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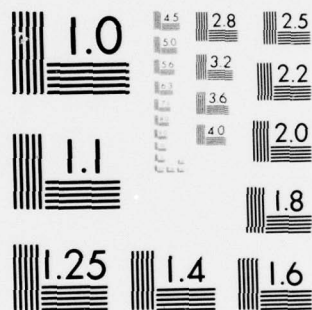
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IMMUNOFLUORESCENCE TECHNIQUE FOR DETECTING ANTIBODIES AGAINST
AFRICAN SWINE FEVER: ITS DIAGNOSTIC VALUE**

C. Sanchez Botija, A. Ordas, J. G. Gonzalez***

The investigation for antibodies of African swine fever acquired great practical interest for diagnosis of chronic forms of the disease in the field as well as the subclinical infections and carriers of the virus surviving at centers of low mortality. In these clinical forms, each time more frequent, they can cause difficulties for the isolation and identification of the virus or for gaining access to pathological material.

Repeated experiments have shown that, in a high percentage of pigs infected with African swine fever and healthy carriers of the virus, the presence of antibodies in the serum coincides with the persistency of the virus in the tissues for long periods of time [17].

Owing to this characteristic of African swine fever, the investigation of antibodies is highly useful for surveying carrier animals whether they are healthy or affected by subclinical infections with discrete lesions.

In order to find the antibodies of African swine fever in the serum of healthy or sick animals suspected of being carriers of the virus, we have preferentially used the complement fixation test. Also, as a parallel technique, we tried the inhibition of fluorescence and precipitation in agar gel.

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The complement-fixation test is a reliable method and has shown a high degree of sensitivity. It has shown the presence of antibodies in a considerable percentage of suspected healthy pigs in which the virus of African swine fever was only found after sacrificing (C. Sanchez Botija, A. Ordas, J. G. Gonzalez [17]). However, this technique requires 24 hours of complex laboratory work in view of the characteristics of the serum of some pigs (precomplementary and anticomplementary capability, requirement for supplementing the complement with calf serum). These circumstances require time-consuming work when an epidemiological survey is made on a great number of animals.

The inhibition of immunofluorescence was first tried on a small number of serums from animals which had recovered from the illness (P. H.

*Numbers in the right margin indicate pagination in the original text.

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Bool, A. Ordas, C. S. Botija [1]). The technique proved to be sensitive and showed antibodies in negative serums with the complement-fixation test. In later testing with serums from pigs with subclinical infections, this method proved to be less sensitive than the complement-fixation test.

The method of precipitation in agar gel was also used but proved to have a sensitivity less than the abovementioned methods.

The inhibition of hemoadsorption was abandoned as a test which could be used for diagnosing carriers in view of the presence in the serum of a number of pigs with nonspecific substances inhibiting the hemoadsorption.

In the last few years, indirect immunofluorescence has been applied in study of the antibodies in various infectious diseases of man and animals [5]. Recently, Ressang and DenBoer [12] used this indirect technique of fluorescent antibodies in order to show in the serum of country pigs the content of antibodies against the virus of the conventional swine fever and have further pointed out the capabilities of this simple and speedy technique in serological research relating to this fever.

The results of the study of antibodies in other illnesses obtained using the indirect technique of fluorescent antibodies as a simple and speedy method have suggested trying this method for studying the content of antibodies of African swine fever, in the serum and its possible application to the serological diagnosis of chronic infections and healthy carriers of the virus.

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In order to evaluate the sensitivity of this technique, the serums were also studied using the complement-fixation test, inhibition of immunofluorescence and precipitation in agar gel. This study was supplemented by postmortem examination of pigs and investigation of the virus in the tissues.

MATERIAL AND METHODS

The material studied by the indirect immunofluorescence method involved serums from 55 pigs in different stages of illness which came from various farms in different regions of the country.

