

# Herd sensitivity in relation to test sensitivity in swine vesicular disease serological tests

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## Summary

After the swine vesicular disease (SVD) outbreaks in 1992 in the Netherlands a national monitoring programme was initiated, testing 12 samples from every pig farm three times per year. In this monitoring a slightly higher cut-off was used than the cut-off agreed on within the European community. The author has analysed the effect of this higher cut-off on the percentage of false positive and false negative results, using information on SVD antibody titres in sera obtained from the monitoring programme and the outbreaks in 1992 and 1994.

The number of false positive results was reduced by 63% when using the higher cut-off. On average the test sensitivity was reduced from 100% to 88%, resulting in a change of the average herd sensitivity from 91.7% to 91.5%, when testing 12 samples per farm. When three samples per farm were tested, the average herd sensitivity changed from 64.9% to 62.9%. The results further indicate that, in contrast to what is generally presumed, there is a relationship between test sensitivity and the prevalence of infection. The results clearly show that sample size is far more important in obtaining a high herd sensitivity than achieving a high test sensitivity.

## Keywords

Outbreak – Monitoring – Sensitivity – Serology – Specificity – Swine vesicular disease.

## Introduction

Swine vesicular disease (SVD) is on the World Organisation for Animal Health (OIE) list of infectious diseases. In the past, the reason for listing SVD was the similarity of the clinical signs of this disease to those of foot and mouth disease (FMD). However, SVD has a limited potential for very serious clinical disease or rapid spread in comparison with FMD. Laboratory diagnosis for SVD is therefore often based on serology followed by virological examination. Serology for SVD virus (SVDV) is therefore very important, and it was decided that in the European Union (EU) the virus neutralisation test should be used as the standard test with a cut-off equal or below the titre of European reference serum (ERS) RS 01-04-94 (5). The cut-off specified by the commission decision applies to the testing of individual pigs; in serosurveillance of herds, a test with lower sensitivity may be used because an

increased number of sera can be collected from each farm to compensate for any reduction in sensitivity.

In 1993, allegations that SVDV-infected pigs had been exported to Italy led to the implementation of a ban on the export of live pigs from the Netherlands to other EU member states (commission decision 93/128/EEC). In response to these export restrictions, the Netherlands introduced a national screening programme for SVDV antibodies. Until October 2004, 12 serum samples were collected and tested for antibodies against SVDV every four months on every pig farm in the Netherlands. These 12 randomly selected samples would enable the system to detect a SVDV serological prevalence of 22.1% with 95% confidence (100% sensitive test in a herd of infinite size). Such a prevalence of 22.1% or more was found on all infected farms that were detected by serology (farms identified as 92-03, 92-04, 92-06 and 96-02, see Table I).

**Table I**  
**Characteristics of the six infected farms from which samples were taken to analyse the sensitivity of the national swine vesicular disease monitoring programme in the Netherlands**

Infected farm	Date of detection	Type of farm	Herd size				Number of sera positive for SVDV	Sampling procedure
			Sows	Boars	Piglets	Fatteners		
92-01	3 July 1992	B	102	3	488	0	3/11	Selective
92-02	13 July 1992	B	92	1	397	11	9/124	All sows, some piglets
92-03	30 July 1992	F	0	0	0	352	20/69	Two pigs per pen
92-04	29 September 1992	B + F	264	2	± 1,000	20	157/272	All sows, and some piglets and fatteners
92-06	27 October 1992	F	0	0	0	365	87/365	All pigs
94-02	18 February 1994	F	0	0	0	22	17/22	All pigs

B: breeding farm

F: fattening farm

SVDV: swine vesicular disease virus

After more than ten years of intensive testing it was decided, at the beginning of 2005, that the level should be reduced to three random samples every four months.

In discussions about the minimal detection level for serological tests, a zero risk policy is often adopted, without acknowledging that a slightly higher cut-off has enormous advantages in minimising the number of false positive results, while hardly compromising the detection level of infected herds. This paper assesses the effect of two different cut-off levels on test specificity, test sensitivity and herd sensitivity, using data obtained during the monitoring programme and on farms infected with SVDV.

