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WORKING DOCUMENT

SWINE VESICULAR DISEASE
(Discussion paper)

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Swine Vesicular Disease (SVD) is a contagious viral disease of pigs. It is characterised by a mild fever and vesicular lesions which appear on the coronary band of the foot, the heels and, less frequently, on the snout, tongue, lips and teats. SVD is not a fatal disease. Typically it is a transient disease which causes a minor loss of condition and weight gain. SVD is considered important only because the lesions it produces are clinically indistinguishable from those of foot and mouth disease. The pig is considered to be the only natural host for the virus. The SVD virus has an extraordinary stability outside the pig; it is very resistant to environmental factors. The spread of disease within herds is primarily by contact of susceptible pigs with infected pigs or with excretions from infected pigs. Faecal contamination is the major source of virus spread. In recent years the spread of SVD over long distances has been linked to the movement of pigs with subclinical infections or the use of inadequately cleaned and disinfected trucks. During the SVD epidemic in the UK in the 1970s swillfeeding was reported to be linked to about 15% of all recorded outbreaks.

SVD situation in the Community

Since 1966, when SVD was recorded for the first time in Europe, it has been present for shorter or longer periods of time in Austria, Belgium, France, Germany, Greece, Italy, the Netherlands, Portugal, Spain and the UK. From 1984-1990 the disease was only reported on 3 occasions.

Since 1991 outbreaks have been recorded as shown below:

	1991	1992	1993	1994	1995
Italy	6	31	12	28	18
Netherlands	0	6	0	3	0
Belgium	0	1	0	0	0
Spain	0	0	3	0	0
Portugal	0	0	0	0	1
Total	6	38	15	31	19

Disease control measures - legal aspects

SVD is a notifiable disease in accordance with the provisions of Council Directive 82/894/EEC.

The measures to control and eradicate SVD are given in Council Directive 92/119/EEC. The main provisions of the Directive are:

- On confirmation of SVD all pigs on the infected holding must be killed, the carcasses destroyed and the site cleaned and disinfected.

- Restriction zones of 3 km radius (protection) and 10 km (surveillance) must be established around each outbreak.
- The presence of disease shall be confirmed:
 - "a) on holdings on which swine vesicular disease virus is isolated either from the pigs or from the environment;
 - b) on holdings containing pigs which are seropositive for swine vesicular disease provided those pigs or others on the holding show lesions characteristic of swine vesicular disease;
 - c) on holdings containing pigs which show clinical signs of disease or are seropositive, provided there is a direct epidemiological connection with a confirmed outbreak;
 - d) on other herds in which seropositive pigs are detected. In the latter case the competent authority shall, before confirming the presence of the disease, undertake further investigations, in particular resampling and retesting with an interval of 28 days at least between collections of samples. The provisions of Article 4 shall continue to apply until such further investigations are completed. If subsequent investigations show no evidence of the disease, although the pigs are still seropositive, the competent authority shall ensure that the pigs tested are killed and destroyed under its supervision or slaughtered under its supervision in a slaughterhouse it has designated in its national territory.

The competent authority shall ensure that on arrival at the slaughterhouse the pigs are kept and slaughtered separately from other pigs and that their meat is exclusively used on the national market."

International trade

SVD is classified by the OIE as a List A disease. By definition, List A diseases:

- have a potential for very serious and rapid spread irrespective of national borders,
- are of serious socio-economic or public health consequence,
- are of major importance in the international trade of animals and animal products.

In accordance with the provisions of the OIE International Animal Health Code, a country may be considered free from SVD when it has been shown that SVD has not been present for at least the past two years. This period may be 9 months for countries in which a stamping-out policy is practised.

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(Discussion paper)

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SVD in intra-Community trade

In recent years SVD has caused problems in trade in production pigs and pigs for slaughter. The first indications of the spread of infection by trade was in August/September 1992 when seropositive pigs were detected in consignments from the Netherlands to Italy. All the herds in the Netherlands that contributed to the consignments were checked without discovering further infection. Some, but not all, of the outbreaks recorded in 1992 in Italy were confirmed on premises which had previously imported pigs from the Netherlands. The outbreak recorded in Belgium in 1992 and outbreaks reported by Spain in early 1993 were traced to pigs of dutch origin. The disease situation called for in-depth epidemiological investigations in all the affected Member States. The investigations in Italy revealed that slaughter pigs of dutch origin had been retained at an Italian abattoir or farm for 3 days or more which may be longer than the minimum incubation time for SVD. In view of these findings the source of infection was never conclusively determined.