On these farms, the history of the illness was characterized by slowly evolving clinical cases, low mortality and generally with symptoms hardly indicating African swine fever. The sick pigs showed cachexia, and some of them had skin lesions in the form of ulcerated nodules. Most of the animals which were sent with the sick ones appeared to be still healthy. The postmortem examination of the latter animals revealed in some the presence of pulmonary lesions. There was also a record of miscarriages and deaths of a number of young animals.

In other farms, the animals were found to be apparently healthy at the moment that they received the serum but there were records of the death of a number of animals at great intervals and of pigs which had recovered from the disease.

The location of the farms in the enzootic area and a number of the symptoms and lesions (of skin and lungs) suggested the possibility of African swine fever.

The laboratory diagnosis on material from the abovementioned farms was always connected with difficulties. The identification of the virus of African swine fever by routine methods (immunofluorescence, hemoadsorption and cytopathogenic effect with culture of leukocytes) had been negative in various cases. It was necessary to make repeated shipments of material from 465 animals until finally it was possible to isolate in one of them the virus following a time-consuming work lasting 8 to 10 days. The concentration of the virus was always weak.

Some strains of the African swine fever virus which had been isolated in these cases had lost the hemoadsorbing capability and others also gave negative results with the immunofluorescence method in the impressions of the tissues. The final identification was made in these last cases by the cytopathogenic effect with leukocyte cultures and by inoculating the pigs. /400

The pigs were sacrificed after the clinical and serological study carrying out each time a postmortem examination. The viscera were removed in order to determine the presence of virus and its concentration in the various tissues as well as its relationship with the presence of antibodies in the serum.

For the tests of the immunofluorescence method, the serums were classified into four lots consistent with the developmental phases of the illness.

- Lot I: Serums from 10 pigs with symptoms and lesions from chronic illness.
- Lot II: Serums from 9 pigs with subclinical infection (apparently healthy but not showing clinical symptoms although having chronic macroscopic lesions in the postmortem examination of the lungs, spleen and ganglia).
- Lot III: Serums from 15 healthy pigs, recovered from the illness several months before receiving the serum.
- Lot IV: Serums from 23 healthy pigs which showed no clinical symptoms and had no postmortem macroscopic lesions but were suspected of being healthy carriers. These pigs had survived without upset at farms where the virus of African swine fever was finally identified.

THE INDIRECT IMMUNOFLUORESCENCE TECHNIQUE

A. Preparation of the Antigen on Cellular Cultures

a. Culture of Leukocytes

Used for these first tests were preferentially cultures of leukocytes

prepared by customary techniques for the identification of the African swine fever virus.

The leukocytes were produced from defibrinated or heparinized blood [6, 7, 14, 15]. The cultures were prepared in ordinary cellular culture tubes without cover slips and in Leighton tubes with cover slips.

The red blood cells taken along during the collection of leukocytes are removed from the cultures at the same time taking away the nutritional liquid after 24 hours of incubation. There is washing with Hank's solution at 37° C and renewal of the medium with the same solution using 50% of standard pig serum. The presence of red blood cells and phagocytosis make difficult reading of the results with the indirect technique. A number of tubes with red blood cells are left for controlling infection by hemoadsorption.

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Cultures of well-developed leukocytes were used ranging in age from four to eight days in order to remove the autofluorescence from a number of series of leukocytes which appears during the first phases of the culture and when they are not well developed.

Other cellular systems (cultures of pig kidney, plexus chorioideus, pig testicle, bovine fetus kidney) were also used.

b. Virus

In order to carry out the infection of the leukocyte cultures, a strain of the African swine fever virus was selected having a known capability of producing abundant inclusions in the cytoplasm of the macrophages. A 20% suspension was used made from the spleen of a pig deceased from an acute African swine fever caused by an experimental infection. The suspension was kept at -70° C in 2 ml ampules. The titer of the 20% suspension after melting was 10^6 DH₅₀/0.2 ml on the leukocyte culture.

For infection of other cellular systems, use was made of a virus suited for culture of pig kidney cells.

c. Infection of the Leukocyte Cultures

Inoculation of each 0.2 ml with a 1/10 dilution of the 20% spleen suspension.

