Swine Vesicular Disease - Report of the Scientific Committee on Animal Health and Animal Welfare adopted 10 August 1998

Request for opinion

The Scientific Committee on Animal Health and Animal welfare was asked to report on the following issues concerning Swine Vesicular Disease.

1. Evaluate recent research developments

2. Evaluate new diagnostic procedures

3. Discuss methods and requirements for surveillance of the disease

4. Discuss the definition of SVD and confirmation of the disease

5. Comment on epidemiological data presented by Member States on reported outbreaks and applied surveillance

Introduction

Swine vesicular disease (SVD) is a contagious disease of pigs, caused by an enterovirus and characterised by vesicles on the coronary band, heels of the feet and occasionally on the lips, tongue, snout and teats. Strains of SVD virus vary in virulence and the disease may be subclinical, mild or severe. In the latter case, at least, the disease has an impact on the well-being of the pigs. SVD has been placed on the A list of diseases of the OIE, because it is clinically indistinguishable from foot and mouth disease (FMD), and any outbreaks of vesicular disease in pigs must be assumed to be FMD until proven otherwise. Because of the subclinical or mild nature of the disease, SVD is often diagnosed solely on the basis of antibody detection. Rather frequently, only one pig in a herd is positive in a serological test; this is called the singleton reactor phenomenon.

1. Recent Research Developments

The following is a summary of research that has recently been completed or which is underway at present.

Most of the research that is in progress within the EU is dealt with in the EU-Fair project (no. PL96-1545), which started one year ago and continues until 2000. The central aim is to improve the diagnosis of SVD and in particular to study in more detail the singleton reactor phenomenon.

Articles recently published or to be published on SVD are:

Brocchi E, Zhang G, Knowles NJ, Wildsen G, McCauley JW, Marquardt O, Ohlinger VF and De Simone F. Molecular epidemiology of recent outbreaks of swine vesicular disease: two genetically and antigenically distinct variants in Europe, 1987-1994., Epidemiol. Infect., 118, 51-61, 1997.

De Clercq K. Reduction of singleton reactors against swine vesicular disease virus by a combination of a virus neutralisation test, monoclonal antibody-based competitive ELISA and isotype specific ELISA. J. Virol. Meth. 70, 7-18, 1998.

Nunez JI, Blanco E, Hernandez T, Gomez-Tejedor C, Martin MJ, Dopazo J and Sobrino F. An RT-PCR assay for the differential detection of viral vesicular diseases in swine. J. Virol. Meth. in press.

Mulder WAM, Van Poelwijk F, Moormann RJM, Reus B, Kok GL, Pol JMA, Dekker A. Detection of early infection of swine vesicular disease virus in porcine cells and skin sections. A comparison of immunohistochemistry and in situ

hybridisation. J. Virol. Meth. 68, 169-175, 1997.

Co-operation is underway between UK and Japanese scientists and they are in the process of mapping a virulence determinant of swine vesicular disease (SVD) virus by infection experiments using chimeric viruses constructed by recombination between virulent (J1) and avirulent (H3/76) infectious copies of Japanese strains of SVD virus.

Although there have been reports to OIE of SVD in Eastern European countries, it is uncertain how much surveillance is carried out. The last reported isolate from this area was from Romania in 1987.

It seems that Eastern European countries are commencing some serological surveys, e.g. Czech republic, Slovakia, Hungary, Poland, Slovenia, Estonia using the officially accepted competitive ELISA from the Brescia institute, which is reported on in the OIE manual. The reference is: Brocchi E, Berlinzani A, Gamba G, and De Simone F. Development of two novel monoclonal antibody-based ELISAs for the detection of antibodies and the identification of swine isotypes against swine vesicular disease virus. J. Virol. Meth., 52, 155-167, 1995.

Reagents for the detection of antibodies to SVD virus have been sent also to Korea and Canada. Madrid (CISA) provided Plum Island (USA) with reagents for diagnostic tests.

The Pirbright lab is sequencing some isolates from Taiwan.

2. Evaluation of new diagnostic procedures

One of the tasks of a Community Reference Laboratory (CRL) will be to discuss, develop and produce standards for virus and antibody detection tests.

