

Pseudorabies and transmissible gastroenteritis: a serological survey in South Africa

R. WILLIAMS¹, J.J. ESTERHUYSEN² and J.T.R. ROBINSON³

ABSTRACT

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Two specific and sensitive, indirect enzyme-linked immunosorbent assays (ELISAs) utilizing a protein G-peroxidase conjugate were developed to detect antibodies to the pseudorabies virus (PRV) and the transmissible gastroenteritis virus (TGEV) in pig sera. Sera from 5,337 pigs, obtained from various abattoirs in South Africa, were tested with both ELISAs. No serological evidence of infection with either PRV or TGEV was found in any of the pigs tested.

INTRODUCTION

Pseudorabies (Aujeszky's disease, mad itch, infectious bulbar paralysis) is an infectious viral disease (Aujeszky 1902) affecting many animal species and occurring in most European countries as well as North and South America (Timoney, Gillespie, Scott & Barlow 1988). This disease, caused by suid herpesvirus 1 (SHV-1), occurs naturally in swine, and as an incidental infection in cattle, sheep, goats, dogs, cats, chickens, mink, rats and racoons, and is highly fatal in all but adult swine. The virus persists in recovered swine as a latent infection, and under certain conditions of stress the virus is reactivated and transmitted to susceptible animals by nasal secretions, saliva, milk, placenta, and also by boars during service (Crandell 1981).

Transmissible gastroenteritis (TGE), first reported in the USA by Doyle & Hutchings (1946), is caused by a coronavirus (TGEV) and is a highly contagious, enteric disease of swine, causing high mortalities in piglets under two weeks of age (Hogg 1982; Underdahl & Torres-Medina 1981). Although swine of all ages are susceptible, most animals over five weeks recover, and in adult swine the disease is often inapparent or mild. Normal transmission occurs by the faecal-oral route, although the respiratory route and air-borne transmission may also be of importance. TGE was first reported in the USA and subsequently in England, other European countries, Japan, Taiwan, Central and South America, and Canada (Timoney *et al.* 1988).

No clinical evidence of either pseudorabies or TGE has yet been reported in South Africa. To substantiate the claim of a national pig population free from PRV and TGEV infection, the South African Meat Board initiated a serological survey in which a total of 5337 pig sera—representing approximately 0,5% of the national pig herd—were collected from various abattoirs throughout South Africa and tested in two separate indirect ELISAs for the presence of antibodies to PRV and TGEV, respectively.

¹ Virology Section, Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa

² Foot-and-Mouth Disease Laboratory, Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa

³ South African Meat Board, P.O. Box 40051, Arcadia, 0007 South Africa

MATERIALS AND METHODS

Viruses and cells

PRV was grown in monolayers of BHK21 cells. TGEV, originally grown on primary pig-kidney cells, was adapted to grow on IB-RS2 cells. All cells were maintained in Vac 1 medium (a modification of Eagle's minimal essential medium), and supplemented with 10% normal bovine serum.

Preparation of antigen

Pseudorabies virus

BHK21 cells were grown in 1-l roller bottles at 37°C. Confluent monolayers were infected with 5 ml of ADV stock virus. When 100% cytopathic effect (CPE) was observed after 24 h incubation, cell-culture fluid was harvested, frozen at -70°C, thawed and clarified by centrifugation at 1 800 g for 30 min. Virus was concentrated by mixing it with an equal volume of saturated (NH₄)₂SO₄ for 1 h at 4°C and centrifugation at 1 800 g for 30 min. The resuspended precipitate was pelleted by centrifugation at 100 000 g for 2 h. The pellets were resuspended in a small volume of 0,01 M Tris and layered onto a 20–50% continuous sucrose gradient. The gradients were centrifuged at 70 000 g for 1 h. Peak fractions of virus, identified spectrophotometrically, were collected, pooled and stored in small aliquots at -70°C.

Transmissible gastroenteritis virus

TGEV antigen on IB-RS2 cells was prepared by the same method as that used for Pseudorabies, except that 100% CPE was observed after 36 h, the virus was pelleted at 150 000 g for 2 h and the resuspended pellets were layered on top of a linear 20–45% sucrose gradient and centrifuged at 70 000 g for 90 min.

Positive control serum

Positive control sera against both PRV and TGEV were prepared in adult pigs housed in an isolation facility of the Foot-and-Mouth Disease Laboratory at Onderstepoort. The pigs were inoculated intramuscularly with 1 ml of purified PRV or TGEV and bled after 21 d. Serum was stored at -20°C.

Study sera

Individual blood samples were collected from 5 337 slaughter pigs at a total of 23 abattoirs situated throughout the whole geographical area of South Africa. The sampled pigs came from 256 regular pig producers and varied in age from 4 to 6 months. Sera were decanted and sent to Onderstepoort where they were stored at -20°C until tested.

