II

*(Acts whose publication is not obligatory)*

# COUNCIL

## COUNCIL DIRECTIVE

**of 11 December 1991**

**amending Directive 80/217/EEC introducing Community measures for the control of classical swine-fever**

(91/685/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 43 thereof,

Having regard to the proposal from the Commission (1),

Having regard to the opinion of the European Parliament (2),

Having regard to the opinion of the Economic and Social Committee (3),

Whereas Directive 80/217/EEC (4), as last amended by Directive 87/486/EEC (5), introduced Community measures for the control of classical swine-fever;

Whereas, during the period covered by Directive 80/217/EEC, this disease has, owing to the measures adopted to combat it, been eradicated in most Member States; whereas, however, certain serious difficulties have been experienced in eradicating the disease in areas with a high density of pigs and in areas containing wild boar;

Whereas, in view of the evolution of the disease, the availability of improved diagnostic methods and the completion of the internal market for 1 January 1993, it is necessary to amend the control measures already taken at Community level to control classical swine-fever;

Whereas these amendments relate to the cleaning and disinfection of infected farms, disease in wild boar, the use of crisis units, movement controls in protection and surveillance zones, emergency vaccination and diagnostic procedures,

HAS ADOPTED THIS DIRECTIVE:

*Article 1*

Directive 80/217/EEC is hereby amended as follows:

1. Article 2 shall be replaced by the following:

   *'Article 2*

   For the purposes of this Directive the following definitions shall apply:

   (a) 'pig': any animal of the Suidae family;

   (b) 'breeding pig': a pig intended or used for reproduction with a view to multiplication of the species;

   (c) 'fattening pig': a pig fattened and intended for slaughter at the end of the fattening period with a view to meat production;

(1) OJ No C 226, 31. 8. 1991, p. 6.
(2) OJ No C 326, 16. 12. 1991.
(3) Opinion delivered on 28 November 1991 (not yet published in the Official Journal).
(4) OJ No L 47, 21. 2. 1980, p. 11.
(5) OJ No L 280, 3. 10. 1987, p. 21.

(d) 'slaughter pig': a pig which is intended for slaughter without undue delay in a slaughterhouse;

(e) 'feral pig': a pig which is not kept or bred in a holding;

(f) 'holding': a holding within the meaning of Article 2 (4) of Directive 90/425/EEC (*), as last amended by Directive 91/174/EEC (**);

(g) 'pig suspected of being infected with classical swine-fever': any pig exhibiting clinical symptoms or showing post-mortem lesions or reactions to laboratory tests carried out according to Article 11, indicating the possible presence of classical swine-fever;

(h) 'pig infected with classical swine-fever': any pig

— in which clinical symptoms or post-mortem lesions of classical swine-fever have been officially confirmed, or

— in which the presence of this disease has been officially confirmed as the result of a laboratory examination carried out in accordance with Article 11;

(i) 'owner or keeper': any person or persons, either natural or legal, having ownership of the pigs, or charged with keeping the said animals, whether or not for financial reward;

(j) 'competent authority': the competent authority within the meaning of Article 2 (6) of Directive 90/425/EEC;

(k) 'official veterinarian': the veterinarian appointed by the competent authority;

(l) 'rendering': the processing of high-risk material in accordance with Directive 90/667/EEC (1)

(m) 'swill': waste from kitchens, restaurants or, as the case may be, from industries using meat.'

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(*) OJ No L 224, 18. 8. 1990, p. 29.
(**) OJ No L 85, 5. 4. 1991, p. 37.
(1) OJ No L 363, 27. 12. 1990, p. 51.

2. in Article 5:

(a) the following shall be added to the seventh ident of paragraph 1:

'The reintroduction of pigs shall take account of the type of farming practised on the holding concerned and must conform to one of the following procedures:

1. as regards open-air pig holdings:

the reintroduction of pigs shall start with the introduction of sentinel piglets which have been checked and found negative for the presence of antibodies against classical swine-fever virus. The sentinel piglets shall be placed, in accordance with the requirements of the competent authority, throughout the infected holding and be rechecked, 21 and 42 days after having been placed on the holding, for the presence of antibodies.

If none of the piglets has developed antibodies against classical swine-fever virus and as soon as the results of the second test are available, with a negative result, full re-population may take place;

2. for all other forms of rearing, the reintroduction of pigs shall take place either in accordance with the measures provided for in paragraph 1 or with the following provisions:

— the reintroduction of piglets shall be based on total repopulation, provided that:

— all the pigs arrive within a period of eight days and come from holdings situated outside the restriction zone,

— no pig may leave the holding for a period of 60 days after the arrival of the last pigs,

— the repopulated herd is subjected to a serological examination in accordance with Annexes I and IV. That examination may be carried out at the earliest 30 days after the arrival of the last pigs.';

(b) paragraph 2 shall be replaced by the following text:

'2. The competent authority may apply the measures provided for in paragraph 1 to other holdings where pigs may have become infected as a result of their location and direct or indirect contact with the infected holding.';

3. the following Article shall be inserted:

*'Article 6a*

1. Immediately after the competent authority of a Member State has information that feral pigs are suspected of being infected, it shall take all appropriate measures to confirm the presence of the disease, by giving information to the owners or keepers of pigs and to hunters, and by investigations of all feral pigs shot or found dead, including laboratory testing.

