

COMMISSION

COMMISSION DECISION

of 4 July 2000

establishing diagnostic procedures, sampling methods and criteria for the evaluation of the results of laboratory tests for the confirmation and differential diagnosis of swine vesicular disease

(notified under document number C(2000) 1805)

(Text with EEA relevance)

(2000/428/EC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 92/119/EEC of 17 December 1992 introducing general Community measures for the control of certain animal diseases and specific measures relating to swine vesicular disease⁽¹⁾, as last amended by the Act of Accession of Austria, Finland and Sweden, and in particular Annex II, paragraph 3 thereof,

Whereas:

(1) It is necessary to lay down at Community level diagnostic procedures, sampling methods and criteria for the evaluation of the results of laboratory tests for the confirmation of swine vesicular disease and a rapid differentiation from foot-and-mouth disease, in order that an improved control of both diseases can be ensured.

(2) Annex III of Council Directive 92/119/EEC lays down the functions and duties of the Community reference laboratory for swine vesicular disease in order to coordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing the disease; these functions and duties include the organisation of periodic comparative tests and the supplying of standard reagents at Community level.

(3) Laboratory tests have been recently developed to ensure that swine vesicular disease is quickly diagnosed and distinguished from foot-and-mouth disease.

(4) The results of the most recent comparative tests performed at Community level suggest, in particular, that reliable tests have been developed to detect the antigen or the genome of swine vesicular disease virus and that these tests can successfully supplement the virus isolation test for the virological diagnosis of swine vesicular disease.

(5) The experiences gained in the control of swine vesicular disease in recent years have resulted in the identification of the most suitable sampling procedures and criteria for evaluation of the results of the laboratory tests for a proper diagnosis of this disease in different situations.

(6) The opinion and recommendations of the Scientific Committee on animal health and welfare on swine vesicular disease have been taken into account.

(7) The measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:

Article 1

1. Member States shall ensure that the confirmation of swine vesicular disease and the differential diagnosis with foot-and-mouth disease are based on:

(a) the detection of clinical signs of disease;

⁽¹⁾ OJ L 62, 15.3.1993, p. 69.

- (b) the detection of virus, antigen or genome in samples of epithelium tissue, vesicular fluid or faeces;
- (c) the demonstration of a specific antibody response in serum samples,

in accordance with the procedures, sampling methods and criteria for evaluation of the results of laboratory tests laid down in the Manual annexed to this Decision.

2. However, the national diagnostic laboratories referred to in Annex II(5) to Directive 92/119/EEC may apply modifications to the laboratory tests referred to in the Manual annexed to this Decision or use different tests, provided that an equal sensitivity and specificity can be demonstrated.

The sensitivity and specificity of these modified or different tests must be evaluated in the framework of the periodic

comparative tests organised by the Community Reference Laboratory for swine vesicular disease.

Article 2

This Decision shall apply from 1 October 2000.

Article 3

This Decision is addressed to the Member States.

Done at Brussels, 4 July 2000.

For the Commission

David BYRNE

Member of the Commission

ANNEX

MANUAL OF DIAGNOSTIC PROCEDURES, SAMPLING METHODS AND CRITERIA FOR THE EVALUATION OF THE RESULTS OF LABORATORY TESTS FOR THE CONFIRMATION AND DIFFERENTIAL DIAGNOSIS OF SWINE VESICULAR DISEASE