## Materials and methods

To evaluate the *specificity* of the monitoring system – a screening enzyme-linked immunosorbent assay (ELISA) (1) followed by a virus neutralisation test – the results of monitoring over the period from 1 January 2003 to 31 December 2004 were analysed.

To analyse the *sensitivity* of the monitoring system, sera from six SVDV-infected farms in the Netherlands (Table I) were analysed by means of the virus neutralisation test (farms 92-01, 92-03, 92-04, 92-06 and 94-02) or an indirect liquid phase blocking ELISA (farm 92-02) (2). The positive sera were divided into two categories: sera with a titre equal to or higher than the mean titre of the ERS, and sera with a titre higher than or equal to two times the mean titre of the ERS (Table II). In some farms sera were taken from all pigs, but in most cases only a proportion of the pig population was sampled. In the analysis, the result of this sample was considered to be representative of the whole pig population on the farm.

In this paper, the data have been used to calculate the probability of detecting infection on a farm in a serological screening programme using either 12 or three samples. For the calculation of this probability, a sample was assumed to be either from an infected swine (chance = prevalence) or from a non-infected swine (chance = 1 – prevalence). The probability that a test on a positive sample will produce a negative result is 1 – sensitivity; the probability that a negative sample will give a negative result is equal to the specificity. Therefore, the probability of detection in an infinite population with a known prevalence of infected swine is 1 – the probability of finding only negative test results:

$$\text{Pr (detection)} = 1 - [\text{prevalence} \times (1 - \text{sensitivity}) + (1 - \text{prevalence}) \times \text{specificity}]^n$$

where n is the sample size.

For the calculation in Table II, the author used a sensitivity of 97.6% for the screening ELISA, which was the level previously determined by Chénard *et al.* (1). For assessing the specificity of the screening programme, the data obtained between 1 January 2003 and 21 December 2004 were used.

To test whether the prevalence on the various farms was equal, a comparison was made of the number of positive and negative samples on each farm, using the Chi square test. The relation between antibody titre of sera with a titre equal to or higher than the titre of the ERS, and an infected farm was analysed by analysis of variance (ANOVA) using titre as the response variable and farm as the explanatory variable. The ANOVA was followed by a multi-comparison analysis using simulation. A standard linear regression analysis was performed on the positive antibody titres and

**Table II**  
**Results of serological tests and probability of detection**

Farm	Number of samples tested	Number of sera with titre		Percentage of sera with titre		Probability of detection			
		≥ ERS	≥ 2 × ERS	≥ ERS	≥ 2 × ERS	12 samples per farm		3 samples per farm	
						≥ERS	≥2 × ERS	≥ERS	≥ 2 × ERS
92-01	11	3	3	27.3%	27.3%	97.6%	97.6%	60.5%	60.5%
92-02	124	9	9	7.3%	7.3%	58.8%	58.7%	19.9%	19.8%
92-03	69	20	20	29.0%	29.0%	98.2%	98.2%	63.2%	63.1%
92-04	272	157	130	57.7%	47.8%	100.0%	99.9%	91.7%	84.8%
92-06	365	87	81	23.8%	22.2%	95.9%	94.7%	54.9%	51.9%
94-02	22	17	15	81.0%	71.4%	100.0%	100.0%	98.5%	96.3%
Mean						91.7%	91.5%	64.9%	62.9%

ERS: European reference serum

prevalence. All statistical analyses were performed in S-Plus®.

## Results

In the period from 1 January 2003 to 31 December 2004, 589,397 sera were tested in the monitoring programme. Resampling the pigs that were found positive together with pen-mates and pigs from adjacent pens showed no evidence of SVDV infection in the Netherlands. The sera reacting positive have therefore to be considered false positive. Of the sera tested in this period, 306 (0.05%) had a titre equal to or higher than the titre of the ERS and 113 (0.02%) had a titre more than or equal to two times the ERS. This reduction of 193 results shows that a slight increase of the cut-off reduces the number of false positive results by 63%. The specificities of the monitoring system were 99.5% when the titre of the ERS was used as cut-off, and 99.8% when the cut-off was equal to two times the titre of the ERS.