2. As soon as confirmation of infection in feral pigs has taken place, the competent authority of a Member

State shall immediately place under official surveillance holdings in the defined infected area and shall in particular order that:

(a) an official census be carried out of all categories of pigs on all holdings; the census must be kept up to date by the owner or keeper; the information in the census must be produced on request and may be checked at each inspection.

However, as regards open-air pig holdings, the first census carried out may be done on the basis of an estimate;

(b) all pigs on the holding be kept in their living quarters or some other place where they can be isolated from feral pigs. The feral pigs must not have access to any material which may subsequently come in contact with the pigs on the holding;

(c) no pigs enter or leave the holding save where authorized by the competent authority having regard to the epidemiological situation;

(d) appropriate means of disinfection be used at the entrances and exits of buildings housing pigs and of the holding itself;

(e) all dead or diseased pigs with classical swine-fever symptoms on a holding be tested for the presence of classical swine-fever;

(f) no part of any feral pig (whether shot or found dead) shall be brought into a holding.

3. Without prejudice to the measures laid down in paragraph 2, Member States shall submit to the Commission at the earliest opportunity a written plan of the measures taken to eradicate the disease in an area defined as infected and the measures applied on the holdings in that area.

The Commission shall examine the plan in order to determine whether it permits the desired objective to be attained and shall approve the plan, if necessary with amendments, in accordance with the procedure laid down in Article 16.

The plan may subsequently be amended or supplemented, in accordance with the same procedure, to take account of developments in the siutation.

4. After the measures provided for in the plan mentioned in paragraph 3 have been approved, they shall replace the initial measures referred to in paragraph 2, on a date which shall be decided upon when approval is given.

5. The plan mentioned in paragraph 3 shall contain information on:

(a) a defined infected area within the territory of the Member State referred to in paragraph 2. When defining the infected area, the competent authority shall take into account:

(i) the geographical distribution of the disease;

(ii) the feral pig population in the area;

(iii) the existence of major natural or man-made obstacles to movements of feral pigs;

(b) the approximate number of groups of feral pigs and their size in the defined area;

(c) specific efforts made to determine the extent of the infection in the feral pig population, by investigation of feral pigs shot by hunters or found dead, and by laboratory testing;

(d) the organization of close cooperation between biologists, hunters, hunting organizations, the wildlife services and veterinary services (animal health and public health);

(e) the reduction of the feral pig population and the issuing of hunting permits; the requirements to be complied with by hunters in order to avoid any spread of the disease; the period adopted for reduction of the feral pig population shall consist of an initial eradication period to be followed by a surveillance period;

(f) the method of removal of feral pigs found dead or shot. In the first phase (eradication period) the removal shall be based on destruction under supervision of the competent authority. In the second phase (surveillance period) the removal shall be in accordance with the requirements laid down by the competent authority;

(g) the epizootiological enquiry which is carried out on each feral pig (shot or found dead). This enquiry must include the completion of a questionnaire which supplies information about:

— the geographical area where the animal was found dead or shot,

— the date on which the animal was found dead or shot,

— the person who found or shot the animal,

— the age and sex of the pig,

— if shot: symptoms before shooting,

— if found dead: the state of the carcase,

— laboratory findings;

(h) disease-prevention measures applicable to the holdings situated in the defined infected area, including the transport and movement of animals within, from and to the area;

(i) the criteria to be applied for lifting the measures taken to eradicate the disease in the defined area and the measures applied to holdings in the area.';

4. the following Article is inserted:

*'Article 7a*

In order to provide full coordination of all measures necessary to ensure eradication of clasiscal swine-fever as quickly as possible and for the purpose of carrying out the epizootiological enquiry, a crisis unit shall be established.

The general rules concerning national crisis units and the Community crisis unit shall be adopted by the Council acting on a proposal from the Commission.';

5. the second subparagraph of Article 8 (2) shall be replaced by the following:

'Where an authorization has been given to remove pigs for slaughter, the competent authority concerned shall ensure that the conditions for removal and slaughtering of pigs fulfil the requirements laid down in Article 9 (4) (f) (i) and that the meat of the said pigs complies with the conditions laid down in Article 9 (4) (g).';

6. Article 9 shall be replaced by the following:

*'Article 9*

1. Immediately after the diagnosis of classical swine-fever has been officially confirmed in pigs on a holding, the competent authority shall establish a protection zone with a radius of at least three kilometres around the outbreak site, which shall itself be included in a surveillance zone of a radius of at least 10 kilometres.

2. When establishing zones, the competent authority must take account of:

(a) the results of the epidemiological studies carried out in accordance with Article 7;

(b) the available serological evidence;

(c) the geographical situation, particularly natural boundaries;

(d) the location and proximity of holdings;

(e) patterns of trade in breeding and slaughter pigs and the availability of slaughterhouses;

(f) the facilities for checking and the nature of the checks employed, whether or not slaughter is carried out on the infected premises.

3. If a zone includes parts of the territory of several Member States, the competent authorities of the Member States concerned shall collaborate to establish the zone.