Incubation at 37° C until the control culture with red blood cells shows hemoadsorption over 50% of the leukocytes (approximately in 48 hours).

d. Selection and Preservation of the Viral Antigen Preparations for Indirect Immunofluorescence

If cultures in Leighton cultures are involved, two cover slips are taken off at 48 hours after inoculation. After having washed with PBS, they are fixed with pure acetone at laboratory temperatures and stained by the direct technique using the anti-African swine fever conjugation according to the customary method (P. H. Bool, A. Ordas, C. S. Botija [1]).

If the cultures from these test cover slips, showing 30-50% of the cells with corpuscles having fluorescent inclusion with specified and quite visible outlines in the cytoplasm of the macrophages, are considered acceptable for the indirect immunofluorescence test, the cover slips are then withdrawn from the remaining tubes. After being fixed with acetone, they are kept at -20°C as a stock of viral antigen on cover slips until their use. The cultures of leukocytes without sufficient corpuscles and with viral antigen in granular form or scattered throughout the cytoplasm are not acceptable for this so-called indirect technique.

When the leukocyte cultures are made in ordinary tubes without cover slips, after 48 hours of post-inoculation and incubation, the liquids from three cultures are collected in a centrifuge tube and another equal volume of PBS is added. The resultant liquid is then centrifuged for 20 minutes at 1000 rpm in order to collect the cellular sediment. The liquid remaining is decanted and the centrifuge is repeated with 10 ml of PBS with a pH of 7.2. Finally, the sediment is homogenized with 0.5 to 1 ml of PBS. This sediment contains a great quantity of macrophages derived from the tube by the cytopathogenic effect of the inoculated virus.

A small drop of the sediment is deposited and spread on two quite clean cover slips. After drying and fixing with acetone, they are stained by the direct method. If these test preparations show the 30-50% of macrophages with well-defined and visible fluorescent inclusions, the cultures are considered to be acceptable, and it is possible to start collecting the sediment from the remaining cultures by means of centrifuging the liquid.

Using the cellular sediment from all the tubes together, preparations are made by means of the extension of small drops on quite clean cover slips. These preparations are dried, fixed with acetone and preserved at -20°C until their use. This method is highly efficient. With a small number of cultures, it is possible to obtain cellular sediment for preparing a great number of cover slips with macrophages containing the viral antigen.

B. INDIRECT STAINING

The anti-IgG rabbit serum of pig and the conjugate of anti-IgG rabbit serum of pig used for the staining were prepared by the methods reported by Bool, et al. [1] in a previous publication.

The test serum in suitable dilution (starting from 1/10) in PBS with a pH of 7.2 is deposited on the cover slips with the viral antigen for 60 minutes at 37°C in a wet chamber using two cover slips for each dilution. The excess serum is removed by three fast washings and from 3-5 minutes in PBS.

Staining is carried out with the rabbit conjugate for 30 minutes at 37°C . The excess of conjugate is taken away with three fast washings and 2 to 5 minutes. Mounting in buffered glycerine with pH 8.6.

Used as controls were cover slips with the viral antigen treated with an anti-African swine fever serum, with standard pig serum and with PBS.

C. INTERPRETATION

The serums are considered as positive when the treated cultures show corpuscles with fluorescent inclusion in the cytoplasm of infected macrophages.

FIXING THE COMPLEMENT

The Cowan [3, 4] technique was used with the modification reported by C. Sanchez Botija, A. Ordas, J. G. Gonzalez [17] in a previous publication.

INHIBITION OF THE IMMUNOFLUORESCENCE

As antigen were used impressions from the spleen of a pig which had died owing to an experimental infection of African swine fever [1]. These impressions showed corpuscles with fluorescent inclusion in the cytoplasm of the macrophages by direct staining.

