African swine fever.
New developments*

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Summary: A.S.F. still poses a threat to the world pig industry, although the disease status in affected countries has markedly improved in recent years.

With regard to epizootiology, diseased pigs are the major source of direct infection, while indirect spread is mainly caused by pork products and mechanical vectors. Information about the role of virus carriers in transmission of the infection is insufficient, but the role of ticks (Ornithodorus genus) and of wild pigs as virus reservoirs is well documented. The incidence and distribution of A.S.F. remain closely linked to pig production and marketing.

The clinical picture of the disease has changed, with an increasing prevalence of subacute and chronic forms, subclinical cases and insidious forms, which makes diagnosis and control more difficult.

The greatest progress was in laboratory diagnosis, for which highly efficient techniques have been developed: direct immunofluorescence and haemadsorption on leucocyte cultures for identification of the viral antigen, indirect immunofluorescence, immuno-electro-osmophoresis and the ELISA test for antibody detection.

In disease control, major aspects of new developments are as follows: cooperation of farmers through sanitary defence associations, sanitary accreditation of farms, creation of A.S.F.-free zones, serological monitoring, official registration of farms, regulations for the creation of new farms, re-planning of extensive pig-raising, permanent abattoir inspection, transformation of «open cycles» to «closed cycles», changes in family holdings and prohibition of farm-to-farm sales.

In the field of research, important advances have been made in the knowledge of the chemical structure, molecular biology and antigenic structure of the virus. Although experiments performed to date for the development of an A.S.F. vaccine have not given satisfactory results, research work undertaken in recent years on A.S.F. immunology could help to clarify the immunological mechanisms of the disease.

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I. — INTRODUCTION

The occurrence of African swine fever (A.S.F.) in new areas of the Americas, Africa and Europe over the past five years has placed further emphasis on the permanent threat this disease represents to the world pig industry.

The significant recrudescence of A.S.F. in 1977 in Spain and Portugal, its occurrence in 1978 in Malta, Sardinia, Brazil, the Dominican Republic and Haiti and then in Cuba in December 1979, created an alarming situation involving the risk of the spread of the disease into other areas of the western hemisphere.

From 1980, a progressive decline in A.S.F. activity and a marked decrease in the number of cases in areas still affected in the Americas and Europe were achieved due to the application of control and eradication programmes in affected countries together with measures adopted in neighbouring countries to prevent the introduction of the disease. This was undertaken with the support of substantial international cooperation.

However, the history of this disease illustrates that A.S.F. commonly has periods of remission followed by periods of recrudescence. Experience has shown that reduced surveillance and the relaxation of measures for the control of the disease in affected or threatened countries have generally been conducive to disease spread in active periods. For this reason, current preventive, control and eradication programmes should be reinforced to a maximum, particularly at the present time when the explosive outbreak which commenced in 1977/78 has been curbed and controlled resulting in a sharp decline in incidence and better eradication possibilities.

Advances have been achieved in current regional control programmes in the field. The progress made in diagnosis, pathogenesis of inapparent infections (carriers), immunology and a better knowledge of the virus with regard to its chemical and biological properties, lend to a short-term positive outlook, with regard to the threat this disease poses for pig populations in numerous countries and for its future eradication from affected areas.

Some of the major aspects of the present A.S.F. status are reviewed in this document, on the occasion of the new « historic » phase of its spread into the western hemisphere. This description will cover recent developments, epidemiology, diagnosis, new scientific discoveries and control procedures in the field.


The Americas.

In Brazil, the first officially reported outbreaks occurred in May 1978 in Paracambi (Rio de Janeiro). The last reported outbreak was in December 1979 and outbreaks eradicated to that date total 226 (1). In 1980, all suspect
cases showed negative results on laboratory tests. No new cases were officially declared in 1981 until July. During the E.E.C./F.A.O. Meeting of Experts on A.S.F. (held in Sardinia from 23-25 September 1981) information was given to the effect that two sporadic cases occurred in August causing deaths in the southern regions of the country. Serological surveillance in three southern States (Sta. Catarina, Rio Grande do Sul and Parana) enabled the detection of 0.3% positive sera collected in abattoirs out of a sample of 49,643 pigs. The positive sera came from the Parana State. The sera from Rio Grande and Sta. Catarina were negative (2). A control programme against A.S.F. is in progress.

The Dominican Republic (having a pig population of 1,400,000) was affected in February/March 1978 by the disease which spread rapidly throughout the country. With the cooperation of the F.A.O., the United States and the International Development Agency, all outbreaks were eradicated and total depopulation was effected. With a view to repopulation, sentinel pigs were introduced in July 1980 in an effort to detect the residual virus in the eastern, central, north-eastern and north-central regions. This programme has not yet been implemented in the north, nor along the Haiti border. Up until September 1981 no cases of clinical disease were recorded and all serological tests of newly-introduced pigs were negative (3).

The disease first appeared in Haiti in December 1978 and remained enzootic in the country during 1979, 1980 and 1981 when a small number of cases presenting clinical signs was observed. A serological survey carried out on 1,368 sera from various origins showed 7% positive cases (accompanied by inapparent infections for the most part) thus illustrating a significant presence of virus carriers. In 1981 the Haiti Authorities, with the cooperation of other countries (United States, Mexico, Canada) and of international Agencies (F.A.O. and I.I.C.A.) initiated an eradication programme against this disease (9).

A.S.F. was discovered in Cuba in 1971 for the first time and then again in January 1980. Case history studies pointed to the possibility that it existed in the Guantanamo province in December 1979. The disease spread to three provinces: Guantanamo, Santiago de Cuba and Holguin, where 56 outbreaks were recorded. The Guantanamo province was completely depopulated to eradicate the disease. In all 173,287 pigs were slaughtered or died, most of which were destroyed but the meat of healthy animals from neighbouring areas was used for human consumption. Since March 1980, no new case has been recorded. In September 1980 a repopulation policy was commenced, initially sentinel pigs were placed in affected areas to check for the presence of any residual virus. This test was completed in December 1980 without any case being found and was later ended with the repopulation of all affected areas (4, 10). No case was recorded in 1981 and the second outbreak is now considered as having been eradicated.
Europe.

In Spain, affected since 1960, the incidence of A.S.F. has decreased considerably in recent years. An alarming increase in outbreaks was recorded in 1977 with 1,780 farms affected, i.e. the highest incidence over the last ten years. From 1978 a progressive decline in the number of cases led to 300 affected farms being declared in 1981 (until October); in other words, the lowest incidence over the last ten years, behind the 1974 record.

The changes in the structure of the pig industry and the application of new ways of orienting the eradication programme (recently reinforced by financial assistance from the E.E.C.) contributed to this favourable change in A.S.F. status.

Portugal has recorded a significant drop in the number of cases during the last few years. After the increased outbreaks in 1977 (5,017 farms affected), a steady fall in the incidence took place during the following years: 2,527 farms affected in 1978, 440 in 1979, 257 in 1980 and 68 during the first seven months of 1981. A control programme is being undertaken with financial assistance from the E.E.C.

In Sardinia, A.S.F. was detected in March 1978 with 19 outbreaks being declared until December of that year. In the following years, 14 outbreaks were recorded in 1979, 42 in 1980 and 4 in 1981 (until October). The disease remains enzootic and is perpetuated by the small pig holding system, open grazing and the ecology of the island.

Malta, with the cooperation of the E.E.C. and the F.A.O., continues her controlled repopulation programme, with no case of A.S.F.

Africa.

Information received through the O.I.E. indicated the following incidence during the period 1978-1981.


One case was reported in Zimbabwe in 1978 and another in 1979. Sudan reported 2 cases in 1978; the Republic of South Africa reported 4 cases in 1978, 1 case in 1979 and 1 case in 1981 (up to September); Mozambique reported one case in 1979; Zambia reported 4 cases in 1979.

In Sao Tome and Principe, the first outbreak of A.S.F. was detected in March 1979 on a farm of 70 animals which were fed food waste from a military barracks close to the farm which received pork meats from Angola. On 4 April, the Luanda Laboratory confirmed the diagnosis (5). In May 1979, more than 7,000 pigs had been slaughtered or died (6). The Government set up a control and eradication programme with F.A.O. assistance. During 1980 and 1981 (until September), no official information on the situation was recorded in the O.I.E. information Bulletins.
In summary, A.S.F. in its epizootic form tends to be on the decline throughout the world.