4. The following measures shall be applied in the protection zone:

(a) a census of all the holdings shall be made as soon as possible; after the establishment of the protection zone these holdings shall be visited by an official veterinarian within not more than seven days;

(b) the movement and transport of pigs on public or private roads shall be prohibited. This prohibition shall not apply to the transit of pigs by road or rail without unloading or stopping. However, in accordance with the procedure may be granted for slaughter pigs coming from outside the protection zone and on their way to a slaughterhouse situated in the said zone;

(c) trucks and other vehicles and equipment, which are used to transport pigs or other livestock or material which may be contaminated (e.g. feedingstuff, manure, slurry, etc.) and which are used within the protection zone, shall not leave:

(i) a holding situated within the protection zone,

(ii) the protection zone,

(iii) a slaughterhouse,

without having been cleaned and disinfected in accordance with the procedures laid down by the competent authority. Those procedures shall provide in particular that no truck or vehicle which has been used in the transport of pigs may leave the zone without being inspected by the competent authority;

(d) no other species of animal may enter or leave a holding without the authorization of the competent authority;

(e) all dead or diseased pigs on a holding shall be notified to the competent authority, which shall carry out any investigations necessary to establish the presence of classical swine-fever;

(f) pigs may not be removed from a holding in which they are kept for 21 days after the completion of the preliminary cleaning and disinfection of the infected holdings as provided for in Article 10; after 21 days, authorization may be given to remove pigs from the said holding:

(i) directly to a slaughterhouse designated by the competent authority, preferably within the protection or surveillance zone, provided that:

— an inspection of all the pigs on the holding has been carried out,

— a clinical examination of the pigs to be moved for slaughter, including the taking of the body temperature of a proportion thereof, has been carried out,

— each pig has been marked by ear marking,

— the pigs are transported in vehicles sealed by the competent authority.

The competent authority responsible for the slaughterhouse shall be informed of the intention to send pigs to it.

On arrival at the slaughterhouse these pigs shall be kept and slaughtered separately from other pigs. The vehicle and equipment which have been involved in the transport of the pigs shall immediately be cleaned and disinfected.

During ante and post-mortem inspection carried out at the designated slaughterhouse, the competent authority shall take into account any signs relating to the presence of the classical swine-fever virus,

(ii) under exceptional circumstances, directly to other premises located within the protection zone provided that:

— an inspection of all the pigs on the holdings has been carried out,

— a clinical examination of the pigs to be moved, including the taking of the body temperature of a proportion thereof, has been carried out,

— each pig has been marked by ear marking;

(g) fresh meat from the pigs referred to in paragraph 4 (f) shall be marked in accordance with the Annex to Council Directive 72/461/EEC of 12 December 1972 on health problems affecting intra-Community trade in fresh meat (*), and subsequently treated in accordance with the rules laid down in Article 4 (1) of Council Directive 80/215/EEC of 22 January 1980 on animal health problems affecting intra-Community trade in meat products (**). This must be done at an establishment designated by the competent authority.

The meat shall be sent to the said establishment on condition that the consignment is sealed before departure and remains sealed throughout the transport.

However, at the request of a Member State, accompanied by appropriate justification and in accordance with the procedure laid down in Article 16, specific solutions may be adopted, in particular with respect to the marking of meat and its subsequent use, and the destination of the processed products.

(*) OJ No L 302, 31. 12. 1972, p. 24. Directive last amended by Directive 89/662/EEC (OJ No L 395, 30. 12. 1989, p. 13).

(**) OJ No L 47, 21. 2. 1980, p. 4. Directive last amended by Directive 89/662/EEC.

5. The measures in the protection zone shall continue to be applied at least until:

(a) all measures laid down in Article 10 have been carried out;

(b) pigs on all holdings have undergone;

(i) a clinical examination which has revealed that they have no signs of disease suggesting classical swine-fever, and

(ii) a serological examination in accordance with Annexes I and IV without the detection of antibodies to the classical swine-fever virus.

The examination referred to in (i) and (ii) shall not take place before 30 days have elapsed after the completion of preliminary cleaning and disinfection measures on the infected holding.

6. The following measures shall be applied in the surveillance zone:

(a) a census shall be taken of all pig holdings;

(b) the movement and transport of pigs on public or private roads, excluding the service roads of holdings, shall be prohibited, unless approved by the competent authority. This prohibition shall not apply to the transit of pigs by road or rail, without unloading or stopping;

(c) trucks and other vehicles and equipment which are used to transport pigs or other livestock or material which may be contaminated (e.g. feedingstuff, manure, slurry, etc.) and which are used within the surveillance zone, shall not leave the zone without having been cleaned or disinfected in accordance with the procedures laid down by the competent authority;

(d) no other species of animal may enter or leave a holding during the first seven days after establishment of the zone without the authorization of the competent authority;

(e) all dead or diseased pigs on a holding shall be reported to the competent authority, which shall carry out any investigations necessary to establish the presence of classical swine-fever;

(f) pigs may not be removed from a holding on which they are kept for seven days after the completion of the preliminary cleaning and disinfection of the infected holding provided for in Article 10; after seven days authorization may be given to remove pigs from the said holding:

(i) directly to a slaughterhouse, designated by the competent authority, preferably within the protection or surveillance zone, provided that:

— an inspection of all the pigs on the holding has been carried out,

— a clinical examination of the pigs to be moved for slaughter, including the taking of the body temperature of a proportion thereof, has been carried out,

— each pig has been marked by ear marking,

— the pigs are transported in vehicles which are sealed by the competent authority.

The competent authority responsible for the slaughterhouse shall be informed of the intention to send pigs to it.

On arrival at the slaughterhouse these pigs shall be kept and slaughtered separately from other pigs.