CHAPTER I

Introduction, objectives and definitions

1. This Manual:
 - (a) provides for guidelines and minimum requirements on diagnostic procedures, sampling methods and criteria for the evaluation of the results of laboratory tests for a proper diagnosis of swine vesicular disease. However, particular emphasis is also on the differential diagnosis with foot-and-mouth disease;
 - (b) integrates the provisions of Annex II to Directive 92/119/EEC, and in particular of paragraphs 4, 7 and 8 of the said Annex;
 - (c) is principally directed towards the authorities responsible for the control of swine vesicular disease. Therefore, emphasis is on the principles and applications of laboratory tests and evaluation of their results and not on detailed laboratory techniques.
2. For the purpose of this Manual the following definitions shall apply:
 - (a) 'seropositive pig' means any pig whose serum has an antibody titre equal to or greater than the swine vesicular disease Reference Serum 4 referred to in Chapter X in the virus neutralisation test used by the National Laboratory;
 - (b) 'singleton reactor' means any single seropositive pig in a holding which yields a positive result in serological tests for swine vesicular disease, but which has no history of contact with swine vesicular disease virus and from which there is no evidence of spread of infection to in-contact pigs. A seropositive pig is confirmed to be a singleton reactor if the conditions referred to in Chapter VIII(C) are fulfilled;
 - (c) 'in-contact pigs' means the pigs which have direct contact, or have had direct contact within the last 28 days, with one or more seropositive pigs or with one or more pigs suspected to be infected with swine vesicular disease virus. In-contact pigs may be, or may have been, in the same pen or in adjacent pens if there is the possibility of pig-to-pig contact between pens.

CHAPTER II

Guidelines for checks on pigs showing clinical signs of swine vesicular disease

1. Member States shall ensure that when the presence of swine vesicular disease virus is suspected in a holding, a statistically significant number of pigs are checked by the official veterinarian as quickly as possible to detect the clinical signs of disease referred to in Chapter IX.
2. Member States shall ensure that when pigs show clinical signs suggesting swine vesicular disease or foot-and-mouth disease, differential diagnosis by means of appropriate sampling and laboratory investigations is carried out as quickly as possible, in accordance with the provisions referred to in Chapters IV, VII and VIII of this Manual.

CHAPTER III

General procedures for sampling and transport of samples

1. Any person entering or leaving pig holdings where there is a suspicion of swine vesicular disease must observe the strictest hygienic measures necessary to reduce the risk of contamination or spread of virus.
2. All pigs sampled must be uniquely marked in such a way that they can be identified for eventual re-sampling. It is recommended that the location of each pig sampled in the holding is recorded together with its unique identification mark, in particular if suspect pigs are sampled.
3. The samples must be sent to the laboratory accompanied by appropriate forms, which will include details of the history of the pigs sampled and the clinical signs observed, if any.
4. Since any vesicular condition in pigs may be foot and mouth disease, special precautions must be taken for the safe packaging of suspected samples. These precautions must be mainly designed to prevent breakage or leakage of containers and the risk of contamination but are also important to ensure that specimens arrive in a satisfactory state. If wet ice is put inside a package escape of water must be prevented. No containers with samples suspected to contain swine vesicular disease virus must be opened once they leave the infected premises and until they arrive to the laboratory.

5. Samples suspected to contain swine vesicular disease virus must only be analysed in a laboratory authorised to handle foot-and-mouth disease virus for diagnostic purposes, in accordance with Community legislation on the control of foot-and-mouth disease, unless foot-and-mouth disease has already been ruled out.
6. All samples can be transported at 4 °C if the anticipated transport time to the recipient laboratory is less than 48 hours, or otherwise they must be maintained at a temperature of not more than -20 °C.
7. For samples destined for the Community reference laboratory from Member States other than the United Kingdom the only permitted method of transport is by airfreight to London (Heathrow) or London (Gatwick) airports. Before shipment the laboratory must be informed by fax ((44-1483) 23 26 21) or e-mail of the details of airline flight, date, expected time of arrival and the airwaybill number so that the parcel can be located on arrival. The parcel must be addressed to:

Institute for Animal Health, Pirbright Laboratory
 Community Reference Laboratory for swine vesicular disease
 Ash Road, Pirbright, Woking
 Surrey GU24 0NF
 United Kingdom, UK

The following information must also be included on the label: 'Animal pathological material of no commercial value. Perishable. Fragile. To be collected at airport by addressee. Not to be opened outside the laboratory.'

Authorised collection of consignments at the airport is made by personnel from the Community reference laboratory, under a special general import permit issued for this purpose by the United Kingdom Ministry of Agriculture, Food and Fisheries. This is a standing arrangement, a separate licence is not required for each importation. The hand carriage of suspected vesicular material into the United Kingdom by unauthorised personnel is not permitted. Courier companies must not be used.

8. Transportation of samples to the national laboratories must be in accordance with the instructions laid down by the competent authority of the Member States.

