Acta vet. scand. 1980, *21*, 318—323.

From the State Veterinary Institute for Virus Research, Lindholm, Kalvehave, Denmark.

A COMPARATIVE INVESTIGATION OF ANTIBODY TO SWINE VESICULAR DISEASE VIRUS USING COUNTER IMMUNO-ELECTROPHORESIS, SERUM NEUTRALIZATION AND DOUBLE IMMUNODIFFUSION TESTS

By

K. J. Sørensen

SØRENSEN, K. J.: *A comparative investigation of antibody to swine vesicular disease virus using counter immunoelectrophoresis, serum neutralization and double immunodiffusion tests.* Acta vet. scand. 1980, *21*, 318—323. — A counter immunoelectrophoresis test (CIET) for assay of antibody to swine vesicular disease virus (SVDV) is described and compared with the serum neutralization test (SNT) and the double immunodiffusion test (DIDT). Using a preparation of complete or empty virus particles as antigen the CIET was found to be more sensitive than the DIDT. The CIET is rapid and economic, and although less sensitive than the SNT it was suggested as an initial large scale screening test for SVD antibody.

SVD antibody; pig; counter immunoelectrophoresis; serum neutralization; double immunodiffusion.

Swine vesicular disease (SVD) has been diagnosed in several European countries as well as in Hong Kong and Japan since its first appearance in Italy in 1966 (*Brooksby* 1975).

The disease has so far never occurred in Denmark, and for confirmation of this disease free status, serum surveys are useful.

Double immunodiffusion tests (*Pereira et al.* 1976), serum neutralization tests and radial immunodiffusion tests with radioactivity labelled antigen (*Golding et al.* 1976) have been applied for assay of SVD antibody. In the present work the counter immunoelectrophoresis test (CIET) is compared with the serum neutralization test (SNT) and the double immunodiffusion test (DIDT).

MATERIALS AND METHODS

Counter immunoelectrophoresis test (CIET)

a) *Antigen.* Preparations of either complete or empty SVD virus particles of the strain UKG 72 were used as antigen. Growth and purification were performed as previously described (*Sørensen* 1977) with some modifications: The virus was grown on monolayers of IBRS-2 cells in 1 l roller bottles. After harvest, $MgCl_2$- and chloroform treatment an equal volume of a 13 % aqueous solution of polyethylene glycol (PEG 6000) was added to the virus suspension. After stirring for at least 1 h on icebath the suspension was centrifuged for 10 min at 20,000 × g. The precipitate was resuspended in 2 ml of a 0.05 M Tris-HCl buffer, pH 7.5, with 0.05 M-$MgCl_2$ by stirring overnight at 4°C, followed by sonication for 15 s (MSE 150 Watt amplitude 10 microns). After clarification by centrifugation for 10 min at 20,000 × g the supernatant was layered as 0.3 ml samples on 4.7 ml gradients of sucrose (approx. 13.5 %—30.5 %). The gradients were formed by freezing (—20°C) a 22 % (w/w) sucrose in 0.05 M Tris-HCl buffer, pH 7.5, with 0.05 M-$MgCl_2$ followed by thawing at 4°C overnight.

Centrifugation was carried out in a Spinco SW 50 rotor for 40 min at 45,000 r.p.m. and 4°C. The gradients were displaced upwards through a measuring cell by a 40 % sucrose solution, and the extinction profiles were recorded at 260 nm. The slower and the faster sedimenting fractions, i.e. the fractions containing empty and complete virus particles, respectively, were collected.

b) *Technique.* The CIET was carried out on 10×10 cm glass plates in a 1.5 mm thick 1 % agarose gel (LSA, Litex Agarose, Denmark) containing 12 % PEG 200 (Hoechst). As buffer served a barbital buffer, pH 8.0, ionic strength 0.02. Parallel rows of wells 3 mm in diameter and 6 mm apart were punched in the gel. Antigen in 15 µl volumes was applied to the cathode wells and similar volumes of 2-fold serum dilutions to the anode wells. Electrophoresis was performed for 1 h at 5 V/cm. After washing overnight in 0.1 M-NaCl the plates were stained with Coomassie Brilliant Blue®. Titers were expressed as the reciprocal value of the highest serum dilution giving a detectable precipitin line. For each batch of antigen the optimal antigen dilution was determined by chessboard titration.

