Original article

Seroprevalence of antibodies to classical swine fever virus and porcine reproductive and respiratory syndrome virus in healthy pigs in Hunan Province, China

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Abstract

Classical swine fever (CSF) and porcine reproductive and respiratory syndrome (PRRS) are responsible for major economic losses and represent a threat to the swine industry worldwide. Routine surveillance serology for CSF and PRRS viruses is critical to maintaining the health status of sow farms in Hunan Province, which is one of the top pig production provinces in China. The aim of our study was to investigate the serological statistics of CSF virus (CSFV) and PRRS virus (PRRSV) in Hunan Province. The cohort serum samples were collected from vaccinated and unvaccinated pigs. Our findings showed that the average rates of CSFV and PRRSV antibody seropositivity were 82.2% (95% CI: 80.1-84.3) and 84.8% (95% CI: 82.5-87.1), respectively, in the immunized group and that these rates were higher than those in the unvaccinated group (58.6% for CSFV and 47.8% for PRRSV). Additionally, the level of CSFV antibody in piglet serum declined gradually with age, whereas PRRSV-specific antibody level increased initially (1 to 2 weeks old) and then declined with age (2 to 4 weeks old). In summary, we investigated the difference in CSFV/PRRSV antibody levels among piglets at various weeks old (1 to 4 weeks) to further establish the duration of maternal immunity in piglets. In addition, routine monitoring of CSFV/PRRSV antibodies in immunized pigs was carried out to evaluate the efficacy of vaccination.

Key words: classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV), pig, seroprevalence, antibody

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Introduction

Classical swine fever (CSF) is listed as a major reportable disease by the World Organization for Animal Health (OIE) and a Class A animal epidemic disease in China (Zhou 2019). CSF virus (CSFV) is a small, enveloped RNA virus in the *Flaviviridae* family, and its genome is approximately 12.3 kb in length (Brown and Bevins 2018). Importantly, CSF is a highly infectious disease that occurs in both piglets and adult pigs via contact, and it causes persistent infections because CSFV can evade host immune surveillance (Goraya et al. 2018). CSF has been endemic in China since the early 20th century. The prevention and control of this disease depends mainly on prophylactic vaccination, such as with the vaccine to the Chinese lapinized strain (Luo et al. 2014). However, compared to the classic virus strain, some newly emerging CSFV isolates can also be epidemic among many immunized pig farms or sporadically isolated from immunized pig farms, and these isolates have shown distinct virulence and clinical manifestations (Zhang et al. 2018, Gong et al. 2019).

Porcine reproductive and respiratory syndrome virus (PRRSV), an enveloped and single positive--stranded RNA virus, belongs to a member of the family Arteriviridae (Han and Yoo 2014). In the early 1990s, it was identified to be the key etiologic agent responsible for porcine reproductive and respiratory syndrome (PRRS) (Montaner-Tarbes et al. 2019). PRRS has been epidemic for more than 20 years in China (Gao et al. 2017, Yin et al. 2021). The use of PRRSV vaccines has provided efficient protection from the virus by reducing clinical signs, decreasing viremia and shortening the duration of viral shedding (Guo et al. 2018). To date, the existing commercial vaccines, consisting mostly of modified live attenuated viruses, provide the highest level of protection (Madapong et al. 2020, Chae 2021, Zhou et al. 2021). However, attenuated vaccines fall short in a variety of areas (Stoian and Rowland 2019). Modified live attenuated viruses may cause chronic or persistent infections and have the potential to revert to virulence, bringing new challenges to PRRSV prevention and control in China.

To accelerate the eradication of CSFV and PRRSV, surveillance serology for both viral infections in the context of the current immunization programs should be performed. Additionally, the use of vaccination needs to be further evaluated to efficiently prevent these virus-associated diseases on farms.

To our knowledge, few studies have examined the serological statistics of these two critical pathogens in Hunan Province, China. Therefore, CSFV and PRRSV antibodies were tested in serum samples from immunized and unvaccinated pigs in different parts of Hunan Province in China from 2017 to 2019 to evaluate the effectiveness of vaccination.

