

## NO SEROLOGICAL EVIDENCE FOR THE PRESENCE OF SWINE VESICULAR DISEASE VIRUS IN SOUTH AFRICA

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### ABSTRACT

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An indirect ELISA incorporating a protein A-peroxidase conjugate was developed for detecting antibodies to swine vesicular disease virus (SVDV) in pig sera. This test and a conventional virus neutralization test were found to be equally sensitive. A total of 2846 pig sera collected from various abattoirs in South Africa were tested using the indirect ELISA. No serological evidence of infection with SVDV in pigs in South Africa was found.

### INTRODUCTION

Swine vesicular disease (SVD) is a vesicular disease of pigs which was first isolated in Lombardy (Italy) in 1966 and described by Nardelli, Lodetti, Gualandi, Burrows, Goodridge, Brown & Cartwright (1968). The disease is caused by an enterovirus which produces vesicular lesions in pigs that are clinically indistinguishable from foot-and-mouth disease.

Although no clinical SVD has been reported in South Africa, the possibility that the virus is present in a subclinical form cannot be excluded since subclinical infections have been recorded (Burrows, Mann & Goodridge, 1974; Donaldson, Ferris, Knowles & Barnett, 1983). Countries importing pig products often required the exporter to prove the absence of infection in the country of origin and sometimes do not accept freedom from clinical disease as proof. Serological surveillance is therefore usually required to substantiate freedom from infection.

In this study pig sera from various abattoirs in South Africa were collected and tested in an indirect ELISA for antibodies to SVDV.

### METHODS AND MATERIALS

#### *Virus and cells*

The SVDV isolate UKG 27/72 was received from the Institute for Animal Health, Pirbright, UK. The virus was grown in monolayers of IB-RS-2 cells using Vac 1 medium (supplemented with 2 % normal bovine serum), which is a modification of Eagle's minimal essential medium.

#### *Preparation of antigen*

IB-RS-2 cells were grown in 1 ℓ glass bottles which were rolled at 37 °C. Confluent monolayers were washed with phosphate buffered saline (PBS), inoculated with 5 ml of virus stock and the cultures rolled for 1 h at 37 °C. The inoculum was then poured off and 80 ml of maintenance medium added. When 100 % cytopathic effect (CPE) occurred, the cell culture fluid was harvested and clarified by centrifugation at 1 800 g for 30 min.

The virus was concentrated by the addition of 50 % saturated  $(\text{NH}_4)_2\text{SO}_4$ , and centrifuged at 1 800

g for 30 min. The resulting precipitate was resuspended in PBS and centrifuged at 90 000 g for 2 h. The pellets were resuspended in 1 ml PBS and layered onto continuous 15-45 % sucrose gradients. These were centrifuged at 140 000 g for 3 h. Peak fractions of virus, scanned at 254 nm with a UV monitor, were collected and stored in aliquots at -70 °C.

#### *Positive control serum*

An adult pig, housed in the isolation stables of the Foot-and-Mouth Disease Laboratory at Onderstepoort, was first pre-bled and then inoculated intramuscularly with 1,0 ml UKG 27/72. The pig was bled 21 days later and the serum separated and stored in aliquots at -20 °C.

#### *Study sera*

Between October 1986 and June 1988 sera from baconers or porkers were collected from various abattoirs in the main pig producing areas of South Africa. The provinces, districts or towns as well as the number of farms from which these sera originated are given in Table 1. The objective was to test 10 sera from each pig farm in the survey.

#### *Virus neutralization (VN) test*

VN tests were carried out in flat-bottomed tissue culture-grade microtitre plates using IB-RS-2 cells according to the method described by Golding, Hedger & Talbot (1976). Serum end-point titres were expressed as the log reciprocal of the dilution which protected 50 % of cultures from  $10^{2.0 \pm 0.5}$  of virus (Karber, 1931).

#### *Preparation of conjugate*

A protein A-peroxidase conjugate was prepared according to the method described by Pain & Suroli (1981). The conjugate was stored in aliquots at -70 °C.

#### *Indirect ELISA*

The indirect ELISA was performed in flat-bottomed plates<sup>1</sup>. SVDV antigen was diluted in carbonate/bicarbonate buffer at pH 9,6 to contain 5 µg/ml of antigen. This was adsorbed to the wells of the plates overnight at 4 °C. Plates were washed in PBS containing 0,05 % Tween 20, which was the washing buffer used throughout. Coated plates were

stored for a period of up to one month at  $-20^{\circ}\text{C}$  until used.

Test sera were diluted 1/16 in PBS containing 0,5 % casein. This single dilution of each serum was then added to duplicate wells. On each microplate, positive and negative control sera that were similarly diluted were included. After addition of sera plates were incubated for 1 h at  $37^{\circ}\text{C}$ , washed and the conjugate, diluted 1/3000 in PBS with 0,5 % casein, was added to all wells followed by incubation for 1 h at  $37^{\circ}\text{C}$ . After a further wash the substrate (o-phenylenediamine in citrate buffer) was added to all wells and the colour allowed to develop for 15 min, after which time the reaction was stopped by the addition of 1,25 M  $\text{H}_2\text{SO}_4$ .

