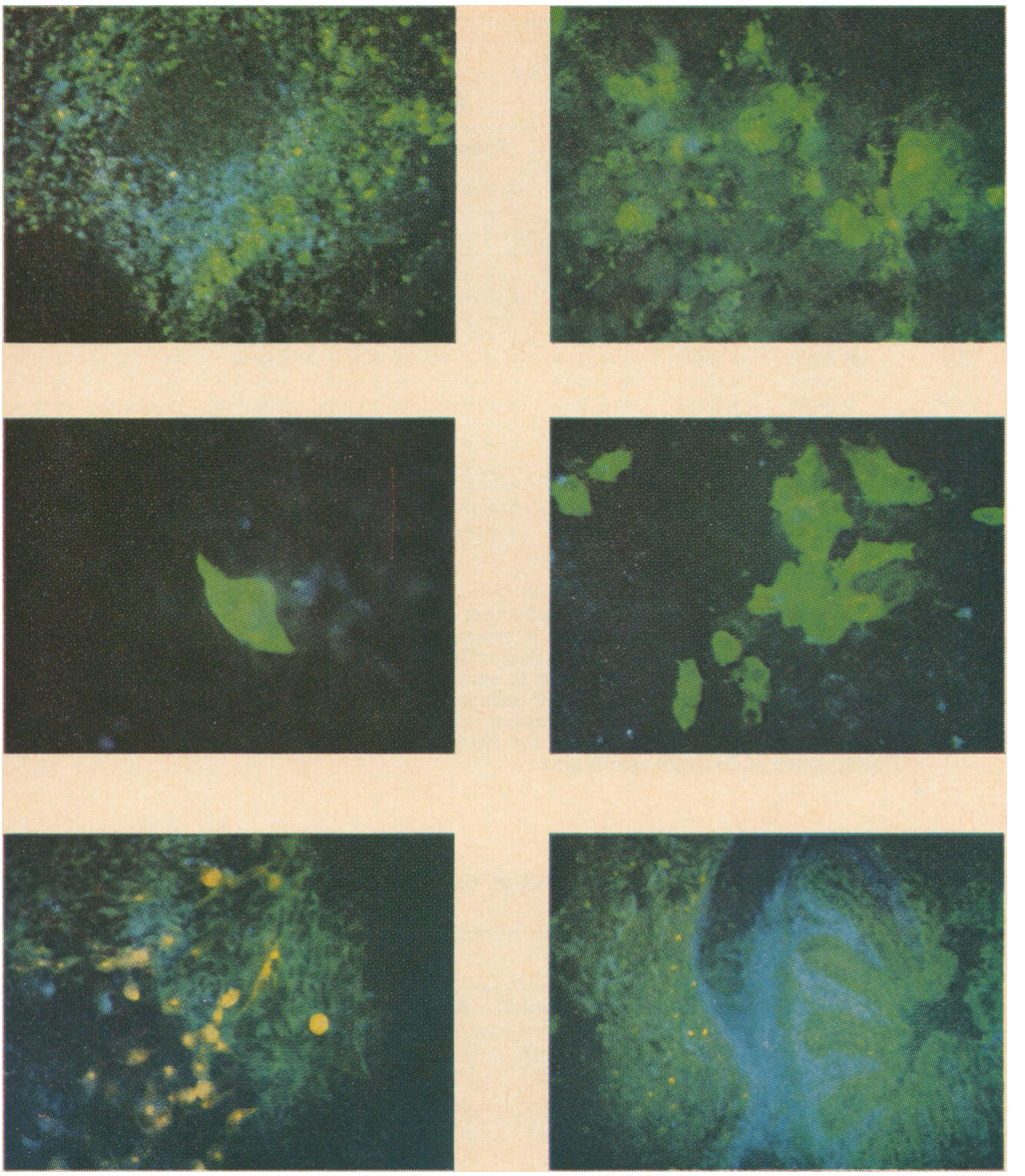

FIG. 1

Top left. — Immunofluorescent (fluorescein isothiocyanate) staining of frozen-cut section of tonsil from pig 145 collected 8 days after exposure to the Spencer strain of ASF virus. Note that the fluorescence is located in the lymphatic tissue surrounding the germinal centers. (x 237).

Top right. — Immunofluorescent (fluorescein isothiocyanate) staining of frozen-cut section of liver from pig 145. Note that the majority of cells showing fluorescence appear as dense fluorescent masses. In rare areas the unstained nuclei are visible. Many granules and globules are dispersed in the parenchyma of the organ between the fluorescent cells (x 592).

Center left. — Immunofluorescent (fluorescein isothiocyanate) staining of pig kidney tissue culture cells examined 1 day after inoculation with ASF, Spencer strain, virus. Note individual elongated cell stained green (x 592).

Center right. — Groups of tissue culture cells stained as above, 5 days after inoculation with ASF, Spencer strain, virus (x 237).

Bottom left. — Pig kidney tissue culture cells inoculated with hog cholera and African swine fever viruses. The slide was stained with hog cholera fluorescein isothiocyanate conjugate (green fluorescence) and with tetramethylrhodamine isothiocyanate African swine fever conjugate (goden orange). When viewed in the fluorescent microscope the latter color appeared brick red but this color was not reproduceable by photography (x 237).

Bottom right. — Immunofluorescent staining of frozen-cut section of lung from a pig inoculated with hog cholera and African swine fever viruses. The preparation was stained with hog cholera fluorescein isothiocyanate conjugate (green fluorescence) and with tethramethylrhodamine isothiocyanate African swine fever conjugate (golden orange). Note the orange cells outside of the bronchus in the alveoli tissue (x 237).

