

- 4 The group did not consider that genuine SR's represented any threat of disease but agreed that they caused problems in international trade.
- 5 The group agreed that the procedure following detection of a positive serological result for antibody to SVD should be identical irrespective of whether it is suspected that the sample originates from a SR or a genuine positive animal.
- 6 The procedure to be followed in the event of the detection of a seropositive animal was discussed under Item 5.

ITEM 5: RECOMMENDATIONS FOR IMPROVED DISEASE CONTROL MEASURES FOR SVD WITHIN THE EU

- i) A draft flow diagram of the procedure to be followed in the event of discovery of seropositive animal(s) was presented by the CRL on behalf of the Commission.

CONCLUSION:

There was unanimous agreement that the procedure was too complex to be resolved by a simple flow chart and that the subject should be referred to the SVD Subgroup of the SVC.

RECOMMENDATION:

The SVD Subgroup should be requested to draw up a set of universal guidelines to be followed by all member states following the detection of one or more seropositive animals.

DISCUSSION OF COMMISSION WORKING DOCUMENT VI/8768/95

The restricted randomized sampling strategy for fattening herds (which are divided into pens) is based on two assumptions:

- (almost) all pigs in a pen will show seroconversion
- the disease is present in 4 to 5 neighbouring pens

If these conditions are met, taking a sample from every 4th pen will be sufficient to detect a "cluster" of infected pens with 95% confidence.

Recent experiments at Pirbright have shown a much lower intra-pen seroprevalence.

CONCLUSIONS:

A sampling strategy for breeding herds must be able to detect a prevalence of 5% (as stated in Doc. VI/8768/95).

Considering the recent evidence for SVD isolates of reduced contagiousness, the restricted random sampling strategy for fattening herds should be reviewed.

RECOMMENDATIONS:

The SVD subgroup of the SVC should study the problem of sampling fattening herds infected with SVD isolates of reduced contagiousness.

ITEM 6: ROLE AND FUNCTION OF THE EU REFERENCE LABORATORY FOR SVD

Dr Mackay presented a report of the work carried out by the CRL for SVD during 1995. The workplan agreed in principle with the Commission for 1996 was outlined. The following points summarise the information given:

DIAGNOSTIC WORK PERFORMED IN 1995

60 SVD strains received and catalogued

58 known isolates

Germany

Russia

Italy

2 new isolates from Portugal

32 samples received for antigen detection from Portugal

24 faeces (2 positive)

4 blood

4 myocardium

245 sera examined

Netherlands

Italy

Belgium

Portugal

Ireland

In addition to diagnostic work the CRL had prepared and distributed a panel of SVD Reference Sera to the Reference Laboratories of the Member States. A report of the results obtained from examina of these sera in attached as Annex 1. Reagents for the 5B7 competition ELISA were produced and distributed.

OBJECTIVES OF THE CRL FOR SVD FOR 1996

1. Complete analysis of reference sera and distribute report of Annual Meeting.
April 1996

2. Carry out standardisation exercise in Ag/Ab detection October 1996
3. Preparation of 5B7 ELISA reagents for distribution to Member States
Ongoing
4. Research and epidemiology
 - Molecular epidemiology
 - Antigenic studies/production of antisera
 - Singleton reactor phenomenon
 - Persistence
 - PCR for antigen detection
5. Organise Third Annual Meeting of SVD Reference Laboratories
February 1997
6. Assist if required in Italian eradication campaign

It was agreed that there was a need for a standardisation exercise in SVD antigen detection. This should involve:

- (1) the distribution of faeces samples 'spiked' with positive faeces from infected pigs to examine the sensitivity of the assay systems used for virus identification (e.g. virus isolation, PCR)
- (2) distribution of dilutions of inactivated SVD virus suspensions for examination in the antigen detection ELISA. Suitable strains and dilutions should be selected to examine both the sensitivity of the various ELISA's in use throughout the Community and the ability of the ELISA's to detect a range of antigenic variants of SVD virus.

Dr Westergaard proposed that the CRL for SVD should host training courses for representatives of the state veterinary service and of the laboratory service of each member state. Courses should last approximately 3 days and should provide training in clinical recognition, collection of samples, epidemiology and laboratory diagnosis of SVD. Each course should involve 2 delegates from each of 5 member states. In this way each MS would have an opportunity to send delegates for training once every 3 years. In addition to training courses the meeting encouraged the production of training videos and recommended that the Commission should sponsor the translation into community languages of videos that already exist and are relevant to SVD and other vesicular diseases.

The CRL agreed in principle to both the standardisation exercise and training courses but indicated that a suitable level of funding would have to be made available from the Commission to enable these activities to be carried out.

CONCLUSIONS AND RECOMMENDATIONS FROM ITEM 6

- 1 The group approved of the work completed by the CRL for SVD during 1995
- 2 The group approved the workplan for the CRL for SVD for 1996
- 3 The group agreed that there was a need for a standardisation exercise on virus isolation and antigen detection involving supply of:
 - faeces samples spiked with different amounts of genuine-positive faeces to standardise the test system used for virus isolation.
 - inactivated virus suspensions to standardize the ELISA used for antigen detection.
4. The group recommends that the CRL organises and hosts annual training courses in SVD clinical diagnosis, epidemiology, and laboratory diagnosis.
5. The group recommends that the Commission should encourage the production and distribution of training videos relating to SVD and other vesicular diseases in the major national languages of the EU

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OUTBREAKS OF SVD AND SURVEILLANCE FOR THE DISEASE DECLARED DURING 1996
DATA AS AT 14.2.96

TABLE 2:

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	2934	39	1518	38	785	37	1416	1
Denmark	0	7946	1	7864	1	-	-	82	0
Finland	0	2275	0	2257	0	18	0	0	0
France	0	1507	0	1507	0	-	-	-	-
Germany	0	742	0	730	0	-	-	12	0
Greece	0	*40	0	-	-	-	-	-	-
Ireland	0	1076	4	1076	4	-	-	-	-
Italy	18	306272	4048	295454	3894	6786	128	4032	26
Luxembourg	0	*100	0	-	-	-	-	-	-
Netherlands	0	797633	713	760518	570	-	-	37115	143
Portugal	1	19211	1093	-	-	12574	NS	6637	NS
Spain	0	185330	0	-	-	-	-	-	-
Sweden	0	1486	0	-	-	-	-	-	-
U.K.	0	1173	1	927	1	11	0	235	0

* Approximate
NS = Not specified

TABLE 3:

SINGLETON - REACTORS E.V.P.

1995

Autonomous Region	Singleton reactors	Samples tested by S.N.C.T.S.A.	Samples tested by PIISA Autonomous regions
ANDALUCÍA	-	1	1.837
ARAGÓN	4	2.664	24.000
ASTURIAS	-	-	-
BALEARES	-	32	351
CANARIAS	-	233	-
CANTABRIA	-	-	383
CASTILLA LA MANCHA	-	867	1.750
CASTILLA Y LEON	1	425	4.096
CATALUÑA	4	4.745	73.057
EXTREMADURA	2	8.405	25.127
GALICIA	6	1.611	8.261
MADRID	2	1.242	2.494
MURCIA	9	7.254	39.988
NAVARRA	-	98	4.023
PAÍS VASCO	-	-	-
LA RIOJA	-	-	1.256
VALENCIA	2	2.989	16.484
TOTAL	30	31.067	203.070

EU Workshop on SVD Serology 1995

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Introduction

Panels of standardised sera were prepared by the Community Reference Laboratory for SVD (CRL) during 1993 and 1994 and were distributed to the National SVD Reference Laboratories of Member States. Analysis of the results revealed differences in the sensitivities of the assays in routine use by the different laboratories. At the Annual Meeting of EU National SVD Reference Laboratories in Brussels in 1995, it was agreed that the CRL should produce and distribute a definitive panel of 6 reference sera that should be used by laboratories to harmonise the sensitivity of assays throughout the EU. This paper analyses the results of examination of this panel of definitive reference sera as reported by the National Laboratories.

In December 1993, the 5B7 MAC-ELISA was accepted as the standard screening test for antibody to SVD virus and the test has now been adopted by the majority of EU Member States. Analysis of the results of the reference sera also provided an opportunity to compare the performance of the 5B7 assay as carried out by different laboratories.

Materials and Methods

A panel of 6 reference sera were prepared by the CRL for SVD, Pirbright and were distributed to the National Reference Laboratories of EU Member States listed in Annex 1. The panel comprised one negative serum (RS1), one strong positive serum (RS2), 3 low-positive sera from animals soon after infection (RS3, RS5 and RS6), and one serum which defines the lowest level of antibodies that laboratories should consistently score positive (RS4). The origins of the sera are detailed in Annex 2. The workshop was divided into two phases. During the first, 'wet' phase of the workshop participants were requested to examine the sera by the virus neutralisation test &/or ELISA used routinely in their laboratory for SVD serology. Laboratories were requested to examine the sera on a number of occasions and to report the results to the CRL for collation. Laboratories were also requested to report, for each of their tests, the "cut-off" titre above which sera were considered positive.