The degree of colour development was recorded with a Titertek Multiscan at 492 nm.

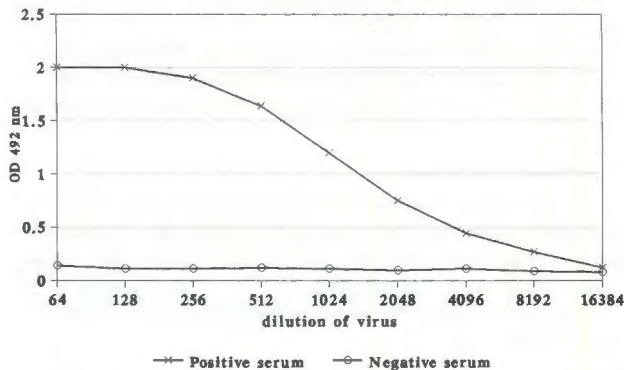


FIG. 1 Titration of a pig antiserum to SVDV using an indirect ELISA

RESULTS

Fig. 1 shows the reactions of the positive and negative control sera in the indirect ELISA. In this test the titre of the positive control serum was taken as that dilution of the serum that gave a reading at least twice that of the negative control serum. Using this criterion the titre of the positive control serum was calculated as  $\log_{10} 3,6$ . By comparison, the neutralizing antibody titre of the serum was  $\log_{10} 3,4$ .

All 2846 pig sera collected at abattoirs and tested in the indirect ELISA were negative for SVDV antibodies, i.e. titres were equal to or less than  $\log_{10} 1,2$ .

TABLE 1 Localities of abattoirs in South Africa from which pig sera were collected, 1986-1988

Province	District or town	No. of farms	No. of sera
Natal	Estcourt	111	1 228
Cape Province	Southern Cape	57	581
Transvaal	Heidelberg	37	348
	Potgietersrus	4	40
	Pietersburg	49	364
	Vereeniging	26	285
Total		284	2 846

DISCUSSION

Armstrong & Barnett (1989) used a liquid-phase blocking sandwich ELISA for testing pig sera for SVDV antibodies. Their results correlated closely with those obtained by virus neutralization. In this investigation an indirect ELISA which produced titres with a positive serum that closely correlated with the VN test results, was used. The indirect ELISA has the advantage that it is a simpler and more rapid test.

The total pig population of South Africa is in the region of 1,2 million (Directorate of Animal Health, 1991). Of these 90,4 % occur in the three provinces included in this survey (Table 1). From these a total of 2846 sera were collected from 284 farms. The Orange Free State (with only 9,6 % of the pigs in the country) was not included.

It could be argued that this represents a rather small sample of the total pig population of South Africa. On the other hand, observations made in the field in the United Kingdom indicated that SVD is a highly infectious disease of pigs with morbidity rates as high as 80 % being reported (Dawe, Forman & Smale, 1973; Burrows *et al.*, 1974). For this reason it is unlikely that infection could be present in South Africa without either serological evidence of its presence being found or the appearance of clinical disease. It is therefore concluded that there is no evidence for the presence of SVDV in South Africa.

REFERENCES

ARMSTRONG, R. M. & BARNETT, I. T. R., 1989. An enzyme-linked immunosorbent assay (ELISA) for the detection and quantification of antibodies against swine vesicular disease virus (SVDV). *Journal of Virological Methods*, 25, 71-80.

BURROWS, R., MANN, J. A. & GOODRIDGE, D., 1974. Swine vesicular disease: virological studies of experimental infection produced by the England/72 virus. *Journal of Hygiene*, 72, 135-143.

DAWE, P. S., FORMAN, A. J. & SMALE, C. J., 1973. A preliminary investigation of the swine vesicular disease epidemic in Britain. *Nature*, 241, 540-542.

DIRECTORATE OF ANIMAL HEALTH, 1991. Livestock census (March 1991). *Department of Agriculture, Republic of South Africa*.

DONALDSON, A. I., FERRIS, N. P., KNOWLES, N. J. & BARNETT, I. T. R., 1983. Comparative studies of United Kingdom isolates of swine vesicular disease virus. *Research in Veterinary Science*, 35, 295-300.

KARBER, G., 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Archiv für experimentelle Pathologie und Pharmakologie*, 162, 480-483.

GOLDING, S. M., HEDGER, R. S. & TALBOT, P., 1976. Radial immunodiffusion and serum neutralization techniques for the assay of antibodies to swine vesicular disease. *Research in Veterinary Science*, 20, 142-147.

NARDELLI, L., LODETTI, E., GUALANDI, G. L., BURROWS, R., GOODRIDGE, D., BROWN, F. & CARTWRIGHT, B., 1968. A foot-and-mouth disease syndrome in pigs caused by an enterovirus. *Nature*, 219, 1 275-1 276.

PAIN, D. & SUROLIA, A., 1981. Preparation of protein A-peroxidase monoconjugate using a heterobifunctional reagent, and its use in enzyme immunoassay. *Journal of Immunological Methods*, 40, 219-230.