Serum neutralization test

The SNT was performed in flat-bottomed tissue culture grade microtitre plates using IBRS-2 cells with a modification of the test described by *Sullivan & Rosenbaum* (1967): The sera were inactivated at 56°C for 30 min before use. With a 0.05 ml dropper pipette 1 drop of 2-fold dilutions was placed in each of 4 wells. One drop of virus suspension in cell growth medium containing 100 TCID50 was then added to each well, and the mixtures were incubated for 1 h at 37°C. Finally 1 drop of the cell suspension (2×10^5 cells per ml) was added to each well, and the plates were sealed with cellulose sealing tape. Incubation was performed at 37°C. Controls in each test consisted of

a titration of a homologous antiserum of known titer, cell controls and a titration of the test virus, from which the actual amount of virus used in the test was calculated. Results were read microscopically. Wells with CPE comprising more than 50 % of the monolayer were considered as infected. Neutralization titers were expressed as the reciprocal value of the serum dilution at the 50 % endpoint calculated by the method of Spearman-Kärber (*Daugherty* 1964).

Double immunodiffusion test

DIDT was performed as described by *Pereira et al.* (1976).

Sera

Pig antisera were obtained by inoculating 4 pigs, Danish landrace of about 50 kg body weight, intravenously with about 10^8 TCID50 of the UKG 72 virus strain propagated in IBRS-2 cells grown in medium supplemented with 10 % pig serum and maintained in serum free medium. Serum samples were collected at different days after infection, pooled and stored at —20 °C until use.

RESULTS

The CIET gave distinct precipitin lines facilitating clear cut endpoint determinations. In Table 1 the titers obtained with 8 pooled serum samples collected at different days after infection of pigs with SVDV as measured by the CIET, the DIDT and the SNT are shown. It appears that in the CIET higher serum titers

Table 1. CIET, DIDT and SNT antibody titers of pooled sera collected at 8 different days after inoculation of 4 pigs with SVDV.

Days after inoculation	CIET titers		DIDT titers	SNT titers
	antigen			
	empty virus particles	complete virus particles		
2	13	3	n.d.*	69
3	32	15	2	259
4	85	24	6	511
9	192	64	12	2620
24	128	64	8	2202
82	43	16	6	2835
109	53	24	10	4840
190	53	16	10	4695

Each titer value represents the mean of at least 2 determinations.
* not detectable.

were obtained with the antigen preparation containing empty particles than with complete particles.

With both antigen preparations antibody was detectable in the serum samples, collected on the 2nd day after infection. The experiment was terminated 190 days after infection, at which time antibody could still be detected. Using the DIDT lower titers were obtained, and the antibody response was first detectable on the 3rd day after inoculation. The differences between the values obtained by the DIDT and the CIET using complete particles as antigen in the CIET are significant ($P < 0.05$ t-test, *Croxton* 1959). The SNT gave higher titer values than the other tests employed. The differences between the values obtained by this test and by the CIET using empty particles as antigen are significant ($P < 0.05$, t-test, *Croxton*). Titration of a sample on 5 different days by the CIET using complete particles as antigen gave reproducible results (mean titer 58, standard deviation 14).

DISCUSSION

The availability of rapid and sensitive tests is of great importance, when extensive serological surveys have to be performed. The SNT is a sensitive test for demonstration of antibody to SVDV. However, it is rather labourious and time consuming. *Golding et al.* (1976) found the radial immunodiffusion test to be convenient and economic. *Pereira et al.* (1976) found the DIDT to be less sensitive than the SNT in detecting SVD antibody. However, they stated that in a survey of pig farms only a small proportion of those, on which the disease had existed, was likely to be missed, when the DIDT was used alone. Thus, the DIDT has been used as an initial screening test in serum surveys for SVD (*Hendrie et al.* 1977) and as a supporting test for the interpretation of positive and inconclusive results obtained with other tests (*Watson & Hedger* 1974, *Golding et al.*, *Hendrie et al.* 1978).

The CIET used in the present work proved to be more sensitive than the DIDT, and the results were found to be reproducible.

