Detection of Asymptomatic Antigenemia in Pigs Infected by Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) by a Novel Capture Immunoassay with Monoclonal Antibodies against the Nucleocapsid Protein of PRRSV\textsuperscript{V}

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Porcine reproductive and respiratory syndrome (PRRS) is rapidly gaining importance as one of the most economically significant diseases in swine worldwide. The PRRS virus (PRRSV) plays a major role in its pathogenesis and causes persistent disease due to factors such as virus genetic diversity, virulence, or modulating the immune system of the swine (8, 10, 29). Since PRRSV is genetically heterogeneous, currently available vaccines cannot provide a completely protective effect (9, 17, 19). Therefore, the rapid diagnosis of PRRSV infections is important for reducing economic loss through timely management and epidemiological control. Currently, reverse transcriptase PCR (RT-PCR) and serological tests are widely performed for the diagnosis of PRRSV infections (1, 6, 15, 18, 26, 27). Although molecular detection, such as that by RT-PCR, provides promising sensitivity and rapid diagnosis, the molecular approach is costly, as it requires specialized laboratory equipment and experienced technicians. Serological testing is a cost-effective tool for the routine diagnosis of PRRS. However, the serological detection is limited by the inability to differentiate between vaccination, primary infection, or reinfection (19). Therefore, the development of effective methods for monitoring and controlling PRRS is necessary. Viral antigen detection methods are convenient and cost-effective and have been used successfully with various infectious diseases (2, 11, 16). During acute virus infection, viral antigens in the blood appear earlier than the antibodies. Thus, rapid and accurate primary screening for the stage of PRRSV infection can be achieved by the detection of the viral antigens rather than by the detection of specific antibodies. In our previous study, we successfully used novel monoclonal antibodies (MAbs) raised against ideal targets to develop the viral antigen detection methods for the diagnosis and monitoring of human disease activity (4, 24, 38). The approach may be adapted to the diagnosis of PRRSV infections. PRRSV is an enveloped positive single-stranded RNA virus that can be divided into two different genotypes, the European strains (EU PRRSV; type 1) and the North American strains (US PRRSV; type 2) (21). PRRSV contains nine known open reading frames (ORFs) (28), and ORF7 encodes the highly conserved viral nucleocapsid (N) protein. This N protein has been identified as the most abundant and immunogenic protein in the virion (35, 36). Currently, several serological tests based on the PRRSV N protein as the antigen...
have been developed and are widely used for the detection of antibodies produced in PRRS from infection with the North American or European PRRSV (23, 27, 34). Thus, the PRRSV N protein may be used to develop viral antigen detection methods. Here, we report on the successful development of an enzyme immunoassay for the PRRSV N antigen using monoclonal antibodies (MAbs) raised against the N proteins of both US PRRSV and EU PRRSV. This assay represents a valuable test for monitoring swine sera for the presence of active PRRSV.

MATERIALS AND METHODS

Viruses and cells. Five field isolates of US PRRSV, identified by sequencing analysis, were kindly provided by the Institute of Veterinary Medicine, Guangdong Academy of Agricultural Sciences. A CH-1R-derived PRRSV vaccine strain was from Weike Biotechnology (Harbin, People’s Republic of China) (37). All PRRSV isolates were propagated in immortalized MA-104 in minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS). Virus stocks were used to infect 70% confluent cell monolayers in MEM supplemented with 5% CO2 in a cell culture incubator until an obvious cytopathologic effect was observed. The viral culture supernatants and cell monolayers then were harvested and kept frozen at –80°C until needed.