Depending on the results supplied to the CRL, the data are expressed as:

1. The mean \log_{10} titre \pm the SD
2. The % inhibition recorded in the screening ELISA (i.e. the 5B7 Mab competition ELISA at a dilution of 1:7.5 unless indicated otherwise)
3. Positive (+), negative (-). Positive sera had titres higher than the cut-off value and negative sera had titres lower. Sera previously classified as doubtful due to low positive or inconsistent titres were defined as positive for the purpose of this analysis.

Results

The detailed results of the individual laboratories using VNT and ELISA are attached as Annex 3. Summaries of the overall results by VNT and ELISA are presented in Tables 1 and 2 respectively.

When examined by VNT, there was unanimous agreement on the interpretation of all 6 reference sera for the 9 laboratories which reported results (Table 1). Variation was observed in the actual titres recorded for the positive sera and in the ranking of the sera by titre, except for RS2 which had the highest titre in all laboratories.

When examined by ELISA, there was full agreement with the interpretation of the negative (RS1) and strong positive (RS2) sera but a minority of laboratories scored the low positive sera RS3, 4 and 5 as negative.

A summary of the results obtained using the 5B7 competition ELISA is presented in Table 3. There was generally good correlation of results and the majority of laboratories scored all 5 positive reference sera positive. However in two laboratories the sensitivity of the test was lower and RS4 and/or RS5 scored negative.

Table 4 presents the results of examination of the reference sera by isotype-specific ELISA at the IZS, Brescia and the IAH, Pirbright.

Conclusions

The VNT is currently the 'gold standard' assay for antibody to SVD virus and is used to confirm the results of ELISA screening. The results of analysis by VNT of the reference sera by the different EU National Reference Laboratories were highly consistent. Although the actual titres differed between laboratories, the classification of sera as negative or positive was unanimous. The VNT therefore continues to serve an important function as the confirmatory test for antibody to SVD virus.

Differences were observed between the sensitivities of the ELISA's used for screening. Most importantly, two laboratories scored RS4 as negative. The majority of laboratories scored RS4 as a low positive serum, confirming the definition of the serum by the CRL as a serum establishing 'the lowest level of antibodies that EU National Reference Laboratories should consistently score positive by ELISA and virus neutralisation'. There is therefore a requirement for laboratories which scored this serum, and the other low positive reference sera RS3 and 5, as negative to increase the sensitivity of their screening ELISA to bring them into line with the remainder of the EU. Both laboratories which scored RS4 as negative use the 5B7 ELISA which correctly scored all 4 low positive reference sera as positive in the majority of laboratories using the test. There is therefore a requirement to harmonise the use of the 5B7 competition ELISA throughout EU National Reference Laboratories. The adoption of a common protocol by all EU Reference Laboratories will help to achieve this aim.

TABLE 1: SUMMARY RESULTS OF EU SVD REFERENCE SERA BY VNT

Reference Serum Number	No. of laboratories scoring the serum positive	No. of laboratories scoring the serum negative
1	0	9
2	9	0
3	9	0
4	9	0
5	9	0
6	9	0

TABLE 2: SUMMARY RESULTS OF EU SVD REFERENCE SERA BY ELISA

Reference Serum Number	No. of laboratories scoring the serum positive	No. of laboratories scoring the serum negative
1	0	13
2	13	0
3	12	1
4	11	2
5	12	1
6	13	0

TABLE 3: SUMMARY RESULTS OF EU SVD REFERENCE SERA USING BY THE 5B7 Mab COMPETITION ELISA

Reference Serum Number	Summary Result				
	No labs +	No labs -	Mean % inhib.	Max % inhib.	Min % inhib.
1	0	9	15	39	1
2	9	0	95	100	80
3	9	0	88	100	70
4	7	2	78	98	42
5	8	1	86	99	63
6	9	0	91	100	76

Table 4: 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

Inverse of the titres of RS1-6 as measured by isotype-specific ELISA at the CRL for SVD, Pirbright and at the IZS, Brescia.

**ANNEX 1: LIST OF PARTICIPATING EU NATIONAL SVD REFERENCE
LABORATORIES**

Country	Laboratory
Austria	Bundesanstalt für Viruseuchenbekämpfung, Wien
Belgium	Institute National de Recherches Vétérinaires, Brussels
Denmark	State Veterinary Institute for Virus Research, Lindholm
Finland	National Veterinary & Food Research Institute, Helsinki
France	Laboratoire Central de Recherches Veterinaires, Paris
Germany	Bundesforschungsanstalt für Viruskrankheiten, Tübingen
Greece	Institute of FMD Exotic Diseases, Athens
Ireland	Veterinary Research Laboratory, Department of Agriculture & Food Abbotstown, Dublin
Italy	Instituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia, Brescia.
Netherlands	ID-DLO, Centraal Diergeneeskundig Instituut Veterinary Department, Lelystad.
Portugal	Laboratorio Nacional de Veterinaria, Lisbon
Spain	Laboratorio de Alta Seguridad Biológica (INIA) Valdeolmos, Madrid.
Sweden	The National Veterinary Institute, Uppsala
United Kingdom	Community Reference Laboratory for Swine Vesicular Disease Institute for Animal Health, Pirbright Laboratory

ANNEX 2: 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 ¹ .
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

¹ 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

ANNEX 3: EU WORKSHOP ON SVD SEROLOGY 1995/96

1. EXAMINATION OF REFERENCE SERA BY ELISA

Country	Assay used	Cut-off	RS1		RS2		RS3		RS4		RS5		RS6							
			+/-	mean	SD	+/-	mean	SD	+/-	mean	SD	+/-	mean	SD						
Austria	LPBE	1.65	-	<0.09		+	3.02	0.09	+	2.28	0.06	+	1.58	0.04	+	2.23	0.04	+	2.65	0.05
Belgium	5B7 ELISA	70%	-	11	3	+	97	0	+	93	1	+	85	3	+	92	1	+	95	0
Denmark	Block. ELISA	70%	-	0	0	+	94	1	+	62	6	+	81	3	+	78	2	+	81	2
Finland	5B7 ELISA	70%	-	0		+	2.51		+	1.9	+	+	1.3	+	1.6	+	1.9	+	1.9	
France	5B7 ELISA	70%	-	15	10	+	80	2	+	72	2	+	42	7	+	63	3	+	78	3
Germany	5B7 ELISA	70%	-	16		+	100		+	90	+	+	87	+	87	+	96	+	96	
Greece																				
Ireland	5B7 ELISA	70%	-	9	4	+	97	0	+	93	0	+	89	1	+	89	1	+	94	1
Italy	5B7 ELISA	70%	-	12	3	+	96	1	+	86	3	+	80	6	+	85	3	+	88	1
Netherlands	LPBE	2.20	-	1.63	0.44	+	3.79	0.13	+	2.95	0.18	+	2.32	0.13	+	2.98	0.16	+	3.22	0.16
Portugal	5B7 ELISA	70%	-	1		+	91		+	70		+	54		+	75		+	76	
Spain	LPBE	1.30	-	<1.3		+	3.04	0.07	+	2.02	0.04	+	1.46	0.06	+	2.18	0.08	+	2.50	0.05
Sweden	5B7 ELISA	70%	-	39	9	+	100	0	+	100	0	+	98	2	+	99	1	+	100	0
U.K. (CRL)	5B7 ELISA	70%	-	16	12	+	100	0	+	97	2	+	89	6	+	94	2	+	98	1

Results of examination of the EU SVD Reference Sera RS1 to RS6 by ELISA. Value shown represents either the log(10) end point titre or, for countries using the 5B7 ELIS the mean % inhibition at the screening dilution (usually 1:7.5)

2. EXAMINATION OF REFERENCE SERA IN THE VIRUS NEUTRALISATION TEST

Country	Assay used	Cut-off	RS1		RS2		RS3		RS4		RS5		RS6							
			+/-	mean	SD	+/-	mean	SD	+/-	mean	SD	+/-	mean	SD						
Austria	VNT	1.50	-	<0.09		+	2.95	0.09	+	2.11	0.11	+	1.99	0.13	+	2.32	0.08	+	2.35	0.08
Belgium	VNT	1.81	-	<1.81		+	3.05	0.09	+	2.15	0.18	+	1.81	0.00	+	2.27	0.12	+	2.69	0.14
Denmark	VNT		-	<0.60		+	2.35		+	2.18		+	1.95		+	2.18		+	1.90	
Finland	VNT	1.60	-	0.00	0.00	+	2.95	0.15	+	1.81	0.29	+	1.90	0.15	+	2.35	0.15	+	2.11	0.29
France	VNT	1.20	-	<0.9		+	2.60	0.17	+	1.20	0.00	+	1.70	0.17	+	1.50	0.00	+	1.80	0.00
Germany	VNT	1.51	-	<0.9		+	3.54		+	2.66		+	2.36		+	2.78		+	3.03	
Greece	NR																			
Ireland	NR																			
Italy	VNT	2.40	-	<0.9	0.00	+	3.24	0.25	+	2.70	0.00	+	2.46	0.39	+	2.58	0.27	+	2.76	0.25
Netherlands	VNT	1.70	-	1.65	0.09	+	3.40	0.23	+	2.45	0.00	+	2.20	0.09	+	2.80	0.09	+	3.20	0.15
Portugal	NR																			
Spain	VNT		-	<1.0		+	2.15	0.11	+	1.30	0.24	+	1.70	0.14	+	1.35	0.20	+	1.65	0.20
Sweden	NR																			
U.K. (CRL)	VNT	1.20	-	<0.9	0.00	+	3.09	0.18	+	1.88	0.30	+	2.10	0.25	+	2.03	0.32	+	2.26	0.31

Results of examination of the EU SVD Reference Sera RS1 to RS6 by VNT. The value shown is the geometric mean log(10) end-point titre reported to the CRL. NR - Either the laboratory does not perform VNT or no results for RS1-6 were reported to the CRL for SVD

DEVELOPMENT OF AN IMMUNO-PCR TEST FOR THE DETECTION OF SVDV IN FAECAL SAMPLES.