Materials and Methods

Study locations and design

Hunan Province is located in the south-central region of China, a farming zone known for high pig production. The sampling area consisted of 8 regions and the regions included the north-eastern (Changsha and Yueyang), northern (Changde and Yiyang), south-eastern (Chenzhou), and southern (Hengyang, Shaoyang, Yongzhou) parts of Hunan Province (Fig. 1). All the vaccinated farms in our study used the same protocols for swine immunization: piglets were routinely immunized once with one commercial PRRSV attenuated vaccine (at two weeks of age) or CSFV attenuated vaccine (at three weeks of age) by intramuscular injection, while sows and boars were immunized with the PRRSV and CSFV attenuated vaccines three times per year. Notably, all unvaccinated piglets were from immunized sows in vaccinated farms, while the other unvaccinated pigs at various growth stages (fattening pigs, nursery pigs, sows and boars) were rare and from an unvaccinated farm. The number of pigs to be sampled per herd was based on the availability of pigs and the willingness of the farm owner. Furthermore, face-to-face interviews were carried out with farmers (>18 years old) who were involved mostly in swine breeding and management and who completed the registration form covering information related to the samples, such as the growth stage, age, and immunity condition of the pigs.

Sample collection

Three milliliters of blood were collected from each pig using a 5-ml syringe and corresponding needle. The blood was transferred into a 5-ml sterile tube, labelled appropriately and allowed to stand overnight. The blood was then centrifuged at $3000 \times g$ for 10 min to separate serum. The serum was then decanted into an appropriately labelled 2-ml plastic serum storage tube and stored at -20°C until use.

Laboratory analysis

Two commercial kits, namely the IDEXX PRRS X3 Ab Test (indirect ELISA for PRRS) and the IDEXX CSFV Ab Test (E2 competitive ELISA for CSF) from IDEXX Laboratories, Inc. (Westbrook, ME, USA), were used to test for the presence of PRRSV and CSFV antibodies, respectively, in serum. The manufacturer's
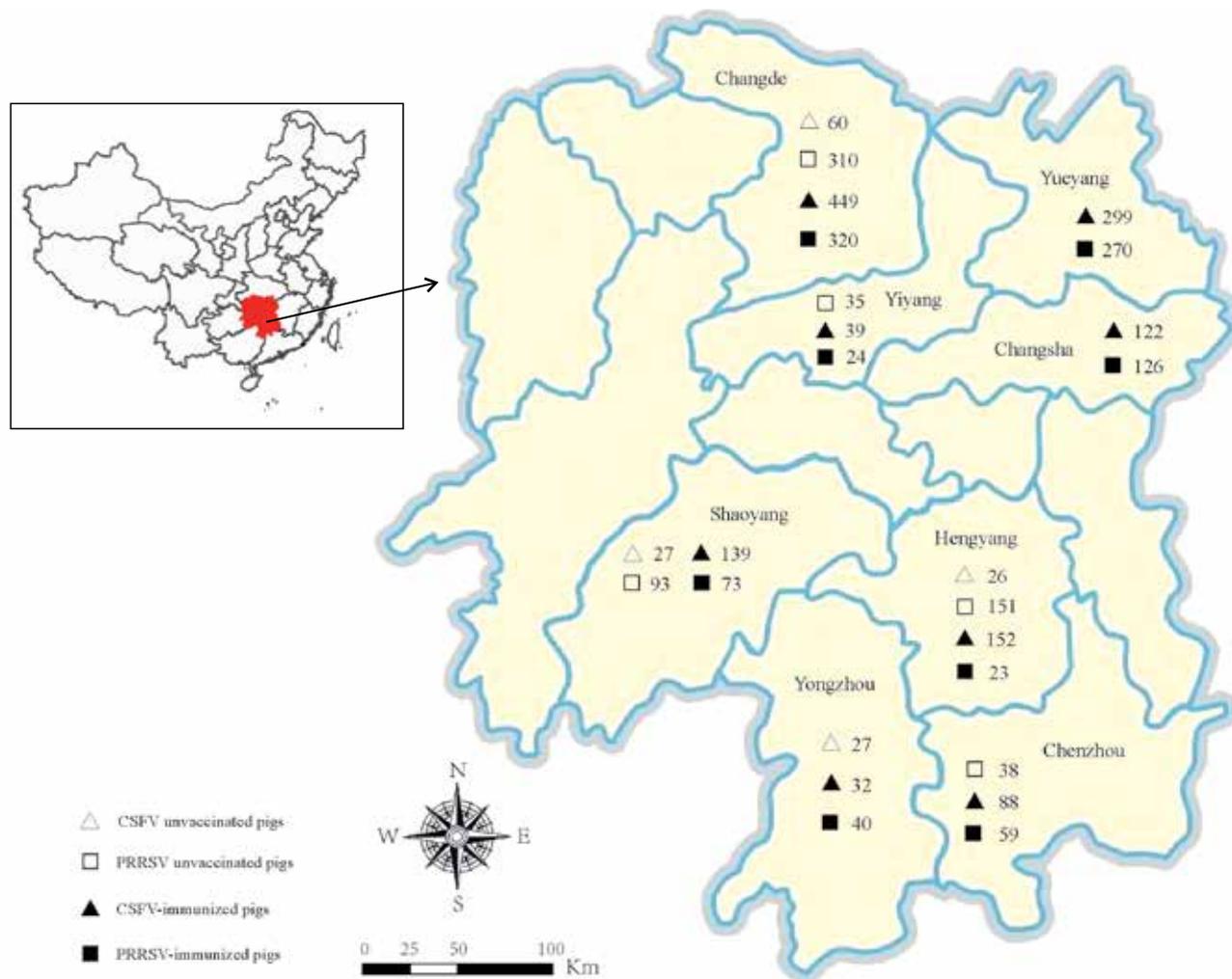
Fig. 1. Geographic distribution of swine herds in this study.