Preparation of recombinant N protein from US PRRSV and EU PRRSV. Total PRRSV RNA was extracted from a US PRRSV strain isolated from a swine farm in the Guangdong province of China. The gene encoding the US PRRSV N protein was amplified from the total PRRSV RNA by RT-PCR with forward primer 5′-ATGCGAAATAACACGGCAGA-3′ and reverse primer 5′-TCATGCTGAGGTTAGCTGT-3′. The amplified N gene then was ligated into the prokaryotic expression vector PGEX-5X-3 (GE Healthcare, Chalfont St. Giles, United Kingdom) in frame and upstream of the glutathione S-transferase (GST) gene coding sequence. In addition, the N gene from EU PRRSV was synthesized according to an NCBI GenBank sequence (M96262; Lelystad virus) and also was cloned into PGEX-5X-3 (GE Healthcare). The recombinant US and EU PRRSV N proteins were expressed in Escherichia coli (BL21) and purified by affinity chromatography using glutathione Sepharose 4B (GE Healthcare) using the GST purification modules according to the manufacturer’s instructions. The US and EU PRRSV N proteins were detected by Western blot analysis with both PRRSV vaccine (CH-1R)-immunized mouse serum and mouse anti-GST MAb. The purified recombinant US and EU PRRSV N proteins and Western blot analysis are shown in Fig. 1. The concentration of protein was determined by the Coomassie Plus protein assay (Pierce Biotechnology, Rockford, IL) according to the manufacturer’s instructions.

Preparation and identification of MAbs against the PRRSV N protein. The preparation and identification of MAbs against the PRRSV N protein were performed as previously described, with some modifications (4, 24, 38). Briefly, 4- to 6-week-old female BALB/c mice first were immunized with subcutaneous injections of one dose of formalin-inactivated recombinant US PRRSV N protein (10 μg) administered at 10-day intervals. The mice then were given four boosters of US PRRSV N protein at 10-day intervals. Ten days later, all mice were bled and the serum was tested by ELISA with the US PRRSV N protein as the coated antigen to determine which animal had the greatest response to US PRRSV N protein. This mouse was given a further intravenous inoculation of 10 μg of US PRRSV N protein in phosphate-buffered saline (PBS), and its splenocytes were fused with NS-1 myeloma cells 3 days later. The hybridoma cell lines were screened primarily by an indirect ELISA with both US PRRSV N protein and PRRSV-infected cell lysates as coating antigens. The screening was confirmed further by an indirect immunofluorescence assay (IFA) that detected MAb binding on MA-104 cells infected with the CH-1R-derived PRRSV vaccine strain, which was performed according to our previously described protocol (5).

The specificity and cross-reactivity of the MAbs were evaluated by Western blot analysis with the recombinant US PRRSV N protein and EU PRRSV N protein. Positive hybridoma cells were cloned by limiting dilution. The isotype of each MAb was determined by the use of a commercially available mouse MAb isotyping kit (Zymed Laboratories, Carlsbad, CA). The purified MAbs from ascitic fluids were conjugated with EZ-Link sulfo-NHS-biotin reagents (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer’s instructions and with horseradish peroxidase (HRP) (Sigma-Aldrich) by the periodate method (33).

Analysis of MAb-binding epitopes. The binding epitopes of MAbs were analyzed by a competition ELISA with the US PRRSV N protein as the coated antigen, as described previously (24). Microwell plates (Costar Corning Inc., Corning, NY) were coated with 100 μl/well of the US PRRSV N protein at a concentration of 1 μg/ml in carbonate-bicarbonate (pH 9.6) coating buffer overnight at 4°C. Afterwards, the wells were blocked with 3% BSA (Sigma-Aldrich) in PBS for 2 h, and then a constant concentration of one of the nonbiotinylated MAbs (50 μl) was incubated with an optimal concentration of different biotinylated MAbs (50 μl) for 1 h at 26°C. After the plates were washed, streptavidin conjugated with HRP (Zymed) was added and plates were incubated for 30 min at 26°C. The plates were washed, and the binding of the biotinylated MAb was detected by the addition of tetramethylbenzidine (TMB; KPL, Gaithersburg, MD). The reaction was stopped after 10 min by the addition of 1 N sulfuric acid, and the plates then were examined in an ELISA plate reader (Bio-Tek, Winooski, VT). An irrelevant, unlabeled MAb was used as a control. The percentage of inhibition was calculated with the equation $\left(1 - \frac{OD_{test}}{OD_{control}}\right) \times 100$, where $OD_{test}$ is the optical density at 450 nm. The results were described as follows: inhibition of >75% represented competitive binding, inhibition from 25 to 75% represented relatively competitive binding, and inhibition of <25% represented noncompetitive binding (24).
The S/P ratio was calculated as \[
\frac{\text{OD}_{450} \text{ of the sample in PRRSV well}}{\text{mean } \text{OD}_{450} \text{ of the normal controls}}
\]. The absorbance was measured at 450 nm: \(\text{OD}_{450}\) of the sample in PRRSV well) / (mean \(\text{OD}_{450}\) of the normal controls).