CHAPTER IV

Sampling procedures in a holding with clinical suspect pigs

1. When the presence of swine vesicular disease virus in a holding is suspected as clinical signs of disease have been observed, appropriate samples from representative groups of pigs showing these signs must be collected for disease confirmation and differential diagnosis with foot-and-mouth disease.
2. In these holdings the preferred samples for diagnosis are epithelium and vesicular fluid from unruptured or freshly ruptured vesicles collected from pigs showing the typical signs of disease, in which swine vesicular disease virus, its antigens or genome can be detected. It is recommended that about five or six of these pigs are sampled.
3. Even if fresh epithelial tissue and vesicular fluid in sufficient quantity (1g or more) are available, the following samples must also be collected:
 - (a) blood samples from the suspected pigs and in-contact pigs for serological testing; and
 - (b) faecal samples from suspected pigs and from the floor of their pen and of adjacent pens for virological testing.
4. The samples must be collected and transported in accordance with the following procedures:
 - (a) for epithelium samples and vesicular fluid:
 - if possible, at least 1g of epithelium tissue from an unruptured or recently ruptured vesicle must be collected. It is recommended that pigs are sedated before samples are collected both to avoid injury to personnel as well as for pig welfare,
 - if transport to the national laboratory is carried out immediately (less than three hours), epithelial samples can be transported dry and kept refrigerated. However, if the time taken is likely to exceed three hours, the samples must be placed in a small volume of transport medium consisting of equal amounts of glycerol and 0,04 M phosphate buffer or other equivalent buffer (hepes), so that the pH is maintained in the optimal range for foot and mouth disease virus survival (pH from 7,2 to 7,6). The transport medium must contain antibiotics for additional anti-microbial activity. Suitable antibiotics and their concentration per ml final are:
 - (i) penicillin 1 000 IU,
 - (ii) neomycin sulphate 100 IU,
 - (iii) polymyxin B sulphate 50 IU,
 - (iv) mycostatin 100 IU,
 - if vesicular fluid can be collected from an unruptured vesicle, this must be kept undiluted in a separate container;

- (b) for blood samples:
- blood samples can be collected for serological or virological tests. However, generally they are collected only from pigs suspected to have recovered from clinical or subclinical infection for antibody detection, as epithelium, vesicular fluid and faecal samples from pigs showing clinical signs of disease are more suitable for virus detection than blood samples. It is recommended that whole blood samples are taken using vacutainers with no anticoagulant and that the vacutainers are transported unopened;
- (c) for faecal samples:
- faecal samples from the floor of premises suspected to contain, or to have contained, pigs infected with swine vesicular disease or faecal swabs and faecal samples from suspected live pigs must be placed in strong, leak-proof containers.

Containers of suspected samples must be disinfected on the outside before being transported to the laboratory. Suitable disinfectants are:

- sodium hydroxide (1:100 dilution),
- formalin (1:9 dilution of a solution of formalin containing a minimum of 34 % formaldehyde), and
- sodium hypochlorite (2 % available chlorine).

These disinfectants must be handled with care.

CHAPTER V

Sampling procedures for swine vesicular disease serosurveillance

1. When sero-surveillance is carried out with the following purposes:
 - (a) for surveillance in holdings where there is no evidence or suspicion that the disease might be present;
 - (b) for surveillance at the slaughterhouse, market, collecting centre or similar place by routine serological sampling;
 - (c) as non-discriminatory surveillance on pigs received from other Member States at the importing holding,blood samples must be collected for serological testing from pigs in accordance either with the provisions laid down in the monitoring or eradication programmes or plans approved in the framework of Decision 90/424/EEC ⁽¹⁾ or Directive 90/425/EEC ⁽²⁾, or, in the absence of these provisions, with the procedures established by the competent authority of the Member States.
2. When sero-surveillance is carried out with the following purposes:
 - (a) for surveillance of holdings located within the protection and surveillance zones, which have been established following confirmation of disease outbreaks in accordance with Annex II(7) and (8) to Directive 92/119/EEC;
 - (b) for surveillance of the holdings referred to in Article 9 of Directive 92/119/EEC,blood samples must be collected for serological testing from the pigs in accordance with the following scheme:
 - in case of breeding holdings, a randomised sampling procedure must be carried out in such a way to detect 5 % prevalence of seroconversion with 95 % confidence;
 - in case of holdings containing fattening pigs only, the sampling procedure must ensure that the total number of samples collected is at least equal to the number required to detect a prevalence of 5 % with 95 % confidence. In any case, the samples must be taken from as many random selected pens as possible;
 - in case of mixed breeding and fattening holdings, each group of pigs living in separate premises must be sampled in such a way to detect a 5 % prevalence of seroconversion with 95 % confidence.

