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Porcine reproductive and respiratory syndrome virus (PRRSV) antibody levels in large swine farms in selected regions of Yunnan province, China*

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Summary

Porcine reproductive and respiratory syndrome (PRRS) is caused by the PRRS virus (PRRSV), and it is a widespread disease that severely affects swine production in all age groups. Detection of PRRSV antibody levels in pig farms is beneficial for immunity evaluations. In this study, a total of 1,206 serum samples of breeding boars, breeding sows, reserve pigs and commercial pigs from 16 large-scale swine farms in 4 different regions of Yunnan province in China were collected during 2019 and detected by indirect enzyme-linked immunosorbent assay (ELISA). The results showed that the average positive rate of PRRSV antibody was 88.32%, among which the antibody-positive rates were 89.03%, 89.18%, 92.11%, and 82.95% in East Yunnan (E. Yunnan), Central Yunnan (Cent. Yunnan), Northwest Yunnan (N.W. Yunnan) and Northeast Yunnan (N.E. Yunnan), respectively ($P > 0.05$). For the different pig categories, the reserve pigs (93.51%) showed much higher antibody-positive rates, followed by breeding sows (92.44%), commercial pigs (87.34%) and breeding boars (85.62%). Statistical analysis revealed that the rates were significantly different among different pig categories ($P < 0.05$). These s results indicated that pig categories were significantly associated with PRRSV antibody levels in this study. All the positive rates in this study fulfilled the requirement of ≥ 70% set by the National Animal Disease Surveillance Plan of China (2011). The study could provide evidence of the antibody response of PRRSV at the farm level.

Keywords: antibody detection, large swine farms, porcine reproductive and respiratory syndrome, PRRS virus

Porcine reproductive and respiratory syndrome (PRRS), also known as blue-ear disease in China, is a highly contagious disease caused by the PRRS virus (PRRSV), which is an enveloped, single-stranded, and positive-sense RNA virus belonging to the family *Arteriviridae* (3). Pigs are the only natural host for PRRSV (13). PRRS is characterized by reproductive failure in sows, as well as respiratory signs and high mortality in piglets and bred pigs (1, 21, 22). Pigs of different ages, breeds and sexes can be infected. Once pigs have been infected with PRRSV, it can cause premature delivery, abortion, stillbirth, weak fetuses or mummified fetuses of pregnant sows and degradation of breeding performance in boars (5, 14). Pregnant sows and piglets under 1 month of age are most susceptible (12). In pigs infected with PRRSV, it is likely to be secondary to other pathogens, which makes the disease very difficult to prevent and control (9). Since the appearance of PRRSV in the late 1980s, the virus has become endemic throughout the world, with only Sweden, Switzerland, Finland, Norway, Australia and New Zealand historically free of the PRRSV as of 2017 (20). The initial outbreak of PRRS was documented in an intensive pig farm in North China at the end of 1995, and in the subsequent 10 years, the disease became a common condition for Chinese pig production (26). At present, the disease has been widespread and is one of the main breeding barriers in large swine

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farms, causing serious economic losses to China's pig industry. It is also becoming the most important disease plaguing the global swine industry (8, 17, 19). The World Organisation for Animal Health (OIE) listed PRRS as a statutory report of animal epidemics, and China has listed it as a level B animal epidemic.

Vaccine immunization is an effective means to prevent various diseases (7). Through regular and planned immunization of healthy pigs, certain immunity can be generated to prevent and control the occurrence and epidemic of PRRS. Animal epidemic prevention and control is a long and complex process. Ensuring the immune effect is the top priority in epidemic prevention and control. A key component of animal epidemic prevention and control is immune antibody monitoring (18, 24). To prevent the failure of immunization and disease outbreaks, swine farms should collect blood samples in a timely manner three to four weeks after immunization to monitor the antibody level after immunization. Regular monitoring of the PRRSV antibody level after immunization of swine herds does not only assess the immune status but also prevent PRRSV infection (4, 10).

