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NOTE

Immunology

Determination of antibody induction by highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) vaccine: A comparison of two ELISA kits

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ABSTRACT. Two commercial porcine reproductive and respiratory syndrome virus (PRRSV) antibody ELISA kits (IDEXX and LSI) are currently in extensive use. To determine which kit is more suitable for the evaluation of HP-PRRSV vaccine efficacy, the two kits were used to test 546 serum samples. The agreement between the results was unsatisfactory, with a kappa statistic of 0.681 and a linear correlation coefficient of 0.665. In tests of samples from experimentally vaccinated and PRRSV-negative herds, IDEXX-ELISA identified antibody-positive conversion earlier and showed a higher specificity compared to LSI-ELISA. The serological profile obtained by neutralization testing was closer to that obtained by IDEXX-ELISA than by LSI-ELISA in the late immunization period. The findings reveal that IDEXX-ELISA is the more suitable for the evaluation of antibody response to HP-PRRSV vaccine and for guiding vaccination strategies.

KEY WORDS: antibody, ELISA, evaluation, highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) vaccine

Porcine reproductive and respiratory syndrome virus (PRRSV) is classified into two genotypes, European (type 1) and North American (type 2) [8], initially identified and reported in 1987 and 1990, respectively [4, 14]. In China, North American genotype strains have predominated since the first report of PRRSV in 1995 [5, 15]. Specifically, since the occurrence of large-scale outbreaks of PRRS and the identification of highly pathogenic PRRSV (HP-PRRSV) in 2006 [7, 12, 13, 16], these latter strains have been more prevalent in China, causing extensive losses to the domestic swine breeding industry [6, 15]. Accordingly, the prevention and control of infections due to HP-PRRSV are critical to the Chinese swine industry. The primary measure to control HP-PRRSV remains immunization with vaccines, and this strategy is currently adopted by most pig farms, with piglets usually being immunized once at 14–35 days old, and sows annually 1–3 times. Antibody determination is used for evaluating and guiding vaccination, and the detection methods most commonly applied in China involve the use of one or the other of two commercial kits: IDEXX HerdChek PRRS Ab X3 (IDEXX Laboratories, Inc., Westbrook, ME, U.S.A.; abbreviated here as IDEXX-ELISA) and LSIVet Porcine PRRS/AS-Serum (Laboratoire Service International, Lissieu, France; abbreviated here as LSI-ELISA). However, to our knowledge, a full comparison of the practicability of the two kits had not been performed before the present one, and it was not known which kit is the more appropriate for the evaluation of HP-PRRSV vaccination programs.

There are significant differences in the two kits. The IDEXX-ELISA utilizes plates coated with the viral nucleocapsid protein while the LSI-ELISA kit uses the viral glycoprotein. The incubation times in the IDEXX-ELISA is 30 min, much shorter than that in the LSI-ELISA (60 min). For IDEXX-ELISA, samples are considered positive if the S/P ratio is ≥0.4 and negative if <0.4, where S/P=(sample OD_{650}−negative-control OD_{650})/(positive-control OD_{650}−negative-control OD_{650}). For LSI-ELISA, the samples are considered positive if the relative index percent (IRPC) is ≥20 and negative if <20, where IRPC=(sample OD_{450}−negative-control OD_{450})/(positive-control OD_{450}−negative-control OD_{450}) ×100.

For the comparative study, a total of 546 serum samples constituted three sets of samples. First, 406 samples were collected from clinical herds immunized with HP-PRRSV vaccine. Of these, 90 were from post-weaning pigs aged 14–28 days (pre-immunization), 105 were from nursery pigs aged 35–70 days (post-immunization), 110 were from fattening pigs aged 90–150 days (post-immunization), and 101 were from sows (post-immunization). Second, 50 samples were collected from sows in PRRSV-negative herds. Third, 90 samples were collected from 10 PRRSV-negative pigs following immunization with an HP-PRRSV vaccine (strain JXA1-R) at 1 week pre-immunization (week −1) and at 1, 2, 4, 8, 12, 16, 20, and 26 weeks post-immunization.