With the aim to avoid the spread of disease and to review the SVD situation in the Community, in the spring of 1993 the Commission adopted a number of Decisions. Decision 93/128/EC introduced a total ban on exports of live pigs from the Netherlands and from Italy whilst Decision 93/177/EC requested all Member States to carry out a serological screening during a 3 month period for SVD antibodies on 50% of the slaughter boars and 5% of the slaughter sows. About 150,000 samples (see Annex II) were examined but no disease detected. The screening programme confirmed that "false positive reactors" or "Singleton reactors" occur at a level of up to 0.1% of samples tested. At that time the definition for "Singleton reactors" was: pigs which yield a positive result in serological tests for SVD but which have no history of contact with, and from which there is no evidence of spread of infection to, in-contact animals.

In January 1994 Italy reported an outbreak at a farm which had received a consignment of pigs from the Netherlands. The source of the infection was never determined.

In February 1994 SVD virus was isolated in the Netherlands from faeces samples taken from a collecting centre at Haarle. The centre was used for the dispatch of pigs entering intra-Community trade. The source of infection was never determined.

In October 1995 Portugal confirmed the presence of SVD virus in a pig herd. The positive animal had been transported in a consignment of boars arriving from the Netherlands in July 1995. Epidemiological investigations were carried out in the Netherlands and Portugal but the source of infection never determined.

In January 1996 Belgium detected antibodies against SVD virus in slaughter pigs (sows) which were accompanied by a health certificate originating in Spain. The laboratory examinations carried out suggested that the sows had recently been exposed to SVD virus. The health certificate was falsified and the source of infection has not been found.

Disease surveillance

Information on surveillance within the Community for antibodies to SVD virus during 1994 and 1995 is given in Annex III and Annex IV respectively.

Two Member States have well-defined ongoing surveillance programmes. These programmes are described in Annex V and Annex VI respectively.

General observations

1. SVD is a very difficult disease to eliminate from intra-Community trade. Although clinical disease is observed in some outbreaks it appears that pigs infected with recent European strains of SVD virus frequently show no clinical signs and can only be detected by serology. This situation is demonstrated by the results of screenings carried out at Sardinia, Italy (Annex VII).
2. In an experiment carried out in 1995 by the Community Reference Laboratory (see Annex VIII) for SVD it was demonstrated that a recent strain of SVD virus could be recovered from the tonsils of pigs and from their secretions and excretions for much longer than has been accepted for historic strains of the virus. Virus excretion can be "reactivated" in pigs from which the agent can no longer be identified by subjecting the animals to physiological stress. Further experiments are now in progress to determine whether the results of this initial study can be repeated using different SVD strains and to identify the site(s) in which the virus persists.
3. The biological reason for the presence of "Singleton reactors" must be clarified.

Experimental investigations carried out in Belgium on two groups of seropositive, virus-negative animals from declared outbreaks of SVD produced no evidence of viral spread or seroconversion in susceptible animals kept in contact for up to 10 months, even when corticosteroids were administered to the seropositive animals. Titres remained high for at least 10 months and piglets born to immune dams were seropositive for varying periods. Studies of Singleton reactors revealed high initial titres (2-3 logs by SNT) which gradually declined over a period of 3-4 months. Interestingly, offspring born to Singleton reactor dams were seronegative.

Problem areas

1. The actions taken by Member States when seropositive pigs occur in herds without clinical signs of disease vary from Member State to Member State.
2. There are currently no tests able to differentiate genuine positive sera from false-positive, "Singleton reactor" sera.

An attempt to overcome this problem is described in Annex IX.

ANNEX I**Number of outbreaks of Swine Vesicular Disease
in Member States 1983-1995**