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Previous reports describe that SVDV can be detected in faeces for some weeks after exposure to infection. The detection of SVDV in faecal samples by conventional virus isolation assays is currently used for the identification of clinically inapparent infection, in the case of poor and/or old lesions, or to check for the presence of virus in seropositive pigs. The isolation of SVD virus from faeces often requires 2 to 4 tissue culture passages and may be adversely affected by the presence of immune complexes and by other interfering enteroviruses.

A polymerase chain reaction (PCR) was investigated as an alternative approach to isolation of virus in tissue culture.

Since faecal samples are known to inhibit *Taq* polymerase, an immuno-PCR was designed, based on the immune capture of SVDV from extracts of faeces by an SVDV-specific monoclonal antibody coated to ELISA plates. After extensive washing to eliminate faecal material, viral RNA was extracted and RT-PCR was performed by standard procedures.

Preliminary experiments enabled selection of primers which provided the highest sensitivity for amplification. The specificity of the selected primers was evaluated by performing PCR on different amounts of genomic RNA from the four known antigenic variants of SVDV, various picornaviruses and a pig cell line. No amplification was detected with FMDV, EMCV, swine enterovirus isolates or IBRS-2 cells, while all the SVDV strains gave the expected product of 280 bp.

To evaluate the sensitivity of PCR and the possible inhibition by faeces of the RT-PCR, serial 10-fold dilutions of partially-purified SVDV, strain Italy 72 were submitted to both direct PCR and immuno-PCR; dilutions of virus were performed either in PBS or in negative faecal extracts. Amplified products detected by gel-electrophoresis showed that as little as 10-100 TCID₅₀ could be detected by both the immuno-PCR and the direct PCR when dilutions of virus were performed in PBS. When virus was suspended in faecal extracts, the immuno-PCR had the same sensitivity whereas 100-fold more virus was necessary to give a visible band using the direct PCR.

A comparison between the two PCR methods and conventional virus isolation for the detection of SVDV in faecal samples, collected at various times following experimental infection with a recent European isolate of SVD virus (Italy R 1072), is shown in Table 1. The sensitivity of the immuno-PCR is comparable to that of tissue culture isolation, whereas the direct PCR is less sensitive and reliable, confirming the inhibitory effect of faeces.

Table 1: Comparison of immuno-PCR, direct PCR and virus isolation for the detection of SVDV in faecal samples following infection

Days post infection	Immuno-PCR	Virus Isolation	Direct PCR
0	-	-	-
2	+	+ (1st passage)	+
4	+	+ (1st passage)	+
6	+	+ (2nd passage)	+
8	+	+ (2nd passage)	+/-
11	+	+ (2nd passage)	+
15	-	+ (2nd passage)	-
22	+	+ (1st passage)	-
25	+/-	+ (1st passage)	-
29	-	-	-
32	-	-	-
36	-	-	-
39	-	-	-
43	-	-	-
46	-	-	-
56	-	-	-

Note: a booster injection was given 36 days post infection

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.

THE DETECTION OF SWINE VESICULAR DISEASE VIRUS BY PCR AND NESTED PCR

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Identification of swine vesicular disease virus (SVDV) is currently carried out by virus isolation in cell culture and typing by ELISA. In the study reported here a reverse transcription-polymerase chain reaction (RT-PCR) amplification method was developed for the detection of SVD viral RNA. A set of primers was synthesized corresponding to parts of the 1C and 1D capsid-coding genes. The RT-PCR using these primers identified all isolates of SVD virus tested, including representatives of 6 phylogenetically distinct groups (Zhang *et al.* unpublished data). When viral RNA was extracted using the QIAGEN RNeasy total RNA kit, the limit of sensitivity was 100 TCID₅₀. A good correlation was observed between the PCR and virus isolation for identification of the agent in clinical samples. The specificity of the RT-PCR was checked using a range of porcine viruses, and was further confirmed by direct sequencing of the PCR-amplified fragments.

A nested PCR was developed to investigate the duration of the infection of pigs with SVD virus. In addition to the original primer set, an outer primer set was designed corresponding to parts of the 1C and 2A regions of the genome. The nested PCR (nPCR) detected down to 0.1 TCID₅₀ and was therefore 1000 times more sensitive than the original RT-PCR. A range of samples including nasal swabs, faeces and tissues were collected from pigs following experimental infection with a recent european isolate of SVDV (WRL ref. ITL 9/93). Examination in parallel by conventional virus isolation and the nPCR demonstrated that the latter technique was considerably more sensitive than virus isolation, especially for the detection of viral RNA in nasal swabs and tissues.

In order to optimise the PCR and nPCR for the specific detection of SVDV genome, a range of PCR buffers differing in pH, potassium chloride concentration and magnesium chloride concentration were analyzed using two different sets of primers. The results showed that both sets of primers functioned best with 10mM Tris-HCl (pH 8.3 or 9.2), 75mM KCl and 1.5mM MgCl₂. Low KCl concentration (25mM) or high MgCl₂ concentration (35mM) decreased the sensitivity of the PCR. Low pH (8.3) and high pH (9.2) conditions increased the yield of the PCR products. The addition of 6 additives (e.g. formamide, DMSO, BSA) did not increase the yield of the desired amplification product. These results demonstrate the importance of optimizing buffer conditions for PCR when maximum sensitivity is required.

Development of new serological tests for swine vesicular disease

A. Dekker, G. Chenard, C. Baars, C. Terpstra

Three ELISAs for antibody detection against swine vesicular disease (SVD) virus are being developed. Two isotype specific ELISAs (IgM and IgG) for epidemiological studies, and a monoclonal antibody (Mab) based screening ELISA. For detection of IgM an antibody capture ELISA was used (fig. 1), IgG was determined in an indirect double antibody sandwich ELISA (fig. 2).

The IgM ELISA was validated using sequential sera taken after intrabulbar infection with SVD virus isolate NL92. Sera were taken 0, 1, 2, 4, 5, 6, 8, 11, 13, 15, and 21 days after infection. The SVD specific IgM response, measured in the ELISA, started at 6-8 days after infection and lasted at least 21 days. However 4 out of 5 pigs had already neutralising antibodies 4-6 days after infection. Therefore the virus neutralisation test is more sensitive to detect early infection. However in an outbreak situation only a few animals will be infected 4-6 days prior to collection of the blood samples, therefore the ELISA will still be a useful tool for the detection of early infection.

The IgG ELISA was validated using 52 sera; 22 negative field sera, 12 pré-infection sera from SPF pigs, and 18 sera taken 14 to 55 days after infection with SVD isolate IT66 (4 sera) or NL92 (14 sera). Using dilutions 1:10 to 1:160 some negative field sera had the same optical density as post-infection sera. Only at dilutions 1:320 or higher a good distinction could be made between negative field sera and postinfection sera. This illustrates the difference between field sera and SPF sera using an indirect ELISA. None of the IT66 postinfection sera (14 and 25 dpi) was positive in the 1:320 dilution but reacted significantly in the lower dilutions compared to negative SPF sera. This indicates that these sera have a lower affinity to the UK72 antigen used in the test. However the IgG ELISA will be used for an epidemiological study on sera collected in the SVD outbreak in 1992 and 1994. For further validation a dilution of 1:312.5 was chosen to test the postinfection sera of the NL92 infection experiment described before. Based on these results the IgG antibody starts 8-12 days after infection (fig. 3). Figure 3 is based on the optical densities of these sera in a 1:312.5 dilution, titration of these sera will presumably give more information.

The third test is a Mab based screening ELISA using the principle described by Brocchi et al. (1995), using a monoclonal antibody produced at the ID-DLO (Lelystad) (fig. 4). This ELISA was validated using two sets of diluted postinfection sera (series 1 against NL92, series 2 against UK72).