During ante and post-mortem inspection carried out at the designated slaughterhouse, the competent authority shall take into account any signs relating to the presence of the classical swine-fever virus;

(ii) under exceptional circumstances, directly to other prenises located within the protection zone, provided that:

— an inspection of all the pigs on the holding has been carried out,

— a clinical examination of the pigs to be moved, including the taking of the body temperature of a proportion thereof, has been carried out,

— each pig has been marked by ear marking.

Trucks and other vehicles and equipment used for the transport of these pigs must be cleaned and disinfected after each transport operation;

(g) fresh meat derived from the pigs referred to in paragraph 6 (f) shall be marked as described in the Annex to Directive 72/461/EEC and subsequently treated in accordance with the rules laid down in Article 4 (1) of Directive 80/215/EEC. This shall be done at an establishment designated by the competent authority.

The meat shall be sent to the said establishment on condition that the consignment is sealed before departure and remains sealed throughout the transport.

However, at the request of a Member State, accompanied by appropriate justification and in accordance with the procedure laid down in Article 16, specific solutions may be adopted, in particular with respect to the marking of meat and its subsequent use, and the destination of the processed products.

7. The measures in the surveillance zone shall continue to be applied at least until:

(a) all measures laid down in Article 10 have been carried out;

(b) the pigs on all holdings have undergone a clinical examination and have been found to have no signs of disease suggesting classical swine-fever;

(c) a serological examination has been carried out by representative sampling of the holdings, to be determined in accordance with the procedure laid down in Article 16 and such sampling has failed to reveal any antibodies to the classical swine-fever virus.

The examinations referred to in (b) and (c) may not take place before 15 days have elapsed after completion of preliminary cleaning and disinfection measures on the infected holding.

8. By derogation from paragraphs 4 (f) and 6 (f), the competent authority may authorize that pigs be moved from the holding to be transported to a rendering plant for rendering or to a place where the pigs are slaughtered in order to be burned or buried. These animals shall be tested at random for the presence of the classical swine-fever virus. The criteria laid down in Annex IV with regard to the collection of blood samples shall be taken into account during such random testing.

All necessary precautions shall be taken to avoid the risk of spreading the virus during such transport, in particular by cleaning and disinfecting the truck after the transport.

9. Where the prohibitions provided for in paragraphs 4 (f) and 6 (f) are maintained beyond 30 days because of an outbreak of further cases of the disease and as a result problems arise in keeping the pigs, the competent authority may, following a reasoned application by the owner, authorize removal of pigs from a holding within the protection or surveillance zone, as the case may be, provided that:

(a) the official veterinarian has verified the facts;

(b) an inspection of all pigs on the holding has been carried out;

(c) a clinical examination of the pigs to be moved, including the taking of the body temperature of a proportion thereof, has been carried out;

(d) each pig has been marked by ear marking;

(e) the holding of destination is located in the protection zone or within the surveillance zone.

Es All necessary precautions shall be taken to avoid the risk of spreading the virus during such transport, in particular by cleaning and disinfecting the truck after the transport.

10. The competent authority shall take all necessary measures, including the use of prominent signs and warning notices and use of media resources, such as the press and television, to ensure that all persons in

the protection and surveillance zones are fully aware of the restrictions in force, and shall take such measures as they consider appropriate to ensure the adequate enforcement of these measures.';

7. Article 10 shall be replaced by the following:

'*Article 10*

Member States shall ensure that:

(a) the disinfectants to be used and their concentrations are officially approved by the competent authority;

(b) the cleaning and disinfection operations are carried out under official supervision in accordance with:

(i) the instructions given by the official veterinarian; and

(ii) the procedure for cleaning and disinfecting an infected holding as laid down in Annex V.';

8. the following Article shall be inserted:

'*Article 10a*

Should classical swine-fever be confirmed in a slaughterhouse, the competent authority shall ensure that:

(a) all pigs in the slaughterhouse are slaughtered without delay;

(b) the carcases and offal of infected and contaminated pigs are destroyed under official supervision in such a way as to avoid the risk of classical swine-fever virus spreading;

(c) cleaning and disinfection of buildings and equipment, including vehicles, take place under the supervision of the official veterinarian in accordance with instructions laid down by the competent authority;

(d) an epidemiological enquiry is carried out in accordance with Article 7;

(e) no pigs are reintroduced for slaughter until at least 24 hours after completion of the cleaning and disinfection operations carried out in accordance with (c).';

9. Article 14 shall be replaced by the following:

'*Article 14*

1. Member States shall ensure that:

(a) the use of classical swine-fever vaccines is prohibited;

(b) the manipulation of classical swine-fever virus for research, diagnosis or manufacture of vaccines shall be carried out only in approved establishments and laboratories;

(c) the storage, supply, distribution and sale of classical swine-fever vaccines in the territory of the Community are carried out under official control.

2. Notwithstanding paragraph 1 concerning the use of classical swine-fever vaccine, it may be decided, when classical swine-fever has been confirmed and threatens to spread, that emergency vaccination may be introduced. In this case, the Member State concerned shall submit to the Commission an emergency vaccination plan which shall include information on:

(a) the disease situation which has resulted in the request for emergency vaccination;

(b) the extent of geographical area in which emergency vaccination is to be carried out;

(c) categories of pigs and the approximate number of pigs to be vaccinated;

(d) the vaccine to be used;

(e) the duration of the vaccination campaign;

(f) the identification and registration of the vaccinated animals;

(g) measures for the movement of pigs and their products;

(h) other matters appropriate to the emergency situation.