The disease prevails in its enzootic form though a substantial decrease in cases has been recorded in Spain, Portugal, Sardinia and Brazil. A.S.F. also remains enzootic in Haiti. No new cases have been reported in the Dominican Republic, nor in Cuba or Malta after the controlled depopulation of pigs and the subsequent repopulation carried out to date.

III. — EPIZOOTIOLOGY

Introduction and spread of A.S.F. in free countries.

The introduction of the virus into a country takes place by importing pork products or live pigs from infected countries.

The origin of the first cases in newly affected countries over the last few years (1978-1980) i.e. Malta, Sardinia, Brazil, Dominican Republic, Haiti, Sao Tome and Principe was attributed to the feeding of pigs with food waste from boats or aeroplanes. In countries with land borders, spread of the disease from affected countries can occur by contact between pigs from countries along the borders or by the uncontrolled transit of infected meat or live animals (this was the case in the Dominican Republic and Haiti).

Once the virus has been introduced, the first cases of disease generally pass unnoticed or are confused with other swine diseases, particularly classical swine fever (hog cholera), especially when the latter is endemic in the country.

In the initial stages, the disease is often transmitted to healthy pigs through contact with the first affected animals. The disease may remain for a while in the zone of the primary outbreak or spread further through the transportation of pigs either in incubation or already showing symptoms. During this phase and generally before A.S.F. has been identified, infected pigs may be slaughtered for family or public consumption and therefore their meat contributes to the spread of the virus. The contamination of vehicles during transportation of affected pigs as well as the spread of the virus by various other vectors such as men, rodents, poultry, insects, etc. are conducive to the spread of A.S.F. in newly affected countries.

When total eradication of the disease has not been achieved in the initial phases and when mortality decreases, chronic cases and recovered animals constitute another source for maintaining the A.S.F. virus. Finally the persistence of the enzootic form of A.S.F. in regions where certain ticks (Ornithodorus) are found, may facilitate the adaptation of the virus to these ticks which become reservoirs of the virus (21). In the same manner, susceptible wild animals can be infected and later contribute to disease spread when they come into contact with domestic pigs.
The delay in diagnosing A.S.F. when introduced into free countries has been the main cause for severe spread on several occasions, thus complicating eradication and incurring heavy economic losses.

In newly affected areas of the Americas, the delay in suspecting the presence of A.S.F. in the field, as well as in diagnosing the disease together with the absence of an appropriate infrastructure and of preventive and control means or programmes against A.S.F. facilitated its spread and hampered the rapid elimination of initial outbreaks.

With regard to this important factor of delayed identification of A.S.F. when it occurs in a previously free country, useful knowledge is gained from information supplied by countries in the Americas where it was detected, which will assist in directing efforts to control this disease.

In the Dominican Republic the disease probably first occurred in February 1979 but was not identified until five months later, in July 1979. In Haiti, A.S.F. was officially reported in January 1979 but it is possible that it was introduced two months before the end of 1978. Finally, in Cuba, A.S.F. was diagnosed on 30 January 1980 by the Havana Laboratory but the disease occurred towards the end of 1979 (10).

This information illustrates that in countries at risk, A.S.F. can remain unnoticed for a long time and can be mistaken for other swine diseases. It is for this reason that efforts are justified in maintaining an appropriate veterinary educational programme in the field, hence facilitating the early suspicion of disease and organised surveillance for the collection of samples from suspect animals which should automatically be subjected to the differential diagnosis for A.S.F.

**Transmission and spread in affected countries.**

In countries where infection has been present for some time, as in Spain and Portugal, sources of direct transmission are: affected pigs, carriers of inapparent infections, ticks and receptive wild animals. In indirect transmission, the sources of virus are pork products and mechanical vectors which are mostly infected delivery vehicles and less often: men whose activities are related to the pig industry (attendants, merchants), other mammals (rodents), poultry and certain insects which may circulate the virus.

The most common transmission mechanisms are contact with affected pigs and with virus carriers and the ingestion of infected feed, the transport in infected delivery vehicles, tick bites and other possible vectors.

Among factors facilitating virus spread is the movement of animals, brought about by production and marketing structures which change with the development of the pig industry and thus represent one of the most important factors in disease spread. This is a serious problem which is further complicated by an increase in the swine population and in trade.
In countries where the disease already exists (Iberian Peninsula), the relative importance of each of the modes of transmission cited above may vary over time, depending on epizootiological conditions, changes in the properties of the virus (strains of higher or lower virulence), changes in the production and marketing systems, farmers' awareness of animal diseases and the application of disease control measures.

A survey carried out in Spain in 1981 on the frequency of different modes of transmission and spread gave the following results: 65% of outbreaks were caused by contact between neighbouring farms; 1.7% were due to food waste; 19% were associated with the introduction of new animals (during incubation period, diseased or carrier animals); 5% were attributed to ticks; 5.8% were due to contact with wild boars (*Sus scrofa ferus*) (15).

A periodic review of the relative importance of each of these modes of transmission is important in countries where the disease exists in order that available resources can be concentrated on counteracting the current causes of transmission and spread.

**A.S.F. incidence, production and marketing systems.**

A.S.F. incidence in countries where infection persists is closely linked to production systems with the different modes of transmission depending on the type of system used.

Production systems involving the highest risks are: small holdings, extensive pig farms and intensive fattening units.

In countries where infection is persistent, the growth in pig production and the excessive concentration of poor farm lay-outs, as well as considerable movement of animals, contribute to the persistence of the disease.

The correlation between the A.S.F. incidence, production structures, geographical areas and major movement of piglets from production areas to areas concentrating on fattening units, has been clearly illustrated in an epizootiological study of A.S.F. in the Iberian Peninsula (26). This study was conducted by the O.I.E. with a view to setting up a technical and financial plan for the eradication of A.S.F. from Europe.

During recent years, Spain has recorded the largest number of cases in extensive farms in the south and south-east followed by intensive fattening units in the north-east (Catalonia) due to the substantial imports of piglets from multiple origins. In Portugal, the greatest number of cases was recorded in small holdings in the centre and north of the country, and to a lesser extent in extensive farms south of the Tago.

Two areas can also be distinguished in Brazil: the southern states where pig farming is highly industrialised and where risk of infection is minimal and the northern area (Para State) which is characterized by small holdings, open grazing, wild pigs and marshland, where risk of transmission and persistence of the disease is greater.
A.S.F. virus carriers.

A.S.F. virus carriers are apparently healthy animals which are persistently infected without showing clinical symptoms (inapparent or subclinical infection). These pigs which have survived virus attack either recovered from more or less serious clinical disorders or since the beginning suffered mild or subclinical infection.

The increase in subacute, chronic and subclinical forms of the disease as well as the higher survival rate following a spontaneous adaptation of the virus together with its lower virulence has facilitated the establishment of carriers of inapparent infection.

At the present time, great importance has been placed on carrier animals in the persistence and spread of A.S.F. However, there is insufficient knowledge on certain aspects necessary to assess the true participation of carrier animals in the spread of A.S.F. These factors include the incidence, distribution and duration of the « carrier » state, their ability to transmit the disease either directly or through feed, the persistence of antibodies, the clinical activation of inapparent infection due to stress factors and transplacental transmission in different conditions (viraemia, clinical activation, highly virulent strains, reinfection, immuno-pathological disorders).

In practice, it is usually difficult to prove that carriers are the source of outbreaks and often some outbreaks of unknown origin are attributed to carriers without sufficient evidence.

Current information on the prevalence and distribution of carriers is limited. Some countries where the disease exists in its enzootic form are conducting control and eradication programmes based on serological surveillance to detect carriers. It is hoped that information thereon will be available in the near future.

In 1979, Brazil conducted a serological survey on the movement of 8,890 pigs from eight States before transport was authorised. A.S.F. antibodies were detected in 7.9 % of sera. In another investigation linked to the control programme, serological surveillance on a sample of 49,643 pigs from three States in southern Brazil revealed 0.3 % positive sera in the Parana State (21). In Haiti, where 1,368 sera were collected during the course of the epizootic and in the absence of a control programme, laboratory tests confirmed 7 % positive cases.