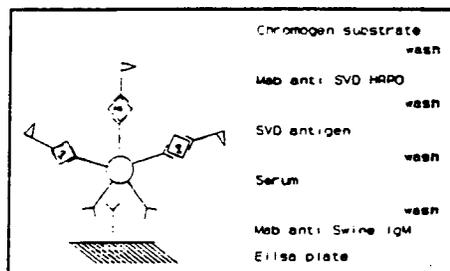


Figure 1: ELISA layout for the detection of SVD specific IgM antibodies.

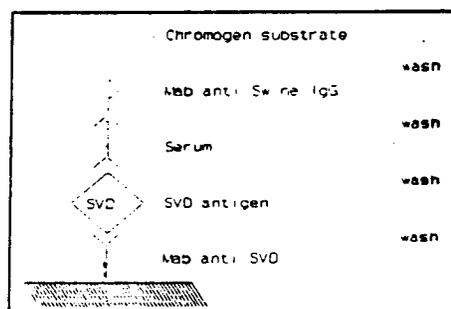


Figure 2: ELISA layout for the detection of SVD specific IgG antibodies

The sensitivity and specificity were compared to the conventional LPBE ELISA, and a Mab 5B7 based LPBE ELISA (Dekker et al. 1995) (Table 1).

Table 1: Sensitivity and specificity of the conventional LPBE, the Mab 5B7 LPBE and the Mab based screening ELISA

Type of test	Series	Results	
		Sens.	Spec.
LPBE (conventional)	1	100	90
	2	95	96
LPBE with Mab 5B7	1	100	89
	2	99	99
Mab based screening ELISA	1	100	96
	2	96	99

The sensitivity and specificity using these sera were comparable in all three tests (table 1). Further validation of the Mab based screening ELISA, the IgM ELISA and the IgG ELISA has to be done using negative field sera collected in the surveillance programme, singleton reactor sera, and sera from the outbreak farms in 1992 and 1994.

It can be concluded that the isotype specific ELISAs will be promising tools in epidemiological research, and that the Mab based screening ELISA will be a good alternative for SVD ELISAs used currently in Europe.

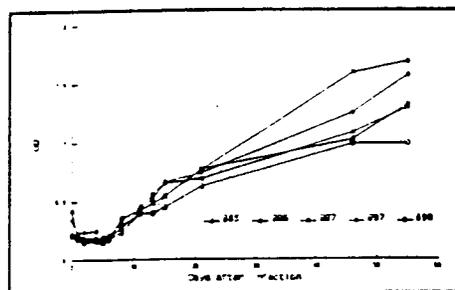


Figure 3: IgG ELISA: Optical density of NL92 postinfection sera in a 1:312.5 dilution

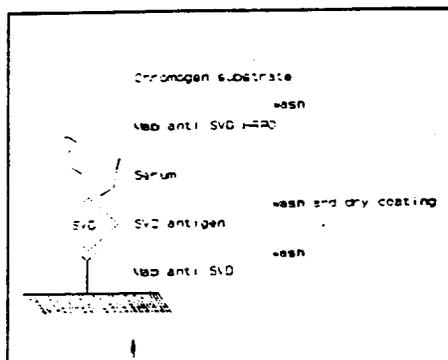


Figure 4: layout of the Mab based screening ELISA

Kimman, T.G., Westenbrink, F., Straver, P.J., Van Zaane, D., Schreuder, B.E. 1987. Isotype-specific ELISAs for the detection of antibodies to bovine respiratory syncytial virus. *Res. Vet. Sci.* 43(2): 180-187

Brocchi, E., Berlinzani, A., Gamba, D., De Simone, F. 1995. 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. Vir. Met.* 52: 155-167

Dekker, A., Chenard, G., Moonen, P., Terpstra, C. 1995. Comparison of two automated liquid phase blocking ELISAs for SVD, using polyclonal and monoclonal antibodies. First annual meeting of EU national SVD reference laboratories, Brussels.

ANNEX 3 (a):

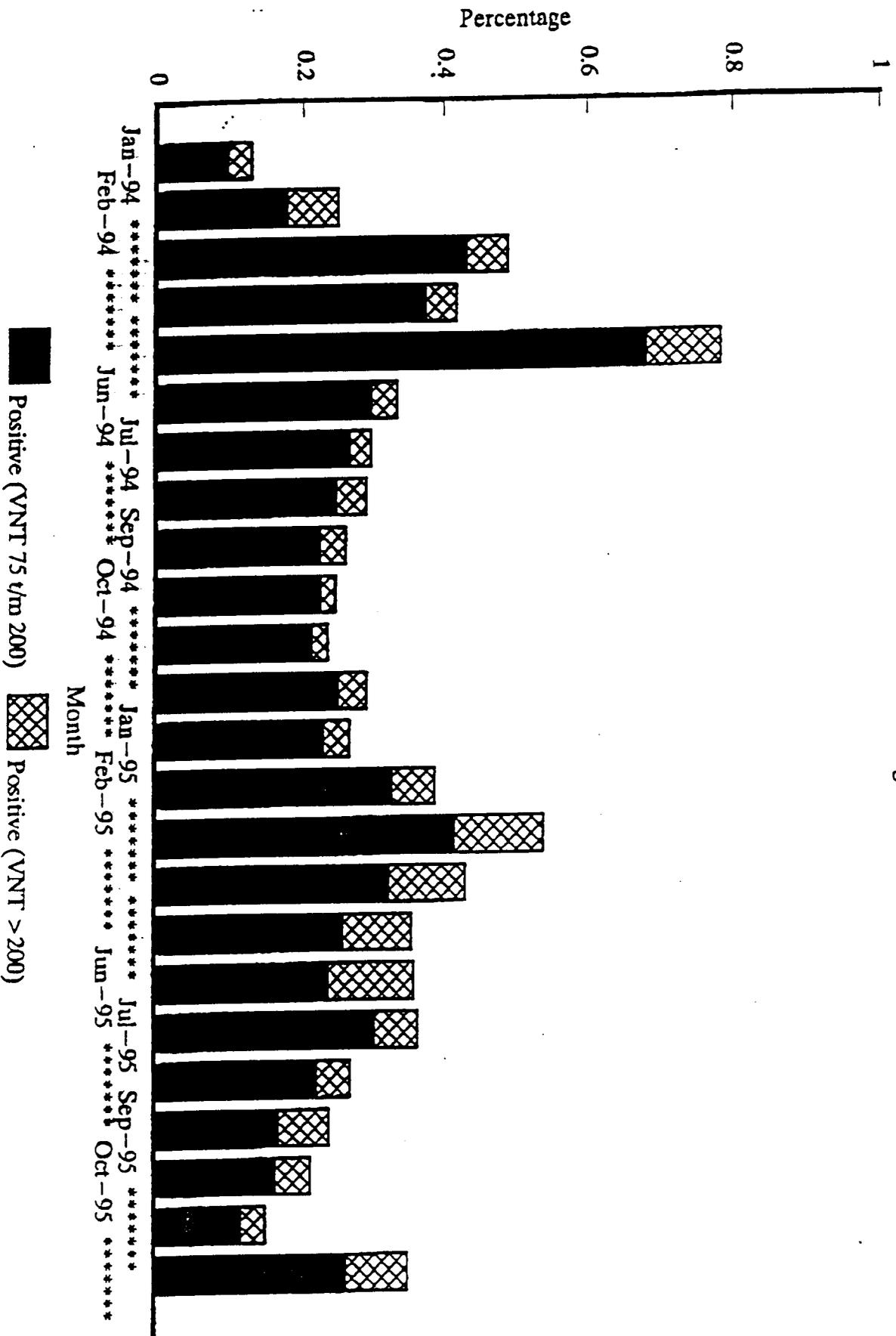
Studies on the prevalence of 'singleton reactor' sera at the ID-DLO, Lelystad

C Terpstra, A Dekker, ID-DLO Lelystad

The attached figure shows the percentage of sera classified as 'singleton reactors' from the national SVD surveillance scheme carried out in the Netherlands. A seasonal pattern was observed in the prevalence of singleton reactors. There was a tendency for the prevalence to increase in spring and to decrease in autumn. To date no explanation has been found for this phenomenon.

Percentage positive SVD sera

Total screening



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Work connected to the campaign for SVD eradication in Italy allowed to identify a number of sera from animals which reacted as "singleton reactors" (RS), among samples submitted for serological survey

This offered the opportunity to study some reactivity features of such sera.

In table 1 information on the origin, number and reactivity of SR sera are summarised. A total of 143 SR from Lombardy and Emilia-Romagna regions has been identified: most of them (approximately 80%) showed Virus Neutralising (VN) titres corresponding to the cut-off level used in our laboratory (1/256) or slightly higher (1/512). Only a minority scored titres 1/1024 or higher, like those commonly found in infected herds.

In case of high titres, the number of reactor pigs corresponded to the number of herds, indicating that just one SR serum was found in one herd. For borderline titres, two SR, very seldom three, can occasionally occur in one herd.