The aim of the study was to evaluate the immune effect against PRRSV after vaccination injections in large swine farms in Yunnan province. Concerning material and method, an indirect enzyme-linked immunosorbent assay (ELISA) method was used in this study to detect PRRSV antibodies in 1,206 serum samples from 16 large swine farms in Yunnan province.

Material and methods

The study sites. The study was carried out in Yunnan province, which is located in southwestern China and shares its borders with Myanmar, Laos and Vietnam. Four regions in Yunnan province, named East Yunnan (E. Yunnan), Central Yunnan (Cent. Yunnan), Northwest Yunnan (N.W. Yunnan), and Northeast Yunnan (N.E. Yunnan), were selected (Fig. 1).

Samples. Blood samples were obtained from 16 large swine farms with a minimum stock count of 300 from 4 regions of Yunnan. All selected pig farms had vaccinated their pigs against PRRSV according to the immunization procedures. Blood samples were randomly collected from 5-10% of the healthy livestock on each farm more than 21 days after the last vaccination. Blood samples were collected from the anterior vena cava of pigs and then centrifuged at 3,000 rpm for 20 min to separate the serum.

Test procedure. Indirect ELISA was performed to detect the level of the serum PRRSV antibody according to the protocol provided by the IDEXX PRRS X3 test kit (IDEXX, Liebefeld-Bern, Switzerland).

Interpretation. The presence or absence of antibody to PRRSV (positive/negative) was determined by calculating the sample/positive (S/P) ratio for each sample.

– If the S/P ratio was less than 0.4, the sample was judged to be negative for PRRSV antibody.

– If the S/P ratio was greater or equal to 0.4, the sample was judged to be positive for PRRSV antibody.

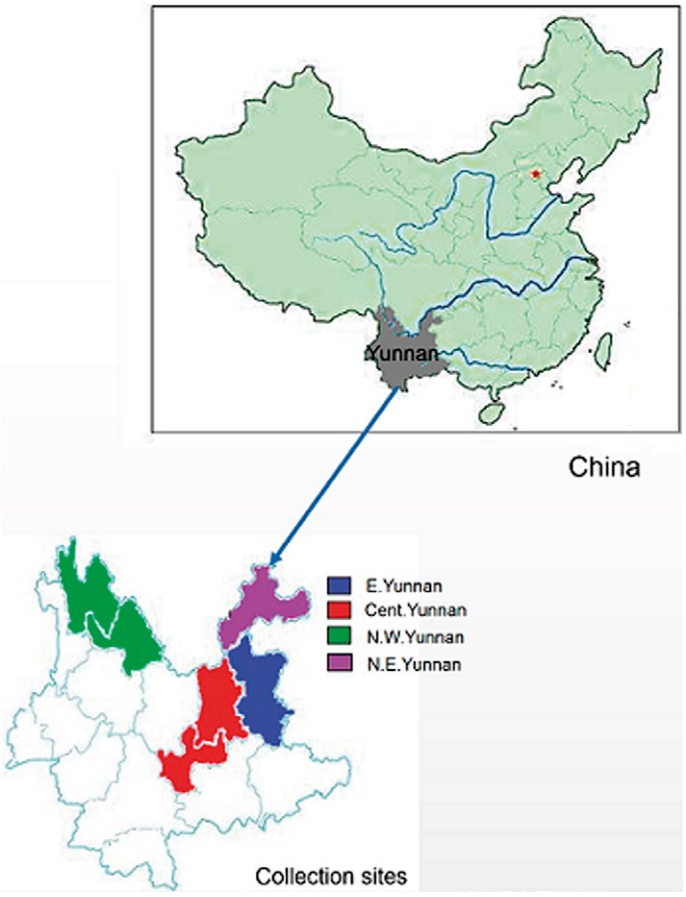

Fig. 1. Diagrammatic sketch of the geographic location of collection sites: East Yunnan (E. Yunnan), Central Yunnan (Cent. Yunnan), Northwest Yunnan (N.W. Yunnan) and Northeast Yunnan (N.E. Yunnan)

– Positivity for the antibody in this investigation was judged as passing, and the positive rate was the pass rate.

