COMMISSION
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Directorate-general for Agriculture
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# SECOND ANNUAL MEETING OF EU NATIONAL SVD REFERENCE LABORATORIES 19-20 FEBRUARY 1996, BRUSSELS

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## LIST OF ANNEXES (INCLUDING DETAILS OF SCIENTIFIC PAPERS)

**Second Annual Meeting of EU National SVD Reference Laboratories**
**19 - 20 February 1996, Brussels**

**Monday 19 February 1996**

**PRESENT**
REPRESENTATIVES OF EU NATIONAL REFERENCE LABORATORIES

**AGENDA**

| | | |
|---|---|---|
| 11.00 | Opening Session | **Welcome addresses**<br>- Commission<br>- CRL for SVD |
| 11.30 | Item 1 | **Standardisation of SVD antigen and antibody detection within the EU**<br>Chairman: Dr D Mackay<br>Rapporteur: Dr A Dekker<br><br>i) Report on results obtained with SVD Reference Sera — CRL for SVD<br>National Representatives<br><br>ii) Standardisation of SVD antibody detection<br>iii) Discussion of requirement for an exercise in SVD antigen detection |
| 13.00 | | LUNCH |
| 14.00 | Item 2 | **Research Session**<br>Chairman: Dr A Dekker<br>Rapporteur: To be announced<br><br>Abstracts to be sent to CRL for SVD by 12th February 1996. |
| 15.30 | | TEA |
| 17.30 | | Close of session |
| 19.30 | | Dinner at Commission Restaurant<br>29, rue de la Science |

**Tuesday 20 February**

**Present**
REPRESENTATIVE OF EU NATIONAL REFERENCE LABORATORIES AND STATE VETERINARY SERVICES

**AGENDA**

09.00 Item 3 **Review of SVD situation in the EU**
Chairman: Dr K De Clercq
Rapporteur: Dr P O'Reilly

i) Report on current SVD situation and surveillance in member states.
Representatives of National Reference Labs/
Representatives of State Veterinary Services

10.30 COFFEE

11.00 Item 4 **The 'Singleton Reactor' phenomenon**
Chairman: Dr P Have
Rapporteur: Dr E Brocchi

i) Information provided to the Commission by Member States
ii) Reports from participants of investigations of the singleton reactor phenomenon

a) Dr D Mackay - CRL for SVD
b) De R Brocchi - IZS, Brescia
c) Dr K De Clercq - NIVR, Brussels, "Detection of antibodies against SVD comparison of the VNT and MAC-ELISA with reference to singleton reactors".
d) Dr D Mackay - CRL for SVD

ii) Recommendations on the procedure to identify 'singleton reactors' and action to be taken subsequently
iv) Recommendations for changes to EU legislation

13.00 LUNCH

14.00 Item 5 **Recommendations for improved disease control measures for SVD within the EU**
Chairman: Dr C Terpstra
Rapporteur: Dr B Haas

i) Sampling strategy for follow-up of seropositives or suspect disease (Commission Working Document VI/8768/95)
ii) Review of EC Directive 92/1191EEC

15.00 Item 6 **Role and function of the EU Reference Laboratory for SVD**

Dr D Mackay

15.30 TEA

16.00 Item 7 **Closing Session**

Chairman: Dr J Westergaard
Rapporteur: Dr D Mackay

1. Agreement of conclusions of the meeting

# REPORT OF THE MEETING

## OPENING SESSION

Dr J Westergaard of the Commission welcomed the delegates and outlined the objectives of the meeting namely:

- Review of the SVD and control measure
- Review of comparative serological testing
- Presentation of scientific papers on the current status of research on SVD
- Review of the the duties of EC Reference Laboratory for SVD

Dr D Mackay welcomed the delegates on behalf of the CRL for SVD. He proposed the Agenda which was unanimously accepted.

A list of delegates is attached as Annex 7. The meeting took place in meeting rooms of the Commission in Brussels.

## ITEM 1: STANDARDISATION OF SVD ANTIGEN AND ANTIBODY DETECTION WITHIN THE EU

**Chairman Dr D Mackay**
**Rapporteur Dr A Dekker**

The results of the EU workshop on SVD serology 1995 were presented. There was unanimous agreement in all Community SVD Reference Laboratories on the classification of the reference sera as either positive or negative when using the virus neutralisation test (VNT). When examined by ELISA some differences between countries were observed in the interpretation of the low positive reference sera (for details see the report by D.K.J. Mackay, G. Wildsen and R. Armstrong, Annex 1). The IZS, Brescia and the IAH, Pirbright also tested the sera in by isotype-specific ELISA with the results shown in Table 1.