Country	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995
Austria	0	0	0	0	0	0	0	0	0	0	0	0	0
Belgium	0	0	0	0	0	0	0	0	0	1	0	0	0
Denmark	0	0	0	0	0	0	0	0	0	0	0	0	0
Finland	0	0	0	0	0	0	0	0	0	0	0	0	0
France	2	0	0	0	0	0	0	0	0	0	0	0	0
Germany	0	0	1	0	0	0	0	0	0	0	0	0	0
Greece	0	0	0	0	0	0	0	0	0	0	0	0	0
Spain	0	0	0	0	0	0	0	0	0	0	3	0	0
Ireland	0	0	0	0	0	0	0	0	0	0	0	0	0
Italy	4	1	0	0	0	1	0	0	6	31	12	28	18
Luxembourg	0	0	0	0	0	0	0	0	0	0	0	0	0
Netherlands	0	0	0	0	0	0	0	0	0	6	0	3	0
Portugal	0	0	0	0	0	0	0	0	0	0	0	0	1
Sweden	0	0	0	0	0	0	0	0	0	0	0	0	0
United Kingdom	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	6	1	1	0	0	1	0	0	6	38	15	31	19

ANNEX II**Swine Vesicular Disease (SVD)****Screening* for SVD virus antibodies, 1993**

Member State	Samples examined	No. positive	
		Screening	Definitive
Belgium	10,924	23	23 (0.2%)
Denmark	19,099	0	0
Germany	19,986	-	30 (0.15%)
Greece	891	12	0
Spain	32,880	-	106 (0.3%)
France	11,702	425	59 (0.5%)
Ireland	1,596	255	2 (0.1%)
Italy	21,811	-	453 (2.0%)
Luxembourg	57	0	-
Netherlands	20,958	466	30 (0.14%)
Portugal	2,403	0	-
United Kingdom	7,537	2,580	0
	149,844		

* Screening within the framework of Commission Decision 93/178/EEC.

ANNEX III**Swine Vesicular Disease (SVD)****Screening for SVD virus antibodies, 1994**

Member State	Samples examined	No. positive ⁽¹⁾ sera	Outbreaks
Austria	3 946	0	0
Belgium	3 026	8	0
Denmark	3 580	0	0
Finland	198	0	0
Germany	2 000 *	0	0
Greece	100	0	0
Spain	78 951	0	0
France	3 000 *	0	0
Ireland	0	0	0
Italy	28 253 **	1 354	28
Luxembourg	0	0	0
Netherlands	832 629	440 ⁽²⁾	3
Portugal	2 000	0	0
United Kingdom	2 025	0	0
Sweden	5 000 *	0	0
		1 802	31

* Approximate

** Only sera tested at Brescia

⁽¹⁾ Positive: Titer > EU Reference Serum (01-04-93).⁽²⁾ Seropositive related to outbreaks.

ANNEX IV

OUTBREAKS OF SVD AND SURVEILLANCE FOR THE DISEASE DECLARED DURING 1995
DATA AS AT 14.2.96

Country	Number of outbreaks	Total number of sera for SVD Serology		Sera for routine surveillance or Pre-export		Sera from imported animals		Sera from investigations of suspect disease or seropositive animals	
		Examined	Positive	Examined	Positive	Examined	Positive	Examined	Positive
Austria	0	105	0	-	-	-	-	-	-
Belgium	0	2 934	39	1 518	38	785	37	1 416	1
Denmark	0	7 946	1	7 864	1	-	-	82	0
Finland	0	2 275	0	2 257	0	18	0	0	0
France	0	1 507	0	1 507	0	-	-	-	-
Germany	0	742	0	730	0	-	-	12	0
Greece	0								
Ireland	0	1 076	4						
Italy	18	306 272	4 048	295 454	3 894	6 786	128	4 032	26
Luxembourg	0								
Netherlands	0	797 633	713	760 518	570			37 115	143
Portugal	1								
Spain	0	185 330	0	-	-	-	-	-	-
Sweden	0	1 486	0	-	-	-	-	-	-
U.K.	0	1 173	1	927	1	11	0	235	0

ANNEX V

THE NETHERLANDS

SUMMARY NATIONAL PIG HEALTH CONTROL PROGRAMME⁽ⁱ⁾

The Dutch pig population is monitored with regard to SVD through the National Pig Health Control Programme. Within the framework of this programme every pigholding is clinically and serologically monitored every four months for notifiable diseases. All pigholdings are registered in a central data-base and only approved holdings are allowed to have pigs and to move pigs. The programme is executed by the non-governmental Animal Health Service. All initial costs are paid by the farmers.

Every year 800.000 sera are tested for SVD. Of these sera 0.1% shows a titre > 1:200. These figures are still within the rate expected for "singleton reactors".

⁽ⁱ⁾ Source: Third and final report on Swine Vesicular Disease, The Netherlands, January 4, 1996.