Data analysis. SPSS 20.0 was used to analyze the significance of the antibody positivity rates and the S/P ratio by the chi-square test and one-way ANOVA, respectively. Statistical significance was defined as a probability value (P) less than the significance level of 5% ($P < 0.05$). The degree of dispersion or coefficient of variation (CV) was used as a measure of the antibody level uniformity of the serum samples. CV = standard deviation (SD)/mean × 100%.

Results and discussion

Overview. Between March and August 2019, a total of 1,206 serum samples from 16 large swine farms were collected, covering breeding, reserve and commercial pigs, as detailed in Table 1. The PRRSV antibody-positive rate was 88.32%, as shown in Table 2, which fulfilled the requirement of ≥ 70% set by the National Animal Disease Surveillance Plan, China (2011).

The PRRSV antibody positive rate analyzed by region. The sample composition by region is shown in Figure 2. The PRRSV antibody test results by region are shown in Table 2. Among them, N.W. Yunnan had the highest pass rate at 92.11%, whereas N.E. Yunnan had the lowest at 82.95%. There were no significant differences observed among the four regions ($P > 0.05$). The average S/P ratio was 1.64 with CV of 38.96%.

Tab. 1. Number of blood samples from regions of Yunnan province, China

Region	No. of pig farms	Number of serum samples				Total
		Breeding boars	Breeding sows	Reserve pigs	Commercial pigs	
E. Yunnan	3	15	87	14	121	237
Cent. Yunnan	10	117	189	58	403	767
N.W. Yunnan	2	13	38	5	58	114
N.E. Yunnan	1	8	30	0	50	88
Total	16	153	344	77	632	1206

Tab. 2. Positive rate of PRRSV antibodies in regions of Yunnan province, China

Region	Number of samples	Percent positive (%)	S/P (Mean ± SD)	Degree of dispersion (%)
E. Yunnan	237	89.03	1.62 ± 0.70[a]	43.21
Cent. Yunnan	767	89.18	1.57 ± 0.54[a]	34.39
N.W. Yunnan	114	92.11	1.92 ± 0.84[b]	43.75
N.E. Yunnan	88	82.95	1.45 ± 0.50[a]	34.48
Overall	1206	88.32	1.64 ± 0.65	38.96

Explanations: a, b – values within each column not sharing a common superscript differ significantly ($P < 0.05$)

Tab. 3. PRRSV antibody test result of different pig categories in different regions

Region	Percent positive (%)			
	Breeding boars	Breeding sows	Reserve pigs	Commercial pigs
E. Yunnan	80.00	87.36	92.86	90.91
Cent. Yunnan	86.32	93.65	93.10	87.34
N.W. Yunnan	92.31	94.74	100.00	89.66
N.E. Yunnan	75.00	96.67	–	76.00
Total	83.41	93.11	95.32	85.98

Tab. 4. PRRSV antibody test result by pig category

Category	Number of samples	Percent positive (%)	S/P (Mean ± SD)	Degree of dispersion (%)
Breeding boars	153	85.62	1.46 ± 0.62[b]	42.47
Breeding sows	344	92.44	1.62 ± 0.59[a]	36.42
Reserve pigs	77	93.51	1.48 ± 0.65[b]	43.92
Commercial pigs	632	87.34	1.65 ± 0.62[a]	37.58
Overall	1206	89.73	1.55 ± 0.62	40.10