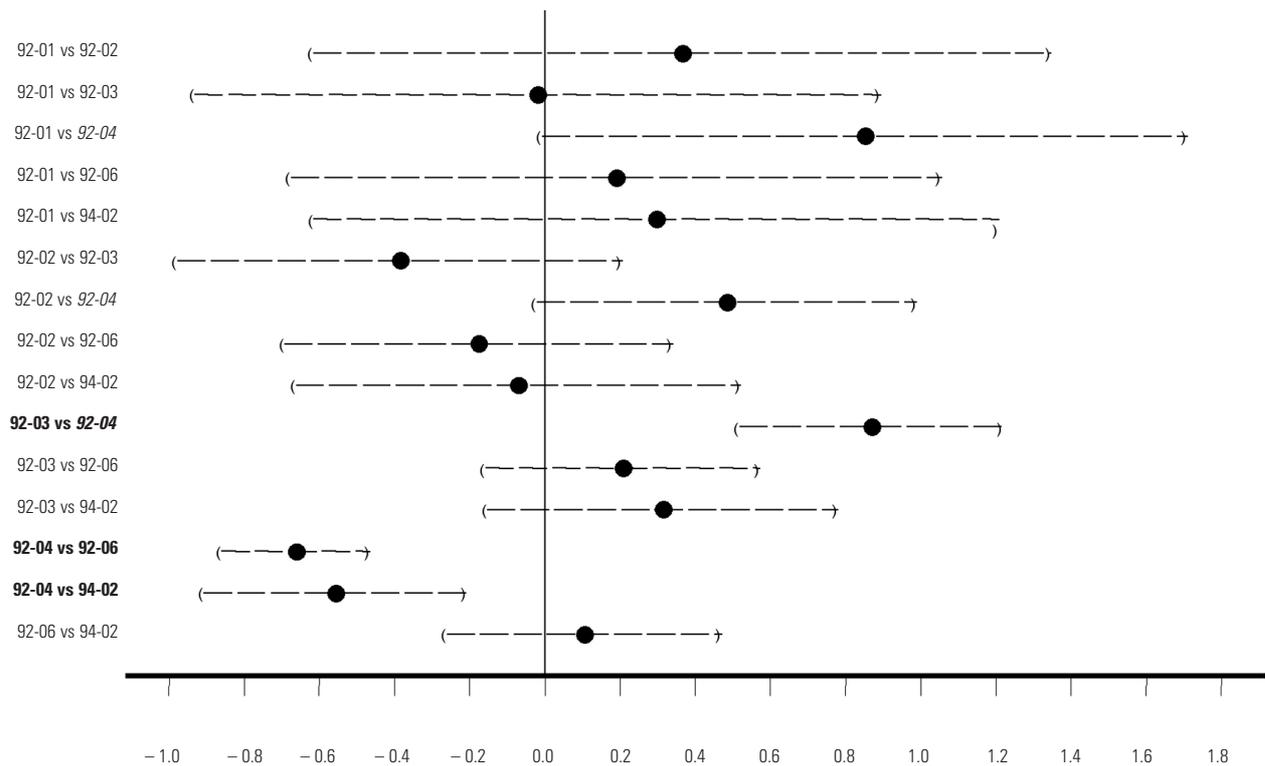
Among the sera collected on outbreaks that had a titre equal to or higher than the ERS, 12% had a titre equal to or 1.5 times as high as the ERS. This indicates that changing the cut-off to a titre equal to two times the titre of the ERS will reduce the sensitivity of the serological test from 100% (the gold standard defined by the ERS) to 88%. However, the probability of identifying infected farms by serological testing of 12 samples when using the high cut-off (≥ 2 × ERS) was on average 91.5%, which was only 0.2% lower than when the EU defined cut-off was used (91.7% at ≥ ERS in Table II). In fact the probability of detection in five out of the six farms was close to 95% or higher when 12 samples were tested, regardless of the cut-off used. When the number of samples was reduced to three, the probability of detection decreased as would be expected, but in most cases was still above 50%. Increasing

the cut-off reduced the probability of detection on average by only 2%.