In Portugal, out of 25,000 apparently unaffected pig sera collected from abattoirs, 0.9 % positive cases were found and 1.4 % farms where confirmed as being infected (18). Out of 20,093 sera collected from 408 farms in various regions in Spain, 0.75 % positive cases and 4 % infected farms were found. The percentage of carrier pigs on farms with inapparent infection varied from 25 % - 80 % (15). In Malta, out of 2,409 sera from 200 farms, collected from an abattoir during the active stage of the disease, 32.5 % positive cases and 12.8 % farms with infected animals were detected.
The percentages are quoted from tests carried out in different periods and under various conditions (sera from farms or abattoirs) and thus only have an indicative value. These percentages may vary depending on the number of tests and the different conditions linked to the epizootiological situation in the area of origin of the pigs subjected to tests, to phases of remission and recrudescence of the disease and to the application of control and eradication programmes.

The duration of the carrier state in present epizootiological conditions which imply a decrease in the virulence of the virus, has not been sufficiently investigated. For many years recovered pigs were considered as remaining carriers for their whole life. However, there is now evidence that a large number of carriers do not excrete virus following a certain period post-infection.

In Portugal, tests carried out on 8 pigs with antibodies which had survived disease and were slaughtered 5 months after infection, showed no infective virus in the numerous organs examined (19). In Spain, in a similar investigation exactly the same results were obtained from several pigs which had recovered from field infection, slaughtered 5 - 10 months post-infection (5). Among pigs which had recovered from an experimental Malta strain infection, the infective virus was not detected between 6 - 16 months after remission of fever (20). These studies should be followed up in order to establish the persistence of the virus.

There is little knowledge on the factors determining carriers ability to transmit disease. Repeated experiments have shown that transmission from carriers without clinical symptoms to healthy pigs is rare but when inapparent infection is clinically activated by stress factors (transportation, deficient feeding, etc.), transmission can take place. However, some observations also show that this transmission by the activated carrier does not occur in all cases.

Recent studies in Spain have shown that carrier pigs of natural infection, with antibodies, did not transmit the disease to susceptible pigs after three months contact between the sixth and ninth month post-infection of the carriers and without disinfection of the premises (15). Tests carried out in Portugal showed that 47 pigs which had survived and had antibodies, did not transmit the disease to four susceptible pigs after contact for one year and without the premises being disinfected. During this challenge, three carriers died from acute A.S.F. by reactivation of infection. Transmission to the four receptive pigs was not observed (19). In the early stages of inapparent infection, 18 days after their temperature had dropped, carrier pigs may transmit the disease through contact (20).

C.A. Mebus and A.H. Dardari (29) observed that two groups of pigs which had recovered from experimental infection with a Brazil and Dominican Republic strain respectively, did not transmit the disease through contact with susceptible pigs, 135 and 110 days post-infection.
With regard to transplacental transmission, Portuguese scientists have recently established that 20 piglet litters, born of carrier sows, possessed maternal antibodies which disappeared 3 months later and the piglets developed normally (19). In Spain as well, the birth of piglets from carrier sows without transmission of the disease was also observed. These results tend to dismiss the importance of transplacental transmission from apparently healthy carrier sows, but this aspect should also be further investigated in the light of present A.S.F. conditions.

Virus concentration in the tissues of the carrier is low, always lower than that found in the affected pigs and its distribution in the organs is irregular. It is often that after a fairly long post-infection period the virus is only found in a lymph node. Recent research in Spain has demonstrated virus concentrations between $10^{0.6}$ and $10^3$ HAD$_{50}$ per gram of tissue in various organs of the carriers, slaughtered 45 days post-infection (15).

Little is known about the persistence of A.S.F. antibodies in carriers apart from the fact that they do persist for long periods. In Spain and Portugal antibodies were detected twelve months post-infection (15, 19). The titre of antibodies in the serum was between 1:160 and 1:1280 five months post-infection and between 1:160 and 1:640 ten months post-infection in investigations conducted in Spain (15) and between 1:400 and 1:6400 five months post-infection in observations made in Portugal (19). Low virus titres and antibody presence undoubtedly limit disease transmission.

**Transmission by ticks.**

Persistence of the disease in areas where the argasid tick of the genus *Ornithodorus* exists, can facilitate the virus adaptation to tissues of the tick which then becomes a reservoir of the virus.

In Spain, the presence of the virus was confirmed in 1963 in the *Ornithodorus erraticus* (present in south and south-west Spain) which was proved to have transmitted A.S.F. to pigs. The virus was isolated in leucocyte culture from ticks originating in farms where pigs had died from A.S.F. (21). The *Ornithodorus* contracts the virus when feeding on blood of A.S.F.-infected pigs. It then transfers the disease when it feeds on blood again. These results were later confirmed in Africa by isolating the virus in the *Ornithodorus moubata* (22, 23, 24). Investigations conducted in Spain ascertained the persistence of the virus in this tick up to eight years (C. Sánchez Botija et al., unpublished results).

These facts suggest the possibility of the establishment of new virus reservoirs among ticks in recently affected areas of Latin America. It has become apparent that research on the distribution of these ticks in free or affected countries is necessary. Research in the United States shows that under experimental conditions the *Ornithodorus coriaceus* (present in the U.S.A.) can retain the virus for 77-118 days (25).
Wildlife and A.S.F. virus reservoirs.

In Africa, warthogs and bushpigs (*Phacochoerus aethiopicus* and *Potamochoerus porcus*) host the A.S.F. virus. In various regions of Africa there is a correlation between the presence of A.S.F. and these wild pigs. Recent surveys conducted in South Africa have shown a close link between the distribution of warthogs with antibodies and areas where A.S.F.-infected ticks (*Ornithodorus*) are to be found (24).

In Europe, the wild boar (*Sus scrofa ferus*) is susceptible to the virus. It generally contracts disease by contact with domestic pigs (27). It can spread the disease to the wild boar population and is also a source of the virus for the domestic pigs.

The possible development of carriers among the wild pig population could create a new virus reservoir among wildlife in southern European countries. Recent work in Spain was undertaken to determine the possible existence of carriers among wild pigs; the presence of the virus and of antibodies was investigated in 84 wild boars from mountain areas in various regions of the country during the last few years. Results obtained were negative. On the other hand, 67 affected or dead pigs found in the same areas were examined. Twenty-three showed virus presence but no antibodies were detected. Three animals demonstrated both the presence of the virus and antibodies (15). These tests did not demonstrate any carriers among the wild boars which seems to indicate that they are presently suffering from acute, fatal A.S.F. Hence their role in the spread of this disease is currently limited to sporadic contact with domestic pigs and to infection of other wild pigs which finally die from the disease.

In Portugal, information collected at the National Veterinary Institute from wild boars found dead and examined between 1975 and 1977 tends to confirm the same situation as experienced in Spain with regard to the mortality due to A.S.F. among wild animals (31).

However, these results do not exclude the possibility of the introduction in the future of mild strains into this wildlife and the creation of carriers as well as new virus reservoirs. These epidemiological surveys should be continued and intensified in Sardinia, Portugal and Spain.

Presence of the A.S.F. virus (ASFV) in pork products (cold meats, cured and preserved meats).

A.S.F. infected meat can be found in pork products. The disease can pass unnoticed in the abattoir: the animals may either be in incubation or may be affected with inapparent forms (carrier animals). Should the virus persist after the products have been processed, these products may be a source for disease spread when food waste is fed to pigs.

For epidemiological and trade purposes it is useful to know just how long the virus persists in pork products after processing. To date, little research has been conducted on the effects of the various processing methods.
In his report presented during the 50th General Session of the O.I.E. (Item II), M.A. Diaz Yubero (63) describes recent work carried out in Spain on the persistence of the A.S.F. virus (ASFV) in both heat-processed products (cooked ham, i.e. York ham) and in products which have not been subjected to heat (i.e. drying, maturing and curing as in sausage, chorizo, filet and dried ham). Results therefrom are summarised below.

Experiments on cooked ham were conducted in 1981 by the Department of Animal Health in the Ministry of Agriculture and the Department of Animal Virology, National Institute for Agronomical Research, Madrid. Ham, weighing from 400-600 grams, from animals infected with $10^{4.25}$ to $10^{5.2}$ HA$_{50}$ doses of ASFV per gram was used. The virus titre in the blood of these animals was $10^6$ HA$_{50}$. A 28% saline solution at 7°C was injected into the ham which was kept at 4°C for twenty hours, then placed in sealed 400 g tins (40 mm high, 140 mm long and 100 mm wide).