The impressions were fixed in hot air for 15 minutes and treated with the test serum for 60 minutes at 37° C. The excess serum was removed by three quick washings and 3 to 5 minutes in PBS. Staining with anti-African swine fever pig conjugate. 30 minutes at 37° C. Remove the excess of conjugate by washings in PBS and mounting in buffered glycerine for its observation.

A standard pig serum and PBS were used as control.

The test serum was considered positive when the treated impressions showed no corpuscles with fluorescent inclusion.

PRECIPITATION IN AGAR GEL

The Ouchterlony plates are prepared with Noble Difco agar at 1.5% in phosphate buffer 0.01 M, pH 7.2.

Each plate has made four groups with seven depressions in each one. Each group has one central depression and six in a circle. The depressions have a diameter of 7 mm and a distance between them of 4 mm.

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Into the depressions of each group are deposited antigen, the test serum (pure and in diluted form) and the control samples with alternations made according to customary technique. The serums are deposited one hour after the antigen.

The plates are put in a wet chamber at ambient temperature. Readings are made at 24, 48 and 72 hours.

The spleen of a pig which had died of acute experimental African swine fever was used as positive antigen. Titer 10^{-6} /0.2 ml of spleen suspension at 20%.

METHOD FOR ISOLATING THE AFRICAN SWINE FEVER VIRUS (IVL - Isolation of Virus in Leukocyte Cultures)

The inoculation of leukocyte cultures was used for isolating the virus. Identification was made by the Malmquist and Hay test (hemoadsorption and cytopathogenic effect), by immunofluorescence (with impressions of tissues and with the cellular sediment from cultures of inoculated leukocytes) and for identifying a number of strains of virus which were nonhemoadsorbing and negative to immunofluorescence, the inoculation of the pig was used.

The concentration of virus in the various tissues was determined on a culture of leukocytes with DI₅₀ (estimated by hemoadsorption and the cytopathogenic effect) and the calculations were made by the Reed and Muench method.

RESULTS

The method of indirect immunofluorescence was applied to the study of the content of antibodies against the virus of African swine fever using the serums from 55 pigs at different developmental stages of illness (pigs with symptoms and lesions of chronic African swine fever, animals with subclinical infection, pigs recovered from the illness, and healthy animals suspected of being carriers). The results compared with other methods are shown in Tables 1, 2, 3, 4 and 5.

Forty-seven serums from 55 pigs examined by the indirect immunofluorescence method (85.4%) resulted positive. The complement-fixation technique used on the serums revealed antibodies in 38 out of 51 cases analyzed (74.5%). The inhibition of fluorescence method was positive in 21 of the 48 cases tested (43.7%) and the precipitation in agar gel method gave lines of precipitation in 15 out of 50 pigs examined by this method (30%).

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The titer of the antibodies revealed by the indirect immunofluorescence method and the complement-fixation test ranged between 1:10 and 1:320.

The comparative serological study of 49 serums using indirect immunofluorescence and the complement-fixation test revealed 41 positives and 8 negatives with the indirect immunofluorescence test and 38 positive and 11 negative with the complement-fixation test. The 8 negative serums with the indirect immunofluorescence resulted positive with the complement-fixation test and the 11 negatives with the complement-fixation tests were positive with indirect immunofluorescence.

The comparison with inhibition of immunofluorescence revealed that three cases negative with the indirect immunofluorescence test were positive with the inhibition of fluorescence method. These three serums were also positive with the complement-fixation test.

In order to determine the relationship between the antibodies of the serum revealed by the method of indirect immunofluorescence and the presence of virus of African swine fever in tissues, 35 pigs were examined by the method of isolating the virus in a leukocyte culture. Of the 35 pigs, 32 contained antibodies shown by the indirect immunofluorescence method.

The virus of African swine fever was encountered in tissues of 24 pigs out of the 35 examined (68.5%) and in 11 pigs the isolation of the virus resulted in a negative. The titer of the virus fluctuated between 10⁻¹ and 10^{-3.5}.