TABLE I. Influence of intervals after exposure to the virus on the detection of ASF (Spencer) viral antigen by immunofluorescence

Pig No.	Exposure time	Duration Pyrexia	Frozen tissue sections: Tonsil	Spleen	Liver	Lung	Kidney	Tissue Culture
138	6 days	1 day	+	++++	+++	ND**	++	+++
162	5 days	1 day	+++	+++	+++	++++	++	+++
142	3 days	1 day	++	++++	++	±	−	++
140	5 days	2 days	++	++++	+++	+++	+	+++
141	4 days	2 days	+++	++++	+++	++++	++	++++
148*	4 days	2 days	++++	+++	+++	+++	++	++++
139	6 days	3 days	+++	++++	+++	++++	++	++++
147*	6 days	3 days	++++	++++	++++	++++	++++	++++
145	8 days	4 days	++++	++++	++++	+++	++++	++++
161	7 days	4 days	++++	++++	++++	++++	++	+++
149*	6 days	4 days	++++	++++	++++	++++	++++	++++
146	8 days	5 days	++++	++++	++++	++++	++++	++++
125	7 days	5 days	++++	++++	++++	++++	+++	++
130	6 days	5 days	++++	++++	++++	+++	+++	++++
Sow 160	7 days	0 day	+++	+++	++	++	++	+++
Sow 156	7 days	2 days	+++	++++	++	+++	+	++
Sow 159	7 days	3 days	++++	++++	++++	++++	+++	++++
Normal 1 to 9	none	none	−	−	−	−	−	−

* : These swine died the night before collection of specimens.
** : Not done because it was impossible to prepare an adequate section; however, typical fluorescent cells were seen in a tissue impression of this lung.
++++ : Innumerable typical fluorescent cells in each field.
+++ : Approximately 25 typical fluorescent cells in most fields.
++ : Approximately 5 to 10 typical fluorescent cells in most fields.
+ : One to 5 typical fluorescent cells in few fields.
± : Fluorescence of doubtful significance.

TABLE II. Detection by immunofluorescence of virus in tissues of swine infected with various ASF viral strains

Virus strains	Pig No.	Exposure time	Duration Pyrexia	Frozen tissue sections: Tonsil	Spleen	Liver	Lung	Kidney	Tissue Culture
Portuguese	129*	9 days	3 days	+++	++++	++++	++++	+	+++
Portuguese	131*	4 days	2 days	++	++	++	++++	++	−
Gasson	156	5 days	2 days	+++	++++	++	+++	+	++
Gasson	157	5 days	1 day	++	+++	++	++	+	+++
Spencer	Sow 159	7 days	3 days	++++	++++	++++	++++	+++	++++
Spencer	Sow 160*	7 days	0 day	+++	+++	++	++	++	+++
Madrid I	166	5 days	3 days	++++	++++	++++	++++	++	++++
Madrid I	167	5 days	3 days	++++	++++	++++	++++	++	++++
Madrid II	176	5 days	1 day	++++	+++	+++	++++	+	++++
Madrid II	175	5 days	2 days	++	++++	+++	++++	++	++++
Vaccine	172	3 days	1 day	−	+++	+	−	−	+
Vaccine	174	4 days	0 day	+	++	−	−	−	++
Vaccine	173	5 days	1 day	−	+	−	−	−	±

* : These swine died the night before collection of specimen.
++++ : Innumerable typical fluorescent cells in each field.
+++ : Approximately 25 typical fluorescent cells in most fields.
++ : Approximately 5 to 10 typical fluorescent cells in most fields.
+ : One to 5 typical fluorescent cells in a few fields.
± : Fluorescence of doubtful significance.

best fluorescence was encountered in the spleen. After 2 or 3 days of pyrexia, the amount of fluorescence was as great in liver, tonsil and lung as in spleen. In the kidney, fluorescence in most cases was in the form of isolated granules in the glomeruli. However, in a few cases of advanced disease the types of fluorescent cells described in the other organs were also seen in the kidney.

Cross-reactivity of the various ASF strains

As shown in Table II, immunofluorescence using conjugated serum 117, derived from the Portuguese vaccine strain, detected viral antigen in frozen tissue sections of swine infected respectively with 5 virulent strains of ASF virus. It also located the virus, without difficulty, in the spleen of 2 of the 3 swine inoculated with the Portuguese vaccine strain. However, only a few fluorescent cells were seen in the spleen of the 3rd animal killed 5 days after exposure to this virus. It was felt that the amount of fluorescence in the 3rd animal was not sufficient to establish a diagnosis. Immunofluorescence applied to tissue culture cells inoculated with spleen from the above animals, supported the results obtained in direct examination of frozen tissue sections. With one exception, pig 131, it detected the virus in the spleen of swine infected respectively with the 5 virulent strains as well as in 2 of the 3 swine inoculated with the vaccine strain. The tissue culture inoculated with spleen 131 collected a few hours after death of the animal was contaminated, which might explain the failure to show virus.

Discussion

Differentiation between hog cholera and African swine fever infection in swine presents no difficulty when the diagnosis is made by immunofluorescence. In addition, the presence of both antigens in experimentally infected swine can be shown by the use of appropriate conjugates specific for each infection. To facilitate the localisation of respective antigens, a pooled HC — ASF conjugate can be utilised. In this case the HC conjugate is prepared with fluorescein isothiocyanate and stains the homologous antigen green, whereas the ASF conjugate is prepared with tetramethylrhodamine isothiocyanate and stains the homologous antigen brick-red.

Heuschele, Coggins and Stone (11) failed to demonstrate specific fluorescent antigen in tissue impressions from swine which survived the acute course of ASF. They concluded that the absence of demonstrable antigens was possibly due to antibody in the tissues that blocked reaction of antigen and labeled antibody. In our study using frozen tissue sections we have shown the presence of antigen in certain organs from swine possessing a very high serum antibody content.

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