ANNEX VI

ITALY

OUTLINE OF THE PLAN⁽¹⁾

1. The plan aims to bring Italian herds to SVD accredited status in order to reach the goal of national freedom from SVD. All herds in the territory of Italy come within the plan except for very small herds (less than 6 sows) which do not trade pigs in the central and northern regions of Italy: Piemonte, Valle d'Aosta, Lombardia, Liguria, Trentino, Veneto, Friuli, Emilia-Romagna, Toscana, Marche, Umbria, Abruzzo, Molise (see attached map). The plan will be in operation for two years.
2. During the first year of the plan all breeding herds will be tested twice and, if free of SVD, will be given accredited status that allows them to sell pigs for breeding or for production.
3. During the plan fattening herds will be considered accredited when they receive pigs only from accredited breeding herds. Small fattening herds (less than 10 pigs) will be encouraged to adopt all in/all out procedures with appropriate disinfection. If they do not apply these procedures and they do not confine their purchases to accredited breeding herds all pigs entering these herds will be tested on entry.
4. Non-accredited herds can only move pigs to slaughter.
5. Accreditation of a herd will depend on the absence of antibodies to SVD in a sample of pigs calculated to reveal a prevalence of 5% or greater (95% probability).
6. If the initial sample of pigs reveals antibodies against SVD all pigs previously sampled in the herd will be retested and faeces samples will be collected (for virus isolation) from pens containing the seropositive pigs.
7. If the faeces examinations reveal the presence of virus the herd will be declared an outbreak herd (Directive 92/119/EEC) and stamped out. If virus is not recovered all breeding pigs in the herd will be tested and seropositive animals will be slaughtered out as and when convenient and the herd retested to achieve accredited status.
8. Accredited status will also be applied to any place where pigs are collected.
9. When all the herds in Italy are accredited the status will be maintained by a surveillance scheme.

⁽¹⁾ Source: Eradication and control programme for Swine Vesicular Disease in Italy, 1995. (Doc. VI/7260/94-Rev.1).

Annex VII

SWINE VESICULAR DISEASE
OVERALL RESULTS OF THE SEROLOGICAL TESTS
CARRIED OUT IN 1995

ASL	DISTRICTS	HOLDINGS TESTED	HOLDINGS POSITIVE	HOLDINGS CREDITED	HOLDINGS WITH SINGLETON REACTORS	SAMPLES TESTED	SAMPLES POSITIVE
1	SASSARI	767	8	65	2	3,776	75
	ALGHERO	529	5	22		1,741	19
	TEMPIO PAUSANIA	531	4	34		1,325	12
2	OZIERI	457	1	5	1	1,992	2
	OLBIA	731	3	132	1	1,814	38
TOT.	PROV SS	3,015	21	258	4	10,648	146
3	MACOMER	314	10	13	1	1,945	61
	NUORO	1,175	15	217	4	7,216	54
	SINISCOLA	1,134	4	755	2	4,459	13
	SORGONO	523	5	264		2,468	24
	ISILI	605	9	10	2	2,085	25
4	LANUSEI	1,563	2	774		8,979	3
TOT	PROV. NU	5,314	45	2,033	9	27,152	180
5	GHILARZA	353	3	3	1	1,217	6
	ORISTANO	505	4	7	4	3,649	34
	ALES	567	27	28	1	3,002	383
TOT.	PROV. OR	1,425	34	38	6	7,868	423
6	SANLURI	392	20	1	4	2,872	242
	GUSPINI	254	6	13	1	4,081	186
	SENORBI	247	16	3	3	2,660	66
7	CARBONIA	246	6	2		1,416	32
	IGLESIAS	333	14	15	2	2,386	168
8	QUARTU S. ELENA	412	7	47	4	4,411	39
	CAGLIARI 1	362	13	40	3	3,377	154
	CAGLIARI 2	126	6		2	1,596	21
TOT.	PROV. CA	2,372	88	121	19	22,799	908
	TOTAL	12,126	188	2,450	38	68,467	1,657