Different sets of tins were heated at 65°C for one hour and at 75°C for one hour, two hours and two and a half hours. The temperature in the depth of the ham reaching 46°C, 60°C, 75°C and 75°C respectively at the end of the above periods. In the tins heated for two and a half hours, the temperature of the ham was maintained at 75°C for thirty minutes. Other tins which were not heat-treated were used as controls. All tins were cooled under running water for fifteen minutes. They were then stored at 4°C.

All heat-treated tins gave negative results. Techniques applied consisted in inoculating pig leucocyte cultures and receptive pigs. These results show that the virus is inactivated when applying commonly used temperatures (70°C to 75°C) and methods for processing the York type cooked ham. Consequently, the risk of disease spread through these products is eliminated. These tests proved that temperatures below the norm (i.e. 46°C) also inactivate the virus.

In uncooked products tests were performed on sausage, chorizo, filet and dry ham processed according to standard methods. The meat of these products contained $10^{3.7}$ to $10^{4.7}$ HA$_{50}$ virus doses per gram. The meat and additives of the sausages were placed in 60 mm diameter pig casings and in 36 mm diameter sheep casings. They were incubated at 22°C and 85% humidity for sixteen hours and then kept in a drying room for thirty days at 17°C.

Tests for the residual virus showed virus persistence in these « raw » products for three to six months. Tests on ham bone marrow were negative after five months.

Results show that the above described raw products may transmit A.S.F. if the waste therefrom is fed to pigs during the period in which the virus persists.

Some types of hams and filets bearing a label of origin (generally processed from Iberian pig meat), need at least nine to twelve months to mature before being marketed. This period is longer than that required to inactivate the virus and hence eliminates the risk of disease spread.
The results from tests undertaken in Spain on the stability of the ASFV in cooked ham which has been heat-treated correspond to similar tests carried out by P.D. McKercher, W.R. Hess and F. Hamdy (64) in 1978 at the Plum Island Animal Disease Center, United States Department of Agriculture.

McKercher et al. examined ham morsels weighing 800 g which had been prepared from meat containing $10^{3.75} \text{ HAD}_{50}$ ASFV per gram. The ham was inoculated with 16% saline solution and soaked in the same solution for twenty-four hours. It was then placed in tins, sealed and heated in a water bath at 37°C. This temperature was gradually increased over three and a half hours, reaching a temperature of 69°C in the ham. The tins were then cooled and stored at 4°C. The virus titre in the cured ham (before heating and two days after slaughter of the pig) was $10^2$ to $10^{3.75}$. The virus was inactivated after heat treatment and was not detected five days later when leucocyte cultures and receptive pigs were inoculated. These results show that the virus is totally inactivated after three and a half hours at 69°C.

The results found in Plum Island and Madrid indicate that the ASFV is inactivated when routine processing methods are used in the York type ham.

McKercher et al. (64) also offered interesting findings on virus persistence in uncooked, smoked or dried products processed from pork containing $10^{3.75} \text{ HAD}_{50}$ ASFV per gram. In these tests the meat was finely minced and mixed with additives, fairly similar to those used in Spain for sausages (salt, sugar, dextrose, sodium nitrite and nitrate, garlic and pepper or red pepper). After forty-eight hours at 4°C, lactic acid was added and the mixture placed in 37.5 mm and 25 mm casings. These preparations were maintained at 20°C and 68% humidity for forty-eight hours. One batch was walnut wood smoked for twelve hours at 32°C and 80% humidity, the other batch for twelve hours at 49°C and 58% humidity. Following this they were incubated at 11°C and 72% relative humidity for a minimum of twenty-five days. Another batch (placed in 25 mm casings) was smoked for only eight hours at 32°C, 85% humidity and dried at 11°C and 72% relative humidity for a minimum of sixteen days.

The virus was found after the smoking and before the drying processes but was not detected after smoking and drying undertaken thirty to sixty days after processing. In those batches which were smoked for twelve hours at 32°C and then at 49°C for twelve hours the virus was inactivated much more rapidly than in the others smoked for twelve hours at 32°C.

Results obtained from these products confirm virus inactivation by smoking and drying, once the period required by the processing methods has elapsed.

Data on ASFV persistence described in this document relates to a limited number of pork products. As a much larger range of pork products exists on the market it would be desirable to pursue and develop research work on ASFV persistence in other types of products.
IV. — CLINICAL CHARACTERISTICS

The clinical symptoms of A.S.F. closely resemble those of classical swine fever (hog cholera) and in practice clinical differentiation between the two diseases cannot be accurately determined. In the field a clinical entity alone may generally be confirmed and that is swine fever. Whether it is African or classical swine fever, if suspected in certain cases, may only be definitively established by the laboratory.

In the current epizootiological situation, A.S.F. may occur in a country and demonstrate clinical signs yet remain unrecognised as it is confused with classical swine fever (hog cholera).

Present clinical forms of the disease are acute, subacute, chronic and subclinical. These different forms may all appear in a single outbreak or farm. The disease generally commences in its acute or subacute forms and is followed by chronic and subclinical cases, depending on the activity of the outbreak and the application of disease control measures. Outbreaks can also commence with chronic cases and inconspicuous clinical signs which develop imperceptibly and are difficult to identify. These cases are less common than the acute or subacute ones but they are most important from an epidemiological point of view as, when they occur in a free area, they may only be detected once the disease breaks out perceptibly with acute cases entailing high mortality rates.

The chronic and subclinical forms have emerged over the years and have become more important due to the decrease in the virulence of the virus.

It is impossible to determine the percentage of the different clinical forms in countries where control measures are applied. If animals are slaughtered early during the course of disease only acute forms are observed as they generally prevail at the beginning of an outbreak. If a slaughter policy is effected later or not at all, subacute, chronic and subclinical cases are found to be most common.

The incubation period in natural conditions varies and depends on various factors (virulence and dose of virus, resistance, etc.). It generally varies from 4-6 days in acute cases and from 6-8 days in subacute cases.

The symptoms of the acute and subacute forms are similar but differ by the intensity and duration of disease: temperature from 40-42°C, devoid of disorders for the first 2-4 days (presymptomatic febrile stage). Inappetence, tremors, adynamia, mild conjunctivitis, circulatory and vascular disorders, constipation, vomiting, intestinal haemorrhaging, dyspnoea, coughing, neuromotor disorders (paresis, ataxia, convulsions). Death takes place 4-6 days after the first symptoms appear in acute cases and 6-10 days in subacute cases. Abortion is common and in a number of cases is the first sign of disease.
The interval between viral invasion and death of the animal has increased in recent years, probably because of a decrease in virus virulence. In acute forms it is between about 12-14 days and in subacute forms from 15-20 days but can, in rare cases, extend to 30 days (covering the incubation period, the febrile stage where symptoms are not noticed, and the period of apparent symptoms).

In chronic cases, the symptoms are as follows: irregular and undulant fever (39-40°C), dullness, inappetence, loss in weight, retarded growth, coughing and arthritis. In some outbreaks lesions of the skin may be observed (nodules, ulcers, necrotic areas and loss of tissue in places), on the ears, snout, trunk and joints. The intensity of these signs ranges from the complete picture given above to simple mild signs like weight loss, coughing and oscillating temperature. The symptomatic period is from 20 to 30 days with alternating periods of remission and activation of symptoms. Some of the affected animals recover and retain subclinical infection while others die at different intervals. These forms can in the end be confused with other swine conditions.

Subclinical cases are associated with field virus strains of low virulence; they were also observed in animals which recovered from acute, subacute or chronic A.S.F. infection and then regained an apparently healthy state (remaining nonetheless carriers of mild lesions and of the virus). These subclinical forms can be reactivated and develop acute symptoms which are generally followed by death. This clinical form includes carriers which have been examined above.

With regard to macroscopic lesions in acute and subacute forms found in enzootic areas, incidence of severe haemorrhagic lesions which are characteristic of A.S.F. has decreased considerably. Lesions resembling those of classical swine fever (C.S.F.) (hog cholera) have increased, as well as the mild lesions of little significance. Lymph nodes are now the most common organs showing the characteristic severe haemorrhagic lesions (42%); in the spleen, characteristic lesions are shown in 18% of cases and in the kidney in 7% of cases.