Indirect ELISA

The procedure, with slight modifications, was based on methods described by Williams, Du Plessis & Van Wyngaardt (1993). Flat-bottomed microtitre plates (Linbro/Titertek, Flow Laboratories, CT) were coated overnight at 4°C with purified virus at a concentration of approximately 10 µg/ml protein in phosphate-buffered saline (PBS), pH 9,6. After the plates had been washed, they were blocked for 1 h at 37°C with 50 µl/well of a blocking solution consisting of 2% Nestlé "Lactogen" milk powder (Food & Nutritional Products, Randburg, RSA), 1% casein hydrolysate, and 1% Tween 20 in PBS. This same solution also served as the diluent for sera and the conjugate. After they had been washed, 50 µl/well of a 1:50 dilution of each serum specimen was incubated at 37°C for 2 h in duplicate wells. After another washing, 50 µl/well of a 1:5 000 dilution of horseradish peroxidase-conjugated protein G (Zymed Laboratories, San Francisco, CA) was incubated at 37°C for 1 h. Following a further wash, the substrate (o-phenylenediamine in citrate buffer) was added to all wells and the reaction was stopped after 20 min by the addition of 1,25 M H₂SO₄. Optical-density (OD) values were recorded with a microplate reader (Bio-Tek EL340 Instruments, Winooski, VT, UDSA) at a wavelength of 492 nm.

Interpretation of results

A positive and a negative control serum were included in each test plate and the positive cut-off point was established at 1,5 times the OD value of the negative control serum. All specimens with OD values equal to or lower than the negative control were considered to be negative. Any sample with an OD value higher than the negative control, but below the positive cut-off point, was considered as "suspect" and was retested with a commercial pseudorabies antibody kit (Index Pseudorabies Virus Antibody Kit, Index Laboratories, Westbrook, Maine 04092, USA) and a TGE antibody kit (Svanova Biotech TGE Test Kit For Detection of Antibodies in Serum, SVANOVA Biotech, S-751 83 Uppsala, Sweden).

RESULTS

Fig. 1 (a) and (b) show the reactions of the positive and negative control sera in the indirect ELISAs for pseudorabies and TGE, respectively.

Of a total of 5 337 pig sera, 5 295 (99,2%) tested negative for antibodies to both PRV and TGEV, while no sera tested positive for either of the two viruses. The remaining 42 samples (0,8%) were "suspect" for either pseudorabies (23 samples) or TGE (19 samples). On retesting with two commercial ELISA kits, all "suspect" samples proved to be negative for antibodies to both viruses.

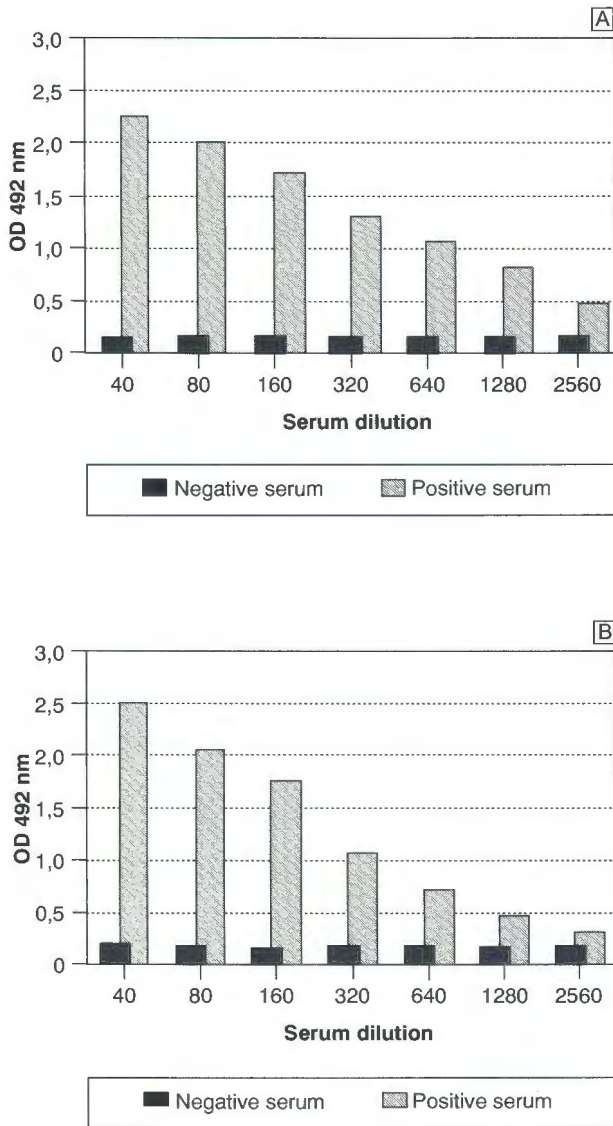


Fig. 1 Titration of serum in an indirect Elisa for (a) PRV and (b) TGEV

DISCUSSION

The fact that no serological evidence of previous contact with either PRV or TGEV was found in any of the pig sera tested, supports the general assumption and previous findings that neither of these two viruses are present in the pig population of South Africa and contradicts a statement by Andrewes in 1964 that pseudorabies is found in South Africa. A pig population free of pseudorabies and TGE could be very important to the pig industry in terms of future export prospects, especially to countries which are also free of these two diseases. It should be appreciated, however, that it also implies a fully susceptible pig population which calls for strict and efficient control of importation.

TABLE 1 Number of samples and localities of abattoirs in South Africa where samples were collected

Province	District or town	No. of samples
Transvaal	Benoni	251
	Germiston	220
	Heidelberg	340
	Irene	3
	Klerksdorp	13
	Krugersdorp	229
	Pietersburg	142
	Pretoria	930
	Vereeniging	500
	Witbank	40
Free State	Bethlehem	80
	Bloemfontein	20
Natal	Escort	830
	Pietermaritzburg	320
Cape Province	Humansdorp	150
	Cape Town	713
	Kimberley	43
	East London	87
	Stellenbosch	426
Total		5 337

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