The identity of amplified fragments was confirmed by sequencing analysis. A PCR product that can be used for discrimination between US and EU strains.

The purified recombinant N protein from US and EU PRRSV was used as the coating antigen and was reacted with purified MAbs against the PRRSV N protein. The absorbance was measured at 450 nm: \(\text{OD}_{450}\) of the sample in PRRSV well) / (mean \(\text{OD}_{450}\) of the normal controls)

Development of MAb-based PRRSV N antigen capture ELISA. To select the best combination of capture and detector antibodies for an antigen capture ELISA, MAbs were paired according to their ability to recognize different epitopes on the N protein. The procedure was modified from that previously described (24, 38). In brief, microwell plates (Costar) were coated with 100 \(\mu\)g/well of each capture MAb at a concentration of 10 \(\mu\)g/ml and incubated overnight at 4°C. After the blocking steps were performed, a series of diluted HRP-conjugated MAb was added and incubated for 30 min at 37°C. After further washes, 100 \(\mu\)l/well of each capture MAb at a concentration of 10 \(\mu\)g/ml and incubated overnight at 4°C. After the blocking steps were performed, a series of diluted HRP-conjugated MAb was added and incubated for 30 min at 37°C. After further washes, 100 \(\mu\)l/well of TMB solution was added, and the reaction was stopped by adding 2N sulfuric acid. The absorbance was determined as described above. Test samples were considered positive if their mean absorbance was greater than twice the mean absorbance of the normal controls.

RT-PCR assay. The extraction and detection of viral RNA in serum specimens were carried out using the QIAamp viral RNA Mini kit (Qiagen, Hilden, Germany).

The assay was calculated as the ratio of the number of clinical samples that tested positive to the total number of samples that were positively identified by virus isolation or RT-PCR. The specificity of the assay was calculated as the ratio of the number of samples that tested negative to the total number of negative samples. The derived data in the figures were tabulated in Microsoft Office Excel 2003 worksheets. A cross-tabulation of test results from both tests was used to assess agreement between the N antigen test and RT-PCR and was calculated using SPSS statistical package version 13.0 using McNemar’s test.

RESULTS

Selection and characterization of MAbs for PRRSV N antigen capture ELISA. Twenty-two hybridoma cell lines that stably produced MAbs initially were established on the basis of their strong positive reactivity with the US PRRSV N protein (Table 1). The cross-reactivity of the MAbs was identified further by testing their reactivity to the EU PRRSV N protein by ELISA and Western blot analysis. Of the 22 MAbs, 12

