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Development of Immunological Test Kit for the Detection of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in Swine

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ABTRACT

Two types of porcine reproductive and respiratory syndrome virus (PRRSV), European (EU) and North American (US), cause an important swine disease called porcine reproductive and respiratory syndrome (PRRS). An enzyme-linked immunosorbent assay (ELISA) for the simultaneous detection of serum antibodies against these two PRRS was developed. A fused protein containing EU PRRSV and US PRRSV nucleocapsid proteins (USEU-rN protein) expressed in *Escherichia coli* were partial purified and used as antigens.

As determined by a checkerboard titration, an optimal dilution of the USEU-rN protein and serum were 1:1600 and 1:40, respectively. The optimal cut-off value for the developed USEU PRRS ELISA was 0.4, with diagnostic sensitivity and specificity of 97.5% and 100%, respectively. Using Two Graph-ROC program testing with 200 positive sera (IDEXX® HerdCheck PRRS ELISA) and with 200 negative sera from PRRS-free country (Denmark), comparison of 1,077 field sera obtained from USEU PRRS ELISA with those from IDEXX® ELISA showed the degree of agreement (k value) was highly obtained at 0.7916. The kit is considered reliable for routine diagnosis, epidemiological surveys and outbreak investigations.

Key words: PRRS, fusion nucleocapsid protein, checkerboard titration, cut-off value, partial purified, k value, ELISA

INTRODUCTION

PRRS is one of the major causes of economic loss in the swine industry (Collins *et al*., 1992; Wensvoort *et al*., 1993). It is characterized by reproductive failure in sows and respiratory distress affecting pigs of all ages. However, this syndrome affects younger pigs more severely. It causes growth reduction, high mortality rate and pneumonia which becomes more severe when co-infected with other pathogens. The etiologic agent for this disease is porcine reproductive and respiratory syndrome virus (PRRSV). It belongs to the order *Nidovirales*, family *Arteriviridae* and genus *Arterivirus* (Cavanagh, 1997; Snijder and Meulenberg, 1998). PRRSV is an enveloped virus with a 15 kb positive single-stranded RNA genome containing nine open reading frames (ORFs), 1a, 1b, 2a, 2b, 3, 4, 5, 6 and 7 (Thanawongnuwech *et al*., 2004). ORF

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1a and 1b encode the viral replicase, ORF 2a to 5 encode membrane-associated glycoproteins, ORF 2b and 6 encode an un-glycosylated protein and ORF 7 encode nucleocapsid protein. Major structural proteins consist of an enveloped glycoprotein (GP_5) of 25 kDa, an un-glycosylated membrane (M) protein of 19 kDa and a nucleocapsid protein (N-protein) of 15 kDa, which are coded by ORF 5, 6 and 7, respectively (Nelson *et al*., 1993; Meulenberg *et al*., 1995). The EU PRRSV was isolated in the Netherlands and designated as Lelystad virus or LV (Wensvoort *et al*., 1991), while the US PRRSV was identified in United States and designated as ATCC VR-2332 (Benfield *et al*., 1992). In addition to the different location of isolates, there is also ample genetic variation among both genotypes. Analysis of the amino acid sequences of the open reading frame (ORF) regions of LV and ATCC VR-2332 confirmed that they were 76% for ORF 2, 72% for ORF 3, 80% for ORFs 4 and 5, 91% for ORF 6, and 74% for ORF 7 (Murtaugh *et al*., 1995). However, nucleotide sequence analysis indicated that viruses are evolved by random mutation and intragenic recombination (Meng *et al*., 1994; Nelsen *et al*., 1999). The antigenic differences between these two isolates have been demonstrated using polyclonal antisera (Wensvoort *et al*., 1992) and monoclonal antibodies (Nelson et al., 1993), suggesting that the US isolates are more diverse while the epitopes among EU PRRSV strains are rather conserved (Drew *et al*., 1995). PRRSV can survive outside the host for more than 4 months at temperatures ranging from -70 to -20 °C but the viability decreases with increasing temperature (Benfield *et al*., 1992).