**SWINE VESICULAR DISEASE
SIZE OF POSITIVE HOLDINGS
(NUMBER OF SOWS)**

Annex VII

ASL	DISTRICTS	HOLDINGS TESTED	HOLDINGS POSITIVE	HOLDINGS WITH SINGLETON REACTORS	SOWS 1->5 POS/TESTED	SOWS 6->50 POS/TESTED	SOWS 51-> POS/TESTED
1	SASSARI	767	8	2	3/550	5/214	0/3
	ALGHERO	529	5		3/441	2/88	
	OZIERI	457	1	1	0/320	0/135	1/2
2	TEMPIO	531	4		3/472	1/59	
	OLBIA	731	3	1	1/658	2/73	
TOT.	PROV SS	3,015	21	4	10/2441	10/569	1/5
3	MACOMER	314	10	1	3/190	7/124	
	NUORO	1,175	15	4	7/922	8/250	0/3
	SINISCOLA	1,134	4	2	4/1065	0/69	
	SORGONO	523	5		3/457	2/66	
	ISILI	605	9	2	5/517	4/88	
4	LANUSEI	1,563	2		1/1361	1/197	0/5
TOT	PROV. NU	5,314	45	9	23/4512	22/794	0/8
5	GHILARZA	353	3	1	2/266	1/87	
	ORISTANO	505	4	4	0/337	4/160	0/8
	ALES	567	27	1	11/436	15/126	1/5
TOT.	PROV. OR	1,425	34	6	13/1039	20/373	1/13
6	SANLURI	392	20	4	10/269	8/113	2/10
	GUSPINI	254	6	1	1/137	5/104	0/13
	SENOBBI	247	16	3	2/112	13/130	1/5
7	CARBONIA	246	6		1/143	5/102	0/1
	IGLESIAS	333	14	2	2/188	12/139	0/6
8	QUARTU S. ELENA	412	7	4	3/202	4/191	0/19
	CAGLIARI 1	362	13	3	3/185	10/163	0/14
	CAGLIARI 2	126	6	2	1/47	5/74	0/5
TOT.	PROV. CA	2,372	88	19	23/1283	62/1016	3/73
TOTAL SARDINIA		12,126	188 1,5%	38 0,3%	69/9275 0,74%	114/2752 4,1%	5/99 5%

THE DURATION OF INFECTION OF PIGS WITH SVD VIRUS

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Two groups of 12 pigs each were challenged with a recent Italian isolate of SVD virus (WRL ref. Italy 9/93). Challenge was performed by intradermal inoculation into the bulb of the heel of 2 animals within each group. The remaining animals were exposed to infection by contact with the inoculated pigs. Pigs were sequentially killed over the next 6 months and a range of tissues collected *post mortem*. The tissues were examined for the presence of SVD virus, and SVD viral genome, by virus isolation in tissue culture and a nested polymerase chain reaction (nPCR) respectively. In addition, samples of blood, nasal swabs and faeces were collected at regular intervals for examination by the same techniques.

All 4 pigs which were inoculated intradermally developed clinical disease. Only 9 out of the 20 in-contact pigs showed clinical signs. The severity of the clinical signs varied considerably between animals. High titres of SVD-specific antibody were detected by ELISA in all pigs showing clinical signs. Of the 11 animals which did not show clinical signs, 2 responded to high titre, 3 gave a weak response and 6 had no detectable antibody to SVD virus for up to 3 months after initial exposure to infection.

SVD virus could be detected in faeces by both virus isolation and nPCR up to 63 days post infection (d.p.i.). Viral RNA could be identified in tonsillar tissue and nasal swabs for the same period. The agent could not generally be found in other organs or tissues beyond 14 d.p.i. with the exception of somatic muscle where viral RNA was detected up to 35 d.p.i..

Neither SVD virus nor viral RNA could be detected in nasal swabs and faeces collected from the surviving pigs beyond 76 d.p.i.. At 119 d.p.i. the remaining pigs from the two initial groups were mixed to form a single group of 6 animals. SVD virus was once again isolated in tissue culture from the faeces of 4 pigs collected 121 d.p.i. (i.e. 2 days after mixing) and the faeces of all 6 animals were positive by nPCR. The reappearance of the agent was associated with an increase in SVD-specific antibody titre in one pig and in seroconversion from negative to positive in a second. The apparent reactivation of SVD virus was short-lived. SVD virus could not be isolated from faeces collected 1 week after mixing (126 d.p.i) although 2 samples were positive by nPCR.

This experiment demonstrates that a recent strain of SVD virus could be recovered from the tonsils of pigs, and from their secretions and excretions, for much longer than has been accepted for historic strains of the virus. Virus excretion can be 'reactivated' in pigs from which the agent can no longer be identified by subjecting the animals to physiological stress. Further experiments are now in progress to determine whether the results of this initial study can be repeated using different strains of SVD virus and to identify the site(s) in which the virus persists. If these further studies indicate that the carrier state is a common sequel to infection with SVD virus, this will significantly affect our understanding of the epidemiology of the disease.