In the chronic forms of the disease, the typical lesions are seen in some cases: pleuritis, pericarditis, pneumonia, arthritis, haemorrhagic lymph nodes, and cutaneous ulceration and necrosis. In other cases, there are only vascular changes in the skin, pulmonary congestion, hyperplastic and oedematous lymph nodes; there are no detectable lesions in the spleen and kidneys. Subclinical cases show oedema and discrete haemorrhages in the lymph nodes.

In newly affected countries — Malta and Sardinia — lesions observed were generally severe and characteristic of the acute form of A.S.F. and of highly virulent strains (28).

In Brazil, Dominican Republic, Haiti and Cuba, A.S.F. lesions were observed in a certain number of acute cases, when the outbreaks first started.
In subsequent stages, lesions resembled those of classical swine fever and, in other cases, were of minor significance for diagnosis.

The initial development of an outbreak of A.S.F. usually commences with the death of a single animal or the development of illness in a few animals of a group. The other animals remain normal for 10 to 12 days. After this period a large number of affected animals die, in character with the acute forms of A.S.F. However, as already indicated, the death rate is either maintained or drops in a spectacular manner. The duration of the course of the outbreaks cannot be clearly stated when control measures are implemented but in general, it depends on the virulence of the strain, the number of animals, environmental conditions, sanitary measures, etc. In outbreaks where the acute forms and high mortality prevail, it can continue for 30-45 days. If the subacute, chronic and subclinical forms prevail, clinical signs can continue for 2 to 4 months. Over a period of some months some animals can deteriorate and die, even though they have shown apparent clinical recovery.

Mortality.

Mortality is an important aspect in the current characterisation of A.S.F. Mortality rates in affected countries from 1978-1981 were very variable from one outbreak to another in different countries and they cannot be determined precisely where early slaughter policies were applied. In general, mortality decreased while the number of survivors increased in contrast with the dramatic forms of disease recorded before. The cause for this drop in mortality was associated with the presence in some countries of virus strains which show low virulence in the field.

To summarise the current situation, it can be recalled that outbreaks were observed where acute forms with high mortality (70-80%) prevailed throughout the outbreak. In other outbreaks a high mortality rate was recorded for the first few days and was then considerably and rapidly reduced over the following days. Finally, mention should also be made of outbreaks of mild clinical cases which are vaguely characteristic, insidious and which initially show low mortality (2 or 3 animals) or no mortality at all.

In Malta, acute forms and high mortality have prevailed. Brazil has noted vast differences between mortality and morbidity, observing the death of all animals and then the death of very few (1 or 2). The first A.S.F. diagnosis in Brazil was of an outbreak where acute cases and extremely high mortality predominated. In general, Brazil, the Dominican Republic and Haiti have all recorded very high mortality rates (80-100%) in the first outbreaks, followed by a marked decrease down to 3-7%. Low mortality rates prevailed in Haiti when there was no programme for disease control. In Cuba, mortality reached 63% in the first outbreaks then dropped to 30% in the final outbreaks (10). Mortality rates in the Iberian Peninsula generally varied from 10-80% from one outbreak to another. As stated in various publications, outbreaks with low mortality and hardly significant mild clinical symptoms were also reported.
V. — DIAGNOSIS

The clinical diagnosis of A.S.F. in countries or areas where classical swine fever (hog cholera) also prevails (as is the case in currently affected European and American countries) is complicated by the resemblance between symptoms and lesions of these two diseases. This is why the definitive diagnosis can only be confirmed by a laboratory.

However, A.S.F. can be suspected or presumed to exist in the field when the disease appears in its peracute or acute forms showing high mortality, symptoms as well as the serious haemorrhagic lesions which have been described repeatedly. But such suspect cases must always be confirmed in a laboratory for them to be differentiated from classical swine fever (hog cholera) or other swine conditions.

On the contrary, the presence of A.S.F. in the field is difficult to suspect when slow and insidious clinical forms develop, accompanied by low mortality and uncharacteristic mild symptoms and lesions. The same applies to subacute forms similar to classical swine fever (hog cholera) which may be endemic in the country. Clinical diagnosis and control thus present considerable difficulties, especially in the initial stage of infection in free countries. This may have been the case in some Latin American countries, as well as in the outbreak which occurred in France in 1974 (30).

The main problem posed by these clinical forms in countries at risk due to the proximity of affected regions is the delay in suspecting A.S.F. presence, in collecting samples and in laboratory diagnosis. Another problem is the risk of the disease passing unnoticed and being confused with other swine conditions until clinical recrudescence and the appearance of acute forms with spectacular development arouse suspicion of its presence.

LABORATORY DIAGNOSIS

As no vaccine against the disease exists and as control depends entirely on strict sanitary measures, the rapidity and reliability of laboratory diagnosis are crucial in controlling A.S.F.

Technology in the diagnosis of A.S.F. has made great progress and now provides a good foundation for the new ways of orientating control and eradication programmes.

Given the current epidemiological situation, diagnosis cannot be performed by using only one technique; an appropriated method for each case is necessary. A series of techniques is available, the application of which depends on: rapidity, simplicity, reliability and sensitivity. The infrastructure of A.S.F. diagnosis necessitates that laboratories be appropriately equipped and that specialists be trained in differential diagnosis techniques.
A.S.F. diagnosis may be carried out for two purposes: identification of the disease in animals showing clinical symptoms (dead or diseased animals) and detection of apparently healthy virus carriers.

Diagnosis of A.S.F. in outbreaks with clinical signs can be performed by identifying the virus and by detecting antibodies. Current techniques are briefly discussed below (details appear in the reference section):

**Virus identification.**

Present methods include: direct immunofluorescence (D.I.F.), haemadsorption reaction and pig inoculation (32). Other methods have been developed but for various reasons they are not as practical for routine diagnosis (agar gel double diffusion test, radio-immuno-assay, enzyme-linked-immunosorbent assay ELISA).

*Identification of viral antigen by direct immunofluorescence (D.I.F.).*

Viral antigen is identified by direct immunofluorescence. It is a rapid, highly sensitive technique for peracute and acute forms which develop both rapidly and fatally. It is performed on smears or on cryostatic sections of spleen, lung, lymph nodes, kidney or tonsils. Its sensitivity for clinical forms is between 70-80% (33). However, this sensitivity has dropped considerably over the past few years, to 40% when diagnosing subacute, chronic and slow or insidious forms which now are most prevalent in enzootic areas (Iberian Peninsula) (32).

The explanation for this reduced sensitivity is the presence of abundant antibodies in animals infected by current virus strains which prevent the staining of the antigenic material with fluorescent antibodies. This technique is also used in addition to the leucocyte culture examination to identify the virus in cases of non-haemadsorbing strains and also to confirm negative results obtained from such cultures inoculated with field samples (32). D.I.F. for identification of viral antigen is complementary to the search for antibodies in the same sample by indirect immunofluorescence (I.I.F.) which will be discussed later. These combined tests enable detection of 85-95% of A.S.F. cases.

*Haemadsorption test on leucocyte cultures (H.A.D.).*

The haemadsorption test is the most sensitive for virus identification (34). However, it is more work- and time-consuming than the immunofluorescence techniques. In laboratories familiar with its use, it is usually reserved for diagnosing suspect cases showing negative results to the D.I.F. and I.I.F. tests.

The test is carried out with cultures of leucocytes from pig blood (35, 36, 38) or bone marrow. The cultures are inoculated with a suspension of spleen, lung or lymph nodes. This method, showing sensitivity at 98.5%, has been used on a large scale and has given satisfactory results (37).
A large number of positive field samples (subacute or chronic forms) necessitate one or two subinoculations into leucocyte cultures to produce the haemadsorption phenomenon. A low percentage of field strains only show cytopathogenic effect (C.P.E.) without haemadsorption (non-haemadsorbing strains). This technique is combined with the D.I.F. test on the cellular sediment of inoculated cultures to confirm negative results and to detect non-haemadsorbing strains (39).

**Pig inoculation.**

Inoculation of pigs for detection of virus in field samples is exceptionally used in countries with experience in other techniques. But this technique is used to confirm the first A.S.F. cases in free countries. Susceptible pigs and those immunised against C.S.F. (hog cholera) are used for the differential diagnosis between A.S.F./C.S.F. Diseased and dead pigs are tested by D.I.F. and H.A.D. In the absence of mortality, a search for antibodies in the serum is made using I.I.F. and immuno-electro-osmophoresis (I.E.O.P.).

