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Short communication

Comparative study of a blocking enzyme-linked immunosorbent assay and the immunoperoxidase monolayer assay for the detection of antibodies to the porcine reproductive and respiratory syndrome virus in pigs

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Abstract

A blocking enzyme-linked immunosorbent assay (ELISA) for detecting antibodies to the porcine reproductive and respiratory syndrome virus (PRRSV) in pig sera was developed and compared with the immunoperoxidase monolayer assay (IPMA), the most widely used serological diagnostic test in Europe. The blocking ELISA was specific and more sensitive than the IPMA when applied to field sera and to sera which were collected early after an experimental infection with PRRSV. Problems with high background activity as observed in IPMA or indirect ELISA were not encountered.

Keywords: Blocking ELISA; Immunoperoxidase monolayer assay; Porcine reproductive and respiratory syndrome virus; (Pig)

In 1991, the Lelystad virus was shown to be the causative agent of the porcine reproductive and respiratory syndrome (PRRS) (Wensvoort et al., 1991) and attempts were made to detect antibodies to this Dutch isolate in pig sera. An immunoperoxidase monolayer assay (IPMA) was developed and has since been used routinely in most European countries. Briefly, porcine alveolar macrophages are seeded in a microtitre

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plate, infected with PRRSV and fixed with paraformaldehyde. Then, test serum is added and specific antibodies are detected by adding rabbit-anti-swine immunoglobulins labelled with horseradish peroxidase and a peroxidase substrate (Wensvoort et al., 1991). Unfortunately, not all batches of macrophages were equally sensitive for a PRRSV infection as described by Vynckier and Pensaert (1993). Furthermore, some sera reacted with the uninfected control macrophages. Sera of sows and piglets younger than 4 weeks in particular caused background staining. Similar problems with field sera were observed by other researchers (Edwards et al., 1992). Albina et al. (1992) developed an indirect ELISA. The test was simpler, cheaper and more sensitive for the early detection of PRRSV antibodies than the IPMA. However, Edwards et al. (1992) reported similar background colour problems as with the IPMA. In the present study, a blocking ELISA was developed and compared to the IPMA to improve the reliability of the serological diagnosis of PRRS.

The procedure followed for the blocking ELISA was a modification of that described by Callebaut et al. (1982). Immunoglobulins, purified from a porcine hyperimmune serum against the Belgian PRRSV strain 92V58 (Vynckier and Pensaert, 1993) were used to coat microtitre plates (Nunc, Intermed, Denmark). This IgG preparation was also labelled with horseradish peroxidase according to the method of Wilson and Nakane (1978) and used as conjugate. The antigen was the 7th passage of the PRRSV strain 92V58 on porcine alveolar macrophages. Cells and medium were harvested by freezing and thawing 3 times and were clarified at $300 \times g$. In preliminary experiments, optimum working dilutions of coating antibodies, antigen and conjugate were determined by chequerboard titrations and used as follows. A microtitre plate was coated by adding 100 μl per well of coating antibodies, diluted in 0.05 M carbonate buffer pH 9.6 and overnight incubation at 37°C. The plate was washed 3 times and antigen was bound to the coated plate by incubation for 4 h at 37°C with 100 μl per well of the PRRSV stock, diluted in phosphate buffered saline (PBS) supplemented with 2.5% fetal calf serum (FCS) and 0.05% Tween-80. After 4 washings, the plate was saturated by adding to each well 10 μl of a PRRSV negative porcine serum and incubated for 1 h at 22°C. The serum samples to be tested were serially diluted twofold starting at 1:2.5 in PBS containing 0.05% Tween-80 and 0.35 M NaCl. A 40 μl volume of each dilution was added to the plate and incubated for 2 h at 37°C and at 4°C overnight. Subsequently, 50 μl of the PRRSV-specific conjugate appropriately diluted in the serum diluent was added to the serum dilutions and incubation was continued for 1 h at 37°C. After washing the plate 5 times and adding 100 μl per well of substrate solution (5-amino-salicylic acid with 0.005% H_2O_2) plates were incubated overnight at 4°C and the optical density of the wells was determined at 450 nm in Titertekplus MS212 (ICN, California, USA). For every serum sample, the optical densities of the dilutions were corrected by a factor. This factor represented the optical density of a 1:2.5 dilution of the same serum, tested in a well to which buffer instead of antigen had been added. A serum dilution was considered positive if its corrected optical density was $\leq 50\%$ of the corrected optical density of a PRRSV negative porcine serum. Titers were expressed as the reciprocal of the highest positive serum dilution.