As the antigen preparation containing empty particles gave higher titers than the preparation with complete virus particles in the CIET, the former was expected to be the antigen of choice. However, in preliminary tests with sera from pigs which were not infected with SVDV, it was found that the fraction of empty

particles reacted with most of the samples, i.e. it probably cross-reacted with antibody to other enteroviruses, whereas the fraction of complete particles did not. Similarly, *Schmidt et al.* (1963) and *Jansen & Maas* (1973), working with coxsackie B5 viruses which are serologically related to the SVD viruses (*Graves* 1973), found that the preparation of empty particles reacted with heterologous antibody in human convalescent-phase serum, whereas the preparation of complete particles did not. Thus the complete particles of SVDV were preferred as antigen.

As the CIET is a rapid and economic test, and as it was found reasonably sensitive, it was suggested as an initial large scale screening test for SVD antibody.

REFERENCES

Brooksby, J. B.: Swine vesicular disease: History of the disease. Vet. Rec. 1975, *95*, 108.

Croxton, F. E.: Elementary Statistics with Applications in Medicine and the Biological Sciences. Dover Publications, Inc., New York 1959, 209—244.

Daugherty, R. M.: Animal titration techniques. *In* R. J. C. Harris (ed.): Techniques in Experimental Virology. Acad. Press Inc., New York 1964, 169—220.

Golding, S. M., R. S. Hedger, P. Talbot & J. Watson: Radial immunodiffusion and serum-neutralization techniques for the assay of antibodies to swine vesicular disease. Res. vet. Sci. 1976, *20*, 142—147.

Graves, J. H.: Serological relationship of swine vesicular disease and coxsackie B5 viruses. Nature (Lond.) 1973, *245*, 314—315.

Hendrie, E. W., J. Watson, R. S. Hedger, L. W. Rowe & Y. J. Garland: Swine vesicular disease: Continuing serological surveys of pigs presented for slaughter in the United Kingdom. Vet. Rec. 1977, *100*, 363—365.

Hendrie, E. W., K. Baker, R. Hedger, G. Davies & M. S. Richards: Swine vesicular disease: Serum surveys 5 and 6. Vet. Rec. 1978, *102*, 126—127.

Jansen, P. & G. Maass: Nachweiss präzipitierende Antikörper gegen Coxsackie virus B5 mit Hilfe der Acetatfolienelektrophorese. (The demonstration of precipitating antibodies against coxsackie virus type B5, using acetate foil electrophoresis). Zbl. Bakt., I. Abt. Orig. A 1973, *224*, 309—315.

Pereira, H. G., L. Rowe & D. Baber: Use of double immunodiffusion (Ouchterlony) test for the diagnosis of swine vesicular disease. Res. vet. Sci. 1976, *20*, 139—141.

Schmidt, N. J., J. Dennis, L. H. Frommhagen & E. H. Lenette: Serological reactivity of certain antigen obtained by fractionation of coxsackie viruses in cesium cloride density gradients. I. Immunology 1963, *90*, 654—662.

Sullivan, E. J. & M. J. Rosenbaum: Methods for preparing tissue culture in disposable microplates and their use in virology. Amer. J. Epidem. 1967, *85*, 424—437.

Sørensen, K. J.: A comparative electrophoretic examination of swine vesicular disease virus and coxsackie B5 virus. Arch. Virol. 1977, *53*, 235—241.

Watson, J. & R. S. Hedger: Swine vesicular disease: A serological survey of pigs presented for slaughter. Vet. Rec. 1974, *95*, 535.

SAMMENDRAG

En sammenlignende undersøgelse for antistof mod swine vesicular disease virus ved hjælp af counter immunoelektroforese, serum neutralisations- og dobbelt immunodiffusionstest.

Der er til bestemmelse af antistof mod SVD virus beskrevet en counter immunoelektroforesetest (CIE test), som endvidere er sammenlignet med en serum neutralisationstest (SN test) og en dobbelt immunodiffusionstest (DID test). CIE testen fandtes under anvendelse af en præparation af enten komplette eller tomme virus partikler som antigen mere følsom end DID testen. CIE testen er hurtig og økonomisk, og skønt mindre følsom end SN testen skønnes den egnet som en indledende screening test.

(Received February 21, 1980).

Reprints may be requested from: K. J. Sørensen, the State Veterinary Institute for Virus Research, Lindholm, DK-4771 Kalvehave, Denmark.