The Commission shall immediately examine the plan in collaboration with the Member State concerned. In accordance with the procedure laid down in Article 16, the emergency vaccination plan may be approved or amendments and additions may be requested before approval is given, especially where marking is concerned.

3. Any Member State which carries out emergency vaccination shall ensure that:

— no live pigs leave the vaccination area except for immediate slaughter in a slaughterhouse designated by the competent authority and situated within the vaccination area or close to that area;

— all fresh pig meat produced from pigs vaccinated during the emergency vaccination bears the stamp provided for in Article 5a of Directive 72/461/EEC and is stored and transported separately from meat not bearing the said stamp.

4. Paragraph 3 shall apply during the emergency vaccination period and for a minimum of six months following completion of the vaccination operations in the affected area.

In accordance with the procedure laid down in Article 16 and before the end of the said six-month period, measures shall be taken to ban:

(a) sero-positive pigs from leaving the holding where they are kept, except for immediate slaughter;

(b) piglets of sero-positive sows from leaving their holding of origin unless being transported to:

— a slaughterhouse for immediate slaughter,

— a holding designated by the competent authority, from which they are to be sent directly to the slaughterhouse,

— a holding after obtaining a negative result from a serological test for antibodies against the classical swine-fever virus.

5. If necessary, the Commission shall adopt rules relating to the production, packaging, distribution and state of the stocks of classical swine-fever vaccines in the Community.';

10. Article 14a shall be replaced by the following:

*'Article 14a*

Veterinary experts from the Commission may, in collaboration with the autorities of the Member State concerned and, in so far as is necessary to ensure uniform application of this Directive, make on-the-spot checks; the Commission shall inform the Member States of the results of such checks.

A Member State in whose territory a check is being carried out shall give all necessary assistance to the experts in carrying out their duties.

The general provisions for implementing this Article shall be determined in accordance with the procedure laid down in Article 16.';

11. The following Article shall be inserted:

*'Article 14b*

1. Each Member State shall draw up a contingency plan specifying the national measures to be implemented in the event of an outbreak of classical swine-fever.

This plan should allow access to facilities, equipment, personnel and all other appropriate materials necessary for the rapid and efficient eradication of the outbreak. It must give a precise indication of the vaccine requirements which each Member State concerned considers it needs in the event of emergency vaccination.

2. The criteria to be applied *mutatis mutandis* for drawing up the contingency plan shall be those laid down in Commission Decision 91/42/EEC of 8 January 1991 laying down the criteria to be applied when drawing up contingency plans for the control of foot and mouth disease in application of Article 5 of Council Decision 90/423/EEC (*).

The Commission may, in accordance with Article 16, amend or supplement those criteria taking into account the specific nature of classical swine-fever.

3. Plans drawn up in accordance with the criteria provided for in paragraph 2 shall be submitted to the Commission not later than 1 January 1993.

4. The Commission shall examine the plans in order to determine whether they permit the desired objective to be attained and shall suggest to the Member State concerned any amendments required, in particular to ensure that they are compatible with those of the other Member States.

The Commission shall approve the plans, if necessary amended, in accordance with the procedure laid down in Article 16.

The plans may subsequently be amended or supplemented, in accordance with the same procedure, to take into account developments in the situation.

(*) OJ No L 23, 29. 1. 1991, p. 29.'

12. Annex I shall be replaced by the following:

*'ANNEX I*

**DIAGNOSTIC PROCEDURES FOR THE CONFIRMATION OF DIFFERENTIAL DIAGNOSIS OF CLASSICAL SWINE-FEVER**

Notwithstanding the period required for antibodies to develop, the following guidelines, standards and minimum criteria are laid down for the diagnostic procedures of classical swine-fever (CSF).

A. COLLECTION OF MATERIALS FOR DIAGNOSIS

1. For virus isolation and antigen detection, tonsil and spleen tissues are considered essential. Preferably at least two other lymphatic tissues should be collected, such as the retropharyngeal, partoid, mandibular or mesenteric lymph nodes together with ileum or kidney. Each sample of the tissue should be placed in a separate sealed plastic bag and labelled. The samples should be transported and stored in leak-proof containers. They should not be frozen but kept cool at refrigerator temperature and tested without delay.

2. Blood samples for virus isolation from leucocytes should be collected from pigs showing signs of fever or other signs of disease. EDTA or heparin should be used as anticoagulants. The samples must be kept cool at refrigerator temperature and submitted to laboratory testing without delay.

3. Blood samples for the detection of antibody as an aid to diagnosis of clinical outbreaks and for the purposes of surveillance should be taken from animals which have recovered from suspect infection and from pigs known to have been in contact with infected or suspect cases. In such suspect holdings, all of the first 20 suspect or in-contact animals, and 25 % of any additional animals, should be sampled. In order to ensure a high prohability of detection of antibody, samples should be collected from each unit of the holding at this level.

## B. THE LABORATORY DIAGNOSIS OF CLASSICAL SWINE-FEVER

The principal basis for the laboratory diagnosis of CSF shall be the demonstration of viral antigen, virus or antibodies in organs or tissue fluids.