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All serum samples were tested for anti-PRRSV antibodies using the two commercial indirect ELISA kits, according to the manufacturers' instructions. The animal experiments were performed with the approval of the Experimental Animal Use and Care Committee, Academy of Military Medical Sciences, China.

To determine the level of agreement between the two commercial kits and the neutralization test, 90 samples sequentially collected from 10 PRRSV-negative pigs following immunization with HP-PRRSV vaccine (strain JXA1-R) were tested by a serum neutralization assay, as described previously, with minor modifications [3, 11]. Briefly, pig sera were inactivated at 56°C for 30 min before serial dilution at 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 in a PRRSV (strain JXA1-R) mixture of 200 $TCID_{50}$ (50% tissue culture infectious dose) containing 5% *guinea pig* serum. The diluted samples were added to 96-well plates in duplicate, with 0.1 m*l* per well. Following incubation at 37°C with 5% CO_2 for 30 min, the serum-virus mixtures were transferred to wells containing *MARC-145* cell monolayers. Control wells containing cells only (no serum, no virus), and virus only (no serum) were included on each plate. The plates were incubated for an additional 72 hr at 37°C in 5% CO_2, at which point the presence or absence of cytopathic effects (CPEs) was recorded. Neutralizing antibody (NA) titers were expressed as the reciprocal of the highest serum dilution showing complete inhibition of CPEs in at least one of the duplicate wells.

Based on the results obtained using the two kits (Table 1) for analysis of the 546 serum samples, the sensitivity and specificity of IDEXX-ELISA were 89.10 and 79.33%, respectively, using LSI-ELISA as the reference method, whereas those of LSI-ELISA were 89.84 and 78.02%, respectively, using IDEXX-ELISA as the reference method. Sensitivity and specificity were calculated using the following formulae: sensitivity=TP/(TP + FN) ×100; specificity=TN/(TN + FP) ×100, where TP, FN, TN, and FP represent true positive, false negative, true negative, and false positive, respectively [2]. Pearson's correlation coefficient, calculated as a measure of the strength of the linear association between the IDEXX-ELISA S/P ratio and LSI-ELISA IRPC value using SPSS software, was 0.665 (Fig. 1). The Pearson correlation coefficient ranges from −1 to 1 with a stronger linear association being reflected by a higher absolute value. The kappa coefficient, calculated as a measure of the strength of agreement between the results for IDEXX-ELISA and those for LSI-ELISA using SPSS software, was 0.681. A kappa statistic of ≥0.75 represents excellent agreement, 0.40 to 0.75 good to fair, and <0.40 poor [1, 9]. Some inconsistency existed in the results, therefore, and the agreement between the two commercial kits was unsatisfactory.

Table 1. Comparison of IDEXX-ELISA with LSI-ELISA by testing 546 serum samples

		IDEXX-ELISA[a)]		
		Positive	Negative	Total
LSI-ELISA[b)]	Positive	327	40	367
	Negative	37	142	179
	Total	364	182	546

No. of serum samples. a) The negative–positive cutoff was 0.4. b) The negative–positive cutoff was 20.

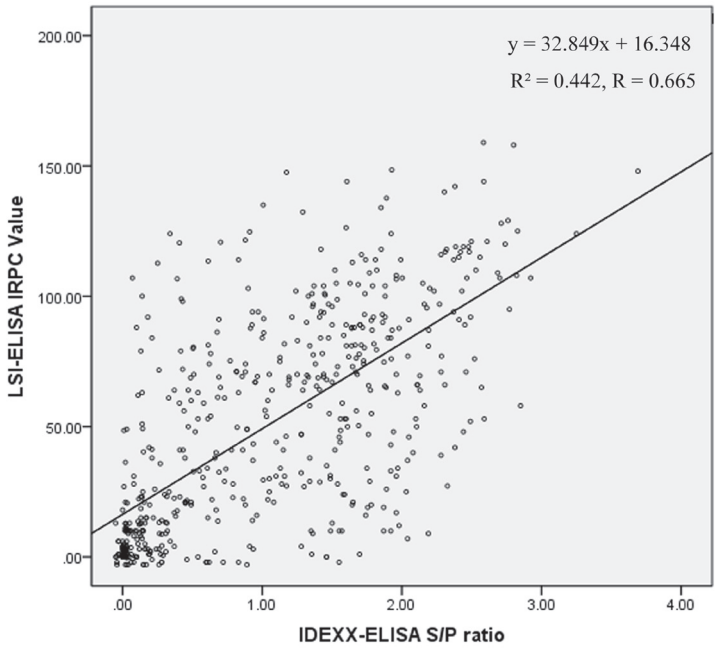

Fig. 1. Pearson correlation between the S/P ratios obtained using the IDEXX-ELISA kit and the relative index percent (IRPC) value obtained using the LSI-ELISA kit for analysis of the 546 serum samples.