Table 1: **Isotype specific antibody titres of the EU SVD Reference Serum Panel**

| SERUM | IgM | | IgG | |
|---|---|---|---|---|
| | BRESCIA | CRL | BRESCIA | CRL |
| RS1 | N | N | N | N |
| RS2 | 4050 | 3200 | 12150 | 1600 |
| RS3 | 1350 | 800 | N | N |
| RS4 | 50 (trace) | N | 450 | 100 |
| RS5 | 1350 | 800 | N | N |
| RS6 | 4050 | 1600 | N | N |

Differences were ovserved in the actual titres obtained in the VNT in various countries. In Spain, Finland, France, and UK the titre of RS3 was lower than the titre of RS4. In the other countries the titre of RS3 was higher than the titre of RS4. There was no obvious explanation for this phenomonem.

## CONCLUSIONS AND RECOMMENDATIONS FROM ITEM 1:

1. The panel of reference sera produced by CRL for SVD should be used by EU National Reference Laboratories to calibrate the VNT and ELISA tests in routine use in their laboratory. Laboratories should score RS 1 as negative and RS 2 to RS6 as positive. If necessary, laboratories should adjust the sensitivity of their tests to score the reference sera correctly. This is particularly important when testing animals for antibody to SVD prior to international trade.

2. The Commission is recommended to propose to the OIE that the EU SVD Reference Sera are adopted as general standards for SVD serology of pigs for International trade.

3. Laboratories should adopt a common protocol for the 5B7 Mab Comptetion ELISA. Laboratories receiving reagents from the CRL are recommended to follow the protocol laid out in the document entitled 'CRL Protocol for the 5B7 Mab Competition ELISA for the Detection of Antibody to SVD virus'. Comments on the protocol, or suggestions for amendments, should be communicated in writing to the CRL.

4. The CRL will continue to supply the reference sera to EU Reference Laboratories on request

## ITEM 2: SCIENTIFIC SESSION

**Chairman Dr A Dekker**
**Rapporteur Dr D Mackay**
Abstracts of the scientific papers are included as Annex 2.

## CONCLUSIONS AND RECOMMENDATIONS FROM ITEM 2

1. PCR is a valuable technique for the detection of SVD viral genome. Further research is recommended to improve the sensitivity, specificity and ease of use of this technique.

2. Further research is indicated to examine the duration of infection of pigs following exposure to recent european strains of SVD virus.

3. Monoclonal antibodies have proved extremely useful in the development of improved serological tests for antibody to SVD virus. Continued development of new assays using Mabs is encouraged.

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## ITEM 3: REVIEW OF SVD SITUATION IN THE EU

**Chairman Dr Kris de Clercq**
**Rapporteur Dr P.J.O'Reilly**

The chairman welcomed the delegates from the different countries.

In his introduction he indicated that there was full agreement between the different national laboratories on the VN test. However the disease was still present in Europe and complacency should be avoided.

The Chairman then organised a Tour de Table on the situation regarding SVD in the different countries and the extent of surveillance carried out. The information presented by delegates or supplied at a later date is summarised in Table 2.

The following report summarises the main points of the discussion that followed the presentation of each countries' report.

### AUSTRA

A small number of sera were tested and all were negative. The Netherlands questioned whether testing animals for export could be construed as surveillance. Surveillance they maintained should be organised.

### BELGIUM

1995

There were no outbreaks of SVD. Two thousand nine hundred and thirty four sera were examined by VNT of which 39 were positive. There were 1518 sera from routine surveillance of which 38 were positive. A part of those sera (785 samples) came from imported pigs of which 37 were positive. Positive sera from pigs imported from Italy had IgG antibodies. Some animals imported from Spain had IgM antibodies indicative of recent infection.

A discussion followed into the possible origin of the pigs imported into Belgium with documentation suggesting a Spanish origin. The main points of this discussion are summarised under the report from Spain.

Following receipt of reports of serologically positive pigs in consignments of animals exported from Belgium to Italy serological examinations were performed on the herds of origin of the positive pigs. On the farms of origin of these animals 1416 animals were retested and only one positive animal was detected.

### DENMARK

7946 sera were tested in Denmark of which 5,000 were part of a systematic screening programme. One sera was found positive with an Elisa titre of 1/72 compared with RS4 of 1/45 . Eighty samples were subsequently tested from the farm of origin and all were negative.

FINLAND

No cases of SVD were recorded in Finland. 2275 sera were tested by ELISA and VN tests and all were negative. There were no singleton reactors. Eighteen imported animals from Norway and Sweden were all negative.

FRANCE 1995

1507 sera were tested using the SN test. All samples were negative.