Using the Chi square test a significant difference was found in the number of positive and negative samples on the different infected farms ( $p < 0.01$ ), indicating that prevalence varied from farm to farm. When comparing the antibody titres of the sera with a titre equal to or higher than the titre of the ERS, significant differences were found among the various farms ( $p < 0.01$ ). Figure 1 shows the average difference, and simulation-based 95% confidence interval, between positive antibody titres between the various farms. In three comparisons a significant difference was seen; farm 92-04 had significantly lower antibody titres than farms 92-03, 92-06 and 94-02. The 95% confidence interval of the difference between antibody titre of the positive sera from farm 92-04 and those of farms 92-01 and 92-02 only just includes zero, indicating that these differences were also almost significant. Regression analysis on the sera with a titre equal to or higher than the ERS is shown in Figure 2. There is an inverse relation between antibody titre and prevalence ( $R$ -squared = 0.15) with a slope significantly ( $p < 0.01$ ) different from zero.

## Discussion

The aim of this study was to evaluate the probability of identifying SVD-infected farms by means of serology when a slightly higher cut-off was used. The big advantage of using this higher cut-off is a 63% reduction in false positive reactions. The probability of identifying the infected farms in a monitoring programme sampling 12 pigs was close to 95%, and higher in five of the six infected farms studied, irrespective of the cut-off used. Only farm 92-02 would have been missed in approximately 41% of the cases, but this farm was only recently infected, as a previous study had shown (3), and had a low serological prevalence.



**Fig. 1**  
Average difference and 95% confidence limits between positive antibody titres on the different infected farms, simulation-based method

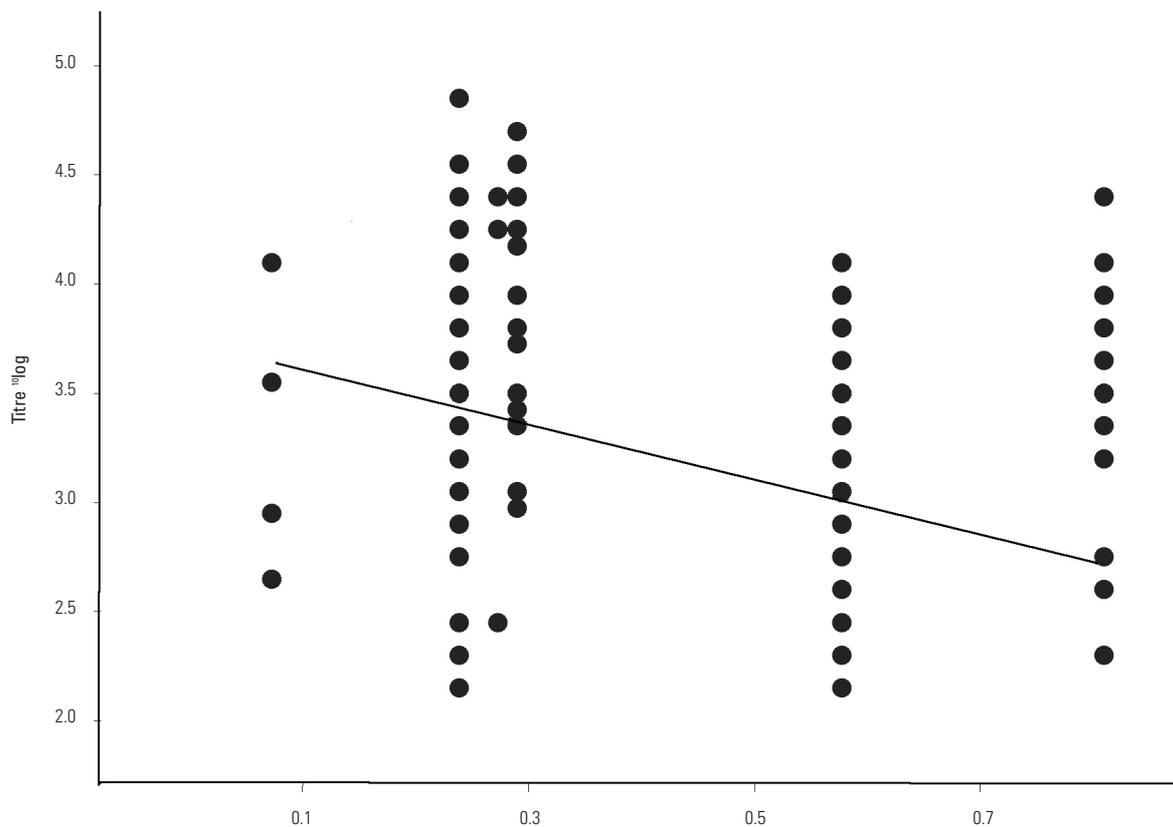
If zero is not included (farm identity in bold), the difference is statistically significant