For the 406 clinical serum samples in set 1, PRRSV antibody-positive rates determined by LSI-ELISA for sows and post-weaning pigs were higher than by IDEXX-ELISA, whereas positive rates for nursing pigs and fattening pigs were higher by IDEXX-ELISA (Fig. 2). For the 50 negative serum samples from PRRSV negative herds, all were negative on testing with IDEXX-ELISA, whereas 3 of the 50 samples were positive by LSI-ELISA. Furthermore, neutralization test assay of the 3 serum samples with inconsistent detection results all produced results consistent with IDEXX-ELISA (data not shown), indicating that the 3 serum samples determined by LSI-ELISA as PRRSV-positive were false positives, and that the specificity of IDEXX-ELISA was higher than that of LSI-ELISA. However, as the 3 false-positive serum samples were all from sows, the lack of specificity may be attributed to non-specific binding in some sow sera. In addition, the two ELISA methods presented significant differences in the serological pattern for serum samples sequentially collected from 10 PRRSV-negative pigs following immunization with HP-PRRSV vaccine (strain JXA1-R) (Fig. 3); following immunization, antibodies were detectable earlier with IDEXX-ELISA, and the positive rate detected by IDEXX-ELISA (10/10) was higher than that detected by LSI-ELISA (3/10) at 2 weeks post immunization. This is in accordance with the finding that positive rates determined by IDEXX-ELISA for sera from nursing and fattening pigs of herds immunized with HP-PRRSV vaccine at ages of 28 days (sera in set 1) were higher than by LSI-ELISA. In the late immunization period, the S/P value showed greater decreases with IDEXX-ELISA relative to LSI-ELISA. By 26 weeks post-immunization, the antibody positivity rate detected with IDEXX-ELISA (6/10) was significantly lower than with LSI-ELISA (9/10), indicating that LSI-ELISA enabled the detection of antibody persistence over a longer duration. The higher rate of detection of antibody positivity in sera from sows and postweaning pigs of the clinical herd (sera in set 1) by LSI-ELISA relative to IDEXX-ELISA may be attributed to this longer duration and the false positive results for sow serum. These data show that IDEXX-ELISA is the more sensitive in detecting antibodies at early time points, and LSI-ELISA at later time points. In general, it is desirable to have an assay that can detect PRRSV antibody at both early and late timepoints if a seroepidemiological survey of PRRSV or PRRSV eradication from infected farms is required. However, for evaluating the efficacy of PRRSV vaccination and for designing an effective immunization program for control of this disease, it is important to consider the relationship between

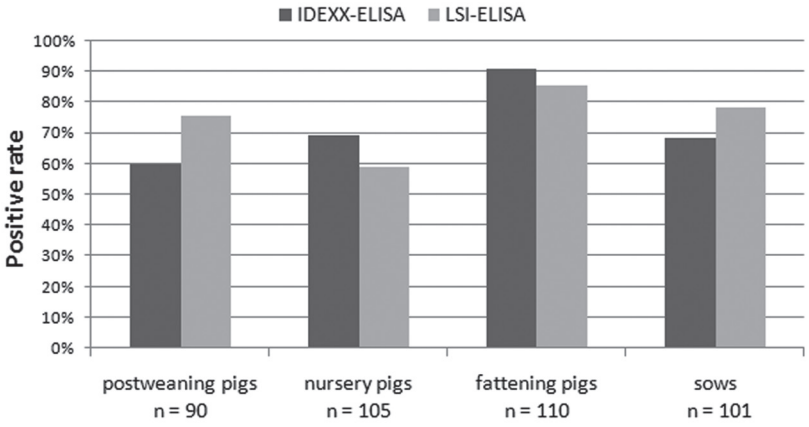

Fig. 2. Positive rate of porcine reproductive and respiratory syndrome virus (PRRSV) antibody detection in pigs of various age groups, as determined by IDEXX-ELISA and LSI-ELISA.