CHAPTER VI

Further actions and re-sampling procedures in case of finding of seropositive pigs

1. In case a single seropositive pig is detected on a holding following the surveillance referred to in Chapter V(1)(a) or V(2), the competent authority shall ensure that:
 - (a) if not already applied, the measures referred to in Article 4 of Directive 92/119/EEC are applied in this holding;
 - (b) a check is carried out in the holding in accordance with the provisions referred to in Chapter II(1);

⁽¹⁾ OJ L 224, 18.8.1990, p. 19.

⁽²⁾ OJ L 224, 18.8.1990, p. 29.

- (c) blood samples are collected for serological testing from:
- the suspect pig,
 - in-contact pigs living in the same and in adjacent pens of the suspect pig; these pigs must be sampled in such a way to detect 50 % prevalence of seroconversion with 95 % confidence in the pen.
2. However, the competent authority may decide to lift the measures referred to in (1)(a) if:
- (a) the epidemiological enquiry carried out in accordance with Article 8 of Directive 92/119/EEC suggests that swine vesicular disease has not been introduced in the holding;
 - (b) no clinical signs of swine vesicular disease have been detected in the holding; and
 - (c) the holding is not located in a surveillance or restriction zone established following a confirmed outbreak of disease or subject to other restrictions applied in relation to a confirmed outbreak of disease,
- and provided that:
- no pigs are moved from the holding for intra-Community trade; and
 - pigs from the holding in question are only moved to a slaughterhouse for immediate slaughter or to another holding from which no pigs are moved for intra-Community trade,
- until the results of the further checks and serological tests indicate that swine vesicular disease can be definitely ruled out.
3. If the checks and the serological tests carried out in accordance with 1(b) and (c):
- (a) give negative results or only the previously positive pig is confirmed positive (singleton reactor), swine vesicular disease can be ruled out. The measures referred to in 1(a) shall be lifted, unless the holding is located in a protection or surveillance zone established around an outbreak of disease where disease eradication measures must stay in force in accordance with Annex II(7) or (8) to Directive 92/119/EEC;
 - (b) indicate that more than one seropositive pig is present on the holding either swine vesicular disease must be confirmed or, if the conditions laid down in Annex II(4) of Directive 92/119/EEC are not fulfilled to confirm the presence of this disease, further samples must be taken from the holding in accordance with the sampling procedures referred to in (4).
4. In case more than one seropositive pig is detected on a holding following the sampling and the serological testing referred to in Chapter V(1)(a), (1)(c) or (2), but the conditions laid down in Annex II(4) to Directive 92/119/EEC are not fulfilled to confirm swine vesicular disease, the competent authority shall ensure that:
- (a) the provisions referred to in Article 4 of Directive 92/119/EEC are applied or shall continue to apply;
 - (b) a check on the holding is carried out in accordance with the provisions referred to in Chapter II(1);
 - (c) blood samples for serological testing are further collected from the seropositive pigs and in-contact pigs in accordance with 1(c);
 - (d) blood samples for serological testing are collected from pigs in the other buildings of the holding in accordance with the procedure referred to in Chapter V(2);
 - (e) a sufficient number of faecal samples are collected for virological tests from:
 - the seropositive pigs,
 - the floor of the pens containing seropositive pigs and adjacent pens,
 - random selected pens from other buildings on the holding.