| Hybridoma (original clone no.) | Isotype | IFA result from cells infected by PRRSV (CH-1R strain) | Reaction with US PRRSV N protein by*: ELISA | Reaction with EU PRRSV N protein by*: ELISA | Epitope group
|-----------------------------|--------|----------------------------------------------------------|--------------------------|----------------------------------|----------------|
| P1 (1A11A13)                | IgG1   | ++ +                                                    | +                        | +                                | I              |
| P2 (V1E73A4)                | IgM    | +                                                       | +                        | +                                | II             |
| P3 (G1C1A2)                 | IgG1   | + ++                                                   | +                        | +                                | III            |
| P4 (V1C1A7A4)               | IgG1   | +                                                       | +                        | +                                | III             |
| P5 (V2A1A2A2)               | IgG1   | +                                                       | +                        | +                                | III             |
| P6 (V1C1A2A1)               | IgG1   | +                                                       | +                        | +                                | III             |
| P7 (G1A88A53)               | IgG1   | +                                                       | +                        | +                                | III             |
| P8 (G1A1A2A2)               | IgG1   | +                                                       | +                        | +                                | III             |
| P9 (G1B37A8)                | IgG1   | +                                                       | +                        | +                                | III             |
| P10 (V1D56A6)               | IgG1   | +                                                       | +                        | +                                | III             |
| P11 (2B11A18)               | IgG1   | +                                                       | +                        | +                                | IV             |
| P12 (V1D24A11)              | IgG1   | +                                                       | +                        | +                                | IV             |
| P13 (V1D16A6)               | IgG1   | +                                                       | +                        | +                                | IV             |
| P14 (1A28A6)                | IgG1   | +                                                       | +                        | +                                | IV             |
| P15 (G2C51A2)               | IgG1   | +                                                       | +                        | +                                | IV             |
| P16 (V1C74A5)               | IgG1   | +                                                       | +                        | +                                | IV             |
| P17 (V1C4C4A1)              | IgG1   | +                                                       | +                        | +                                | IV             |
| P18 (V1B3A4)                | IgG1   | +                                                       | +                        | +                                | IV             |
| P19 (G2E14A21)              | IgG2a  | +                                                       | +                        | +                                | V              |
| P20 (G2E38A2)               | IgG1   | +                                                       | +                        | +                                | V              |
| P21 (V1E50A1)               | IgG1   | +                                                       | +                        | +                                | V              |
| P22 (V2E49A6)               | IgG1   | +                                                       | +                        | +                                | V              |

*: The purified recombinant N protein from US and EU PRRSV was used as the coating antigen and was reacted with purified MAbs against the PRRSV N protein. The absorbance was measured at 450 nm: \(\text{OD}_{450}\) of the sample in PRRSV well) / (mean \(\text{OD}_{450}\) of the normal controls). Traditionally produced MAbs initially were established on the basis of their strong positive reactivity with the US PRRSV N protein (Table 1).
MAbs had cross-reactivity to the EU PRRSV N protein. The results demonstrated that the 12 MAbs recognizing cross-epitopes on both the US and EU PRRSV N proteins could be useful for developing an antigen capture ELISA for the detection of both genotypes. According to our previously described protocol (24, 38), a two-site sandwich antigen capture assay requires a pair of MAbs that are capable of binding to discrete, nonoverlapping epitopes on the antigen. Thus, the selection of MAbs with distinct epitope binding was done with competition experiments. On the basis of the five groups of epitopes identified among the 22 MAbs listed in Table 1, all combinations of MAbs were evaluated in a sandwich assay. The most effective pair of capture and detection MAbs was selected primarily based on the sensitivity in the detection of both US and EU PRRSV N proteins. Ultimately, the pairing of MAb P6 as a solid-phase immobilized capture antibody and MAb P15 as a labeled detecting antibody gave the highest combination of detection sensitivity in PRRSV-infected cell culture supernatants and in known concentrations of serially diluted recombinant N protein from the two PRRSV genotype strains (Fig. 2).