The Institute for Animal Health (Pirbright) was the CRL for FMD between 1990 and 1995, and is the designated CRL for SVD. Since 1995 there has been no officially designated CRL for FMD, and since 1996 there has been no funding for the work carried out by the Pirbright lab in its role as the CRL for SVD. The designation of a CRL for vesicular virus diseases is crucial as is adequate financial support to enable it to fulfil its functions.

Standardisation of testing within the EU should be on the basis of demonstrating compliance with defined standards and not on the use of particular tests. The standards that currently exist or are proposed by the CRL for SVD are attached as Annex I.

The EU must adopt the international standards as described in the OIE manual, but may set higher standards than those described in the OIE manual. Six EU reference sera have already been accepted (Annex I).

The setting of standards fits in well with quality control procedures and proficiency testing according to OIE guidelines. It is recognised that standardisation is easier for antibody tests than for virus detection tests. However, standards for virus detection should also be developed by the CRL.

Several different polymerase chain reactions (PCR) have been developed and some have been published recently. References are as follows:

Lin F, Mackay DKJ, Knowles NJ. Detection of swine vesicular disease virus RNA by reverse transcription polymerase chain reaction. J. Virol. Meth. 65, 111-121, 1997.

Nunez JI, Blanco E, Hernandez T, Gomez-Tejedor C, Martin MJ, Dopazo J and Sobrino F. An RT-PCR assay for the differential detection of viral vesicular diseases in swine. J. Virol. Meth. in press.

They use different RNA extraction procedures and different primers. However, there is still reservation on the use of the PCR as the only test for making a diagnosis (see under 4), because it is not yet extensively validated. The PCR must still be considered as an additional test. The PCR will be evaluated within the EU-FAIR research project, e.g. the PCR will be compared with virus isolation.

The Pirbright lab intends to organise a ring test on virus isolation, antigen detection tests and PCRs. More research on PCR is recommended.

For antigen detection most countries use an ELISA based on the use of polyclonal antibodies; the Brescia lab uses a pool of monoclonal antibodies. Antigen detection is performed on epithelial (lesion) samples, or on passages in cell culture. At least 10^5 $TCID_{50}$ of virus is needed to become positive in the antigen ELISA. This or a higher quantity is usually present in epithelial suspensions, but little is known of the virus quantity in faeces. It is also a task of the EU-Fair project to characterise new monoclonal antibodies for use in diagnostic tests.

The 5B7 ELISA is the standard screening test for antibody to SVD virus in the EU. A number of different countries have now developed MAb-based assays and compared their performance to the 5B7 ELISA. Correct scoring of the EU SVD reference serum panel is also essential if tests other than the 5B7 ELISA are to be used within the EU.

Pirbright has developed a test using Mab C70 with good results, it is not yet in routine use. Lelystad has developed a monoclonal-based competition ELISA using MAb CVI-124-11. Madrid employs a liquid phase blocking ELISA, with IgG from pig hyperimmune serum as catching antibody and MAb 9B11 as indicator antibody. Lindholm (Denmark) is using a blocking test with a guinea pig antiserum as catching and a biotin-labelled rabbit antiserum as indicator antibody. It was mentioned that a sequential testing of sera in different ELISAs can reduce the number of false-positive reactions, provided that a serum is scored negative if one of the two ELISAs scores it negative. All laboratories use the virus neutralisation test for confirmation of sera that are positive in ELISA.

Another described immunoassay is the isotype-specific ELISA for detection of SVD IgM and IgG antibodies (Brocchi et al., 1995), which can be employed for a more refined interpretation of results. Moreover, it has been reported (Brocchi, Berlinzani, De Clercq et al., report of the Brussels meeting, February 1996) that in most singleton reactor sera antibodies to SVD virus are only of the IgM isotype and that singleton reactor pigs do not develop an IgG response. A recent publication on this topic is: De Clercq K et al. Reduction of singleton reactors against swine vesicular disease virus by a combination of a virus neutralisation test, monoclonal antibody-based competitive ELISA and isotype specific ELISA. J. Virol. Meth. 70, 7-18, 1998.

In several countries it has been found that the frequency of false-positive results by the ELISA used is only 1 to 3 positives out of 1,000 sera tested, which indicates a very high specificity of the tests or combination of tests used. The way to deal with these positive results is an important issue which needs to be resolved.