Changing from 12 to three samples has enormous consequences for the probability of detecting SVDV infection. The fact that no infected farms have been detected in more than ten years shows that the hygienic control measures implemented on animal transport vehicles are effective. A reduction in the frequency of sampling was therefore reasonable. The 12 samples are still collected every four months, for Aujeszky's disease monitoring, but only three random samples are tested for SVDV antibodies. This will result in a slower detection of infection on farms if SVDV is introduced. However, pigs can also be sampled at slaughter, and there the pigs that have been on the farm for the longest time and that have the highest chance of being infected will be sampled, thus increasing the chance of detection.

The probability of identifying infected farms in a serological monitoring programme that samples 12 pigs was on average only reduced by 0.24% when a higher cut-off was used. This is lower than would have been expected (1.7% reduction) if the observed prevalence (prevalence times test sensitivity) had been used in the calculation. This indicates that the test sensitivity on the different infected farms varied.

Table II shows that in just one case (infected farm 92-04) the number of positive samples at a high cut-off (130) was much lower than would be expected (prevalence times sensitivity:  $157 \times 0.88 = 138$ ). This indicates that there might be a relation between the prevalence found on the different infected farms and the antibody concentration in the samples, and consequently between prevalence and the test sensitivity on a herd level. Not only were the prevalences found on the different infected farms significantly different, but also the antibody titres found on infected farm 92-04 were significantly lower than the titres found on infected farms 92-02, 92-03 and 92-06.

Given these results, an analysis of whether there was a linear relation between prevalence and antibody titre gives interesting results. Although the slope differed significantly from zero, only 15% of the variation in the data could be explained by the model. Therefore, the contact structure, timing of infection in relation to sampling, weight of challenge, virulence of the viral strain, breed, age and susceptibility of the hosts can be assumed to be different among the various infected farms. These factors might influence both the prevalence and antibody titre. The inverse relation found between prevalence and antibody



**Fig. 2**  
**Prevalence on infected farms in relation to sera with a titre equal to or higher than the titre of the European reference serum ( $2.15^{10}\log$ )**

titre is probably based on a confounding rather than a direct relationship.

For a monitoring programme, the herd-level sensitivity is more important than the animal-level sensitivity. The results show that reduction of herd-level sensitivity was minimal although the change in cut-off reduced the test sensitivity from 100% to 88%. Any reduction in herd sensitivity would be small, as could be calculated using the formulae given by Martin *et al.* (4). However, the reduction in herd sensitivity found in the infected farms was even smaller if calculated by using the observed prevalence; this was because the distribution of positive sera was not the

same on all infected farms. The assumption that prevalence and test sensitivity are independent is therefore not true here. This is probably also the case in other diseases. When evaluating tests for animal disease control programmes too much emphasis is often mistakenly placed on sensitivity alone. The purpose of the programme, herd size and sample size are also important. Even for screening 'individual animals' for the purposes of international trade, the highest degree of sensitivity is unnecessary where such individuals are actually part of groups.