Of the 24 pigs which turned out to have virus in the tissues, 23 (95.8%) had in the serum antibodies which could be shown by the indirect immunofluorescence test and all of the 24 pigs (100%) were positive with the complement-fixation test. Of the 11 negative cases with isolation of the virus, 9 were positive and 2 negative upon searching for antibodies using the indirect immunofluorescence method. In those 11 negative pigs, 9 were positive and 2 negative with the complement-fixation test. The two serums which were negative with indirect immunofluorescence were positive with the complement-fixation test and the two serums negative with the complement-fixation test were positive with indirect immunofluorescence.

The comparative study of the content in antibodies of pig serums at different developmental stages of the African swine fever revealed the following results:

Serums from Lot I

Collected from 10 sick pigs with symptoms of chronic African swine fever. Results in Table 2.

The indirect immunofluorescence test showed antibodies in the 10 serums (100%). The complement-fixation test was positive in six cases (60%). The inhibition of fluorescence was positive in six cases (60%) and the precipitation in agar gel was positive in 5 (50%).

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The titer of the fluorescent antibodies was 1:160 with four serums, 1:320 in two, 1:80 in two and 1:40 in two serums. The fixing antibodies of the complement were included within the same scale of values.

Pigs number 53 and 55 with a titer of 1:320 and number 20 with a titer of 1:160 by the indirect technique were negative with complement-fixation.

The virus of African swine fever was encountered in the tissues of nine pigs (90%).

Serums of Lot II

Collected from nine pigs with subclinical infection (without symptoms but with discrete lesions of pneumonia checked postmortem). Results in Table 3.

The indirect immunofluorescence test revealed antibodies in nine serums (100%), the complement-fixation test was positive in five out of eight examined (62.5%), the inhibition of fluorescence test was positive in six out of the nine tested (66.6%) and the precipitation in agar gel

was positive in four (44.4%). The titer of the fluorescent antibodies was 1:160 in one serum, 1:80 in one serum, 1:40 in five serums and 1:10 in one serum. The titers of the complement-fixation test were similar.

The virus of African swine fever was encountered in the tissues of six pigs (66.6%).

Serums from Lot III

Collected from 15 apparently healthy pigs recovered from the disease. Results contained in Table 4.

The indirect immunofluorescence test was positive in 12 pigs (80%) with titers of 1:20 in seven pigs and 1:10 in five pigs. The three negative serums with the indirect immunofluorescence test were positive with the complement-fixation test. In 12 pigs out of the 14 tested (85.6%), the complement-fixation test revealed antibodies. The inhibition of fluorescence test was positive in six serums out of the six tested (100%) and the precipitation in agar gel was positive in one pig (12.5%).

The virus was isolated from the tissues of seven out of eight pigs examined (87.5%).

Serums from Lot IV

Collected from 23 healthy pigs suspected of being carriers of the African swine fever virus. They do not show either clinical symptoms or lesions in the postmortem examination. Results are listed in Table 5.

The indirect immunofluorescence test was applied to 21 serums with 16 resulting positive (76.1%) and five negative. The five negative ones were positive with the complement-fixation test. The titers of the antibodies determined by the indirect immunofluorescence test fluctuated between 1:10 and 1:320. /407

The complement-fixation test was positive in 17 serums out of 19 tested (89.4%), the inhibition of fluorescence test was positive only in six out of the 23 (26%) tested and the precipitation in agar gel was positive in five serums out of 23 tested (21.7%).

The African swine fever virus was encountered in the tissues of two pigs out of a lot of eight which were examined (25%).

The two pigs which were positive on isolation of the virus had fluorescent antibodies.

DISCUSSION

→ The tests carried out using the indirect immunofluorescence method showed the possibility of using it to reveal and evaluate the content in antibodies of the serum against the African swine fever virus.

A high percentage (85.4%) of the animals analyzed showed a clear response in fluorescent antibodies in the course of the chronic forms

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of the infection (clinical or subclinical) as well as during the latent phases of the virus (carriers).

