

AN ELISA FOR THE DETECTION OF SERUM ANTIBODIES TO BOTH TRANSMISSIBLE GASTROENTERITIS VIRUS AND PORCINE RESPIRATORY CORONAVIRUS

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SUMMARY

A competition ELISA utilizing a mAb directed towards a peplomer protein epitope common to TGEV, PRCV and related feline and canine coronaviruses is described.

INTRODUCTION

Transmissible gastroenteritis (TGE) is a disease of pigs caused by a coronavirus and characterized by a watery diarrhoea often fatal to sucking piglets. In Britain, it has occurred as periodic epizootics, the last of which was in the winter of 1980/81. The virus may also persist within endemically infected herds (Pritchard, 1987) and in these circumstances fatalities may be much less common. At this laboratory, large numbers of samples from scouring pigs have been examined for the presence of TGEV over many years and using a variety of methods; but very few positive identifications have been made in the last 8 years. Porcine respiratory coronavirus (PRCV) is antigenically very similar to the virus of TGE, but it spreads aerogenically and causes mainly inapparent respiratory infections (Pensaert *et al.*, 1986). It is known to have been present in Britain at least since 1986 (Brown & Cartwright, 1986) and it had already become widespread in the national herd by 1987 (Brown & Cartwright, unpublished survey results).

Indirect ELISAs for TGE serology were first reported before the appearance of PRCV (Nelson & Kelling, 1984). Antibodies to TGEV and PRCV show complete cross-neutralization and are therefore both detected by virus neutralization tests (VNTs) using laboratory strains of TGEV. Such tests (Paton, 1989) have been widely used, both for diagnosis in cases of respiratory or enteric disease and for export certification purposes. Serological differentiation between TGEV and PRCV is now possible using competition ELISAs and monoclonal antibodies (mAbs) to TGEV-specific epitopes (Callebaut *et al.*, 1989, van Nieuwstadt & Boonstra, 1990). However, the VNT continues to be necessary for investigation of respiratory disease. Furthermore, it is more sensitive than differential ELISAs examined at this laboratory and it has generally been retained as the preferred method for the export certification of individual animals. This communication describes a competition ELISA utilizing a mAb (6A.C3) directed towards a peplomer protein epitope common to TGEV, PRCV, and a number of related feline and canine coronaviruses, but not to other porcine coronaviruses (Sanchez *et al.*, 1990). Indirect fluorescent

antibody tests using rabbit anti-mouse conjugate confirmed that the mAb recognized all TGEV/PRCV strains tested. These comprised five British isolates of TGEV and two of PRCV, five TGEVs from Bulgaria, three TGEVs from the Netherlands and a single PRCV isolate from the USSR.

MATERIALS AND METHOD

ELISA antigens prepared by detergent treatment (octyl- β -D-glucopyranoside: Aldrich) of LLCPK1 cells, infected with the Purdue strain of TGEV or mock-infected, were coated onto alternate wells of flexible polystyrene ELISA plates (Falcon) with 0.1 M bicarbonate buffer pH 9.6. A full description of the preparation of these antigens is given in a report on experiences with a TGEV-specific, serological test that distinguishes TGEV from PRCV and which utilizes the same antigens (Brown & Paton, 1991). Fifty microlitres of a 1 in 5 dilution of test serum were added to each of duplicate pairs of wells and left overnight at room temperature. The remainder of the test could be completed within an hour, all incubations being at 37°C. A 1/10 000 dilution of mAb 6A.C3 in PBS pH 7.2 with 0.05% Tween 20 (PBST) and 10% ox serum was dispensed into all wells without removing the test serum and left for 10 min. After washing, bound 6A.C3 was detected by a goat anti-mouse peroxidase conjugate (Nordic: 1/2000 for 30 min) and a tetramethyl benzidine/hydrogen peroxide substrate (10 min). The substrate reaction was stopped with 2 M sulphuric acid. PBST was used for washing the plates between stages and as the serum diluent. The conjugate diluent was PBST plus 5% negative pig serum.

RESULTS

The ELISA value was expressed as a percentage of the result obtained with a negative control serum calculated from net optical densities (optical density of positive antigen wells minus that of negative ones). The threshold value for the test was established using 279 VNT negative (titre < 2) sera from 64 farms, all of which had no history of TGEV/PRCV infection. Subtracting three standard deviations from the mean of these results (Savigny & Voller, 1980) gave a cut-off value of 50.21%. VNT positive sera from field cases ($n=167$) and from pigs experimentally infected with TGEV or PRCV ($n=35$) all scored positive in ELISA (values: 1–49%). Despite the good qualitative agreement between ELISA and VNT, no significant correlation was detected (coefficient=0.072) between ELISA values and VNT titres for positive sera. Sera collected at abattoirs during 1989, from 231 sows, were examined by ELISA and 160 (69.25%) were positive. A recent serological survey of British pigs by TGEV-specific ELISA indicated a seroprevalence of only 0.6% (Brown & Paton, 1991), suggesting that most TGEV/PRCV antibody positive samples reflect PRCV infection and that such infections remain common.

CONCLUSIONS

The ELISA is very similar to the VNT with respect to sensitivity and specificity but is much quicker and cheaper to perform. Export certification involves processing large numbers of sera concomitantly and the semi-automation that can be achieved using the

ELISA technique would be a considerable benefit. For differential diagnosis, this ELISA could be used in conjunction with a TGE-specific test and indeed with minimal modification both could be performed side by side on the same antigen coated plate. Since the mAb 6A.C3 is directed towards an epitope that is highly conserved amongst TGEV/PRCVs, the risk of false negative results due to antigenic diversity amongst these viruses appears to be low.

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REFERENCES

- BROWN, I.H. & CARTWRIGHT, S.F. (1986). *Veterinary Record* **119**, 282.
BROWN, I. H. & PATON, D. J. (1991). *Veterinary Record* (in press).
CALLEBAUT, P., PENZAERT, M. B. & HOOYBERGHS, J. (1989). *Veterinary Microbiology* **20**, 9.
NELSON, L. D. & KELLING, C. L. (1984). *American Journal of Veterinary Research* **45**, 1654.
PATON, D. J. (1989). In *Manual of Recommended Diagnostic Techniques and Requirements for Biological Products for Lists A and B Diseases*, Vol. 1, B53. Paris: Office International Des Epizooties.
PENSAERT, M. B., CALLEBAUT, P. & VERGOTE, J. (1986). *Veterinary Quarterly* **8**, 257.
PRITCHARD, G. C. (1987). *Veterinary Record* **120**, 226.
SANCHEZ, C. M., JIMENEZ, G., LAVIADA, M. D., CORREA, I., SUNE, C., BULLIDO, M. J., GEBAUER, F., SMERDOU, C., CALLEBAUT, P., ESCRIBANO, J. M. & ENJUANES, L. (1990). *Virology* **174**, 410.
SAVIGNY, D. & VOLLER, A. (1980). *Journal of Immunoassay* **1**, 105.
VAN NIEUWSTADT, A. P. & BOONSTRA, J. (1990). *Proceedings of the 10th International Pig Veterinary Society*, Lausanne, p. 265.

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