## Rapport entre la sensibilité du troupeau et la sensibilité du test dans le cadre des épreuves sérologiques de détection de la maladie vésiculeuse du porc

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### Résumé

Un programme national de surveillance a été lancé aux Pays-Bas à la suite de l'apparition de foyers de maladie vésiculeuse du porc en 1992. Dans le cadre de ce programme qui prévoyait le prélèvement trois fois par an dans chaque élevage de 12 échantillons destinés à être testés, la valeur seuil utilisée était légèrement supérieure à celle convenue au sein de la Communauté européenne. L'auteur analyse l'effet de cette augmentation de la valeur limite sur le pourcentage de faux positifs et de faux négatifs, en utilisant les données sur les titres d'anticorps dirigés contre le virus de la maladie vésiculeuse du porc dans les sérums obtenus dans le cadre du programme de surveillance et des foyers survenus en 1992 et en 1994.

Le nombre de résultats faussement positifs était réduit de 63 % en utilisant une valeur seuil supérieure. En moyenne, la sensibilité de l'épreuve est passée de 100 % à 88 %, ce qui a entraîné une modification de la sensibilité moyenne du troupeau, laquelle est passée de 91,7 % à 91,5 %, lorsque 12 prélèvements par élevage étaient étudiés et de 64,9 % à 62,9 % lorsque 3 prélèvements par exploitation étaient testés. Les résultats confirment que, contrairement à ce que l'on suppose généralement, il existe un rapport entre la sensibilité de l'épreuve et la prévalence de l'infection dans toutes les exploitations. Les résultats indiquent clairement que la taille des échantillons est beaucoup plus importante pour obtenir une sensibilité élevée du troupeau que pour parvenir à une sensibilité élevée de l'épreuve.

### Mots-clés

Foyer – Maladie vésiculeuse du porc – Sensibilité – Sérologie – Spécificité – Surveillance.



## Sensibilidad de los rebaños en relación con la sensibilidad de las pruebas serológicas para la enfermedad vesicular porcina

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### Resumen

Después de los brotes de enfermedad vesicular porcina ocurridos en 1992 en los Países Bajos, se puso en marcha un programa nacional de vigilancia conforme al cual se analizaban 12 muestras de cada explotación porcina tres veces al año. En dichos análisis se utilizó un valor umbral ligeramente superior al convenido en el seno de la Unión Europea. El autor ha estudiado los efectos de esa diferencia sobre el porcentaje de falsos positivos y falsos negativos, utilizando para ello los datos sobre títulos de anticuerpos obtenidos gracias al programa de vigilancia y durante los brotes de 1992 y 1994.

El uso de un valor umbral más alto redujo en un 63% el número de falsos positivos. En promedio, la sensibilidad de la prueba pasó del 100% al 88%, lo que

indujo un cambio en la sensibilidad media de los rebaños, que pasó del 91,7% al 91,5% al analizar 12 muestras por granja. Cuando en lugar de 12 se analizaron tres muestras por granja, la sensibilidad media de los rebaños pasó del 64,9% al 62,9%. Los resultados indican que, a diferencia de lo que suele pensarse, existe una relación entre la sensibilidad de la prueba y la prevalencia de la infección en una granja determinada. Además, ponen claramente de manifiesto que el tamaño de la muestra es mucho más importante para obtener una elevada sensibilidad de los rebaños que para incrementar la sensibilidad de las pruebas.

**Palabras clave**

Brote – Enfermedad vesicular porcina – Especificidad – Sensibilidad – Serología – Vigilancia.



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