**Diagnosis by antibodies detection.**

The search for antibodies for A.S.F. diagnosis is applied to dead animals, chronic and subclinical cases. Methods used are : I.I.F. to detect antibodies in tissues collected from dead animals or in the serum of suspect cases, the I.E.O.P. and ELISA (enzyme-linked immunosorbent assay).

**I.I.F. test for tissue antibody detection.**

In the present course of A.S.F. development, most pigs which die from the disease demonstrate specific antibodies and the virus in tissues. The identification of antibodies extracted from tissues is a new, rapid, sensitive and specific method of great efficiency for A.S.F. diagnosis, particularly in subacute and chronic forms which prevail in enzootic areas (32).

Over the past few years, the I.I.F. test has proved more sensitive for detecting tissue antibodies in the diagnosis of subacute and chronic cases than the D.I.F. test on the same tissues when detecting viral antigen. The ability to detect antibodies in the tissues is especially advantageous for rapid diagnosis because the first field samples received in the laboratory are tissues collected from dead animals.

Antibodies may be extracted from different organs (spleen, lymph nodes, lung) but they should preferably be detected in exudates or plasma that may emerge spontaneously from tissues submitted to the laboratory (32). Monolayers of cell lines infected by an adapted A.S.F. virus are used as the antigen (40). This method has enabled the detection of an average 88.8% of all A.S.F. positive cases in Spain between 1978-1981. In Portugal, the average detected in 1979 and 1980 was 80.5% of all cases.

The combined use of the I.I.F. and D.I.F. techniques increased the efficiency of rapid diagnosis in Spain to 98% out of all A.S.F. cases (annual average for 1978-1981) and in Portugal, to 91% for 1979-1980 (19).
I.I.F. test for serum antibody detection.

Small-scale serological diagnosis of chronic or subclinical cases is performed by using the I.I.F. test (40). It is both the most sensitive and most specific technique and has been recommended as a reference technique to confirm positive cases which have undergone the I.E.O.P. and ELISA tests.

Antibody detection for identifying carriers.

The immuno-electro-osmophoresis (I.E.O.P.) test is used for large-scale serological investigation as it is a rapid, uncomplicated, low-cost method. It was recommended in the conclusions of the Consultation Meeting of O.I.E. Experts in serological techniques for detecting A.S.F., held in Paris on 29 and 30 March 1979. This method may also be used for diagnosing chronic cases. It was adapted by I.C. Pan et al. (41, 42). It is not as sensitive as the I.I.F. and ELISA techniques but is sufficiently sensitive and specific for group diagnosis, that is, for detecting farms or herds with carriers. The antigen is obtained from a cell line culture infected with the A.S.F. virus. The quality of the antigen is an essential factor for specificity and should be standardised. As some negative sera give false positive reactions, the I.I.F. test should be performed to confirm positive cases. Spain, Portugal, Brazil and Malta are currently using this technique in serological surveys in support of control, eradication and repopulation programmes. Purified antigens presently used in Spain and Portugal have considerably increased the specificity of this method.

Antibody detection using the ELISA technique.

The ELISA test was adapted to detect A.S.F. antibodies (43, 44, 45). The possible automation of this method offers a considerable advantage for large-scale serological tests. Its sensitivity is similar to the I.I.F. and greater than the I.E.O.P. The test was recently standardised through joint efforts between the Madrid, Pirbright, Lisbon and Alfort laboratories. In the future, it could provide a valuable tool for serological screening. The application of this method is at an experimental stage.

GENERAL PROCEDURES FOR ROUTINE DIAGNOSIS

For individual routine diagnosis it is recommended that field samples be examined according to the following sequence of procedures and depending on results (32):

1° Antibody detection on exudates or on tissue samples by I.I.F. With this test, 85-88% of A.S.F. cases are detected in enzootic areas.

2° Viral antigen detection by D.I.F. on tissue smears from negative cases found in the preceding test. With this test, an additional 10-12% A.S.F. positive cases are detected. This test may also be applied simultaneously with the above I.I.F. test or even before. The use of both techniques enables detection of 95-98% of A.S.F. cases.
3° For the H.A.D. test, inoculation to leucocyte cultures with samples negative to the I.I.F. and D.I.F. tests. With this technique 2-4% additional cases are detected.

4° Viral antigen detection on leucocyte cultures negative to the H.A.D. test, with or without C.P.E. The D.I.F. test is used on cellular sediments to detect non-haemadsorbing or non-cytopathogenic strains.

5° Subinoculation on fresh leucocyte cultures using the negative leucocyte cultures from the preceding test to confirm the absence of A.S.F. virus.

For the differential diagnosis from classical swine fever (hog cholera) and Aujeszky's disease of negative cases in the second phase, organ sections with fluorescent conjugates of C.S.F. and Aujeszky's disease are examined and PK15 cell line cultures inoculated.

Leucocyte cultures showing negative results to the H.A.D. and D.I.F. tests with A.S.F. conjugates are examined with Aujeszky's disease conjugates in cases where a cytopathogenic effect has been recorded.

In epidemiological surveys for the detection of carriers, for disease surveillance and for the monitoring of A.S.F. free areas and farms, the search for antibodies is conducted by the I.E.O.P. and positive cases are confirmed by the I.I.F.

Finally, it should be noted that studies leading to the standardisation of reagents and technical procedures used in A.S.F. diagnosis should be followed up and encouraged.

VI. — DISEASE CONTROL

As there is no vaccine against A.S.F., control is based on early diagnosis, the application of strict sanitary measures in infected countries and on stringent measures for the protection of free countries.

The basic rules for control of the disease on national and international levels are described in various documents and recommendations adopted during several international meetings which have been held over the past twenty years. The most important documents and meetings giving the essential standards for control are quoted in the O.I.E. Director General's Report on the Activities of that Organisation (in May 1978). This document also includes recommendations from the International Meeting of Experts in veterinary administration and control in the field, convened with the approval of the O.I.E. and held in Avila in March 1978. Reports and working documents from the following Meetings should also be mentioned: Mexico (December 1978) and Panama (October 1979) convened by the F.A.O.

In the current situation, the basic disease control norms are still those formulated by the O.I.E./F.A.O. Emergency Meeting held in Paris from 17-20 January 1961 when A.S.F. invaded the Iberian Peninsula.
However, although the fundamental criteria remain the same, due to experience gained in control in the field over the last twenty years, changes which have occurred in the early characteristics of the disease, the appearance of new clinical forms, the evolution and development of production and marketing structures, scientific progress achieved in epidemiological knowledge and particularly the advances made in diagnosis, former rules have been revised and the orientation of control and eradication programmes has been updated.

These new orientations include the reinforcement of some of the classical norms and new sanitary measures which necessitate modernised legislation, adapted to present scientific and practical knowledge.

Firstly, the classical A.S.F. control norms will be reviewed and then, the major aspects of new developments in orienting control and eradication programmes will be indicated.

ESSENTIAL CLASSICAL NORMS FOR THE CONTROL AND ERADICATION OF A.S.F.

The norms recommended for control, formulated in 1961 and enforced over the last twenty years, are as follows:

— Compulsory notification of the disease, whether clinical or suspect.
— Quarantine of affected farms and neighbouring farms.
— Sample collection and despatch to the official laboratory for diagnosis.
— Compulsory slaughter of all pigs on an affected farm (sick, suspect and healthy animals).
— Disposal of carcasses and contaminated products, disinfection, disinsectisation and desinfestation.
— Control of swine movement in infected areas, prohibition of removal of pigs from infected zones.
— Disinfection of vehicles.
— Prohibition of fairs and markets according to the location of the outbreaks.
— Ban on feeding pigs with uncooked domestic and abattoir waste.
— Introduction of a small group of sentinel pigs for virus detection before total repopulation permitted.
— Prohibition of pig farms annexed to restaurants, cantines, abattoirs, meat factories, knackers, etc.
— Control of ticks and other vectors in extensive farming areas.
— Vaccination against classical swine fever (hog cholera) with identification of animals.
— Organisation of a surveillance service in the field including specialised personnel and organisation of a laboratory diagnosis service with specialised personnel and equipment for differential diagnosis of swine fevers.