Faecal samples collected in accordance with the first and the second indents above must be examined as soon as possible. In case these samples are negative but the results of the serological tests suggest that swine vesicular disease virus might have spread to others buildings, the faecal samples collected in accordance with the third indent above must also be examined;

If following these further checks and tests the conditions laid down in Annex II(4) of Directive 92/119/EEC are not fulfilled to confirm the presence of swine vesicular disease, the seropositive pigs shall be killed or slaughtered in accordance with the provisions referred to in Annex II(4)(d) of Directive 92/119/EEC. However, if further pigs have been found seropositive in addition to the ones already found seropositive following the previous sampling, the provisions and procedures laid down in (a), (b), (c), (d) and (e) shall be further applied *mutatis mutandis*.

5. Without prejudice to the measures referred to in Article 9 of Directive 92/119/EEC, in case one or more seropositive pigs are detected following the surveillance activities referred to in Chapter V(1)(b) or V(1)(c), the competent authority shall ensure that:
- where necessary and feasible, appropriate further checks including collection of samples are carried out to confirm or rule out swine vesicular disease in the place where these pigs have been detected, taking into account the local situation;
 - the measures referred to in Article 4 of Directive 92/119/EEC are applied in the holding of origin of these pigs;
 - a check is carried out in the holding of origin of these pigs in accordance with the provisions referred to in Chapter II(1); and
 - blood samples are collected for serological testing from the pigs in the holding of origin of the seropositive pigs, in accordance with the provisions referred to in Chapter V(2).
6. However, the competent authority may decide to lift the measures referred to in 5(b) if:
- the epidemiological enquiry carried out in accordance with Articles 4 and 8 of Council Directive 92/119/EEC suggests that swine vesicular disease has not been introduced in the holding;
 - no clinical signs of swine vesicular disease have been detected in the holding;
 - the holding is not located in a surveillance or restriction zone established following a confirmed outbreak of disease or subject to other restrictions applied in relation to a confirmed outbreak of disease,
- and provided that:
- no pigs are moved from the holding for intra-Community trade, and
 - the pigs are only moved from the holding to a slaughterhouse for immediate slaughter or to another holding from which no pigs are moved for intra-Community trade,
- until the results of the further checks and serological tests carried out in the place where the seropositive pigs had been detected and in the holding of origin indicate that swine vesicular disease can be definitely ruled out.

CHAPTER VII

Principles and applications of virological tests and evaluation of their results

A. Detection of virus antigen

1. An indirect sandwich ELISA has replaced the complement fixation test as the method of choice for the detection of swine vesicular disease viral antigen. The test is the same as that used for foot-and-mouth disease diagnosis. The tests for the two diseases must be performed at the same time, unless foot-and-mouth disease has already been ruled out. It is recommended in particular on samples of epithelium or fluid from vesicular lesions, in which both swine vesicular disease and foot-and-mouth disease viruses can be present at high titers in acutely infected pigs and detected in a few hours ⁽³⁾.

Duplicate rows in multiwell ELISA plates are coated with rabbit antiserum to swine vesicular disease virus and to each of the seven serotypes of foot and mouth disease virus. These are the trapping sera. Test sample suspensions are added to each of the rows. Appropriate controls are also included. Homologous guinea-pig detection serum is added in the respective rows at the next stage followed by rabbit anti-guinea pig serum conjugated to an enzyme such as horse-radish peroxidase. Extensive washing is carried out between each stage to remove unbound reagents. A positive reaction is indicated if there is a colour reaction on the addition of chromogen and substrate. With strong positive reactions this will be evident to the naked eye, but results can also be read spectrophotometrically, in which case an absorbance reading 0,1 above background indicates a positive reaction.

2. Alternative monoclonal antibody-based ELISA systems, using selected monoclonal antibodies as trapping antibody and peroxidase-conjugated monoclonal antibodies as detecting antibody, may be used for swine vesicular disease antigen detection and for differential diagnosis with foot-and-mouth disease in epithelium samples, vesicular fluid or infected tissue culture.
3. A monoclonal antibody-based ELISA can be used to study antigenic variation among strains of swine vesicular disease virus. Tissue-culture grown viral antigens are trapped by a rabbit hyperimmune anti-serum to swine vesicular disease adsorbed to the solid phase. Appropriate panels of monoclonal antibodies are then reacted and the binding of monoclonal antibodies to field strains is compared to the binding of monoclonal antibodies to the parental strains. Similar binding indicates the presence of epitopes shared between the parental and the field strains.