3. Methods and requirements for surveillance for SVD

A first question which arises is whether all member states should do surveillance for the presence of antibodies against SVD? Serosurveillance studies are often not scientifically valid, at a high confidence level. Demonstrating freedom from infection requires different approaches (statistical methods) than determining prevalence of infection. A bad surveillance system is worse than having no surveillance. For performing a scientifically sound surveillance, in general you need to sample enormous numbers (more than 10-100,000s) of pigs. An OIE working group is now drafting guidelines for surveillance of infectious diseases. Most countries that have no history of SVD would not be inclined to perform such a survey. On the other hand, the situation in Italy has shown that many infections can run a subclinical course. Also experimental infections performed in Madrid (with the Spanish 1993 isolate), Brescia, Lelystad and Pirbright with recent isolates induced no or very mild clinical signs in pigs. Therefore, clinical inspection only may not suffice to detect cases of SVD virus infection. Often, clinical signs become evident only in stressful conditions, e.g. transport. Italy has carried out surveillance for almost 4 years now. The Netherlands is performing one where all farms are clinically and serologically monitored three times a year, resulting in a total of 800.000 samples being tested each year. This programme gives 95% certainty that there is no infected farm, assuming that on an infected farm there is more than 25% prevalence.

In addition to the above surveillance, all suspicious cases and epidemiological links to outbreaks are subjected to clinical, virological and serological examinations.

The Committee recommends the EU commission to evaluate the progress of the Italian eradication programme, on the

basis of a thorough report from the Italian authorities. The eradication programme in Italy comprises the detection of SVD virus, also from faeces, in seropositive herds, followed by stamping out of herds from which virus is isolated and controlled slaughter of all seropositive pigs in herds where virus is not detected. Regional labs in Spain do carry out a surveillance program, of which the details are not known. A positive aspect of serosurveillance studies is that it enhances the awareness of veterinary authorities and practitioners and gives the lab the opportunity to do the diagnostic tests on a regular basis.

The Committee recommends the EU commission to repeat the 1993 exercise (at least every 5 years), to review the results and depending on the results take further action if required. In the meantime, the member states can be asked by the commission to provide information on their SVD status and how they apply surveillance.

The Committee considers it adequate to put more effort in the eradication of SVD virus from Italy, if this would be the sole "pocket" of infection of SVD in the EU. A firm action at present could prevent much damage in the future.

Member States currently adopt different sampling strategies for SVD in terms of general surveillance for the disease, as a follow up to the detection of serological reactors, and in the event of suspicion of the disease. The current legislation as defined in Commission Decision 92/119/EEC is not sufficiently detailed to ensure harmonisation of approach. DGVI of the Commission therefore proposed in working document VI/1794/96 the methods and requirements for performing serological sampling for SVD after finding a seropositive animal. However, this document is a discussion paper and has never been finalised such that its recommendations could be implemented in legislation. The Committee considers that it is important to complete this discussion document and recommends that this be done.

4. The definition of SVD and confirmation of the disease

This issue is interpreted as: when is the diagnosis of SVD definitely made?. The working group agrees that a diagnosis of SVD is made, when

1. on holdings the SVD virus is isolated from one or more pigs or the environment;

2. on holdings where clinical signs/lesions of SVD are apparent and antigen/genome of the virus has been detected in samples;

3. on holdings where pigs are present from which samples contain antigen/genome and pigs are present that possess antibodies against SVD virus;

4. on holdings where clinical signs/lesions of SVD are apparent and pigs are present that have antibodies against SVD virus;

5. on holdings where there is a direct epidemiological connection with a confirmed outbreak and where pigs show clinical signs/lesions of SVD, or have antigen/genome in samples taken from pigs, or are seropositive;

6. on holdings wherein seropositive pigs or antigen/genome of SVD are detected. In these cases the competent authority shall, before confirming the presence of the infection, undertake further investigations. These further investigations should be in line with annex IX of the document VI/1794/96 which is attached as Annex II.

Points 1-6 above describe when the diagnosis is made, not the action that should be taken.