The new orientations meet the need of a global policy for the control of A.S.F.

The major aspects of these new orientations are as follows:

— Active participation with the farmer/breeder, to ensure his cooperation in a general control and eradication programme. Encouraged by the Government, it is not only based on adequate compensation but also on the farmer’s responsibility through Breeders’ Associations, the aim of which is animal health improvement. These Associations or Groups of Sanitary Defence should be encouraged by having special privileges accorded to them by the Government (credit facilities for transforming farms to ensure a complete cycle in the production system, credits for improving health conditions in farm buildings, the development of local plans for the control of A.S.F., etc.).

— Accreditation of farms (farms under sanitary control and farms under special sanitary protection).

— Accreditation and demarcation of A.S.F.-free zones, ensuring disease absence for at least six months.

— With various means of assistance, encouragement to change farm structures in order to achieve the complete pig production cycle, to transform small holdings into larger farms with good hygiene conditions.

— Laying down of conditions for the approval of new independent fattening units, thereby avoiding mixing animals of different origins and reducing massive animal movement.

— Serological detection of A.S.F. virus carriers, with a view to protecting A.S.F.-free zones, to the accreditation of farms under sanitary control and under special sanitary protection. This also applies to herds belonging to Breeder Associations for sanitary defence and to epizootiological surveys. This constitutes a new and important aspect for control and eradication programmes and enables special investigation of the carrier problem. Serological surveillance requires the backing of a laboratory infrastructure with specialised personnel.

— Registration of pig farms and authorisation of new farms, subject to specific hygienic and location requirements.

— Re-planning and transformation of extensive farms.

— Control of pig movement in free areas.

— Permanent abattoir inspection, slaughter of pigs in approved abattoirs.

— Prohibition of farm-to-farm sales.

— Setting up of processing factories for carcasses, abattoir waste and domestic food waste.
These new orientations have been integrated in their entirety in the control and eradication programme under way in Spain, which is partly financed by the E.E.C. Some new measures, such as recording free zones, accreditation of farms and serological control are also applied in the programme to be used in Brazil. These new measures have been welcomed by farmers in Spain and the spectacular improvement of the sanitary situation is attributed to their success.

PROTECTIVE MEASURES IN FREE COUNTRIES

— Strengthening of disease control measures laid down for the importation or transit of domestic and wild pigs from A.S.F.-infected countries, as well as refrigerated or frozen pork meats and products, except those which have been heat-treated with animal health guarantees.

— Strict surveillance of airports and frontiers to avoid the possible introduction of food and food waste.

— Disposal of all food waste from aircraft, boats and all vehicles.

— Information notices for the public in customs posts on the risks of disease introduction by food or food waste.

— Availability of specialists trained in the latest diagnostic techniques.

PREVENTIVE MEASURES IN COUNTRIES AT RISK, NEIGHBOURING AFFECTED AREAS

In addition to the general protective measures indicated above for free countries, the following measures have been recommended:

— Reinforcing field surveillance of endemic swine infections which could be confused with the clinical, subacute, chronic or insidious forms of A.S.F., particularly of C.S.F. (hog cholera) outbreaks. In all suspect cases, material should be sent to the laboratory.

— Vaccination against C.S.F. when endemic.

— Informing veterinarians and farmers so they may be aware of any possible disease occurrence, therefore avoiding any delays in suspecting A.S.F.

— Organisation of an official infrastructure which is necessary in the field and laboratory to ensure rapid diagnosis. Specialised personnel should be used in field control and trained personnel familiar with laboratory techniques for the A.S.F./C.S.F. differential diagnosis. For trade purposes, a system based on identifying and keeping records of all pigs should be provided for.

— Laboratory tests of all suspect field samples for differential diagnosis, especially with C.S.F. (hog cholera) if the latter is endemic in the country at risk.
— Training of required personnel in differential diagnosis techniques in official laboratories of countries familiar with these techniques. It is recommended that laboratories in free countries use standard techniques and reagents.

VII. — RESEARCH AND PROGRESS

The most significant progress in A.S.F. control has been achieved in the field of laboratory diagnosis, as described in Section V, « Diagnosis ». In recent years immunofluorescence methods have been developed and in particular the indirect immunofluorescence test for rapid individual A.S.F. diagnosis based on detection of antibodies in the tissues of dead animals (80). Immuno-electro-osmophoresis (I.E.O.P.) (41, 42, 80) is used to identify carriers, to accredit farms and/or zones as A.S.F.-free and to conduct serological surveys. Finally, the enzyme-linked immunosorbent assay (ELISA) (43, 44, 46) has enabled improving the quality of antigens (46, 57), standardising and automating serological screening.

Interesting progress has also been achieved on the chemical structure, molecular biology and antigenic structure of the virus.

A.S.F. virus is a DNA virus with an icosahedral symmetry. It multiplies in the cytoplasm (47, 48, 49) after DNA synthesis in the nucleus (50). The viral genome is linear and double-stranded with a molecular weight (MW) of 10^8 daltons (51, 52, 53). With polyacrylamide gels, at least 28 polypeptides have been identified in the intracellular virus with molecular weights ranging from 11,500-243,000 daltons, 14 of which were localised in the envelope, 3 of these were glycoproteins showing a molecular weight of 89,000, 56,000 and 51,000 daltons. Out of the 28 proteins, 6 were antigenic against hyperimmune serum and their molecular weights were 172,000, 162,000, 146,000, 73,000, 34,000 and 12,000 respectively; 5 of the 28 proteins are present in greater amounts than the others (VP-172, VP-73, VP-42, VP-15 and VP-12). Among these 5 proteins, 3 are antigenic (VP-172, VP-73 and VP-12) (54). Other scientists who applied less sophisticated techniques found a lower number of structural proteins (59, 60) but molecular weights observed corresponded to those mentioned above.

In the infected cell, at least 39 virus-induced proteins were identified by using various radioactive markers. Their molecular weight ranged from 9,500 to 243,000 daltons. Two of these 39 proteins, PI-73 and PI-12, are present in greater amounts than the others; 3 are glycoproteins and 8 are phosphoproteins; and 21 are antigenic in vitro with hyperimmune serum (55, 56).

An important progress should be pointed out: the main protein VP-73 was purified recently from infected cell extracts and it is used as highly specific antigen for antibody detection in the ELISA test. When this protein is inoculated into the pig it induces antibodies, but the pig is not protected against infection (57, 58).
In Spain, the study of proteins induced in the infected cell by different isolates has enabled the identification of a natural mutant which induces a different protein with a MW of 13,500 daltons (56).

Recent work on the structure of the viral genome made with restrictive enzymes clearly showed differences between several strains which had been examined (Lisbon 60, V-65, Haiti, Brazil and Dominican Republic). This technique could be useful in the identification of strains with different functional properties (61).

With regard to the replication of the viral genome, it was shown that the DNA syntheses in the nucleus of the infected cell and moves towards the cytoplasm (50). The nucleus is essential for A.S.F. virus replication (81).

Work in genetic engineering has recently been undertaken in Spain. The ECO R1, Kpm I and Sal I enzymes produce in the A.S.F. virus DNA 28, 16 and 14 fragments of dimensions between 0.3-21.0, 1.3-39.0 and 0.5-32.3 pairs of kilobases respectively. Out of the 28 fragments, 20 which represented 70% of the total genome were cloned in the vector phage λ WES, λ B. These fragments were arranged on a physical map of the viral genome by hybridisation (62).

With regard to the finding of an A.S.F. vaccine for control purposes, tests performed to date have not given satisfactory results. Future research requirements were reviewed during the F.A.O./E.E.C./T.A.C. Meeting held in Rome on 19-20 December 1978 (82) and during the F.A.O. Meeting held on 12-14 December 1979, where three priority topics were selected: virus characterisation, immunology and vaccines. These requirements remain top priority today. During these Meetings, it was emphasised that further research on these three topics be conducted for the development of a vaccine.

Little is known about A.S.F. immunology but further knowledge has been acquired in recent years.

Several experiments carried out in different laboratories have confirmed that those pigs which survive natural infection or inoculation of partially attenuated strains, generally withstand reinfection with the homologous virus strains. This is not the case with heterologous strains. Resistant pigs are generally ASFV carriers.