⁽³⁾ Positive ELISA results are associated with the presence of at least 10^5 TCID₅₀ (tissue culture infectious doses) of virus in the sample.

B. Isolation and growth of virus

1. As a routine, clarified suspensions of samples of epithelium, vesicular fluid or faeces suspected to contain swine vesicular disease virus must be inoculated onto sensitive cell cultures. If the quantity and quality of samples from vesicular lesions submitted for examination is insufficient for immediate examination by ELISA, the growth of virus in tissue culture will be necessary to amplify viral antigen.
2. To isolate and grow virus, clarified epithelial suspension is inoculated onto monolayer cultures of IB-RS-2 cells. Two dilutions of epithelial suspension, one high (1/500) and one low (1/10) should be used to avoid interference with virus growth by interferon, the release of which will interfere with the growth of swine vesicular disease virus. For virus isolation only antibiotics are added to maintenance medium. For differential diagnosis from foot-and-mouth disease virus, primary bovine thyroid cells, or baby hamster kidney cells (BHK-21) must also be inoculated.
3. If a cytopathic effect develops, the supernatant fluid must be harvested from positive cultures when the effect is complete and used in the ELISA for virus identification. Negative cultures must be inoculated on fresh tissue cultures at 48 or 72 hours and this blind passage examined up to 72 hours later. In the absence of cytopathic effect after a further blind passage the sample can be declared negative for the presence of live virus.
4. Suspensions of faecal samples can be processed as described in 1. As there is generally less virus in faeces than in epithelium, it is essential that in the absence of a cytopathic effect in the first two passages, a third blind passage is included.
5. The simultaneous inoculation of a porcine cell line and one of the above-mentioned tissue culture systems (preferably primary bovine thyroid cells) is a useful guide as to whether vesicular samples contain swine vesicular disease virus or foot and mouth disease virus, as the former will only grow in cells of porcine origin. However, foot-and-mouth disease virus isolates with a prolonged history of transmission between pigs may also preferentially grow in porcine cell culture systems.

C. The polymerase chain reaction (PCR) for genome detection

1. Nucleic acid recognition methods can be used to detect swine vesicular disease viral genome in clinical material using the PCR and to establish relationships between isolates of swine vesicular disease virus by determining the nucleotide sequence of part of the genome. Techniques using the PCR have been developed to improve the sensitivity of diagnosis. Slightly different reverse transcriptase-PCR procedures have been described using primers corresponding to highly conserved regions in the 1C and 1D genes.
2. The PCR technique is rapid (the results are usually available within 24 hours), detects all genotypes of swine vesicular disease virus, and is sufficiently sensitive for use on samples collected from cases of suspect clinical disease.
3. Where sub-clinical infection is suspected, or when samples are collected after the resolution of clinical disease or when processing faecal samples, enhanced RT-PCR techniques, like nested RT-PCR, immune-PCR, ELISA-PCR and more elaborate RNA extraction methods produce a detection system at least as sensitive, but considerably more rapid, than multiple passage on tissue culture.
4. By sequencing approximately 200 nucleotides within the 1D gene which codes for the major structural protein VP1, it is possible to group strains of swine vesicular disease virus according to their sequence homology, and epidemiologically relate strains causing disease in different regions or at different times.

D. Evaluation of the results of virological tests

The detection of antigens or genome of swine vesicular disease virus by means of ELISA and PCR has the same diagnostic value as virus isolation.

However, virus isolation must be considered as the reference test and must be used as confirmatory test when necessary, in particular if a positive ELISA or PCR result is not associated with:

- (a) the detection of clinical signs of disease,
- (b) the detection of seropositive pigs, or
- (c) a direct epidemiological connection with a confirmed outbreak.

CHAPTER VIII

*Principles and applications of serological tests and evaluation of their results***A. Virus neutralization (VN) test**

1. The quantitative VN micro-test for swine vesicular disease virus antibody detection is performed with IB-RS-2 cells or an equivalent cell system in flat-bottomed tissue culture grade microtitre plates.
2. Virus is grown in IB-RS-2 cell monolayers and stored either at -20°C after the addition of 50 % glycerol or at -70°C without glycerol. The sera are inactivated at 56°C for 30 minutes before testing.