Each seropositive animal was re-sampled generally 10 to 20 days after the first bleeding.

Blood and faeces, collected from SR and from close stabled pigs, were tested for the detection of antibody and virus respectively.

The number of SR sera still positive at 2nd bleeding is reported in table 1.

Most animals became negative within short time, even when the titre recorded at first sampling was high. Only 15 out of 143 animals were confirmed positive, with the tendency to score lower titres.

In all SR, IgM isotype was the only antibody ever detected and faeces samples were ever negative.

As far as the percentage of SR is concerned, the 143 SR sera derived from 70,406 tested pigs (0.2%); the number of herds with SR was 122 out of 1632 examined (7.47%).

Remarks attached to table 1 summarise findings common to all the SR sera.

Additional analysis was done by examining SR sera in Western Blotting test (WB). The aim was to ascertain if SR reactivity could be directed to one particular viral protein, indicating a "status" specific for SR.

For this purpose WB was previously standardised by using known sera either from experimentally infected animals or from the field; the pattern of reactivity of sera collected during the IgM phase production was compared to the one of SR sera.

Specific sera showed in WB test a selected reactivity of IgM to VP1 and, only in some cases, also a faint reactivity to VP2 and VP3.

SR sera showed many different patterns of IgM reactivity, ranging from negative to reactivity against all viral proteins, with no selection for VP1 and no correlation with VN titres.

It appeared that WB pattern of SR reactivity was clearly different from the one of specific sera.

WB and previous results suggest that SR reactivity is more probably due to a general stickiness of untyped IgM than to a specific immunological response of pigs to infectious agents antigenically related to SVD virus.

Table 1 : SINGLETON REACTORS - 1995 - LOMBARDIA and EMILIA-ROMAGNA REGIONS

	Virus neutralisation test						
	256	512	1024	2048	4096	8192	TOTAL
N° pigs	81	31	13	14	3	1	143
N° herds	71	26	13	14	3	1	122
pigs still							
pos. at 2nd	8	3	2 (= ↓)	0	2 (= ↓)	0	15
bleeding							

REMARKS

- ALL SERA SHOWED ONLY IgM - MOSTLY LOW NEUTRALISING TITRES
- SWITCH TO IgG NEVER OBSERVED
- ONLY 15 OUT OF 143 PIGS STILL POSITIVE AT SECOND BLEEDING (10-20 DAYS LATER)
- FAECES ALWAYS NEGATIVE
- ONE (seldom 2/3) SEROPOSITIVE PIGS PER HERD
- NEVER OBSERVED SEROCONVERSIONS IN OTHER PIGS

Detection of antibodies against SVD: comparison of the VNT and MAC-ELISA with reference to singleton reactors.

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During serological screening for SVD by VNT, from time to time one (or two) animal(s) is (are) found positive on a pig farm. In this case the pig farm is blocked (animals stand still) and the same animal is blood sampled again together with 50 other animals in the neighbourhood (same pen and adjacent pens). When all other 49 pigs are negative, the positive one is considered as a singleton reactor. This means that the animal is negative for SVD but gives a false positive reaction in the laboratory test.

During the screening of 1992 and '93 about 60.000 animals were checked for the presence of antibodies against SVD by VNT. In this way 0.28 % singleton reactors (SR) were found. Although this is a low percentage, it is quite embarrassing for the blocked farm and in international trade.

The question was raised whether by analysing data from different tests performed on the same serum, the number of SR would be less without risking false negatives. Therefore the results of 185 pigs in VNT, MAC-ELISA and isotype specific ELISA were analysed. The animals were selected on basis of their VNT result. As well negatives, as positives and SR were taken in the study. The origin of the pig sera is given in Table 1.

The VNT test was performed with SK₆ cells and SVDV UK 72. The cut-off used, was at 1.81 log₁₀ based on reference serum RS 01.04.93. The MAC-ELISA and isotype specific ELISA were performed as described by Brescia. The cut-off used of the MAC-ELISA was at 80% inhibition.

In Fig. 1 sera were ranked by their titre in VNT. Sera with titre below the cut-off were considered negative. Two SR reference sera 1993 turned out negative in our VNT. Sera with titre \geq cut-off were positive. Fourty-nine of the positive sera came from SR. Most of the SR had a low VN titre.

Each serum is presented with its percentage of inhibition (% inhib.) at serum dilution 1:7.5 in the MAC-ELISA. Most of the sera negative in VNT had a % inhib. below 20%. All non SR sera positive in the VNT had a % inhib. $>$ 80%, with the exception of 1 serum (No 87) with 70%. Most SR had a low % inhib. (lower then 50 to 60%). Only 13 SR sera had a % inhib. $>$ 80% in the MAC-ELISA (Table 2). So the MAC-ELISA is more specific then the VNT.

In Fig. 2 some of the real positives and SR with % inhib \geq 50. at serum dilution 1:7.5 are presented with their % inhib. at serum dilution 1:22.5. With the exception of 4 sera (No 60, 61, 62, 87) all real positives had a high % inhib. at both serum dilutions. Most SR on the contrary had a low % inhib. at the 1:22.5 serum dilution, even those with a high VN titre. Of the 13 SR with a high % inhib. at the 1:7.5 serum dilution only 3 sera (No 96, 112, 139) had also a high % inhib. at the 1:22.5 serum dilution. But the 4 real positives fulfilling also this high/low criterion represented a danger of false negatives.

Some SR and positives were examined in the isotype specific ELISA. Table 3 makes clear that in SR, antibodies against SVDV were only of the IgM isotype. The majority of the real positives had IgG's. Positives from very recent infections had IgM's or IgM + IgG's when the infection was a few days older. Fig. 3 shows that the IgG titre of the positives was variable but in most cases quite high. Also the IgM titre of the real positives was high (\geq 3.0), with the exception of 3 sera (No 89,100,106) with low IgM titre (but sera No 106 has also IgG). Strikingly, SR had low IgM titres. Only 2 SR represented an exception on this (sera No 112,139). Fig. 4 shows more clearly the high/low difference in IgM titre between real positives and SR.

When results of the 3 tests are combined, a profile of real positives and SR can be put together. Table 4 shows such a profile and the profile of possible exceptions as mentioned above. As IgG was only found in real positives it could not be a point of confusion but it helped differentiating SR from positives. The profile of SR's differs clearly from the one of real positives. Only one type of exception (SR exception 2) had a profile that could not be differentiated from real positives.

In table 5 the above criteria and profiles are applied to all mentioned exceptions. Two sera (No 112,139) had a profile that resembles the one of positives. All other sera had at least one criterion that didn't fit. From the SR's with high % inhib. in the 1:22.5 serum dilution, one (No 96) had a low IgM titre which doesn't correspond with a real positive having a high % inhib. in both dilutions. From the 4 possible false negatives based on this high/high % inhib. criterion for real positives, 3 had a high IgM titre and one (No 87) had IgG's both criteria pointing more to real positives. Positives with low IgM titre (sera No 89,100,106) had a high % inhib. at both serum dilution's.

Based on these criteria and profiles only two SR of the 51 in study needed in fact further investigation.

Table 1: Origin of pig sera used in this study (Neg.= negative; SR= singleton reactor; Pos.= positive; Ref.= reference; VNT=virus neutralisation).

	VNT		
	Neg.	SR	Pos.
Ref. Sera 93	5	3	4
Ref. Sera 94	5		9
Ref. Sera 95	1		5
SVD Outbreak	11		13
Screening	7	33	
Pre-export	1	6	
Post-import	13	9	44
Slaughterhouse sera Italy			16
Total	43	51	91

Table 2: Results in virus neutralisation test and in MAC-ELISA (SR= singleton reactor).

	VNT	MAC-ELISA
Total	185	185
Negative	43	82
SR	51	13
Positive	91	90

Table 3: Results of some singleton reactors (SR) and positives in the isotype specific ELISA.

	SR	Pos.
IgM	28	10
IgG		34
IgM + IgG		1

Table 4 : Profile of real positives and singleton reactors based on the results in VNT, MAC-ELISA and isotype specific ELISA.

		VN titre high	% inhib. 1:7,5 >80%	% inhib. 1:22,5 >80%	IgG present	IgM present	IgM titre high	Serum number
Positive	Real Pos.	+	+	+	+ or/and	+	+	
	Exception 1	-	+	-	-	+	+	60/61/62
	Exception 2	-	+	-	+	-	-	87
	Exception 3	- (±)	+	+	+/-	+	-	89/100/106
Singleton Reactor	Real SR or	-	-	-	-	+	-	
		+	+	-	-	+	-	
	Exception 1	±	+	+	-	+	-	96
	Exception 2	+	+	+	-	+	+	112/139

Table 5: Results of different tests of sera representing an exception on the profile of SR or positives.