Provided a stock of antigen is available, the indirect immunofluorescence technique can be used as a quick routine method for serological diagnosis of African swine fever. The method offers the practical advantage of a diagnosis in a few hours by a simple and sensitive technique.

Its interesting practical application is the diagnosis of chronic infections (clinical or subclinical) and of latent states (healthy carriers).

In these forms of African swine fever, the serological diagnosis is quicker and has more capabilities than isolation of the virus from the tissues. The presence in so-called clinical forms of antibodies and substances which inhibit multiplication of the virus as well as the weak concentration of the latter make difficult its identification and requires long periods of time.

In the cases studied with chronic infection, the method of indirect immunofluorescence revealed antibodies in 100% of the animals (19 serums from Lots I-II). In comparison, the complement-fixation test and inhibition of immunofluorescence method showed antibodies in a percentage of the abovementioned serums appreciably less (60 and 64.4%, respectively). These results suggested that, in the chronic form, even though it may be with symptomatology or not apparent, the diagnosis by the indirect immunofluorescence method can be the method of choice.

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In the animals which have recovered from the disease (serums from Lot III), the sensitivity of the indirect immunofluorescence method (12 positive results out of 15 serums) was slightly less than that of the complement-fixation test (12 positive out of 14). The immunofluorescence inhibition method was positive in six serums tested of this group.

In the healthy animals, suspected of being carriers (serums from Lot IV), the complement-fixation test revealed a percentage of positive cases (17 out of 19 serums, 89.4%) appreciably greater than those encountered using the indirect immunofluorescence method (16 out of 21, 76%). The inhibition of immunofluorescence and the precipitation in agar gel methods proved to be poor in operation in this group of serums.

A high percentage of the animals analyzed (in different states of chronic infection) contained fluorescent antibodies and complement-fixers in the serum and simultaneously virus of African swine fever in the tissues.

These results showed the possibility of applying the indirect technique of immunofluorescence together with the complement-fixation test and the inhibition of immunofluorescence method with the epidemiological survey of antibodies and carriers of the African swine fever virus.

It is advisable to point out that the indirect immunofluorescence method for investigation of African swine fever antibodies is a swift and

(cont. p. 10)

simple method on condition of having available a stock of antigen with acceptable characteristics allowing the readout of results. +

The most sensitive cells for preparation of stocks of antigens (cover slips with cells infected with virus) are the leukocyte cultures. Nevertheless, the distinct series of these cultures of leukocytes show differences with respect to the degree of development, phagocytosis, nonspecific fluorescence and formation of viral inclusions. The phagocytosis of the red blood cells can create difficulties in reading out the results. For this reason, it is necessary to set up the stock of antigen, make a prior selection of well-developed cultures, 465 days in age, free of phagocytosis and nonspecific fluorescence and with 30-50% of cells having a quite visible specific viral inclusion.

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Other cellular systems have also been used. These would include cultures made from cells of pork kidney, kidney of bovine fetus as well as the plexus chorioideus. However, these cells are not sensitive to the natural virus, and it is necessary to use for the infection virus which has been adapted to these cultures. Likewise, it is necessary for making the stock of antigen to make a selection of cultures with characteristics acceptable for being able to observe the fluorescent viral inclusions in positive cases.

TABLE 1

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Results of the investigation for antibodies of the African swine fever using the indirect immunofluorescence and other methods.

Pig serums from various farms having chronic African swine fever, subclinical infections and healthy carriers.

Method	Cases Examined	Positive	%
Indirect immunofluorescence (IIM)	55	47	85.4
Complement-fixation test (CFT)	51	38	74.5
Inhibition of immunofluorescence method (IFM)	48	21	43.7
Precipitation in agar gel (PAG)	50	15	30.0
Isolation of the virus in leuko- cyte culture (IVL)	35	24	68.5

TABLE 2

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Investigation for antibodies of African swine fever using indirect immunofluorescence and other methods.

Serum from pigs with chronic African swine fever (Lot I).