In the case of inconclusive results, the tests shall be repeated on the same samples. Additional samples should be collected from the same source if clinical suspicion continues.

Serological tests for the detection of antibodies may be used as an adjunct to diagnosis in cases of suspect CSF. If the demonstration of viral antigen or virus isolation has not been successful on material derived from animals giving rise to suspicion of CSF or with material from holdings which have had contact with cases of CSF, tests for the detection of antibody shall be applied to blood samples from animals which are no longer suspect and from those suspected of having been in contact with the disease.

1. **Demonstration of viral antigen**

For the demonstration of viral antigen in organ tissues, a direct immune labelling system should be used on thin cryostat sections (up to five microns) of tonsils and tissues of other organs as specified in A (1). The diagnostic reagent must be a pestvirus-specific polyclonal antiserum to CSF virus, labelled with a fluorochrome, enzyme or biotin, according to the following cirteria:

(a) hyperimmune serum shall be prepared from pigs which are free from infections or the serum of which is free from any antibody which could affect the specifity or quality of the reactions;

(b) labelled immunglobulin prepared from CSF hyperimmune pig serum as specified under (a) shall have a minimum working titre of 1/20 as determined in CSF virus infected cell cultures and confirmed by check tests on tissue sections. The working dilution of the conjugate shall combine a maximum of signal with a minimum of background staining.

Any sample showing specific cytoplasmic reaction shall be considered positive for pestivirus. In such cases, further tests must be carried out as described in B (3).

2. **Virus isolation and identification in cell cultures**

(a) Virus isolation from tissue samples is performed on susceptible cell cultures of PK15 or other equally susceptible cell lines. Organ suspension from a suspected animal should be inoculated at a dilution of 1/10.

(b) Virus isolation from blood samples, collected and handled as indicated in paragraph A (2), is performed by the inoculation of cell cultures with buffy coat suspension reconstituted to the original blood volume.

(c) For detection of viral antigen in the cytoplasm of inoculates, such cell cultures shall be treated with labelled polyclonal antiserum. The staining should be applied at intervals from 24 to 72 hours from the time of inoculation.

(d) Positive cultures should be subject to differential diagnostic tests as specified in B (3). Negative results after the first cell culture passage may require second or even more passages in order to isolate the virus.

3. **Monoclonal antibody typing of pestivirus isolates**

(a) Duplicates of tissue cryostat sections or cell cultures which give positive reactions with polyclonal antiserum as described in B (1) and (2) shall be further examined by labelled monoclonal antibodies to distinguish the CSF virus from the bovine virus diarrhoea (BVD) or border disease (BD) viruses.

(b) Only monoclonals which have been officially recommended by the Community Referernce Laboratory for Classical Swine-Fever should be used.

(c) The monoclonals should be grouped into four panels according to the following criteria:

| Panel number | Reactivity |
|---|---|
| 1 | All pestiviruses |
| 2 | All CSF viruses |
| 3 | CSF vaccine strains |
| 4 | All BVD/BD viruses |

Each panel may be represented by either a single monoclonal or a mixture of the competent monoclonal antibodies, provided that the spectrum of reactivity corresponds to that given above.

(d) The interpretation of the reaction patterns is summarized as follows:

| Panel | Interpretation |
|---|---|
| 1 2 3 4 | |
| + + − − | CSF confirmed |
| + + + − | CSF vaccine strain |
| + − − + | BVD/BD virus |
| + − − − | Virus unclassified, further tests required |
| + + − + | |
| + + + + | |
| − − − − | |

## C. DETECTION OF ANTIBODIES TO CLASSICAL SWINE-FEVER VIRUS

The detection of CSF virus antibodies in blood samples is carried out to assist in the diagnosis of swine-fever in holdings containing pigs showing clinical signs of the disease or in pigs believed to have had contact with infected pigs. It may also be carried out for the purpose of surveillance or for surveys in herds of unknown status.

For these purposes, blood samples should be subjected to an approved test.

The following tests are approved for use and must be carried out with the inclusion of positive and negative serum controls.

The virus strains to be used for serological tests should be agreed at a meeting of the National Swine Fever Laboratories (NSFL), and issued as required by the Community Reference Laboratory for Classical Swine Fever to the NSFL, upon request.

All test procedures used must be shown to give satisfactory results with CSF reference sera supplied by the Community Reference Laboratory for Classical Swine-Fever.

### 1. The virus-neutralization test

This test is based on the determination of the neutralizing 50 % endpoint. Cultures are inoculated with mixtures of diluted serum and a constant amount of virus after a specified incubation period at 37°C. The results are based on the absence of any viral replication detectable by an immune labelling system. Either neutralization-immunofluorescence (NIF) or the neutralizing peroxidase-linked antibody (NPL) assays must be used. Detailed protocols will be supplied by the EC Reference Laboratory for CSF as required.

For screening purposes, the sera are initially diluted 1/10. When a full titration is necessary two-fold dilutions of serum starting at 1/10 are prepared. Each dilution is mixed with an equal volume of virus suspension containing 100 ($\pm$ 0,5 $\log_{10}$) infectious doses (TCID 50). At least two cultures are used for each dilution. After an appropriate incubation period the cell cultures are fixed and viral antigen is detected by an immune labelling system. The results are expressed as the reciprocal of the initial serum dilution at which half the inoculated cell cultures fail to show any specific labelling. A point between two dilution levels is estimated.