Serum number	P/SR	VN titre	% inhib. 1:7,5	% inhib. 1:22,5	IgM titre	IgG present
60	P	1,81	84	42	3,5	-
61	P	1,81	86	54	3,75	-
62	P	1,81	87	49	3,5	-
87	P	2,28	72	46	<2,0	+
96	SR	2,41	96	85	2,48	-
112	SR	2,71	94	91	3,1	-
139	SR	3,01	95	94	3,5	-
89	P	2,28	92	89	2,6	-
100	P	2,58	90	82	2,5	-
106	P	2,58	97	89	2,4	+

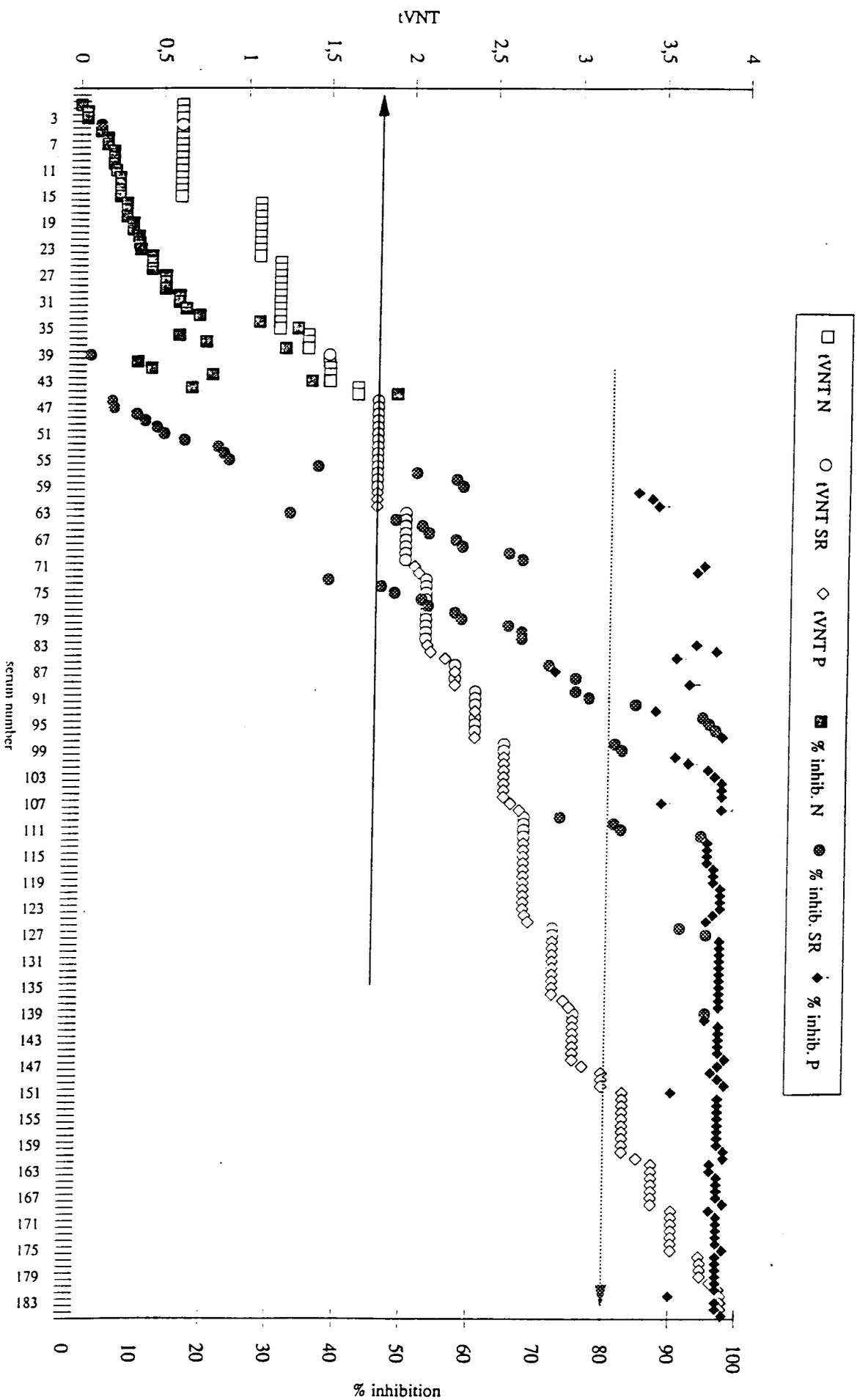


Fig. 1: Virusneutralisation titre (VNT) and % inhibition (% inhib.) in MAC-ELISA is given for 185 pig sera tested against SVDV. The cut-off for VN test (VNT) = 1.81 and for MAC-ELISA = 80%. N= negative; P= positive; SR= singleton reactor.

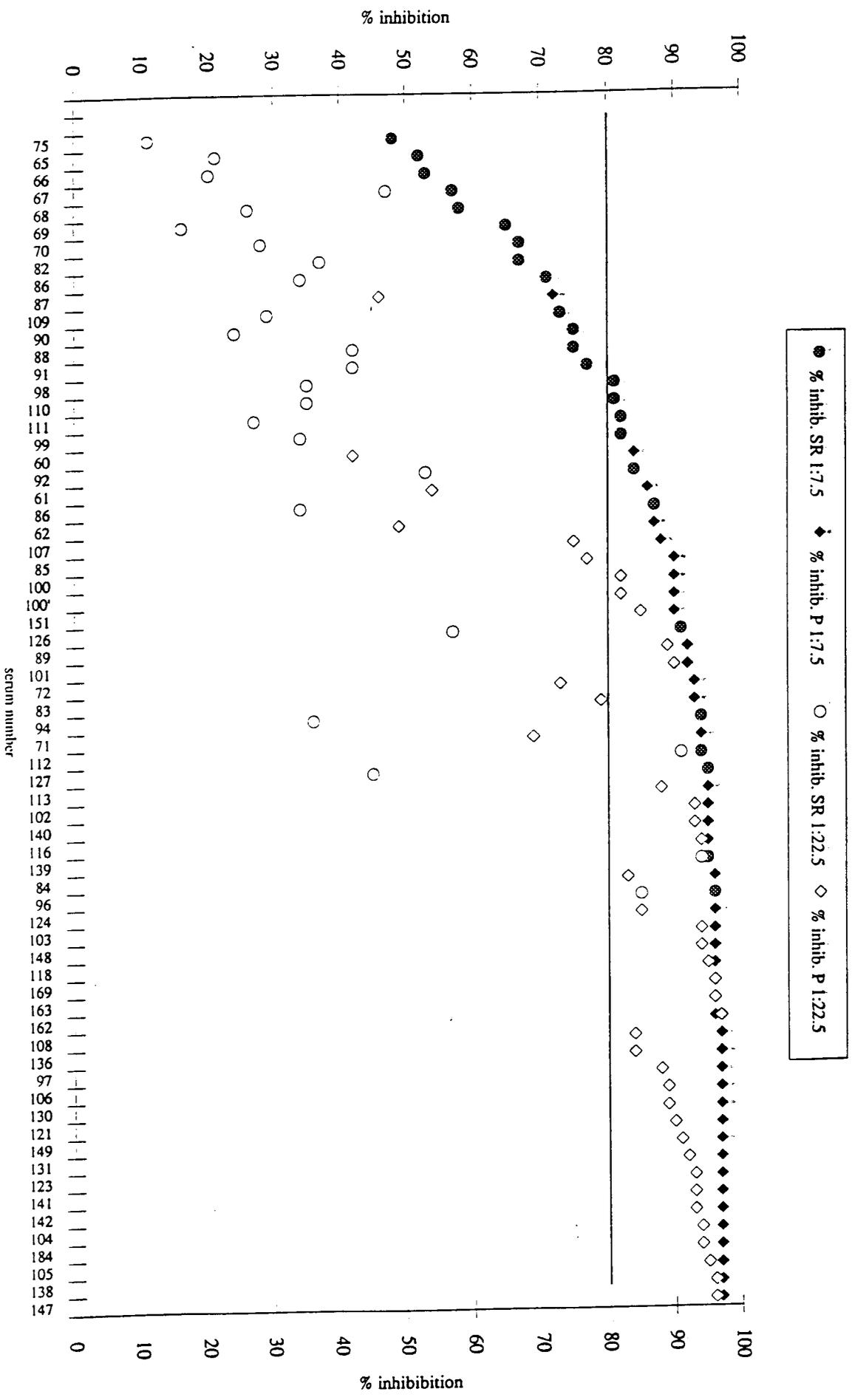


Fig. 2 : Percentage (%) inhibition in the SVD MAC-ELISA of singleton reactors (SR) and positives (P) at serum dilutions 1:7.5 and 1:22.5.