Farm	Pig No.	IIM	CFT	IFM	PAG	Macroscopic lesion	IVL titer *
A	1	1:160	1:80	0	0	P. Pi	3.5
A	2	1:160	1:160	+ 1:2	0	P. Pi	3.0
A	3	1:160	1:320	+ 1:10	0	P. Pi	2.5
A	33	1:40	0	0	+ 1:2	P	3.5
A	39	1:40	0	0	+	P	2.5
B	51	1:160	1:40	+ 1:2	+	P	3.5
B	52	1:80	1:20	+ 1:2	+	P	3.5
B	53	1:320	0	+ 1:2	+	P	1.5
B	54	1:80	1:20	+	0	P	0
B	55	1:320	0	0	0	P	3.5
Examined		10	10	10	10		10
Total pos.		10	6	6	5		9
% of pos.		100	60	60	50		90

IIM = indirect immunofluorescence method

CFT = complement-fixation test

IFM = inhibition of immunofluorescence method

PAG = precipitation in agar gel

IVL = isolation of virus in leukocyte culture. Macroscopic lesion in the organ indicated. In other organs without lesions.

0 = negative

+

P = focal lesion of pneumonia with necrosis.

Pi = ulcerated nodules on the skin.

*Titer expressed as log 10 DI₅₀CL

TABLE 3

Investigation of antibodies of African swine fever using the indirect immunofluorescence and other methods.

Serums from pigs with subclinical infection (Lot II).

Farm	Pig No.	IIM	CFT	IFM	PAG	Macroscopic lesion	IVL titer*
A	4	1:40	1:20	0	0	P	0
A	5	1:80	1:80	0	0	P	1'2
A	8	1:40	1:10	+ 1:5	0	P	0
A	10	1:40	N	+	+ 1:2	P.B.	1'5
A	15	1:160	1:320	+ 1:5	+ 1:10	G.P.	2'0
A	21	1:20	0	0	0	P	0
A	35	1:40	0	+	0	P.B.	1'2
A	36	1:10	1:40	+	+	G.P.	2'2
A	38	1:40	0	+ 1:5	+	P	1'2
Examined		9	8	9	9		9
Total pos.		9	5	6	4		6
% positive		100	62'5	66'6	44'4		66'6

IIM = indirect immunofluorescence method

CFT = complement-fixation test

IFM = inhibition of immunofluorescence method

PAG = precipitation in agar gel

IVL = isolation of virus in leukocyte culture. Macroscopic lesion in the organ indicated. In other organs without lesions.

0 = negative

+

P = focal lesion of pneumonia with necrosis

B = congestive areas in the spleen

G = lymphatic ganglion

*Titer expressed as log 10 DI₅₀CL.

TABLE 4

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Investigation of antibodies of African swine fever using the indirect immunofluorescence method.

Serums from pigs apparently healthy recovered from the disease (Lot III).

Farm	Pig No.	Mos. recd. **	IIM	CFT	IFM	PAG	Macroscopic lesion	IVL titer*
C	721	3	1:10	1:40	N	0	0	20
C	722	3	1:20	1:10	+ 1:2	0	0	15
C	723	2	1:10	1:20	N	+ 1:2		N
C	746	2	1:20	1:20	N	N	0	20
C	748	2	1:20	1:20	N		N	N
C	749	2	1:20	1:10	+ 1:2	O	N	N
C	775	3	1:10	N	N	N	O	20
D	724	3	1:10	0	+ 1:2	O	P.B.	25
D	725	3	0	1:10	+ 1:2	±	P	15
D	726	3	0	1:10	+ 1:2	O		N
D	737	3	1:20	1:40	N	N	O	O
D	738	3	0	1:40	N	N	N	N
D	751	3	1:20	1:40	N	N	O	12
E	734	2	1:10	1:20	N	O	O	N
E	735	2	1:20	0	+ 1:2	N	N	N
Examined			15	14	6	8		8
Total positive			12	12	6	1		7
% of positive			80	85.6	100	12.5		87.5

IIM = indirect immunofluorescence method

CFT = complement-fixation test

IFM = inhibition of immunofluorescence method

PAG = precipitation in agar gel

IVL = isolation of virus in leukocyte culture. Macroscopic lesion in the organ indicated. In other organs without lesions.