B. ELISAs

1. The ELISA for detection of antibodies is a monoclonal antibody-based competitive ELISA. If the serum sample contains antibodies to swine vesicular disease virus, the binding of a selected peroxidase-conjugated monoclonal antibody to virus antigen will be inhibited.

In this ELISA the swine vesicular disease viral antigen is trapped to the solid phase using monoclonal antibodies; then the sera samples are incubated at appropriate dilution, followed by the addition of the peroxidase-conjugated monoclonal antibody. Then, the inhibition of the monoclonal antibody binding is measured by means of a substrate and chromogen.

2. An indirect trapping ELISA using isotype-specific monoclonal antibodies to detect swine IgG or IgM specific for swine vesicular disease virus is helpful in assessing the time of infection in the pig or on the infected premise.

In the isotype specific ELISA the viral antigen is trapped to the solid phase using an antigen-catching antibody. If the serum sample contains antibodies to swine vesicular disease virus, they are detected using an anti-pig IgG or an anti-pig IgM monoclonal antibody conjugated with peroxidase. Then, this binding is measured by means of a substrate and chromogen.

The isotype-specific ELISA may also help in distinguishing singleton reactors from true positive pigs, as referred to in (C).

C. Application of serological tests and evaluation of results

1. The VN test and the ELISA are the recommended serological tests. Chapter X lists the reference sera, which are available from the Community reference laboratory in order that standardised serological tests are carried out in the Community.

The VN test must be considered as the reference test, but has the disadvantage that it takes 2 to 3 days to complete and requires tissue culture facilities.

The ELISA is more rapid and can be more easily standardised. The monoclonal antibody competition ELISA is the most reliable swine vesicular disease antibody ELISA described to date. It is recommended as a screening test on a large number of samples.

However, the VN test must be used as confirmatory test when necessary, in particular after first detection of positive samples in a holding. Pigs positive by ELISA, but negative by VN test can be disregarded.

2. The presence of a singleton reactor (*) may be suspected where a single seropositive pig is detected and where the following criteria are met:
 - (a) there are no clinical signs of disease on the holding;
 - (b) there is no relevant history of clinical disease on the holding;
 - (c) there is no history of contact with a known outbreak of disease.
3. A pig is confirmed singleton reactor when:
 - (a) follow-up testing does not identify other seropositive pigs;
 - (b) sampling performed on in-contact pigs after first detection of the singleton reactor does not reveal sero-conversion;
 - (c) antibody titre on repeated sampling remains constant or declines.

(*) A small proportion of singleton reactors can be detected by any of the current serological tests for swine vesicular disease. The factors responsible for singleton reactors are unknown. Serological cross-reactivity with swine vesicular disease virus might arise due to infection with another, as yet unidentified, picornavirus or may be due to other non-specific factors present in the serum.

4. However, the following additional criteria and principles must also be considered for the confirmation of a singleton reactor:
- (a) singleton reactors occur at a prevalence of approximately 1 per 1 000 pigs;
 - (b) sera from singleton reactors generally have the following profile:
 - low VN test antibody titre,
 - borderline positive in the monoclonal antibody-based competition ELISA,
 - exclusively IgM and no IgG in the swine vesicular disease isotype-specific ELISA ⁽⁵⁾.

CHAPTER IX

Clinical signs and features of swine vesicular disease

Swine vesicular disease is a contagious disease of pigs caused by an enterovirus of the picornaviridae family, which can be a sub-clinical, mild or severe vesicular condition depending on the strain of virus involved, the route and dose of infection, and the husbandry conditions under which the pigs are kept. Additional stress factors such as transport, mixing with other pigs and temperature extremes could also predispose to the development of clinical signs.

It is characterised by a mild fever and vesicles on the coronary band, the bulbs of the heel, skin of the limbs and less frequently the snout, lips, tongue and teats. The morbidity rate may be as high as 100 % but mortality is very low or nil.

Infection can develop in an inapparent or mild form showing only a transitory decline in the general appearance of pigs but leading to the development of virus neutralising antibodies in a few days ⁽⁶⁾.