Explanations: a, b – as in Tab. 2

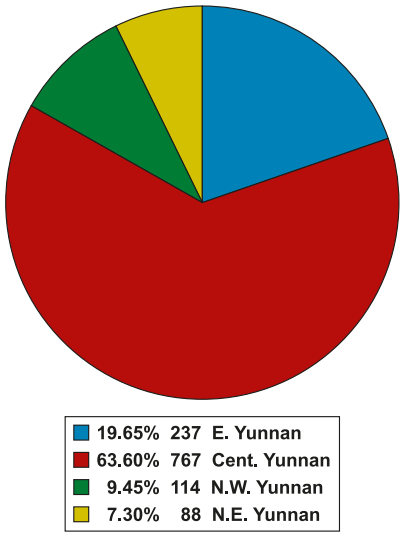

Fig. 2. Sample composition by region. A total of 1,206 serum samples were detected in 4 different regions of Yunnan province in China. The sample quantity and percentage in each region are shown. East Yunnan (E. Yunnan), Central Yunnan (Cent. Yunnan), Northwest Yunnan (N.W. Yunnan) and Northeast Yunnan (N.E. Yunnan).

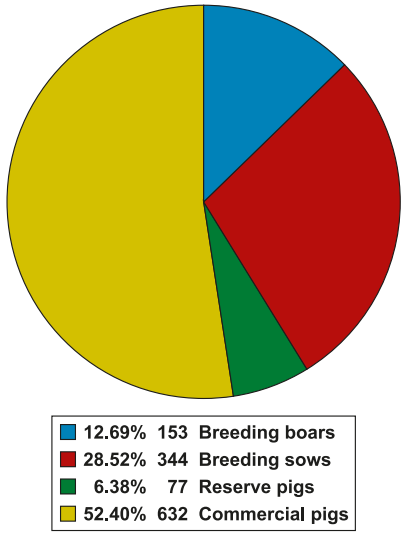

Fig. 3. Sample composition by category. A total of 1,206 serum samples were detected from breeding boars, breeding sows, reserve pigs and commercial pigs. The sample quantity and percentage in each category are shown

The S/P ratio of N.W. Yunnan was significantly higher than the other three regions.

The statistical results of the positive rate of PRRSV antibody of different pig categories in different regions are shown in Table 3. Breeding boars showed the highest antibody positive rate of 92.31% from N.W. Yunnan farms and the lowest antibody positive of 75.00% from N.E. Yunnan. Breeding sows from N.E. Yunnan enjoyed the highest antibody positive rate of 96.67%, followed by N.W. Yunnan of 94.74%, Central Yunnan of 93.65%, E. Yunnan with the lowest positive rate at 87.36%. Reserve pigs in N.W. Yunnan had the highest antibody positive rate at 100% and E. Yunnan the lowest at 92.86%. Commercial pigs in E. Yunnan had the highest antibody pass rate at 90.91% and N.E. Yunnan the lowest at 76.00%. The positive rate of 4 pig categories in 4 regions all met the requirement of ≥ 70%. The different pig categories in different regions had no significant difference ($P > 0.05$).

The PRRSV antibody positive rate analyzed by pig category. The pig category composition is shown in Figure 3. The PRRSV antibody test results by category are shown in Table 4. The results showed that much higher antibody-positive rates were found for reserve pigs (93.51%), followed by breeding sows (92.44%), commercial pigs (87.34%) and breeding

boars (85.62%). Statistical analysis revealed that the antibody-positive rates of PRRSV were significantly different among different pig categories ($P < 0.05$). Our results showed that pig categories were significantly associated with PRRSV antibody levels in this study.

Antibody detection methods mainly include indirect hemagglutination tests (IHAs), immunofluorescence assays and ELISAs (6). Each detection method has its own advantages and disadvantages and adapts to different scenarios. Although the IHA is simple and fast to operate and does not require special instruments, its stability is not high, and the test results are determined to be greatly affected by human factors (16). Immunofluorescence assays have the advantages of simple operation, high sensitivity, direct observation of antigen localization, etc., and have important application value in the screening and identification of monoclonal antibodies. However, due to its long time for cell culture and virus proliferation, it requires high technical ability of personnel and is not suitable for large-scale clinical detection. ELISA is a simple, rapid, specific and sensitive test with low requirements for technicians and instruments. It has been widely used for screening and identification of monoclonal antibodies (2).