0 = negative

+ = positive

P = focal lesion of pneumonia with necrosis

B = congestive areas in the spleen

* Titer expressed as log 10 DI₅₀CL

**Time transpired from clinical recovery until obtaining serum.

TABLE 5

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Investigation of antibodies of African swine fever using the indirect immunofluorescence method.

Serum from healthy pigs from centers of African swine fever (suspected of being carriers) (Lot IV).

Farm	Pig No.	IIM	CFT	IFM	PAG	Macroscopic lesion**	IVL titer *
A	5	1:80	1:80	0	+ 1:2		0
A	7	1:10	1:40	0	0		0
A	9	1:40	0	0	+ 1:2		N
A	11	0	1:40	0	0		N
A	12	0	1:100	0	0		0
A	13	1:80	>1:40	+	0		N
A	14	1:40	1:80	0	0		N
A	16	1:80	N	0	+		N
A	18	1:10	1:40	0	0		N
A	19	N	1:10	+	0		N
A	20	1:160	0	+	+ 1:2		0
A	22	N	1:20	+ 1:2	0		N
A	23	1:160	1:10	0	0		N
A	24	1:10	1:20	0	0		0
A	25	0	1:40	+	0		N
A	27	0	1:10	0	0		N
A	28	1:80	1:40	+ 1:2	0		N
A	29	1:20	1:80	0	0		N
A	30	1:20	1:80	0	0		N
A	31	0	1:80	0	0		0
A	32	1:20	N	0	0		N
A	41	1:320	N	0	0		3'5
A	42	1:80	N	0	+		2'0
Examined		21	19	23	23		8
Total pos.		16	17	6	5		2
% positive		76'1	89'4	26	21'7		25

IIM = indirect immunofluorescence

CFT = complement-fixation test

IFM = inhibition of immunofluorescence

PAG = precipitation in agar gel

IVL = isolation of virus in leukocyte culture

N = not performed

0 = negative

+ = positive

* Titer expressed as log DI₅₀CL.

**This lot of animals showed no macroscopic lesions in the organs after being sacrificed.

SUMMARY

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Preliminary investigations with indirect immunofluorescence technique for detecting antibodies against African Swine Fever virus (A.S.F.) in serum of pigs from the field were described.

These studies have been conducted to find a simple and rapid method for the diagnosis of the chronic form of A.S.F. and the carriers, who are increasing in enzootic areas, giving rise to difficulties in the eradication of the disease.

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Preliminary results indicated that the indirect immunofluorescence technique may be a valuable procedure in laboratory investigations on A.S.F. antibodies and a simple method for rapid serological diagnosis of chronic A.S.F. and carriers.

The indirect fluorescent antibody test detected antibodies in 100% of the sera from pigs with chronic A.S.F. and in 74.5% of the carriers. The titres were between 1:10 and 1:320.

From a total of 55 studied pigs, including animals in different stages of the chronic disease and carriers, 47 (85.4%) had fluorescent antibodies in the serum.

A high percentage (68.5%) of animals analyzed had fluorescent antibodies in the serum and simultaneously A.S.F. virus in tissues. From 24 pigs with virus in tissues, 23 (95.8%) had fluorescent antibodies in the serum. The virus titres in the different tissues were between 10^1 and $10^{3.5}$.

The sensibility of the test was compared with others serological methods (complement-fixation test, inhibition of the immunofluorescence and agar-gel diffusion).

The indirect immunofluorescence test could be a valuable method for the epidemiological survey of antibodies against A.S.F. and the detection of carriers.

The antigen used for the test were buffy coat cultures infected with field A.S.F. virus which presented viral intracytoplasmic inclusions in the macrophages stained on coverslip. Other cellular systems were also used which were infected with cell adapted virus strains.

The details of the utilized techniques are described.

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