Because of the subclinical or mild nature of the disease, it is often first suspected following serological tests for disease surveillance or export certification. Recent European outbreaks of swine vesicular disease have been characterised by less severe or no clinical signs, diagnosis frequently being dependent on serology.

However, clinical signs of swine vesicular disease are indistinguishable from those of foot-and-mouth disease. Any vesicular condition must be treated initially as suspected foot-and-mouth disease and differential diagnosis must be obtained as quickly as possible.

The incubation period of swine vesicular disease in individual pigs is usually between two and seven days, after which a transient fever of up to 41 °C may occur, but clinical signs may become evident in the holding after a longer period. Vesicles then develop on the coronary band, typically at the junction with the heel. These may affect the whole coronary band resulting in loss of the hoof. More rarely, vesicles may also appear on the snout, particularly on the dorsal surface, on the lips, tongue and teats, and shallow erosions may be seen on the knees. Affected pigs may be lame and off their feed for a few days.

Younger pigs are more severely affected, although mortality due to swine vesicular disease is very rare, in contrast with foot-and-mouth disease in young stock.

Nervous signs have been reported, but are unusual. Abortion is not a typical feature of swine vesicular disease. Cardiac failure due to multifocal myocarditis can be a feature of foot-and-mouth disease and encephalomyocarditis, especially in young piglets, but does not occur in swine vesicular disease.

Recovery is usually complete in two to three weeks, with the only evidence of infection being a dark, horizontal line on the hoof where growth has been temporarily interrupted.

Affected pigs may excrete virus from the nose and mouth and in the faeces up to 48 hours before the onset of clinical signs. Most virus is produced in the first seven days after infection, and virus excretion from the nose and mouth normally stops within two weeks. Virus can be isolated from the faeces for up to 20 days after infection, although it has been reported present for up to three months. It can persist for a considerable period of time in the necrotic tissue associated with ruptured vesicles and in the faeces.

⁽⁵⁾ Specific IgG alone or both IgG and IgM are usually detected in serum samples from swine vesicular disease virus infected pigs, whereas sera from singleton reactors generally contain IgM only. Specific IgG will not be detected in serum samples from pigs infected with swine vesicular disease virus during the previous 10 to 14 days, although specific IgG should be detected in a second blood sample. However, recently infected pigs cannot be reliably distinguished from singleton reactors before their immune response switches from IgM to IgG production. See also Chapter IX and footnote 7.

⁽⁶⁾ Specific IgM can be usually detected in the blood from two to three days post infection and disappear after about 30 to 50 days; specific IgG can be usually detected in the blood from 10 to 14 days post infection and last for some years. The Ig isotype can be determined by means of the ELISA described in Chapter VIII(B)(2).

CHAPTER X

Swine vesicular disease reference sera

Reference serum	Origin	Comment ⁽⁷⁾
1	Normal pig serum (NPS)	Negative control serum.
2	Serum collected 21 days post infection (dpi) from a pig infected with swine vesicular disease virus strain UKG 27/72 (neat)	Strong positive control serum.
3	A 1:10 dilution in NPS of a serum collected five dpi from a pig infected with swine vesicular disease strain Italy 8/94	A low-positive serum from a pig soon after infection with a recent European isolate of swine vesicular disease virus. The serum has been diluted to give a low positive result in ELISA and VNT.
4	A 1:40 dilution of a serum collected 21 dpi from a pig infected with swine vesicular disease virus strain UKG 27/72	A low-positive serum defining the lowest level of antibodies that EU National Reference Laboratories should consistently score positive by ELISA and virus neutralisation. Equivalent to serum RS 01-04-94 ⁽⁸⁾
5	Serum collected four dpi from a pig infected with swine vesicular disease virus strain UKG 27/72 (neat)	A low-positive serum from a pig soon after infection.
6	Serum collected five dpi from a pig infected with swine vesicular disease virus strain UKG 27/72 (neat)	A low-positive serum from a pig soon after infection.

⁽⁷⁾ These comments relate to the testing of individual pigs. For sero-surveillance the sensitivity of the test used should be taken into account.

⁽⁸⁾ i.e. a serum with a titre sufficiently greater than the cut-off that it should always score positive by ELISA and VN test in repeated testing.