PRRS is a disease that is difficult to eradicate, and the most direct and rapid way is to vaccinate animals to protect them from PRRSV (7). Since the epidemic of PRRSV in China in 2006, PRRS has been included in mandatory immunization programs, and PRRS immune antibodies are regularly monitored (10). PRRSV antibodies are generally monitored by ELISA. Biernacka et al. (2) compared six different ELISA kits and found that different kits had significant differences in specificity and diagnostic sensitivity. However, in the farms with early PRRSV infection, the performance difference was almost negligible, with the sensitivity of the IDEXX kit being the best. Sattler et al. (23) compared three commercial PRRS ELISA kits, among which the IDEXX kit was considered to be reliable and sensitive. In this investigation of PRRSV antibody levels, an IDEXX ELISA kit was selected.

The positive rates of PRRSV antibody in E. Yunnan, Cent. Yunnan, N.W. Yunnan and N.E. Yunnan were 89.03%, 89.18%, 92.11% and 82.95%, respectively. The positive rates of PRRSV fulfilled the requirement of $\geq$ 70% set by the National Animal Disease Surveillance Plan of China (2011). The results showed that the positive rates of all 4 pig categories met the requirement of $\geq$ 70%. The positive rate of boars in N.W. Yunnan was the highest (92.31%), and the positive rate of boars in N.E. Yunnan was the lowest (75.00%). Antibody dispersion, generally expressed by the CV, is an important indicator to measure the uniformity of the antibody detection value of a pig herd. The smaller the dispersion is, the better the outcome for the pig herd. It can reflect the uniformity of the antibody level of pig farms after immunization, which can be used to evaluate the vaccine effect and provide a reference for the overall immunization of large-scale swine farms. The overall dispersion of pigs of different pig categories in different areas was 38.96%, and the immune effect was qualified. However, there were differences in antibody levels between different regions and categories. The S/P ratio of antibody in N.W. Yunnan was significantly higher than that in the other three regions ($P < 0.05$). The S/P ratios of antibodies in reserve pigs and breeding boars were significantly lower than those in sows and commercial pigs ($P < 0.05$). The level of immune antibodies was different between different pig categories ($P < 0.05$), so it was necessary to adjust the immune procedure to improve the level of antibody protection.

The variance in the serum antibody levels might be the result of multiple factors, such as inconsistent vaccination procedures, differences in autoimmunity, maternally derived antibody interference, etc. In addition, factors such as vaccine quality, operational proficiency, pig health status and breeding management level also affect the level of pig immune antibodies (15). Therefore, a wider range of samples and a larger number of samples are required. Wei et al. (25) conducted a study on the immune sequence, and the results showed that when the PRRS vaccine was given first and the classical swine fever (CSF) vaccine was given later (the two were separated by more than 1 week), the immune effect was better. Jing et al. (11) showed that vaccination against foot-and-mouth disease (FMD) and CSF first and giving the PRRS vaccine one week later resulted in higher antibody levels and fewer immune side effects for the three diseases. Therefore, the immunization procedure should be formulated according to the actual situation of local epidemic diseases, and different immunization procedures should be formulated for reserve pigs, commercial pigs, boars and sows.

In this study, 1,206 serum samples of boars, sows, reserve pigs and commercial pigs from 16 large-scale swine farms in 4 regions of Yunnan Province were tested. The results showed that the PRRS immune protection level of 16 swine farms was relatively high, and the test results met the requirement of $\geq$ 70% set by the National Animal Disease Surveillance Plan of China (2011). However, there were some regional differences and pig category differences in the test results. Each swine farm needed to adjust the immunization procedure, strengthen the immune density according to the actual situation, and carry out the detection of immune antibodies after vaccination to ensure the immune effect.

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