The serological survey of swine sera in Thailand during 1988-1996 showed the earliest detection of the seropositive animals in 1989, which were increased annually from 8.6% in 1991 to over 56% in 1996 (Damrongwatanapokin *et al*., 1996). PRRSV was first isolated from the suckling and nursery pigs in Thailand in 1996 and identified as the US genotype (Damrongwatanapokin *et al*., 1996). However, both US and EU PRRSV may exist in Thai swine population. To obtain an efficient and rapid tool for monitoring and controlling this disease, an enzyme-linked immunosorbent assay (ELISA) for the simultaneous detection of serum antibodies against both PRRSV types was developed using recombinant nucleocapsid-protein as antigen.

MATERIALS AND METHODS

RNA extraction and amplification

Viral RNA was extracted from cell cultures (MARC-145) infected with either US or EU PRRSV (Virological Laboratory, Faculty of Veterinary Medicine, Kasetsart University) using denaturing (D) solution (4M guanidine thiocyanate, 25 mM sodium citrate, 0.1 M 2-mercaptoethanol and 5% N-laurylsacosine), phenol-chloroform and isopropanol precipitation (Chomezynski and Sacchi, 1987). The entire nucleocapsid (N) gene or ORF 7 of the two PRRSV genotypes were amplified by one-step reverse transcription-polymerase chain reaction (RT-PCR), using nucleocapsid specific primers W7EU1 (5'GGA TCC ATG GCC GGT AAA AAC CAG AG') and W7EU2 (5'GTC GAC TTG CAC CCT GAC TGG CGG A 3') for EU PRRSV, W7US1 (5' AGA TCT ATG CCA AAT AAC AMC GGC ARG 3') and W7US2 (5'GGA TCC TGC TGA GGG TGA TGC TGT G 3') for US PRRSV. The W7EU1, W7EU2, W7US1 and W7US2 primers contained *Bam*HI, *Sal*I, *Bgl*II and *Bam*HI (underlined) restricted sites, respectively. Cycling conditions were set at 45 °C for 1 h, 94 °C for 5 min and 35 cycles of 94 °C for 30 s, 53 °C for 30 s and 72 °C for 1 min, with final extension at 72 ° C for 7 min.

Cloning and sequencing of N-gene

The RT-PCR products were ligated into pDrive cloning vectors. The resulting plasmids

were designated as pDrive_7EU and pDrive_7US. The pDrive_7EU was then cleaved with *Bam*HI and *Sal*I, purified by using the OIAquick gel extraction kit (Qiagen) and ligated into the *Bam*HI and *Sal*I sites of the expressed plasmid pQE30 (Qiagen). The ligation product was transformed into *E. coli* and screened by PCR assay using specific primers. The positive plasmid obtained was designated as pQE30_7EU.

The pDrive_7US was then cleaved with *Bgl*II and *Bam*HI, purified and ligated into the *Bam*HI sites of the pQE30_7EU. The ligation products were also transformed into *E. coli*. The clones containing ORF 7 of both US and EU PRRSV were selected and the plasmids isolated from the positive clones were designated as pQE30_7USEU (Figure 1). The sequences of the insert (7USEU-N gene) were confirmed by DNA sequencing with pQE30 forward and pQE30 reverse primers and alignment was done with the published sequences (GenBank).

Protein expression and analysis

For protein expression, the plasmids pQE30_7USEU and pQE30 were transformed into *E. coli* strain M15 (pREP4) (Qiagen). Overnight cultures of the transformant were diluted 1:50 in Luria-Bertani medium (LB medium) containing 100 μg/ml amplicillin and 25 μg/ml kanamycin and incubated for 3 h at 37 °C in a horizontal shaking incubator. When the optimal density (OD) at 600 nm reached 1.0, protein expression was induced by adding 1 mM isopropyl-β-D-thiogalactopyranosidase (IPTG) to the culture