5. Comment on epidemiological data presented by Member States on reported outbreaks and applied surveillance

No recent data were available to comment on. The working group recommends that member states are requested to provide epidemiological data on SVD and the surveillance system they apply.

6. Miscellaneous

Information on the SVD situation in countries outside the EU is essential for the control of SVD within the EU, and a ring test should also include labs from e.g. Eastern Europe.

A statement was put forward that the CRL should have more responsibility in harmonisation and standardisation of control of SVD. The CRL should also organise an annual SVD meeting for representatives of National laboratories of EU member states and EU candidate member states from Eastern Europe, e.g. Poland, the Czech Republic, Slovakia, and Hungary. This group recommends that adequate funding is provided by the commission to fulfil these functions. Adequate funding of a CRL in the short term could reduce the costs in the long term due to trade disputes between member states and the costs of compensation in the event of outbreaks. National Reference laboratories must meet a set of requirements with regard to ISO standards and quality assurance, as concluded on a recent meeting in Vienna.

7. Recommendations

1. The Committee recommends to designate a community reference lab for vesicular virus diseases and to support it financially.

2. The Committee recommends to evaluate the progress of the Italian eradication programme, on the basis of a thorough report from the Italian authorities.

3. The Committee recommends to repeat the 1993 exercise, consisting of a serological surveillance in member states.

4. Each EU member state should be asked by the commission to provide information on their SVD status and how they applied surveillance.

5. The working document VI/1794/96, which is a discussion paper, should be finalised and adopted into community legislation.

8. Summary/Conclusions

1. Evaluate recent research developments.

Most of the recent research developments arise from the EU-FAIR project on SVD, of which the aim is to improve the diagnosis of SVD with particular emphasis on eliminating the problems caused by the singleton reactor phenomenon.

2. Evaluate new diagnostic procedures.

Antigen detection and genome detection of SVD virus have recently been developed. Pending results of validation studies these tests are not yet recommended as definitive diagnostic tests. However, under appropriate conditions, they may be included in lab diagnosis of SVD. The results of these validation studies are not expected to be available for at least 2 years.

3. Discuss methods and requirements for surveillance of the disease.

Surveillance for SVD should be done by testing sera for antibodies, at least every 5 years. It is, however, not yet agreed on what the most optimal method/strategy is.

4. Discuss the definition of SVD and confirmation of the disease.

SVD should be confirmed when at least one of five described situations occurs.

5. Comment on epidemiological data presented by Member States on reported outbreaks and applied surveillance.

Insufficient up to date data was presented to the Committee.

Acknowledgements

This report of the Scientific Committee on Animal Health and Animal Welfare is substantially based on the work of a working group of the Committee.

The working group was chaired by Prof. J.T. van Oirschot. The members of the group are listed below.

Dr. Brocchi, Dr. De Clercq, Dr. Dekker, Dr. Mackay, Dr. Sorensen, Dr. Gomez-Tejedor, Prof. van Oirschot (chairman, rapporteur).

Annex I - ORIGINS OF EU SVD REFERENCE SERA

Reference Serum	Origin	Comment
1	Normal pig serum (NPS)	Negative control serum
2	Serum collected 21 d.p.i. from a pig infected with SVDV strain UKG 27/72 (neat)	Strong positive control serum
3	A 1:10 dilution in NPS of a serum collected 5 d.p.i. from a pig infected with SVDV strain Italy 8/94	A low-positive serum from a pig soon after infection with a recent European isolate of SVD 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 d.p.i. from a pig infected with SVDV 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 (i.e. a serum with a titre sufficiently greater than the cut-off that it should always scores positive by ELISA and VNT in repeated testing)
5	Serum collected 4 d.p.i. from a pig infected with SVDV strain UKG 27/72 (neat)	A low-positive serum from a pig soon after infection
6	Serum collected 5 d.p.i. from a pig infected with SVDV strain UKG 27/72 (neat)	A low-positive serum from a pig soon after infection

Annex II - 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 RS4 (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). 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 declines (Singleton reactor sera generally have the following profile: - low VNT titre, - borderline positive in the 5B7 competition ELISA, - exclusively lgM and no IgG in the SVD isotype-specific ELISA).

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.