GERMANY

742 sera were tested of which 730 were for export. Twelve samples submitted from a regional laboratory due to clinical findings were also negative. There is no SVD in Germany.

GREECE 1995

Forty serum samples from pigs were tested using the VN test. All samples were negative.

IRELAND

1076 sera samples submitted were tested using the 5B7 ELISA. Four samples were positive and were submitted to Pirbright for testing. The ELISA results were confirmed and the VN titres ranged from 32-64 which is less than RS4. 356 samples were collected from the three farms of origin including in one case the original animal. All results were negative.

ITALY

There were 18 confirmed outbreaks of SVD in Italy during 1995. A detailed report of the epidemiological situation and of progress with the eradication campaign is included as Annex 4.

LUXEMBOURG

100 samples collected from breeding animals were tested in Belgium for SVD with negative results.

NETHERLANDS

**Summary of surveillance for SVD in the Netherlands 1995**

1. Serological sampling

| | NUMBER OF SAMPLES | POSITIVE ELISA | VN TITRE 1/51-1/199 | VN TITRE >1/200 |
|---|---|---|---|---|
| HERD CONTROL | 760518 | 9672(1.3%) | 1935 | 570(0.07%) |
| TRACING | 37115 | NT | 963(2.6%) | 143(0.39%) |
| TOTALS | 797633 | 9672 | 2898(0.36%) | 713(0.09%) |

Since the 1st September 1995 all testing for routine screening was performed in the regional laboratory of Boxtall using the LPBE. This testing was controlled by the ID-DLO, Lelystad. All positive samples and samples from follow-up of suspect premises were confirmed at the ID-DLO, Lelystad using the VN

2. Virus isolation

587 faecal samples collected for virus isolation and all were negative.

**Follow-up of the detection of serological positive in Portugal**
A report had been circulated at the SVC detailing the findings of the action taken on the herds of origin of the animals exported to Portugal which were subsequently found to be serologically positive. There was no indication of the presence of SVD in the Netherlands. There was no evidence of reactors on the farms from whence the seven transportations of animals to portugal were initiated nor was there evidence of illegality.

**Testing Programme**
Details of the National Surveillance Scheme for SVD were provided. The surveillance programme operated by the Netherlands is based on the "Herd control regulation for notifiable diseases" and includes;-

A1. Three annual clinical examination of herds with more than 5 pigs.

A2. Three annual samplings of 7-12 samples screened using the LPBE. All positive samples are tested by the VN test at four dilutions of 1;50 to 1;200. If a titre of 1;200 or greater is obtained then 50 to 60 samples (confidence 95% that a prevalence of 5% of seropositive animals will be detected). If > = 3 pigs have a titre of 1/50 to 1/200 or one pig with a titre > = 200 then further samples are taken for both serology and for virus isolation.

B Tracing suspect contacts
Exporting herds are always sampled following detection of seropositive animals in the country of destination following exportation of animals. These are sampled at 50 to 60 samples per herd so as to give a 95% confidence that a prevalence of 5% of seropositive animals will be detected.

C. Virological isolation attempts apply:
-1. to suspect herds

- 2. export collection centres (45 in total) from which 10 samples are collected each month.

D. Movement Controls
A farmer cannot move pigs unless he has a sticker for Transport forms. These stickers are supplied on the basis of serological testing surveillance results. These stickers are valid for four months

PORTUGAL

One outbreak of SVD was reported in Portugal. The outbreak had been linked to the importation of pigs from the Netherlands (see above). A detailed epidemiological report of the SVD situation in Portugal was provided subsequent to the meeting and is attached as Annex 5.

Detection of seropositive animals imported from the Netherlands
There was a discussion of the chronology of the importation of pigs from the Netherlands and the subsequent detection of seropositive animals. It transpired that the pigs which were ultimately detected as seropositive were imported on 27th of July and were sampled on 8/11 August some 10 days later. Final confirmation of the positive serological status of these animals was not obtained until October. Pigs imported on to farms with no native pigs were found to be seropositive. It was unclear whether the farms had been examined on a random basis and whether or not it was possible that the animals could have become infected on the premises or during transport. The IZS, Brescia reported that some of the samples were IgG seropositive indicating that they had been infected for some time.
The Portuguese authorities were recommended to carry out a wide-scale, random survey for the presence of antibody to SVD to ascertain whether or not undetected infection existed in the country. The Portuguese delegate stated that all samples taken from slaughter pigs in the past three years have been negative.

SPAIN

No outbreaks of SVD had been detected during 1995.