Sensitivity and specificity of the PRRSV N antigen capture ELISA. To evaluate the sensitivity of the N antigen capture assay, replicates of serially diluted recombinant US and EU PRRSV N proteins of known concentrations were analyzed. As shown in Fig. 2, a standard curve for the US and EU PRRSV N proteins was constructed. Bovine serum albumin (BSA) was used to establish the baseline for the assay, and a sample was considered positive if the OD$_{450}$ was two times greater than that of BSA. With these criteria, the minimal amount of recombinant US and EU PRRSV N proteins detected with this assay was approximately 4 (0.4 ng/test) and 8 ng/ml (0.8 ng/test), respectively. The specificity of the PRRSV N antigen capture ELISA was analyzed further by testing the presence of N protein in the culture supernatants from PRRSV isolates and other porcine viruses. Serial dilutions of supernatants from cell cultures infected with the PRRSV CR-1R strain and five other PRRSV isolates from the field in Guangdong were analyzed. Culture supernatants from porcine pseudorabies virus (PRV) and foot-and-mouth disease virus (FMDV) also were analyzed. The results are presented in Fig. 3. Only the serial dilutions of PRRSV-infected cell culture supernatants gave a positive signal. None of the PRV or FMDV culture supernatants detected in the N antigen capture assay. The results indicate that the MAb-based antigen capture assay is specific for the detection of PRRSV and has no cross-reactivity with other porcine viruses, like PRV and FMDV.

Detection of PRRSV N antigen in tissue specimens from PRRSV-infected pigs. The PRRSV N antigen capture ELISA was used to detect the N antigen in 8 lung tissues from pigs infected with PRRSV, as confirmed by both PRRSV isolation and RT-PCR, and 16 lung tissues from healthy pigs from food markets. PRRSV N antigen was detected in all 8 supernatants of ground lung tissue by the N antigen capture ELISA, with absorbance ratios of 22.2 to 78.6, while the 16 lung tissues of healthy pigs tested negative by this assay. Traditional RT-PCR also was performed, as a reference, with these lung tissue specimens. The amplification, with primer pairs described previously (18), yielded a 135-bp amplified product within ORF7 in all 8 lung tissues from PRRSV-infected pigs, while the 16 lung tissues from healthy pigs yielded negative results. The amplified fragments were confirmed by sequencing analysis to belong to the North American type. The results are summarized in Fig. 4. Both the sensitivity and specificity of the assay for detecting the N antigen in these lung tissue samples were 100% when the RT-PCR results were used as a reference.

Detection of PRRSV N antigen in serum specimens from field pigs. A total of 466 field serum specimens collected from swine farms were analyzed. Each serum sample was processed into three aliquots for use in the PRRSV N antigen capture ELISA, RT-PCR, and the serologic immunoglobulin G (IgG) test (a commercial IDEXX HerdChek® PRRS 2XR test kit), respectively. The percentage of specimens positive for antibodies against PRRSV, as determined by the IDEXX HerdChek PRRS 2XR ELISA, was 78.97% (368/466), despite all swine previously having been immunized twice. It is very interesting
that when the PRRSV N antigen capture ELISA was used to detect PRRSV N antigen in these 466 serum specimens, which were obtained from asymptomatic animals, the N antigen was detected in 58 of 466 (12.45%) specimens. Traditional RT-PCR was performed as a reference with the 466 serum specimens, and PRRSV N RNA was detected in 70 of 466 (15.02%) specimens. The sequence analysis of ORF7-amplified fragments determined them to be highly homologous, with 94 to 96% nucleotide identities to the PRRSV strain of CH-1a (Chinese isolate). The results of the three assays are summarized in Table 2. Of the 58 N antigen-positive (N antigen\(^+\)) samples, 47 were concordant with the RT-PCR test (i.e., N antigen\(^+\), RT-PCR\(^+\)), leaving 11 sera that were discordant with the RT-PCR test (N antigen\(^-\), RT-PCR\(^+\)). Comparing the sensitivities of the two assays, 47 of 466 sera tested positive for both N protein and viral RNA, 385 tested negative for both N protein and viral RNA, 11 had detectable N protein but were negative by RT-PCR, and 23 had detectable viral RNA but were negative by the N antigen assay. The statistical analysis results demonstrated a relatively good correlation between the two assays, with 92.7% agreement (\(k = 0.693\)). Of the 368 PRRSV antibody-positive sera, 43 were positive by both N antigen and RNA tests. Four sera were positive by both the N antigen and RNA tests but negative by the antibody test. Of the 58 N antigen and 70 PRRSV RNA-positive serum specimens, 51 and 64 specimens, respectively, also were positive for PRRSV antibodies. Collectively, these data demonstrate that although vaccinated pigs have antibody responses, active PRRSV infections are present in some swine farms. The RT-PCR assay was more sensitive than the N antigen assay; although the antigen capture ELISA is relatively convenient and rapid.