ANNEX IX SEROLOGICAL TESTING FOR SWINE VESICULAR DISEASE

Within the context of point 4(d), Annex II of Council Directive 92/119/EEC Member States shall, before confirming the presence of SVD, carry out certain investigations, in particular, re-sampling and retesting seropositive animals. With the aim of harmonising the investigations undertaken by Member States, the following definitions and "guidelines for sampling, analysis of results and further action" should be taken into account.

DEFINITIONS

A positive serum

A serum which has a titre equal to or greater than the EU SVD Reference Serum RS4¹ in the virus neutralisation test (VNT) used by the National Reference Laboratory.

A singleton reactor

- A. The presence of a singleton reactor may be suspected where a single animal whose serum gives a titre equal to or greater than the EU SVD Reference Serum RS4 by VNT is detected and where the following criteria are met:
1. There are no clinical signs of disease on the holding.
 2. There is no relevant history of clinical disease on the holding.
 3. There is no history of contact with a known outbreak of disease.
- B. Singleton reactor is confirmed when:
1. Follow-up testing does not identify other seropositive animals.
 2. When repeated sampling, performed on in-contact animals, does not reveal seroconversion over a period of 28 days after first detection of the singleton reactor.
 3. The titre on repeated sampling remains constant or declines².

In-contact animals

Animals which have contact, or have had contact within the last 28 days, with the seropositive animal. In-contact animals 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.

¹ RS4 - A 1:40 dilution of a serum collected 21 d.p.i. from a pig infected with SVDV strain UKG 27/72 and included in a panel of sera distributed to all National Swine Vesicular Disease Laboratories. The panel of sera has been used to harmonise the sensitivity of assays in use throughout the EU.

² Singleton reactor sera generally have the following profile:

- low VNT titre,
- borderline positive in the 5B7 competition ELISA,
- exclusively IgM and no IgG in the SVD isotype-specific ELISA.

GUIDELINES FOR SAMPLING, ANALYSIS OF RESULTS AND FURTHER ACTION

I. STRATEGY FOR SAMPLING

General

1. All animals sampled must be uniquely marked in such a way that they can be identified for re-sampling (eg eartag).
2. The location (ie building, pen) of each animal sampled must be recorded together with its unique identification mark.

Four levels of testing are recognised.

LEVEL 1 GENERAL SURVEILLANCE (WHERE UNDERTAKEN)

- a) Routine surveillance programme for SVD where there is no evidence or suspicion that the disease might be present on the premises.
- b) Surveillance at the slaughterhouse, market or collecting centre by routine serological sampling or by non-discriminatory sampling of pigs for slaughter received from other Member States.
- c) Non-discriminatory sampling of animals received from other Member States at the importing holding.

LEVEL 2 INVESTIGATION OF SINGLE SEROPOSITIVE ANIMALS

A limited and focused re-sampling which follows the detection of suspect singleton reactors on a holding sampled at Level 1 or Level 3(a). The following animals are sampled:

- the suspect animal,
- pen-mates within the last 28 days at a level to give 95% confidence of detecting seroconversion in 50% of the animals in the pen (up to 5 animals per pen),
- animals in adjacent pens during the last 28 days at the same level of confidence as pen-mates.

LEVEL 3 TARGETED SURVEILLANCE

- a) For surveillance of premises within the 3 km protection and 10 km surveillance zones of declared outbreaks.
- b) Performed on the importing holding (if any) and on the holding of origin of seropositive pigs detected at Levels 1b and 1c.

The sampling strategy depends on the type of holding.

Breeding holdings

- i) A randomised sampling procedure to give 95% confidence of detecting a 5% prevalence of seroconversion

Fattening holdings

- ii) A 'restricted randomised sampling procedure'. This must involve the collection of at least one sample taken at random from every fourth pen. In any case, the sampling frequency 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.

Breeding and fattening holdings

Herd test must include testing of each group at the levels indicated in paragraphs i) and ii) above.

LEVEL 4 WHOLE HERD TESTING

Carried out when suspicion of infection arises from serological investigations into the holding. Level 4 testing is **always** combined with collection of faeces for virus isolation.