Forty-one sera of pigs, known to be negative for PRRSV antibodies, were used to examine the specificity of the blocking ELISA. Twenty-one of these were collected from

Table 1
Correlation of PRRSV antibody detection in IPMA and blocking ELISA

Test applied	Sera from experimentally infected pigs ($n = 50$)		Field sera of piglets ($n = 90$)		Field sera of sows ($n = 76$)	
	No.pos [a]	No.neg [b]	No.pos	No.neg	No.pos	No.neg
Blocking ELISA	41	9	43	47	70	6
IPMA	36	14	14	76	53	23

[a] No.pos = number of sera with PRRSV antibodies.
[b] No.neg = number of sera without PRRSV antibodies.

pigs at slaughter in 1982, which was long before the emergence of PRRSV. Thirteen sera were taken from PRRSV negative specific pathogen free (SPF) pigs and the remaining sera were from SPF pigs, hyperimmunized against porcine rotavirus, Aujeszky's disease virus, transmissible gastroenteritis virus of swine, porcine respiratory coronavirus, porcine epidemic diarrhoea virus, influenza viruses and haemagglutinating encephalomyelitis virus of swine, respectively. All sera were negative for PRRSV antibodies in the blocking ELISA, confirming the specificity of the test. No cross-reaction occurred between PRRSV and the other porcine viruses mentioned.

The sensitivity of the blocking ELISA related to that of the IPMA was determined by testing 50 sera of pigs collected between 3 and 42 days after experimental inoculation with various European strains of PRRSV. Results are shown in Table 1. The 7 sera taken 3 days after inoculation were negative both in IPMA and ELISA. Five out of the 7 sera collected 7 days after inoculation were negative in IPMA but positive in ELISA, whereas the remaining 2 sera were negative in both tests. Thirty six sera, taken between 10 and 42 days after inoculation, were positive both in IPMA and blocking ELISA. The antibody titers in IPMA were on average 37 times higher than in ELISA probably because the IPMA is an indirect test. In IPMA several peroxidase labelled rabbit-anti-swine immunoglobulins may bind to each PRRSV-antibody molecule in the serum sample and amplify the signal, but there is no such amplification in the ELISA. The sensitivity of the blocking ELISA compared to the IPMA was optimal, as all sera positive in IPMA were also positive in blocking ELISA: sensitivity = (number of sera positive both in blocking ELISA and IPMA)/(number of sera positive in IPMA) = 36/36 = 1. The finding that sera obtained early (7 days) after inoculation were often positive in the blocking ELISA but not in the IPMA may be due to a more efficient detection of early formed IgM antibodies by ELISA. The immunoglobulin-specific conjugate used in IPMA to detect antibodies in the serum sample, is known to have a higher affinity for IgG than for IgM. This may explain the inefficient detection of IgM in IPMA. On the contrary, in the blocking ELISA all types of immunoglobulins appear to compete with the virus-specific conjugate and to be detected with similar efficiencies.

As further evaluation of the blocking ELISA, field sera were collected from piglets with maternal antibodies. At the age between 4 and 12 weeks, blood was taken from piglets of 12 litters on closed breeding-fattening farms, where a previous PRRSV infection had occurred. When tested in IPMA, 35 out of 125 sera caused background staining and interpretation of the result was not possible. The latter sera originated

mainly from pigs younger than 6 weeks old. Background colour was not observed in the blocking ELISA. Of the remaining 90 sera, 43 were positive in the blocking ELISA compared to 14 in IPMA (Table 1). Sera that were positive in IPMA were also positive in ELISA. Seventeen sera of pigs at the age of 6 weeks and 12 sera of pigs at 10 weeks were negative in IPMA, but positive in the blocking ELISA. Apparently, the blocking ELISA was more sensitive in detecting waning maternal antibodies to PRRSV. The reason for this difference between the blocking ELISA and the IPMA is not clear.

Furthermore, a total of 88 blood samples were taken from sows in a slaughterhouse and on closed breeding-fattening farms. Twelve sera caused non-specific reactions in IPMA, while in the blocking ELISA no background staining occurred. Of the remaining 76 sera 53 were positive both in IPMA and ELISA, whereas 17 sera were positive only in ELISA (Table 1).

It was concluded that the blocking ELISA was more effective than the IPMA for the detection of active and maternal antibodies in pigs based on the markedly higher numbers of samples of field sera and sera collected after an experimental infection scored positive. Furthermore, background staining in the IPMA often complicated the interpretation of the results when testing sera of sows and of piglets younger than 6 weeks. In the blocking ELISA problems with background colour were never observed. This implies that the blocking ELISA is a specific, sensitive and reliable diagnostic test for the detection of PRRSV antibodies.

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