The mechanism of resistance to the homologous virus is not known. Neutralising antibodies play no role in resistance; they have not been detected in numerous tests on serum of resistant pigs (65, 66). The role of precipitating antibodies, inhibiting antibodies revealed in the HAD test (78) and complement fixing antibodies remains unclear. By the same token, an increase in these antibodies during chronic infection produces hypergammaglobulinaemia (79). Furthermore, pigs which survive natural infection retain their capacity to develop neutralising antibodies against other viruses. These findings suggest that the humoral immune system remains unchanged during development of chronic cases of A.S.F. and that immune response of the pig
(without neutralising antibodies) depends more on the structure and properties of the virus than on defects in the immune system. Recent findings on immune response of pigs are given below.

In a study on various parameters of humoral and cellular immunity (hypergammaglobulinaemia, fluctuations in the total number of leucocytes, T and B cells) in chronically infected pigs, Hess et al. (68) observed lymphocytosis accompanied by a marked increase in T and B cells 7-28 days post-infection (p.i.). The most significant change was the increase in « null » lymphocytes. These results suggest that chronically infected pigs retain their humoral and cellular immune response capacity during infection.

With regard to A.S.F. cellular immunity, Schimizu et al. (1977) (70), using the leucocyte migration inhibition test, proved delayed hypersensitivity. It was concluded that the cellular immune system does not change in chronically affected pigs.

Immune response was studied in A.S.F.-infected pigs by Wardley et al. (1980) (71), who determined its relationship with quantitative and qualitative changes in lymphocyte populations. The reduction of B cells was greater than that of T cells. Blastogenesis tests were performed revealing sensitised leucocytes in the blood ten days p.i. with an attenuated virus. Pigs infected with the virulent virus died before showing response.

In 1981, Sánchez Vizcaíno et al. (72) studied the function of lymphocytes and cellular immunity in pigs affected by a partially attenuated A.S.F. virus. Seven days post-inoculation lymphopaenia and a reduction of T cells were observed together with depression of lymphocyte function evaluated through blastogenesis induced by mitogens and a viral antigen. The decrease in number and function of lymphocyte T was proportionally greater than for lymphocyte B. The study on the phagocytic function showed reduced monocyte activity in the blood while neutrophilic lymphocytes maintained normal phagocytic activity.

In the paper on A.S.F. immunity presented by Wilkinson et al. (77) for the 50th General Session of the O.I.E., recent studies on cellular immunity were described. Blastogenic tests were undertaken on macrophages and lymphocytes in the presence of mitogens and viral antigen. Mitogen response was eliminated by the attenuated virus but stimulated by the virulent virus. In vitro tests were conducted to determine the humoral mechanism of the destruction of infected cells as well as of the reduction in virus quantity. It was observed that lysis of these cells is invoked by the complement system and that in cytotoxicity tests with antibodies, neutrophilic lymphocytes also reduce the virus quantity in vitro.

In 1981, Sánchez Vizcaíno et al. (73) made comparative studies on humoral and cellular immunity among adult pigs and piglets inoculated with viruses of high and low virulence. Four days after adult pigs had been inoculated with highly virulent strains, it was noted that mitogenic response, T cells and
the total number of leucocytes decrease progressively until the animal dies. However, when strains of low virulence are inoculated, the mitogenic response and the number of leucocytes increase. Conversely, piglets inoculated with strains of high and low virulence show total depression of the blastogenic response in regard to non-specific mitogens and viral antigen and they all die.

Variations in immune response reported by various scientists (changes in subpopulations of lymphocytes) are probably linked to different material and methods used (differences in virus strains, degree of virulence, age of animals).

In 1981, Slauson et al. (76) reported new findings in A.S.F. immunopathogenesis. Immunocomplexes in the kidney and the development of E immunoglobulins were revealed two weeks after inoculation of pigs with a partially attenuated virus. Specific degranulation of leucocytes associated with antibodies produced an aggregate of platelets and the release of vasoactive amines which participate in the process of immunocomplex deposits in A.S.F.

Recent research devoted to the development of an inactivated vaccine has contributed to current knowledge on immune response to these antigens. In 1981 Bommeli et al. (84) vaccinated pigs with a suspension of A.S.F.-infected pig spleen, inactivated by a non-ionic detergent (n-octylglucoside). Seven out of ten pigs tolerated the homologous virus but remained carriers and transmitted infection when in contact with other pigs. Reinfection of the survivor pigs with a heterologous virus produced chronic infection and death of 70% of pigs in 9-18 days. A serological survey using the ELISA method only revealed antibodies after challenge with the virulent virus.

Wilkinson et al. (77) (supra) offer the following results from recent tests performed with inactivated antigens with a view to determining the immunogenic activity of antigens induced by the virus on the surface of the infected cell. A.S.F.-infected alveolar macrophages (Malta 1978 virus) were used as the antigen, fixed with glutaldehyde and Freund's adjuvant was added. Residual virus was inactivated by acetyleneimine. ELISA tests performed for antibody detection twenty-eight days after vaccination with this antigen gave negative results and all vaccinated pigs and non-vaccinated controls were inoculated with a homologous live virus which did not invoke apparent clinical disorders in either group. However, pigs which had been vaccinated with fixed infected cells showed a more rapid and greater (1/12,000) antibody response than the non-vaccinated controls (1/1,500) fourteen days after inoculation with the homologous virus. Wilkinson et al. suggest that the more rapid serological response of vaccinated animals was induced by the immunogenic activity of the fixed infected cells. Tests with these antigens should be pursued to determine whether antibodies induced by these surface cell antigens play a role in the resistance mechanism with regard to homologous virulent viruses.
Finally, one must recognise the clear effort made by veterinary services in recent years to control disease in the field and by research laboratories which through their findings contribute to prevention, control and eradication programmes against A.S.F.

International cooperation has played an important role in the progress achieved: results obtained in the field and laboratory are indicative of the importance of maintaining and strengthening this cooperation.

Advantage should be taken of current regression of the disease to accelerate and strengthen A.S.F. eradication programmes.

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Appendix

50th GENERAL SESSION OF THE O.I.E.

RESOLUTION NO. XV

AFRICAN SWINE FEVER (A.S.F.). NEW DEVELOPMENTS

Considering that the recent spread of A.S.F. to new areas once more highlights the permanent threat this disease represents to pig farming, notably in countries where A.S.F. has been endemic for many years;

Having examined the Report entitled « African swine fever. New developments » (document 50 SG/7), Item II of the Agenda, and having heard the presentation of this Report during the Third Plenary Session;

Taking into account the discussions and conclusions on this Item

THE COMMITTEE

RESOLVES

1. To draw the attention of Member Countries to the recommendations contained in document 50 SG/7 emphasising in particular the need to implement the following measures:

A. In free countries.

a) reinforce sanitary measures prohibiting the importation or transit of pigs which come from an area which is infected with A.S.F. as well as of refrigerated or frozen pork products, apart from those which have been industrially processed and which present all necessary sanitary guarantees (O.I.E. Bulletin, 55, 1-2, 1961). It is also recommended that greater surveillance of small-scale transactions and trade be enforced;

b) the public should be made aware of the risks that A.S.F. implies when introduced into free countries;
c) in countries at risk, the necessary infrastructures should be established to enable early diagnosis and control of the disease.

B. In countries where the disease exists.

Control and eradication programmes should be reinforced by:

a) officially promoting cooperation between farmers and organisations dealing with sanitary defence;

b) accrediting pig farms;

c) creating free areas;

d) using serological screening methods to protect free areas to accredit the sanitary status of farms and to conduct serological surveys;

e) officially registering pig farms;

f) re-organising structures of the pig industry;

g) protecting free areas by applying the same measures used in free countries.

2. To recommend that tests be conducted under field conditions before officially adopting any new laboratory techniques for serological surveillance purposes.

3. To recommend to Governments of infected countries that A.S.F. prevention, control and eradication programmes be intensified when the disease shows a decline and that research on virus identification, immunology, pathogenesis, diagnosis and vaccines be encouraged as far as possible.

4. To emphasise the need to improve epidemiological information on A.S.F. spread in the African continent, as well as stress the economic importance of disease spread.

5. The Committee of the O.I.E. expresses its gratitude to Dr. C. Sánchez Botija for the excellent paper he presented and fully endorses the content thereof.

(Adopted by the International Committee of the O.I.E. on 29 May 1982).

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REFERENCES

(see p. 1024)