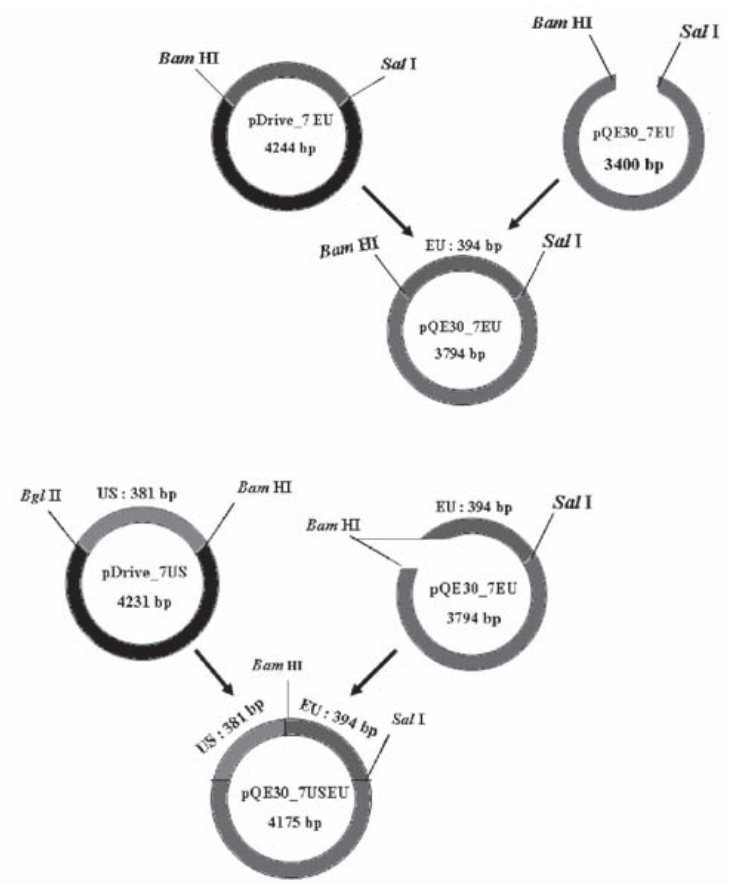

Figure 1 Diagram of cloning strategy and map of pQE30_7USEU.

medium. After further incubation for 4 h at 37 °C, the bacterial cells were harvested by centrifugation at 4000 ×g for 15 min at 4 °C, stored at -20 °C until use. The expressed proteins (7USEU-rN and pQE30 proteins) from 1 ml of the initial culture was determined for protein solubility by resuspending in 200 µl of 1x Sodium chloride-Tris-EDTA (STE) buffer (10 mM NaCl, 10 mM Tris-HCl pH8.0, 1mM EDTA pH 8.0) and lisozyme was added at a final concentration of 100 µg/ml. It was incubated on ice for 30 min, centrifuged at 4000 ×g for 20 min. Both the soluble and insoluble protein (inclusion bodies) fractions were analyzed on a 15% SDS-PAGE and stained with Coomassie brilliant blue.

Partial purification of protein

For partial purification, the cell pellets from the initial bacterial culture of 50 ml were washed three times with 3 ml of 8 M urea, collected by centrifugation at 4000 ×g for 20 min at 4 °C and resuspended in 4 ml of 1× STE buffer, 300 µl of 10% sarcosine. Lysozyme was added at a final concentration of 30 mg/ml and the solution was further sonicated three times for 1 min each at 5 amplitude microns. Cell debris was removed by centrifugation at 4000 ×g for 20 min at 4 °C. The supernatant was collected and 300 µl of 10% Triton X-100 was added. The partial purified protein lysates was analyzed on a 15% SDS-PAGE and Western blot was performed using strong positive serum (IDEXX® HerdCheck PRRS ELISA). The protein concentrations were determined by using Bradford assays (Bradford, 1976). The 7USEU-rN and pQE30 expression proteins were used as antigens for PRRS ELISA development.

Enzyme-linked immunosorbent assay (ELISA)

The lysed 7USEU-rN (positive antigen) and pQE30 proteins (control antigen) were diluted in a coating buffer (0.007 M Na_2CO_3, 0.017 M $NaHCO_3$, pH 9.6). One hundred microliters of the diluted antigens were added into each well of a 96-well microtiter plate (Maxisorb Immunoplates; Nunc, Roskilde, Denmark). The control antigens were in the even numbered columns, while positive antigens were in the odd numbered columns and incubated for 15 h or overnight at 4 °C.