GUIDELINES FOR SAMPLING, ANALYSIS OF RESULTS AND FURTHER ACTION

1. 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 lb and lc.

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 lc

- 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

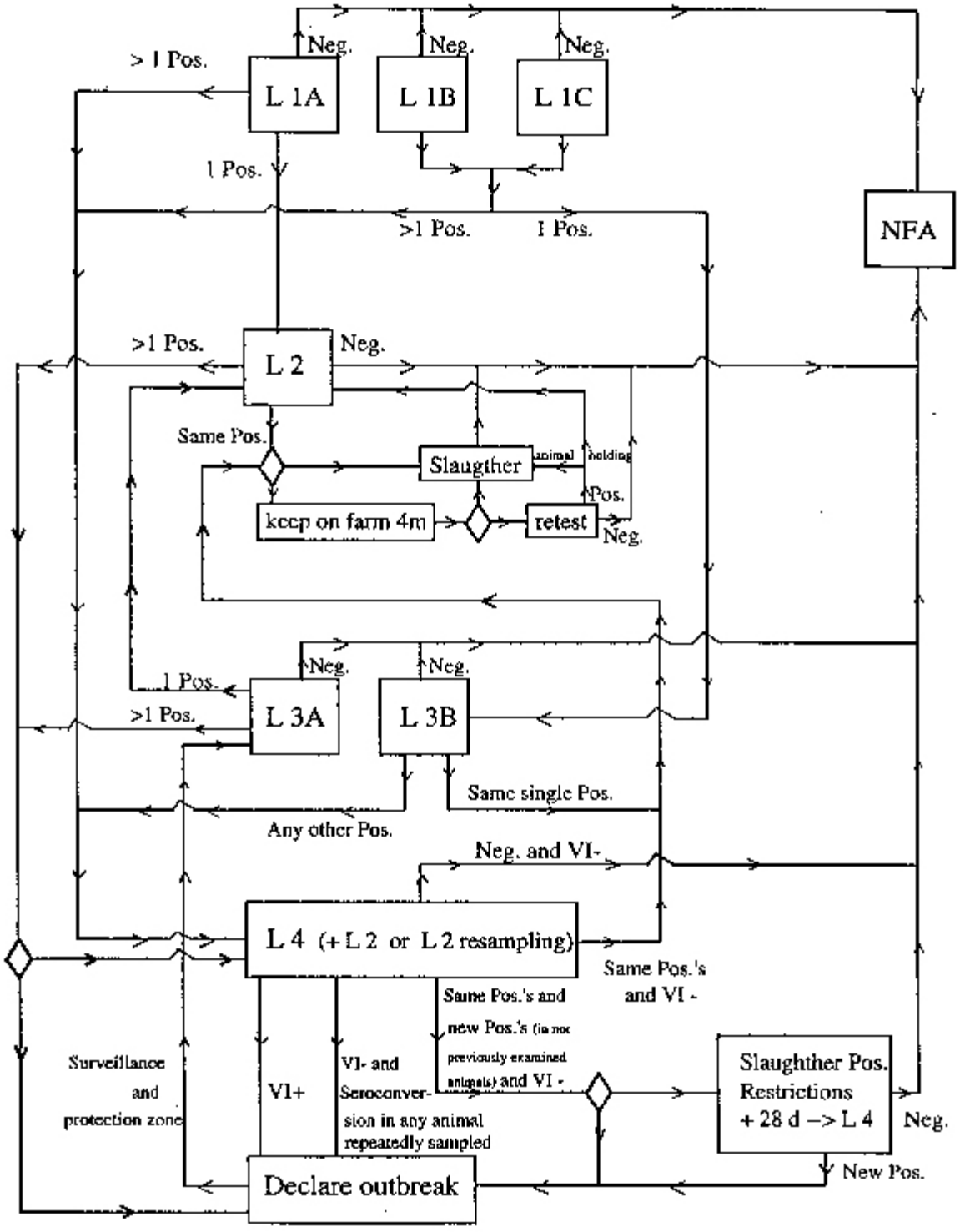
- declare outbreak and stamping out.

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.



L = level; NFA = No further action; Pos.= seropositive; Neg.= seronegative; VI = virusisolation; m = month; d = days.