The sampling strategy depends on the type of holding.

Breeding holdings

- i) A randomised sampling procedure to give 99% confidence of detecting a 5% prevalence of seroconversion

Fattening holdings

- ii) A 'restricted randomised sampling procedure'. This must involve the collection of at least one sample taken at random from every fourth pen. In any case, the sampling frequency must ensure that the total number of samples collected is at least equal to the number required to detect a prevalence of 5% with 99% confidence.

Breeding and fattening holdings

Herd test must include testing on each group at the level indicated in paragraphs i) and ii) above.

Faeces samples should be collected:

- a) Pooled samples from pens containing seropositive animals.
- b) From the seropositive animals themselves.
- c) From every fourth pen from each building on the holding.

Samples collected under a) and b) should be examined without delay. Samples collected under c) should be examined if samples under a) and b) are negative but non in-contact seropositive animals are detected.

Level 4 sampling must be combined with Level 2 sampling of pens containing seropositive animals and adjacent pens. Animals previously sampled at Level 2 as 'in-contacts' should also be re-sampled.

II. POSSIBLE OUTCOMES AND FURTHER ACTION TAKEN

LEVEL 1

1. All negative
 - No further action.
2. 1 sample positive
 - 2.1 Level 1a
 - Impose movement restrictions (Article 4 of Directive 92/119/EEC),
 - Immediate re-sampling of holding at Level 2.
 - 2.2 Level 1b and 1c
 - Impose movement restrictions (Article 4 of Directive 92/119/EEC) and disinfection of place (slaughterhouse, market, collecting centre),
 - Immediate re-sampling of holding at Level 3.
3. More than one positive
 - Impose movement restrictions (Article 4 of Directive 92/119/EEC) and disinfection of place (slaughterhouse, market, collecting centre),
 - Immediate re-sampling of holding at Level 4.

LEVEL 2

1. All animals seronegative
 - No further action,
 - Lift restrictions.

2. Same animal positive as at Level 1 and all other animals seronegative
 - Slaughter seropositive pig and lift movement restrictions on rest of holding, OR,
 - Keep the seropositive pig under restrictions on farm for maximum period of 4 months then compulsory retest or slaughter of the seropositive pig. Lift movement restrictions on rest of holding. In case the seropositive pig remains seropositive after 4 months: slaughter the pig and retest at Level 2.

3. Same animal seropositive and other seropositive animals identified
 - Test herd at Level 4,
 - Member State may declare outbreak with stamping out.

LEVEL 3

Premises are already under movement restrictions

Level 3a

1. All negative
 - No further action,
 - Lift restrictions.
2. One seropositive sample
 - Immediate re-sampling at Level 2.
3. More than one positive sample
 - Re-sample holding at Level 4 or declare outbreak with stamping out.

Level 3b

1. All negative
 - No further action, lift movement restrictions.
2. Same single seropositive and all other animals seronegative
 - Slaughter seropositive pig and lift movement restrictions on rest of holding,
OR,
 - Keep the seropositive pig under restrictions on farm for a maximum period of 4 months then compulsory retest or slaughter of the seropositive pig. Lift movement restrictions on rest of holding. In case the seropositive pig remains seropositive after 4 months: slaughter the pig and retest at Level 2.
3. Any other positive sample
 - Re-sample holding at Level 4.

LEVEL 4

VIRUS ISOLATION POSITIVE

- Declare outbreak and stamping out.

VIRUS ISOLATION NEGATIVE AND ONE OF THE FOLLOWING

1. All animals seronegative
 - No further action,
 - Lift any movement restrictions.
2. Same animal(s) seropositive as at previous testing, all other animals seronegative
 - Slaughter seropositive(s) and lift movement restrictions on rest of holding, OR,
 - Keep the seropositive pig on farm for a maximum period of 4 months then compulsory retest or slaughter. Lift movement restrictions on rest of holding. In case the seropositive pig remains seropositive after 4 months: slaughter the pig and retest at Level 2.
3. Where any animal, during a series of repeat samples, undergoes seroconversion
 - declare outbreak and stamping out.
4. Previous positives remain with detection of new seropositives amongst animals not previously examined
 - option to declare outbreak and stamping out. or,
 - compulsory slaughter of all seropositive animals and retesting at Level 4 after 28 days.

If retesting at Level 4 shows:

- no more seropositives; lift restrictions,
- more seropositives; declare outbreak and stamping out.