2. **The enzyme-linked immunosorbent assay (Elisa)**

Competitive, blocking and indirect techniques may be used on any suitable support.

It is recommanded that the tests used should minimize cross-reactions with BVDV and other pestiviruses. However, the test system must ensure identification of all CSF infections, and at all stages of the immune response to infection.

*Antigen*

The antigen should be derived from or correspond to viral proteins of one of the recommended CSF virus strains. Cells used to prepare antigen should be free of any other pestivirus infection.

*Antisera*

Polyclonal antisera for competitive or blocking assays should be raised in pigs or rabbits by infection with one of the recommended CSF virus strains or with the lapinized C strain. Monoclonal antibodies should be directed against or correspond to an immunodominant viral protein of CSF virus. Indirect assay should use an anti-porcine immunoglobulin reagent which detects both lgG and lgM.

The sensitivity of the Elisa should be high enough to score positive any serum reacting in the neutralization test and also reference positive sera as issued by the Community Reference Laboratory for CSF.

The Elisa procedure may be used only with serum or plasma samples derived from individual pigs.

If the Elisa procedure used is not CSF-specific, positive samples should be further examined by differential tests, as specified in section E.

## D. EVALUATION OF THE RESULTS OF LABORATORY TESTING

1. The demonstration of CSF virus antigen in organ tissues or cell cultures after virus isolation from tissue samples following the techniques defined in B (1), (2) and (3) shall form the basis of confirmation of the presence of the disease, except in the case of a reaction demonstrated to be due to vaccinal virus specified according to B (3). The demonstration of BVD/BD antigen according to B (3) shall rule out suspicion of CSF provided that there are no other grounds for such suspicion.

Following unusual or unexpected results of monoclonal typing according to B (3), pestivirus isolates shall be considered unclassified and the herd of origin regarded as suspect pending further testing. This may include submission of the virus to a reference laboratory for characterization and serological investigations on the herd of origin.

2. Following the detection of antibody reactive with CSF virus, the herd of origin shall be regarded as suspect.

   (a) In order to rule out the suspicion of CSF raised by the detection of antibody, the test described in Section E shall be used to distinguish between CSF-reactive antibody which may have been induced by other pestiviruses and such antibody due to CSF virus itself. All original samples shall be retested by the differential test.

   (b) If suspicion cannot be ruled out on the first differential test, a further test shall be carried out at least 30 days later to follow up the possible spread of infection. All of the first 20 animals on the suspect holding shall be sampled, and 25 % of any additional animals.

3. **Interpretation of serological results**

A virus neutralization titre of $\geq$ 1/10 in any pig, together with clinical or epizootiological evidence giving rise to suspicion of disease, shall constitute a positive diagnosis. A titre of $\geq$ 1/10 in any pig without clinical or epizootiological evidence gives rise to suspicion of disease and should be followed by differential diagnostic procedures.

The same criteria should be applied for any pig giving a positive Elisa result.

## E. SEROLOGICAL PROCEDURES FOR THE DIFFERENTIAL DIAGNOSIS BETWEEN CLASSICAL SWINE-FEVER AND OTHER PESTIVIRUSES

1. Tests for the differential diagnosis of CSF and other pestivirus infections are based on parallel testing of the sera with both CSF and BVD/BD virus strains, using fully comparable methods.

   The CSF and BVD/BD virus strains for use should have been officially approved (see section C). To rule out the suspicion of CSF raised by the detection of antibody, blood samples should be examined by comparative end-point titrations for neutralizing antibody against CSF virus and BVD/BD virus.

   In blocking Elisa, a comparison of percentage blocking with CSF and BVD/BD antigens may be used.

2. The results of the comparative serological tests using refrence strains of CSF and other pestiviruses shall be interpreted as follows:

   (a) if the comparative tests show that more than one pig has antibody to CSF virus with no antibody to other pestiviruses, the test result is considered positive for CSF;

   (b) if the comparative tests show that the titres to CSF virus are equal to or higher than the titres to other pestiviruses in more than one of the pigs, there shall be suspicion of CSF and differentiation shall proceed as follows:

   — those pigs which show neutralizing titres against CSF virus which are higher than or equal to the titres against other pestiviruses shall be slaughtered. Their tissues and, if pregnant, their foetuses, shall be subjected to examination for CSF antigen or virus, following the procedure defined in B (1), (2) or (3),

   — if CSF virus antigen or virus is detected, CSF shall be confirmed,

   — if the examination defined in the second indent fails to reveal the presence of CSF antigen or virus, the holding shall be considered as suspect until a further set of blood samples collected at least 30 days later has been subjected to further comparative tests,

   — if these subsequent comparative tests show all animals to have significantly (four-fold or greater) higher titres against BVD/BD virus than against CSF virus, suspicion shall be ruled out,

   — if one or more animals show a titre against CSF virus which is equal to, or higher than, its titre to BVD/BD virus, the result shall be considered positive for CSF;

   (c) if the BVD/BD titres are such as not to exclude the possibility of CSF, the holding shall be considered as suspect and be retested after at least 30 days.

## F. THE DIFFERENTIAL DIAGNOSIS OF AFRICAL SWINE-FEVER (ASF)

ASF cannot be differentiated from classical swine-fever by either clinical or post-mortem examinations and both of these diseases should be considered in the differential diagnosis of any acute febrile heamorrhagic syndrome of pigs.