**Table 2. Results of detection of PRRSV N antigen, PRRSV RNA, and PRRSV antibodies in 466 field serum specimens obtained from asymptomatic pigs**

| Antigen and/or RNA test result\(^a\) | No. of sera | No. of PRRSV antibody test results\(^b\) |
|--------------------------------------|-------------|----------------------------------------|
|                                       |             | Positive | Negative |
| N antigen\(^+\), RT-PCR\(^+\)       | 47          | 43       | 4        |
| N antigen\(^-\), RT-PCR\(^-\)       | 11          | 8        | 3        |
| N antigen\(^-\), RT-PCR\(^+\)       | 23          | 21       | 2        |
| N antigen\(^+\), RT-PCR\(^-\)       | 385         | 296      | 89       |
| Total                                | 466         | 368      | 98       |

\(^a\) N antigen\(^+\) = positive for N antigen assay; RT-PCR\(^+\) = positive for PRRSV N RNA; N antigen\(^-\) = negative for N antigen assay; RT-PCR\(^-\) = negative for PRRSV N RNA.

\(^b\) PRRSV antibodies detected by the commercial IDEXX HerdChek\(^\text{®}\) PRRS 2XR test kit.

**DISCUSSION**

The pig is a major farm animal in China, because pork always has been an important source of animal protein in the typical Chinese diet. Thus, porcine diseases in Chinese farms have great economic and social impact and are closely monitored and controlled by the relevant local and national agencies. These efforts have resulted in a greater understanding of major pathogens like PRRSV, as well as the discovery of novel potential pathogens, like the porcine hokovirus, which may be associated with PRRSV (14). Since 2006, outbreaks of PRRS in parts of China were suggested to be caused by a highly pathogenic Chinese-type PRRSV strain (31, 32), which has led to the deaths of about 1 million pigs and the infection of 3.8 million pigs according to official statistics. The rapid antigenic variation and persistence of the virus have made the control of outbreaks difficult and, at times, impossible in some environments.

In this study, we successfully developed a PRRSV N antigen capture ELISA for the detection of PRRSV in sera or supernatants of ground tissues. With the optimization of the assay conditions, the microwell-format ELISA could detect purified recombinant US and EU PRRSV N protein at concentrations as low as 0.4 and 0.8 ng/test, respectively. Using this antigen capture ELISA, we found that the PRRSV N antigen was detectable in serum specimens obtained from asymptomatic animals, indicating that a significant percentage of active PRRSV exists in asymptomatic pigs, despite previous immunization. Of the 466 serum samples analyzed by our PRRSV N antigen capture ELISA, 12.45% (58/466) were positive. For the IDEXX HerdChek PRRS 2XR test kit, a routinely used method for PRRS diagnosis via the detection of antibody against the PRRSV N protein, the positive detection rate was 78.97% (368/466); however, this finding may simply indicate the effect from past immunization, which cannot be differentiated from primary infection or reinfection. RT-PCR currently is the gold standard for the diagnosis of active infection, and in this study 15.02% (70/466) of sera were positive by this method. Furthermore, among the eight lung tissue slurries that tested positive by RT-PCR, all also tested positive by N antigen capture ELISA.

It is important that 70 of 466 sera from clinically healthy pigs were RT-PCR positive for PRRSV despite previous vaccination. For the 466 sera, 47 were positive by both antigen and RT-PCR assays, 11 were positive by antigen test only, and 23 were positive by RT-PCR only. These results are discordant, but a statistically significant difference was not found between the two method’s results using McNemar’s test. Although RT-PCR appeared to be more sensitive than our antigen detection
assay, our test is much easier to perform in the field setting or at the herd level, with little risk of amplicon carryover contamination.