The optimal antigen and serum concentrations were determined by checkerboard titration (Dea and Tijssen, 1989) using a strong positive serum (IDEXX® HerdCheck PRRS ELISA or IDEXX® ELISA). The serum was two-fold serially diluted from 1:40 to 1:1,280 in a sample diluent (sonicated solution containing pQE30 transformed *E.coli* protein in 1x phosphate-buffered saline). The antigen was diluted in a coating buffer from 1:50 to 1:6,400, and incubated in the wells at 4°C for 15 hours or overnight. The plates were washed twice with 1x phosphate-buffered saline having 0.05% Tween 20 (PBST) pH 7.5 and blocked with 150 µl/well of 5% skim milk (5% dry skim milk in PBST) for 1 h at 37 °C. The skim milk was then removed and 100 µl of each diluted serum was transferred to the antigen-coated well. After incubation for 1 h at 37 °C, the plates were washed three times with 1x PBST. Subsequently, 100 µl of protein G-horseradish peroxidase conjugate (Zymed, San Francisco, Calif) diluted to 1:30,000 (v/v) in 1% skim milk (1% dry skim milk in 1x PBST) was added to each well and the plates were further incubated for 1 h at 37 °C. After washing the plates as described above, the 100 µl of TMB (3,3',5,5' tetramethylbenzidine) substrate (ZYMED®) was added and incubated for 10 min at room temperature and the OD were determined at 650 nm using an ELISA reader (Rosys anthos lucy 2). The ΔODs (the well containing positive antigens OD minus the well containing negative antigens OD) were calculated and expressed as signal-to-positive (S/P) ratio by using the following formula: $S/P\ ratio = (\Delta OD_{sample} - \Delta OD_{Neg})/average(\Delta OD_{Pos} - \Delta OD_{Neg})$, where OD_{sample} is the swine serum sample OD, OD_{Pos} is the positive control OD and

OD_{Neg} is the negative control OD.

The optimal cut-off value for ELISA was determinded by using Two Graph-ROC program. Two hundred positive sera as determined by IDEXX® ELISA and two hundred negative sera from PRRS-free country (Denmark) were used for setting the cut-off. Intra-test repeatability was assessed by testing six IDEXX® ELISA positive sera at the dilution of 1:40 for six times at different positions on a single ELISA plate. Inter-test repeatability was assessed by testing 32 IDEXX® ELISA positive sera at dilution of 1:40 for four ELISA plates. Coefficients of variation (CV) and 95% confidence interval were calculated for each serum sample. For PRRSV antibody testing, the IDEXX® ELISA was used as a reference assay to validate the USEU PRRS ELISA. The serum samples was tested on our ELISA field and the results were compared on case-by-case basis with those of IDEXXÆ ELISA. The kappa (κ) quotients were determined to assess the extent of agreement between the data obtained by USEU PRRS ELISA and those obtained by IDEXX® ELISA.

Performance of the obtained USEU PRRS ELISA was also determined by comparing with the IDEXX® ELISA and IPMA on the 156 unknown field sera. The IPMA was carried out following the standard protocols (Wensvoort *et al.*, 1991). The sensitivity and specificity of each test were calculated.

RESULTS

RT-PCR, cloning and sequencing of N gene

The amplification of ORF7 gene using specific primers gave 394 bp fragments for EU PRRSV and 381 bp fragments for US PRRSV. They were cloned into the protein expression plasmid pQE30 and screened by PCR using pQE30 forward and W7EU2 reverse primers. The PCR product contained 811 bp (Figure 2A). The positive clone was designated as pQE30_7USEU. The sequence of the insert was confirmed by DNA sequencing with pQE30 forward and pQE30 reverse primers. The nucleotide and amino acid sequences of pQE30_7USEU aligned to those of