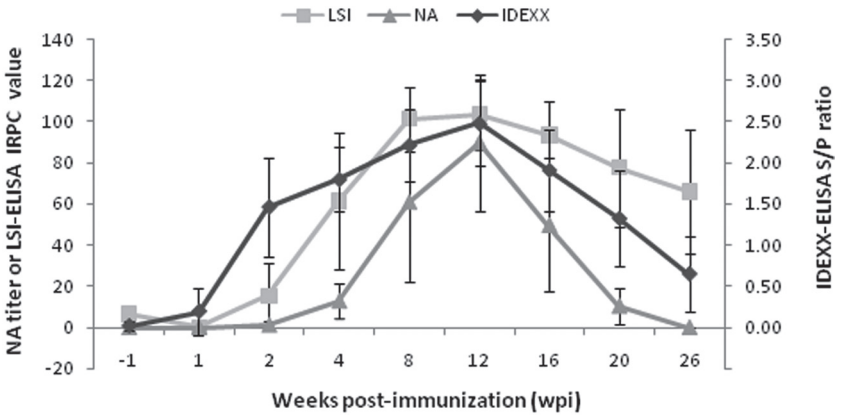

Fig. 3. Serological profile of porcine reproductive and respiratory syndrome virus (PRRSV) antibodies for 10 PRRSV-vaccinated experimental pigs, obtained by IDEXX-ELISA, LSI-ELISA, and neutralization assay. The data are shown as the means ± standard deviations.

immunoprotection against PRRSV and the anti-PRRSV binding antibodies detected by an ELISA in addition to considering the time range over which an ELISA provides positive results.

Despite the more complex procedure involved, the PRRSV antibody neutralization test is of significant value as a reference method for determining whether a particular animal is resistant to PRRSV infection. Recent studies have shown that high levels of PRRSV neutralizing antibodies (NA) elicit broad and strong cross-protection against different PRRSV strains [10]. Therefore, it was necessary to compare results obtained by the two ELISA methods with those obtained using the neutralization test. The serological profile of HP-PRRSV antibodies for the 10 experimental pigs obtained via the 3 methods showed that the duration of PRRSV antibody persistence, as determined by the neutralization test, was substantially shorter than that deteremined by the ELISAs. NAs were not detected until the 4th week post immunization and followed a pattern of antibody rise and decline more closely related to that determined by IDEXX-ELISA than by LSI-ELISA (Fig. 3). The test results of the serum samples collected between the 4th and 26th weeks post immunization, which delineate the onset and disappearance of the NA response, showed that the Pearson correlation coefficient between the NAs and the IDEXX-ELISA (0.519) was higher than that between the NAs and LSI-ELISA (0.393).

These findings indicate that, of the two ELISAs, IDEXX-ELISA is the more suitable for evaluating the antibody response to the HP-PRRSV vaccine, and is therefore more helpful for designing an effective immunization program. The reasons are as follows. First, IDEXX-ELISA detected antibody earlier than LSI-ELISA: it could therefore help to provide an earlier assessment of whether vaccination had produced an immune response. Second, during the late immunization period, the rapid decrease and low antibody levels determined by IDEXX-ELISA correspond well with NA levels, both indicating low resistance to the virus and the requirement for booster immunization. However, positive antibody titers were obtained by LSI-ELISA with a moderate level of antibody persistence detected during the late immunization period, even at times when NA titers were negative. Accordingly, the use of LSI-ELISA to evaluate immunity may falsely indicate adequate immunoprotection, resulting in postponement of the booster immunization which, in turn, could result in an increased risk of infection with wild-type virus.

Furthermore, as IDEXX-ELISA enables earlier detection of antibody than LSI-ELISA, this makes it more suitable for the diagnosis of early infection, which is of particular importance for the surveillance of PRRSV-negative herds.

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