National Surveillance Scheme
A document detailing the operation of the Spanish National SVD Surveillance Scheme was provided subsequent to the meeting and is attached as Annex 6.
A summary of the results of the surveillance scheme was provided subsequent to the meeting and is included as Table 3. The meeting expressed surprise that in the initial results presented, although 185,330 samples had been examined no serological positive were detected. In the Table subsequently provided (Table 3) 30 samples were classified as 'Singleton Reactors' but no information was provided as to how this classification was reached, the number of holdings that these 30 samples originated from or of follow-up action on the herds of origin.

Samples were collected

1. For surveillance - initially 10-15% of the animals present
   - all the animals present if positive were detected
2. Pre-export
3. Post - import

Initial screening of samples for the surveillance scheme is performed in the laboratories of the autonomous communities using an ELISA kit supplied by the Spanish National SVD Reference Laboratory in Valdeolmos (INIA). Training in the use of the kits is also provided by INIA but quality control exercise had not yet started. All positive or doubtful samples are sent to INIA for confirmation in the VNT. Serologically positive animals were slaughtered and the herd retested.

Detection of seropositive animals following importation of pigs from Spain

Following the detection of seropositive pigs at random post-import testing of animals exported from Spain to Belgium, there had been a meeting between officials from Belgium and Spain. It was disclosed that the animals had travelled under false certificates which had been prepared before the animals had been assembled. Once they were identified as positive in Belgium, the animals had been slaughtered and the meat destroyed.

The Official Veterinarian Dr H Cabello reported that several groups of animals which had allegedly come from Spain were seropositive for SVD and CSF. He had personally visited the region from whence these pigs allegedly originated and found that false certificates had been issued by an official veterinarian signing blank Certificates. This Veterinarian has since been suspended and he has been interviewed by the police. No ANIMO notice had been sent to Belgium concerning this consignment of pigs. Four herds were debarred from engaging in intercommunity trade. Ten percent of animals and subsequently all animals in these herds were sampled and were all negative for SVD antibody. These results have been communicated to Belgium and to the Commission. All herds within the 3Km zone have been tested and found to be negative. Dr Cabello therefore concluded that the animals reputedly exported from Spain had not originated in the herds from whence they reputedly had come. The animals were also positive for CSF. Spain does not have CSF nor has it vaccinated against CSF for 10 years. They have a good sanitary system for the control of ASF and CSF.

Dr Cabello confirmed that only approved farms could send pigs for intra-community trade.

SWEDEN

1486 serum samples collected from the ADV surveillance programme at the rate of two samples per herd were tested using the ELISA with negative results.

UK

1173 sera from pigs prior to export were examined as were 11 sera from imported animals. One serum had a titre greater than the RS4. Movements were suspended from the herd of origin for 28 days. The animal and all 235 pigs present on the same premises were re-tested with negative results.

## RECOMMENDATIONS AND CONCLUSIONS FROM ITEM 3

1. All countries should carry out organised surveillance.

2. The National reference laboratories should ensure the quality of SVD testing carried out by regional Laboratories.

3. National laboratories are in close agreement regarding test standards particularly concerning the Virus Neutralisation (VN) test

4. Most member states are free of Swine vesicular Disease virus.

5. International trade should be controlled on a non-discriminatorial basis as the origin of imported animals is not always certain due to false certification.

## ITEM 4: THE 'SINGLETON REACTOR' PHENOMENON

A series of papers were presented detailing the findings of several laboratories with regard to the 'Singleton Reactor' (SR) phenomenon. The papers are attached as Annex 3

## RECOMMENDATIONS AND CONCLUSIONS FROM ITEM 4

1. The definition of a SR animal remains unchanged i.e. sera originating from a pig which gives a positive result in serological tests for antibody to SVD but which has no history of contact with SVD virus and from which there is evidence of spread of infection to in-contact animals. Singleton reactors occur at a prevalence of approximately 1 per 1000 animals and may have titres by VNT ranging from borderline to strong positive. On resampling SRs may show a decreasing or constant titre

2 Sera can not be classified as originating from SR animals based on serological techniques alone. The animal of origin must fulfill all of the criteria in (1) above before it can be classified as a SR. Investigations in a number of laboratories indicate that sera from SR's are characterised by the presence of exclusively SVD-specific IgM and no IgG and by the fact that there is no isotype switch forn IgM to IgG on repeated sampling.
**Recommendation**
Further work on a wider range of SR from more Member States is required to confirm these conclusions

3 In some countries clusters of seropostive animals are detected on certain holdings which fulfill all of the criteria for SR's except that they are not 'single'. The term SR should not be used in these cases of unexplained seropositives.
**Recommnedation**
The term 'singleton reactor' should be limited to animals that fulfill all the necessary criteria including the prerequisite that they are, in fact, 'single'