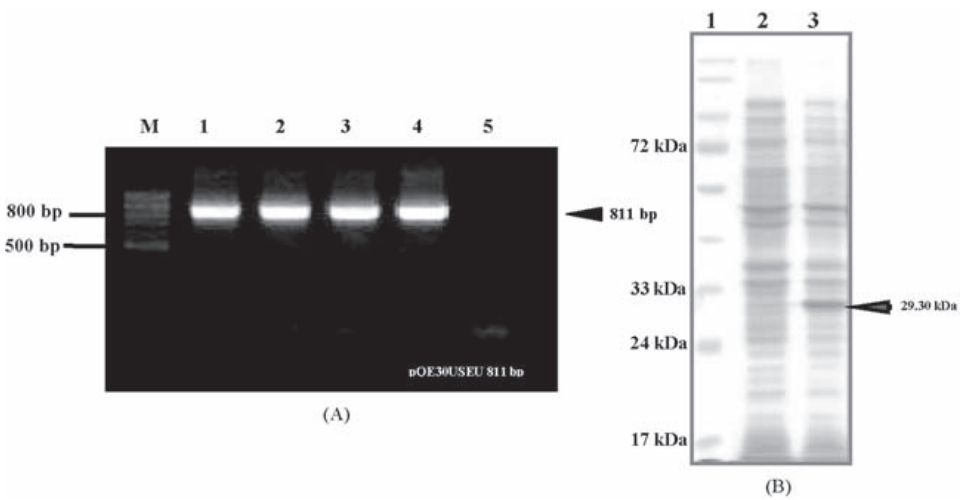

Figure 2 Analysis of plasmid pQE30_7USEU showing (A) PCR products of positive clone amplified by using pQE30 forward and W7EU2 reverse primers giving 811 bp in length (lanes 1 to 4), negative control (lane 5) and 100 bp molecular weight marker (M). (B) Protein on SDS-PAGE without IPTG-induced (lane 2) and with IPTG-induction (lane 3) showing the 29.30 kDa.

ATCC VR-2332 and LV (GenBank accession no.U87392 and M96262) gave 95% and 96.41% similarity (Figure 3).

Expression and partial purification of rN-protein

The plasmid pQE30_7USEU and pQE30 were transformed into *E. coli* strain M15 (pREP4) for IPTG-induced expression of rN protein. The resulting USEU-rN protein was found to be an insoluble protein fraction. This protein was then further lyzed with a lysis buffer containing 10% sarcosine and gave 29.30 kDa band after being electrophoresed through 15% SDS-PAGE and stained with Coomassie blue (Figure 2B). Western blot analysis using strong positive serum (IDEXX® HerdCheck PRRS ELISA) confirmed the identity and the molecular mass of the USEU-rN protein. This protein concentration was obtained at 1.69 mg/ml by Bradford assay and used as a positive antigen. The pQE30 protein was also similarly lysed, having final concentration of 1.64 mg/ml, and used as a negative antigen.

Indirect ELISA using partially purified USEU-rN protein

The optimal dilutions of the antigen and the test serum by indirect ELISA were determined by checkerboard titration. By using the strong positive serum, the optimal dilutions yeilding high specific reactivity but low background were obtained at 1:40 for the serum. The optimal dilution of antigen was 1:1600, which corresponded to a final concentration of 1.10 μg/ml. Negative antigen was applied at the same dilutions as the USEU-rN protein. The optimal cut-off value for the USEU PRRSV ELISA was 0.4 as determined by ROC analysis using IDEXX® ELISA positive sera and true negative sera at 97.50% sensitivity and 100% specificity. These data were subsequently used to generate Two Graph-ROC curves of both S/P ratio from positive and negative sera (Figure 5).

The validation of the USEU PRRS ELISA

The validity of the USEU PRRS ELISA was assessed by intra-test repeatability experiment

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CLUSTAL W (1.83) multiple sequence alignment

U87392-M96262-ORF7      MPNNNGKQQKRKKGDGQPVNQLCQMLGKIIAQQNQSRGKGPGKKNKKKNPEKPHFPLATE
USEU-ORF7               MPNNNGKQQKKQKGNGQPVNQLCQMLGRIIAQQNQSRGKGPGKKNKKKNPEKPHFPLATE
                        **********::**:************:********************************

U87392-M96262-ORF7      DDVRHHFTPSERQLCLSSIQTAFNQGAGTCTLSDSGRISYTVEFSLPTHHTVRLIRVTAS
USEU-ORF7               DDVRHHFTPSERQLCLSSIQTAFNQGAGTCTLSDSGRISYTVEFSLPTHHTVRLIRVTAS
                        ************************************************************

U87392-M96262-ORF7      P--SAMAGKNQSQKKKKSTAPMGNGQPVNQLCQLLGAMIKSQRQQPRGGQAKKKKPEKPH
USEU-ORF7               PSAGSMAGKNQSQKKKKNTAPMGNGQPVNQLCQLLGAMIKSQRQQPRGGQAKKRKPEKPH
                        *  .::************.**********************************:******

U87392-M96262-ORF7      FPLAAEDDIRHHLTQTERSLCLQSIQTAFNQGAGTASLSSSGKVSFQVEFMLPVAHTVRL
USEU-ORF7               FPLAAEDDIRHHLTQTERSLCLQSIQTAFNQGAGTASLSSSGKVSFQVEFMLPVAHTVRL
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U87392-M96262-ORF7      IRVTSTSASQGAS
USEU-ORF7               IRVTSTSASQGAS
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Figure 3 Alignment of amino acid sequence of the USEU-N gene with ATCC VR-2332 (U87392) and LV (M96262) showing 96.41% similarity. The amino acid in frame was a short linker at the conjunction between US- and EU-N genes.