Our findings suggest that the PRRSV vaccine is not completely protective. First, there are pigs dying with PRRSV infections, as demonstrated by positive RT-PCR and antigen tests. Second, asymptomatic healthy pigs also can have antigenemia or viremia in their sera, which is detectable with these more sensitive tests. These asymptomatic pigs may serve as important reservoirs for the transmission of PRRSV to uninfected pigs. Moreover, they may be shedding escape mutants, which one day can exacerbate vaccine failure. The present vaccine is unlikely to be completely effective, because the wild virus is affecting and immunomodulating monocyes and macrophages, which are central players in the pig’s immune system (3, 12, 39). Thus, the vaccine-conferrered immunity can be dampened down once the intrusion by the wild-type virus is sufficient to downregulate the original immunity (19).

During PRRSV outbreaks, the commercial antibody detection kit (IDEXX HerdChek® PRRS 2XR test kit) has been useful for the surveillance and detection of the immune effect of PRRSV vaccines (23, 26, 27). However, this commercial antibody detection kit is not useful in our setting, because prior immunization resulted in 78.97% antibody positive in the 466 serum samples. The test cannot differentiate between vaccination and reinfection. In addition, a large number of diagnostic serological assays have been developed over the years that utilize different viral antigens (22, 34). However, serological diagnosis often is confounded by the failure to distinguish between antibody induced by infection and those arising from other means, such as maternal antibodies or vaccine-induced antibodies, although it might be possible to differentiate the serological response induced by vaccines from that caused by disease to a limited extent (7). Moreover, antibody production varies among pigs with respect to the strength of the immune response, the time of response, and the waning of antibody levels. In these respects, the direct detection of antigen has significant advantages over serological assays by avoiding the reliance on the host’s production of antibodies.

Another limiting factor of current PRRSV serological assays is the inability to discern the stage of infection in affected pigs. The presence of chronic PRRSV infections is difficult to detect clinically, but it may have significance in disease prevention and infection control (20, 32). The proper delineation of the various clinical forms of PPRSV infection is done best by combining multiple diagnostic assays to obtain a staging profile. For this purpose, the currently developed PRRSV N antigen capture ELISA is a promising option as an adjunct to the serological tests. This is based on our assessment of the antigen capture ELISA, which was found to have results comparable to those of RT-PCR. As we did not specifically examine the use of the antigen capture ELISA in pigs chronically infected with PRRSV, further evaluation is needed to demonstrate the role of antigen detection in staging PRRSV infections.

An important finding of the present study is that most of the samples from the diseased pigs were positive by both N antigen capture ELISA and RT-PCR. This indicates that vaccines do not necessarily work well in pigs or against reinfection. Therefore, antigen monitoring is particularly important. Findings from this study indicate that N antigen capture ELISA can be a useful adjunct for the early diagnosis of PRRSV infections and is a good monitoring tool, even after vaccination.

This paper represents the first report of antigen detection by antigen capture ELISA in the peer-reviewed literature. Previous attempts at antigen detection for PRRSV infections were limited largely to the use of immunostaining of tissues from infected animals (25, 30, 34). By using well-characterized MAbs with high affinity and high specificity to N of PRRSV, no evidence of cross-reaction was found when tested with other important pig viruses, such as PRV and FMDV. The overall performance of the present assay is similar to that of other antigen capture ELISAs developed for other viruses, such as dengue virus (24, 38) and severe acute respiratory syndrome coronavirus (4, 13). In summary, these findings suggest that a specific MAb-based PRRSV N antigen capture assay can be used reliably for the early diagnosis of PRRSV infections as an alternative to the more traditionally used virus isolation and RT-PCR methods. Although the detection of PRRSV N by antigen capture assay was slightly less sensitive than the detection of viral RNA by RT-PCR in this study, the N antigen capture assay has the advantage of being a quantitative assay, which can be implemented more easily in the field setting. Other future developments include testing the application of the assay in other accessible specimens, such as saliva, urine, feces, and semen.

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