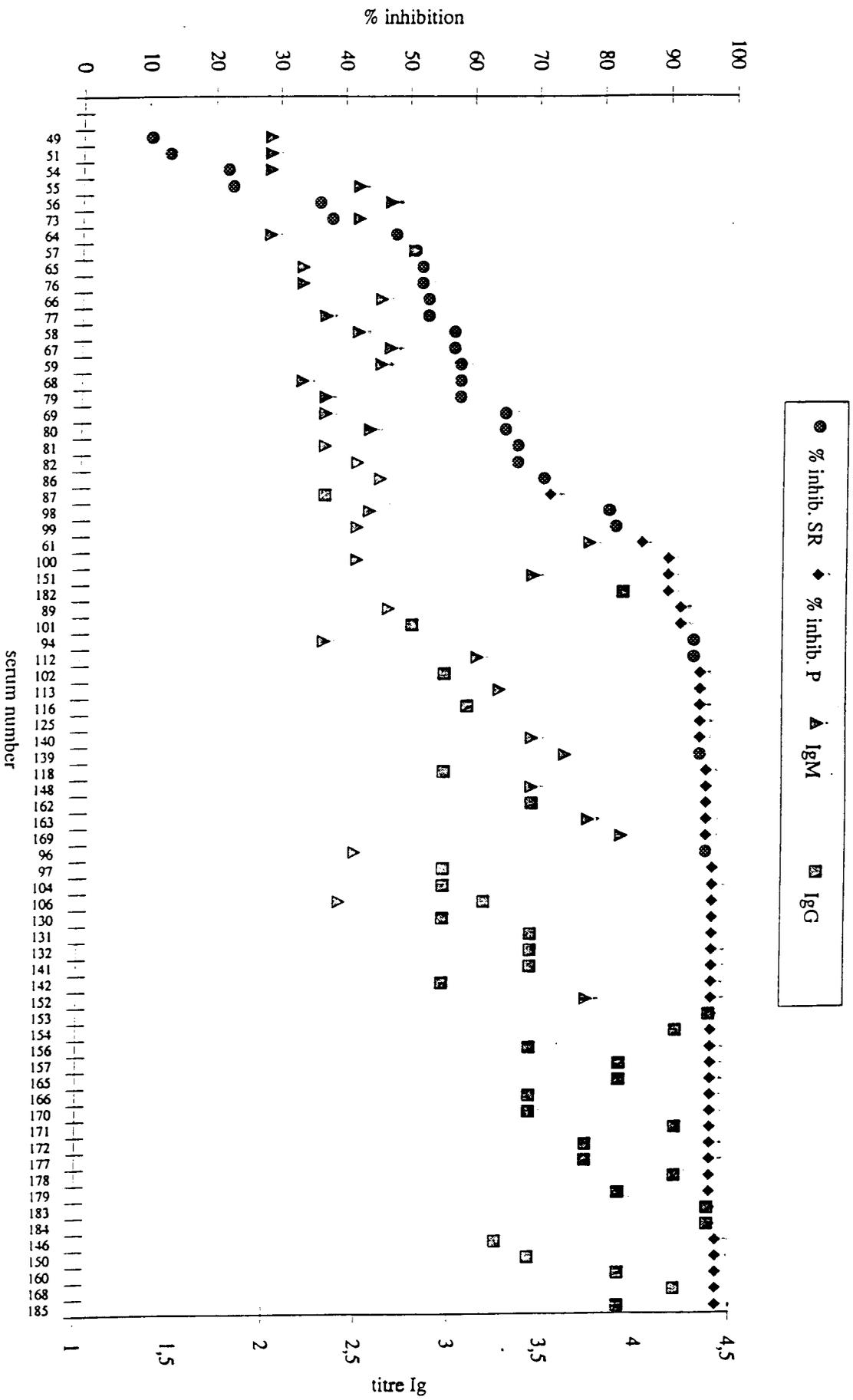


Fig. 3: Percentage (%) inhibition in the SVD MAC-ELISA of singleton reactors (SR) and positives (P) with corresponding IgM or/and IgG titre in the SVD isotype specific ELISA. Ig= Immunoglobuline.

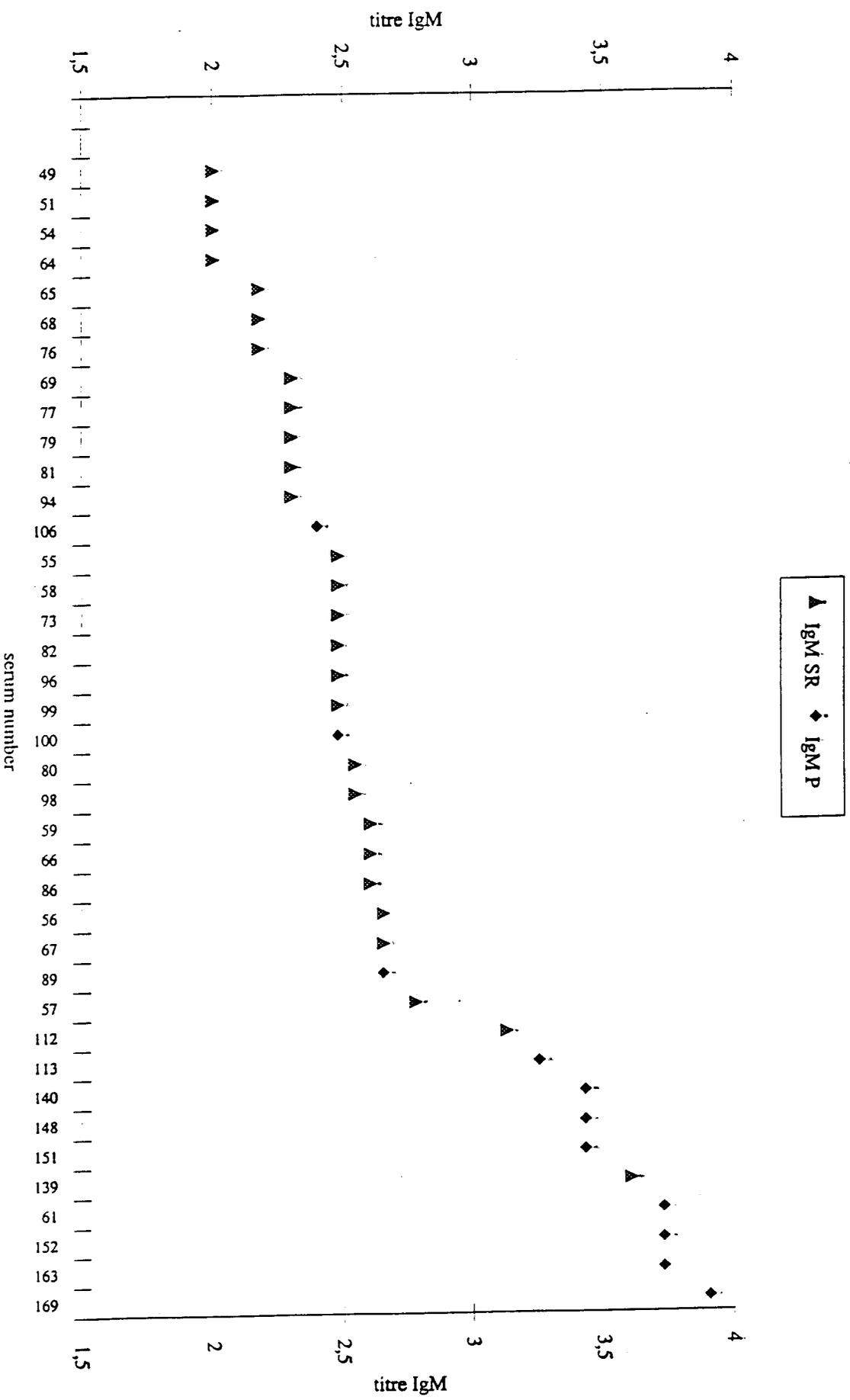


Fig. 4 : IgM titre in the SVD isotype specific ELISA of singleton reactors (SR) and Positives (P).

Preliminary characterisation of 'Singleton Reactor' Sera

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Preliminary characterisation was performed on a limited number of singleton reactor (SR) sera to investigate the factors responsible for their reactivity in serological tests for antibody to swine vesicular disease (SVD). Sera classified as SR's were examined for antibody to SVD virus (SVDV) using the 5B7 MAC-ELISA (Brocchi et al. 1995) and the virus neutralisation test (VNT), using as antigen in both tests the SVDV strain UKG 27/72. Ninety six (96) sera were received from the ID-DLO, Netherlands. When examined at the ID-DLO, these sera were positive by liquid phase blocking ELISA (Dekker et al. 1995) and had VNT titres between 1:50 and 1:200 i.e. the sera were positive for antibody to SVDV but had titres lower than the EU SVD reference serum RS4. When examined at the CRL for SVD using the 5B7 MAC-ELISA, 50 sera (52%) were negative, 44 (46%) were low positive (i.e. positive at 1:7.5 dilution and negative at 1:22.5) and 2 were high positive (i.e. positive at both dilutions). When examined by VNT 62 sera (65%) were negative, 27 (28%) were low positive or doubtful (titre greater than 1:11 and less than 1:45) and 6 (6%) were positive (titre greater than or equal to 1:45). Twenty four (24) SR sera were received from the IZS, Brescia, Italy. When examined at the IZS, the sera were positive in both the 5B7 MAC-ELISA (i.e. more than 70% inhibition at the 1:7.5 dilution) and the VNT i.e. the sera had a titre greater than or equal to that of RS4. When examined at the IZS using an isotype-specific ELISA (Brocchi et al. 1995), all 24 sera contained only SVD-specific IgM and no IgG. At the CRL for SVD examination by VNT classified 5 sera as negative, 13 as doubtful and 6 as positive (see criteria above). For sera classified as doubtful or positive in both the Brescia and Pirbright VNT's, there was a significant correlation between the titres in the two tests ($R=0.571$, $P=0.05$).