(n = 6, R = 6) resulting in CVs of 5.089 and 95% CI of 4.14 to 6.04. Inter-test repeatability (n = 32, R = 4) gave CVs of 7.645 and 95% CI of 6.96 to 8.34. These results are considered acceptable based on the validation of serological assay reported by Jacobson (1998). The sensitivity of USEU PRRS ELISA was further determined by testing with the sera obtained from pigs experimentally infected with either EU or US PRRSV. It was found that the developed ELISA

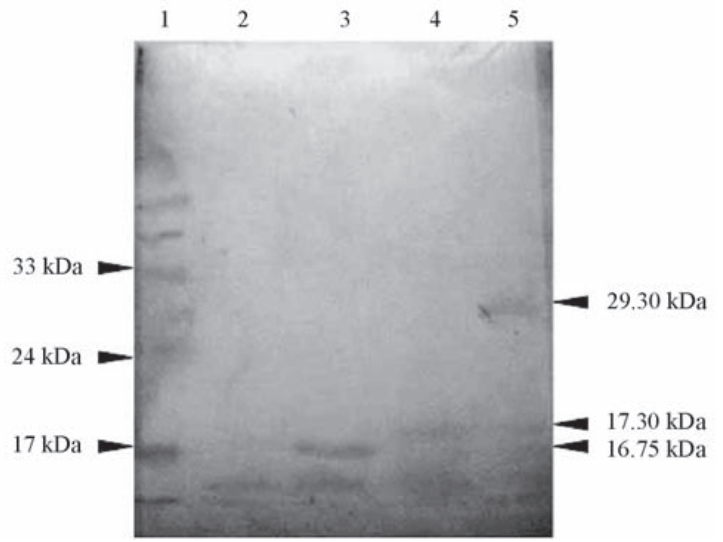

Figure 4 Western blot analysis of pQE30 protein (lane 2), EU- (lane 3), US- (lane 4) and USEU-rN protein (lane 5) using anti-PRRSV serum. The Western blot was performed following SDS-PAGE on 15% polyacrylamide gel. The sizes of marker protein in kilodaltons were indicated (lane 1).

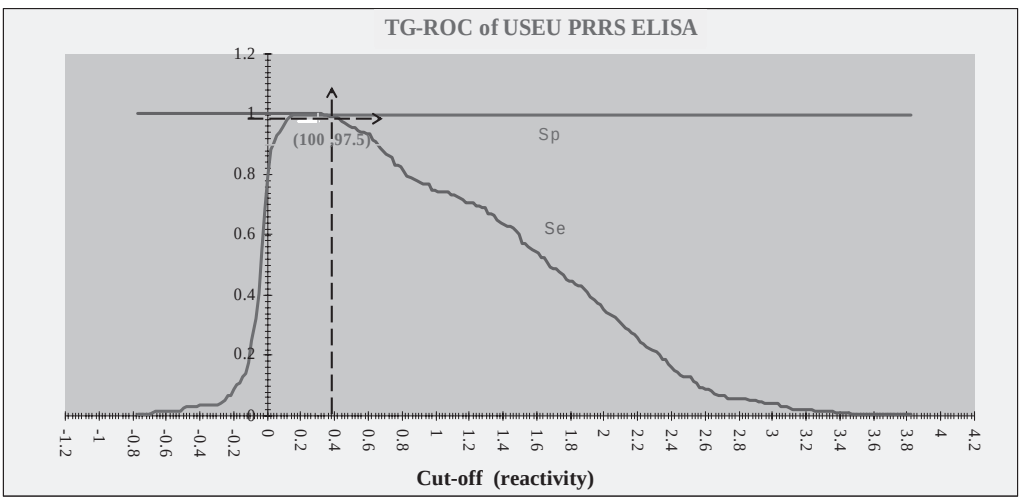

Figure 5 TwoGraph-ROC analysis of USEU PRRS ELISA calculated from testing 200 positive (n = 200) and 200 negative sera (n = 200). The cut-off value was 0.4, having sensitivity (blue) and specificity (red) at 97.50% and 100%, respectively.

could detect specific antibodies in all sera from the infected pigs collected at day 7 of post-infection.