Laboratory tests are essential to distinguish between the two diseases. A positive diagnosis in an ASF-free country should be based on the isolation and identification of ASF virus.

The principal basis for the laboratory diagnosis of ASF shall be the demonstration of virus, viral antigen or antobodies in organs and tissue fluids.

In the case of inconclusive or negative results of at least two tests on samples from animals giving rise to suspicion of ASF or with material from holdings which had contracts with cases of ASF, additional material should be collected in the same holding and from animals which have been in contact with the disease.

1. **Demonstration of viral antigen**

   For the demonstration of viral antigen, the direct immunofluorescence technique or other suitable techniques shall be applied to thin cryostat sections of organ tissues or smears, or on sediments from leucocyte cultures. The procedures used are similar to those described for CSF, except that ASF-specific reagents are used.

2. **Virus isolation and identification**

(a) *Haemadsorption (HAD) test*

The HAD test is carried out by inoculating either 10 % tissue suspensions or blood collected in the field from suspect pigs into primary pig leucocyte cultures or by preparing leucocyte cultures from the blood of febrile pigs inoculated at the laboratory or collected in the field. Haemadsorption consists of the attachment of large numbers of pig erythrocytes to the surface of infected cells and confirm ASF diagnosis.

(b) *Pig inoculation*

A pool is made with aliquote for each 10 % tissue suspension and 2 ml inoculated intramuscularly into each of four pigs: two of these should be vaccinated against CSF and two unvaccinated. Pigs should be examined daily for increase of rectal temperature and onset of clinical signs for 21 days. If fever develops, blood samples should be collected for preparation of leucocyte cultures for the HAD test (autorosette and inoculation of primary pig leucocyte cultures). If no clinical signs develop, blood should be taken for detection of antibodies after the 21 day observation period.

G. DETECTION OF ANTIBODIES INDUCED BY ASF-VIRIS IN BLOOD SAMPLES AND TISSUE FLUIDS

The detection of antibodies in samples of serum or tissue fluid is carried out to assist in the diagnosis of ASF in holdings containing pigs showing clinical signs suspicious of disease or in pigs believed to have had contact with ASF-infected pigs. It may also be carried out for the purpose of surveillance or for surveys in herds of unknown status.

For these purposes, samples should be subjected to an approved test.

The following are approved for use and must be carried out with the inclusion of appropriate positive and negative serum controls.

(a) Indirect immunofluorescence (IIF) test;

(b) Elisa'.;

12. The following Annexes shall be added after Annex III:

*'ANNEX IV*

**SEROLOGICAL SCREENING OF PIGS IN THE PROTECTION ZONE AND SURVEILLANCE ZONE FOR DETECTION OF ANTIBODIES AGAINST CLASSICAL SWINE-FEVER VIRUS**

The programme for serological screening shall take into account the transmission of classical swine-fever and the way pigs are kept, e.g. a reference to whether pigs are kept in groups or not.

1. **Serological screening of pigs kept in a group**

A group is two or more pigs kept in direct contact.

*Sampling of groups*

| | |
|---|---|
| — If 20 or fewer than 20 pigs in a group: | — two pigs. Where the group consists of a sow with piglet, only the sow shall be sampled, |
| — if more than 20: | — two pigs + 5 % of the remainder. |

All groups shall be sampled.

2. Serological screening of pigs kept individually; this includes pigs kept in close proximity to each other but having no direct contact, e.g. tethered sows.

*Sampling procedure*

| Number of pigs | Pigs to be tested |
|---|---|
| fewer than 20 | all |
| 20 — 100 | 20 + 20 % of the remainder |
| more than 100 | 20 + 10 % of the remainder (at least 36); |

*ANNEX V*

**PROCEDURE FOR CLEANING AND DISINFECTING AN INFECTED HOLDING**

I. PRELIMINARY CLEANING AND DISINFECTION

(a) As soon as the carcases of the pigs have been removed for disposal, those parts of the premises in which the pigs were housed and any parts of other buildings, yards etc. contaminated during slaughter or post-mortem examination should be sprayed with disinfectants approved for use in accordance with Article 10.

(b) Any tissue or blood which may have been spilled during slaughter or post-mortem or gross contamination of buildings, yards, utensils etc. should be carefully collected and disposed of with the carcases.

(c) The used disinfectant shall remain on the surface for at least 24 hours.

II. FINAL CLEANING AND DISINFECTION

(a) Grease and dirt should be removed from all surfaces by the application of a degreasing agent and washed with cold water.

(b) After washing with cold water as described in (a), further spraying with disinfectant should be applied.

(c) After seven days the premises should be treated with a degreasing agent, rinsed with cold water, sprayed with disinfectant and rinsed again with cold water.

(d) Manure and used bedding should be stacked to heat, sprayed with disinfectant and left for 42 days. Slurry should normally be stored for 42 days after the last addition of infective material. This period may be extended if the slurry has been heavily contaminated.'

*Article 2*

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive not later than 1 July 1992. They shall fortwith inform the Commission thereof.

When the above measures are adopted by the Member States, they shall contain a reference to this Directive or shall be accompanied by such reference at the time of their official publication. The procedure for such reference shall be adopted by Member States.

*Article 3*

This Directive is addressed to the Member States.

Done at Brussels, 11 December 1991.

*For the Council*

*The President*

P. BUKMAN