A subset of SR sera from the Netherlands, Italy and the UK were examined in serum neutralisation tests (SNT) against a recent Italian isolate of SVD virus, ITA 9/93 (Table 1). Sera were also reacted with monoclonal antibody escape mutants (MAR's) produced by selection of variants of ITA 9/93 able to escape neutralisation by one of a panel of Mabs raised against SVDV. The serum neutralisation tests measured the difference in titre of a virus with and without the presence of the test serum at a dilution of 1:100. The 13 sera selected for further examination were positive in the VNT with a titre greater than 1:45 (\log_{10} 1.65) and were positive in the 5B7 MAC-ELISA. Only 5 of the 13 sera had an SNT titre greater than the cut-off value of 1:4 (\log_{10} 0.6). This probably reflects a lower sensitivity of the SNT as compared to the VNT. SNT titres were not consistently lower with any of the MAR's as compared to the parental strain ITA 9/93. However, the titres of the majority of the sera were lowest against the 5B7 MAR and 3 sera (B3, 953/35 and S27R/95) showed a significantly lower titre against the 5B7 MAR than against the parental ITA 9/93 strain. This suggests that a significant component of the SVD-specific antibody in these 3 sera was directed against the antigenic site recognised by the 5B7 MAb. As this site was present on the parental strain but not on the 5B7 MAR the sera showed a higher SNT titre against the parental strain than against the MAR. Sera were also reacted in the SNT against Coxsackie B5 (CB5) virus. Only 7 out of the 13 sera had titres greater than 1:4 indicating that not all SR sera reacted with CB5 virus.

The same panel of 13 sera was examined by Western blotting using as antigens the capsid proteins of SVDV separated by PAGE. Patterns of reactivity were variable and 7 out of the 13 sera did not react at all in Western blots. Of the 3 capsid proteins, only VP1 was clearly identified by all sera giving a positive reaction. In addition, VP's 2 and 3 were weakly identified by some of the sera reacting against VP1.

Conclusions

1. There is disagreement between laboratories in the interpretation of SR sera. Sera classified as 'singleton reactors' by one laboratory might be classified as doubtful or negative in another. As comparative tests have shown that there is good agreement between the 3 laboratories involved in interpretation of genuine positive (reference) sera, differences in interpretation of SR sera presumably arose due to differences in the specificities of the tests used.
2. Of the antigenic sites identified to date, only the site recognised by the Mab 5B7 is clearly associated with reactivity in some SR sera. This finding is consistent with the observation that the use of the 5B7 MAC-ELISA reduces but does not eliminate the problem of SR sera in serological testing for SVD.
3. Not all SR sera react with CB5 virus suggesting that infection of pigs with CB5 virus is unlikely to be a major cause of SRs
4. Reaction in Western blotting is variable and, when present, is usually directed against VP1.
5. Further studies using a wider range of SR's are required to confirm or refute these preliminary findings.

References

- Brocchi et al. (1995) *J. Virol. Meth.* **52** 155-167
Dekker et al. (1995) *J. Virol. Meth.* **51** 343-348

TABLE 1: EXAMINATION OF SINGLETON REACTOR SERA

SERUM	ELISA	VNT	SERUM NEUTRALISATION					WESTERN BLOTTING		
			ITL/9/93	C29-M	5B7-M	4H3-M	CB5	VP1	VP2	VP3
RS1 (N)	<7.5	<0.9	0.3	0.6	0.6	0.45	0.3	-	-	-
RS2 (P)	1400	1549	1.95	1.5	1.35	1.5	1.65	+	+	+
B3	<7.5	45	1.05	1.5	0.3	0.45	0	-	-	-
C9	13	45	0.45	0.45	0.15	0.6	0.15	-	-	-
D8	13	45	0	0.3	0.15	0.6	0.45	-	-	-
D9	13	45	0.6	1.05	0.9	1.35	0.75	+	+/-	+/-
G5	<7.5	90	0.3	0.15	0.6	0.6	0	+	+/-	+/-
G12	<7.5	45	0.3	0.3	0.15	0.45	0	+	+/-	-
1170/8	POS	64	0.3	0	0.3	0.75	0	-	-	-
307/13	POS	45	0.15	1.05	0	0.15	0.6	-	-	-
953/35	POS	90	1.8	1.35	0	1.2	1.65	-	-	-
2493/24	POS	64	0	0.45	0	0.15	0.75	+	+/-	-
4123/7	POS	>1400	1.05	1.35	1.05	1.35	1.5	+	+/-	-
4229/9	POS	208	0	0.75	0.3	1.05	1.5	-	-	-
S27R/95	N.D.	256	1.95	1.5	1.35	1.5	1.2	+	-	-

Sera were examined in the 5B7 MAC-ELISA; the virus neutralisation test (VNT); the serum neutralisation test against SVDV strain ITA 9/93 and the MAR mutant strains of ITA 9/93 produced using Mabs C29, 5B7 and 4H3 and against Coxsackie B5; and in Western blotting against PAGE-separated capsid proteins of SVDV strain ITA 9/93. The sera examined were the EU SVD reference sera RS1 (negative) and RS2 (positive), 6 SR sera from the Netherlands (B3 to G12), 6 SR sera from Italy (1170/8 to 4229/9) and 1 SR serum from the UK (S27R/95).

Report presented at the 1996 Annual Meeting of EU SVD Reference Laboratories by Dr E Brocchi and Dr A Berlinzani, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia, Brescia, Italy

During 1995, 18 outbreaks of SVD have been recorded in Italy, their geographic distribution and chronological occurrence (shown by a progressive number) are shown in the annex 1.

Five outbreaks occurred in Northern Italy, 4 out of them in Mantua province (Lombardy) and 1 in Emilia-Romagna region. Thirteen outbreaks occurred in Southern Italy. As far as chronology is concerned, these outbreaks occurred within the period from January to March in Abruzzo, Molise, Campania and Emilia regions. The outbreaks in Lombardy occurred in April, June and July; the last two outbreaks occurred in November and December in Basilicata region.

In some cases the epidemiological correlation studies enabled to identify the primary outbreaks (bold-face character in the annex 1). In only 6 out of the 18 outbreaks SVD virus was isolated from epithelium and faeces; in the others lesions were absent and virus was found only in faeces. Outbreaks 1 to 9 and outbreak 11 in South Italy were correlated each other. The outbreak 95/10 in Campania region was connected with the 95/12 in Emilia Romagna region: the owner of the two different infected farms was the same and some pigs were moved from the southern to the northern farm.

The last two outbreaks in Basilicata region were correlated each other as well.

In Lombardy the first outbreak (95V3) was a case of reinfection regarding a big fattening herd in which SVD virus was isolated the first time in November 1994. Two months after the outbreak, when stamping-out, cleaning and disinfection procedures were accurately accomplished, 40 sentinel pigs were introduced. They were tested twice resulting always negative for the presence of SVDV antibodies. Nevertheless, when the farm was completely restocked in March 1995 with 3000 pigs, the disease appeared clinically. These pigs came from a seronegative herd.

The other 3 outbreaks in Lombardy were correlated. The first outbreak (95/14) was detected through serological surveillance on occasion of the eradication campaign. The first sampling in the herd was negative while the second one revealed seropositive pigs showing only IgM isotype. Epidemiological investigations following these outbreaks have been carried out. A final 117 herds (4032 sera) have been checked within the protection and surveillance zones to accomplish the tracing-back. Only 2 herds have been found infected, corresponding to the outbreaks 95/15 and 95/16. In these farms the owner and hands were the same. In the first one (95/15) SVD specific IgM and IgG isotypes were demonstrated and the SVD virus was isolated from epithelium and faeces. In the second one (95/16) only antibodies of IgG isotype were found at serological control and SVD virus was not isolated. Since the presence of only IgG isotype suggests a previous infection, the outbreak 95/16 was consider as a primary one.

The antigenic characterisation of SVDV isolates from the 18 outbreaks occurred in 1995 was not yet performed with the existing complete panel of Mabs but only with 3 selected Mabs: the first of them recognises all SVDV strains but the isolate Italy 1966. the second MAb recognises all European strains from 1992, while does not react with any of the previous ones, and the third MAb does not recognise the small group of italian strains from 1988 to 1991. All 18 SVDV isolates showed identical antigenic profiles, overlapping to the ones of other European strains occurred from 1992 to 1994.