Comparison of 1,077 field sera tested by using USEU PRRS ELISA with those using IDEXX® PRRS ELISA showed the degree of agreement (κ value) at 0.7916, which is considered a substantial value (Landis and Koch, 1977). It was found that 683 samples were positive (true positive) while 294 were negative (true negative) as tested by both kits. However, 77 samples were negative by the USEU PRRS ELISA but positive by the IDEXX® ELISA (false negative). On the contrary, 23 samples were positive by the USEU PRRS ELISA but negative by the IDEXX® ELISA (false positive). Collectively, the number of sera identified as positive and negative sample were 760 and 317, respectively, by IDEXX® ELISA, and 706 and 371, respectively, by the USEU PRRS ELISA. The IDEXX® ELISA, therefore, identified more samples as positive (n = 760) and less as negative (n = 317) compared to those of USEU PRRS ELISA showing 706 positives and 371 negatives.

Performance of the USEU PRRS ELISA

The 156 unknown field sera were used to determine the performance of the USEU PRRS ELISA in comparison to the IDEXX® ELISA and IPMA (Table 1). The sensitivity and specificity of the three tests were assessed. When the IPMA was set as a reference, the USEU PRRS ELISA gave higher specificity (80.24%) but lower sensitivity (85.33%) than those obtained by using the IDEXX® ELISA as a reference (78.13% specificity and 91.67% sensitivity). However, both the USEU PRRS ELISA and the IPMA performed almost equally well when the IDEXX® ELISA was set as a reference (Table 1). Similarly, the comparison of IDEXX® ELISA and the IPMA with the USEU PRRS ELISA as a reference resulted in the sensitivities of 74.47% and 80% and higher specificity of 95.16% and 85.53%, respectively. The ranges of differences in sensitivity and specificity indicate the disparity between USEU PRRS ELISA and each reference test.

The correlation of the USEU PRRS ELISA with IDEXX® ELISA and IPMA were calculated using either IDEXX® ELISA or IPMA as the reference. The results gave close correlation among these two tests (82.69%) as shown in Table 2.

DISCUSSION

An enzyme-linked immunosorbent assay (ELISA) for the simultaneous detection of serum antibodies against EU and US PRRSV was developed by using fusion nucleocapsid proteins (USEU-rN) expressed in *E. coli* as antigens. The viral recombinant protein expressed in *E.coli* have

Table 1 Comparison of sensitivity and specificity of the USEU PRRS ELISA, IDEXX® ELISA and IPMA for the detection of antibodies against PRRSV.

Test	Sensitivity and specificity of the following reference test (n = 156)					
	USEU PRRS ELISA		IDEXX® ELISA		IPMA	
	Sensitivity[a] (%)	Specificity[b] (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
USEU ELISA	-	-	95.89	71.08	85.33	80.24
IDEXX® ELISA	74.47	95.16	-	-	91.67	78.13
IPMA	80	85.53	93.75	72.37	-	-

[a]Sensitivity = 100 × number of positives in both test and reference test/total number of positives in the reference test

[b]Specificity = 100 × number of negatives in both test and reference test/total number of negatives in the reference test

been frequently used for differential diagnosis between vaccinated and infected animals or to design type-specific serological assay. Previous researchers have reported the use of recombinant N-protein as an ELISA antigen for the detection of PRRSV antibodies but using only N-protein of single type of virus (Denac *et al*., 1997; Dea *et al*., 2000; Witte *et al*., 2000; Seuberlich *et al*., 2002). In our study, combined 7USEU-N gene were successfully inserted into pQE30 which is a prokaryotic expression vector. The vector contains an IPTG-inducible promoter and was designed for synthesis of foreign protein fused to a hexahistidine tag which expressed in *E. coli* strain M15. This is the first time that N genes of both US and EU straining of PRRSV were simultaneously cloned and expressed as a single protein. This fused protein from both strains gave 29.30 kDa in size corresponding to a more or less the combined molecular mass of which N-protein of each type approximately 15 kDa (Nelson *et al*., 1993; Meulenberg *et al*., 1995; Seuberlich *et al*., 2002; Brafoed *et al*., 2004). Comparison of the amino acid sequence of USEU-rN protein, EU PRRSV (GenBank accession no. M96262) and US PRRSV (GenBank accession no. U87392) showed a high degree of homology (96.41%) indicating the authenticity of this combined antigen.

It is interesting to find that the extract of rN-protein using 8 M urea, 10% sarcosine, lysozyme, sonication and 10% Triton X-100 was successfully used as antigen while all other research groups based their results on only purified rN-protein (Denac *et al*., 1997; Dea *et al*., 2000; Witte *et al*., 2000; Seuberlich *et al*., 2002). Checkerboard titration was also performed to determine the optimal dilution of antigen by using the strong positive serum which turned out to be 1:1600. At this dilution, high reactivity and low background reaction was obtained. We found that the high background reaction of antigen could be eliminated by absorbing the sera with a sample diluent (pEQ30 protein resuspended in PBST and sonicated three times). In addition, this rN-protein obtained from one round of partial purification (4.3 ml) was sufficient to coat 716 plates or test with a total of 68,800 sera samples. Based on the selected cut-off value of 0.4 reactivity, the developed test kit also revealed a very high sensitivity (97.50%) and specificity (100%).

To validate the developed USEU PRRS ELISA, a total of 1,077 swine sera were tested. It was found that 706 sera were seropositive but 371 were seronegative for PRRSV, comparing to 760 positive and 317 negative as tested by IDEXX® ELISA. However, both ELISAs showed correlation value of higher than 90% and kappa (k) value gave a substantial result (0.7916). These values are close to those of K8-ELISA as reported by Dea *et al*. (2000) but higher than using KSU ELISA (Witte *et al*., 2000), making USEU PRRS ELISA more suitable as a diagnostic test kit. Comparison of the three test kits also showed that the USEU PRRS ELISA was almost equally effective as IPMA when the IDEXX® ELISA was used as a reference. Similarly, comparison of USEU PRRS ELISA with the IDEXX® ELISA using IPMA as a reference resulted in almost equal

Table 2 Sensitivity, specificity and correlation of the USEU PRRS ELISA compared to IDEXXÆ ELISA and IPMA (n=156).

Referencemethod	Specificity (%)	Sensitivity (%)	Correlation* (%)
IPMA vs USEU PRRS ELISA	80.24	85.33	82.69
IDEXX® test kit vs USEU PRRS ELISA	95.89	71.08	82.69

* Correlation between two tests = (number of positive cases in both tests + number of negative cases in both tests)/ total number of cases (experimental and reference test)

performance concerning specificity but somewhat lower sensitivity (Table 1). However, this developed kit was closely correlated (>82%) with the IDEXX® ELISA and IPMA when these two kits were used as reference tests as shown in Table 2. Furthermore, the sensitivity of USEU PRRS ELISA is additionally supported by testing the sera from pigs experimentally infected with either EU or US PRRSV. The results showed the early detection of virus at seven days after infection which agreed with those reported by Wenvoort *et al*. (1993) using IPMA as a test kit. Although several test kits have been previously developed using PRRSV antigen and different testing technique, i.e. rn PRRS ELISA (Denac *et al*., 1997), K8-ELISA (Dea *et al*., 2000) and EU PRRS ELISA (Seuberlich *et al*., 2002) against rN-protein from EU PRRSV and KSU ELISA (Witte *et al*., 2000), US PRRS ELISA (Seuberlich *et al*., 2002) against rN-protein from US PRRSV, our technique has proven to be more accurate, easy to use, rapidly done, cost less and more appropriate for large-scale survey.

CONCLUSION

USEU PRRS ELISA is a simple, reliable, sensitive and specific test for screening a large number of swine sera for the presence of anti-PRRSV antibodies. This study is the first test using both PRRSV types (US and EU PRRSV) fused into *E.coli* as a recombinant antigen. The partially purified rN-protein was effective as antigen and could be used at 1:1600 dilution as determined by checkerboard titration. Their specificity and sensitivity were comparable to those of conventional and commercialized kits but cost less and easy to use. Thus, it is suitable for large scale serological examination, routine diagnostics, epidemiological surveys and follow-up investigations for outbreaks.

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