Map (enlarged area), with location of Hunan Province in China highlighted in red and herds in different parts of Hunan labelled as follows: Changsha, Hengyang, Shaoyang, Yueyang, Changde, Yiyang, Chenzhou, and Yongzhou. A hollow triangle (△) or square (□) represents pigs not immunized for CSFV or PRRSV, respectively. A filled triangle (▲) or square (■) represents pigs immunized for CSFV or PRRSV. The number of samples in each group is shown with the corresponding shape.

Note: The geographical maps used in this figure are from network material (website: http://www.51yuansu.com/); the geographical maps are free of copyrights.

instructions were strictly followed, and the results were read and interpreted using an ELISA reader at a wavelength of 450 or 650 nm. For the PRRSV test, samples were considered positive if the S/P ratio was ≥0.4 and negative if the ratio was <0.4, where $S/P = (sample_{OD650} - negative\ control_{OD650}) / (positive\ control_{OD650} - negative\ control_{OD650})$. For the CSFV test, samples were considered positive if the blocking % was ≥ 0.4 and negative if it was ≤ 0.3, where blocking % = $(negative\ control_{OD450} - sample_{OD450})/negative\ control_{OD450} \times 100$.

Data analysis

A database was built into a Microsoft Excel spreadsheet and included information on the samples collected in the field and the laboratory results. The data were then cleaned in Microsoft excel and exported for analysis in SPSS software (version 20, Chicago, USA). Confidence intervals were calculated in independent proportion to assess where a population parameter probably fell between a range of values. A pairwise test using Bonferroni's adjustment was subsequently performed to determine which differences among groups were significant. All P values are two-sided, and if the value was below 0.05, the results were considered statistically significant and labelled with different superscripts. The pairwise test was not applied when one or more of the cells had an expected count less than 5.

Results

For CSFV antibody detection, 1460 blood samples were obtained from pigs that were (n=1320) or were not

Table 1. Seroprevalence-specific rates of CSFV in pigs by sampling location.

Regions	CSFV antibody-positive sera from unvaccinated pigs (%)			CSFV antibody-positive sera from immunized pigs (%)		
	Total samples evaluated (n)	Percentage of positive (%)	95% CI	Total samples evaluated (n)	Percentage of positive (%)	95% CI
Changsha	n/a	n/a	n/a	122	76.2 [A]	68.6-83.9
Hengyang	26	30.8 [A]	11.8-49.8	152	84.2 [A]	78.3-90.1
Shaoyang	27	74.1 [B]	56.4-91.7	139	80.6 [A]	73.9-87.2
Yueyang	n/a	n/a	n/a	299	86.6 [A]	82.7-90.5
Changde	60	90.0 [B]	82.2-97.8	449	81.1 [A]	77.4-84.7
Yiyang	n/a	n/a	n/a	39	84.6 [A]	72.8-96.5
Chenzhou	n/a	n/a	n/a	88	73.9 [A]	64.5-83.2
Yongzhou	27	0 [C]	n/a	32	96.9 [A]	90.5-103.2
Total	140	58.6	50.3-66.8	1320	82.2	80.1-84.3

Note: 95% CI means confidence interval; n/a means not available, different superscripts within a column indicate significantly different group mean percentage of positive antibodies (P<0.05) while same superscripts within a column indicate no significant difference in regions.
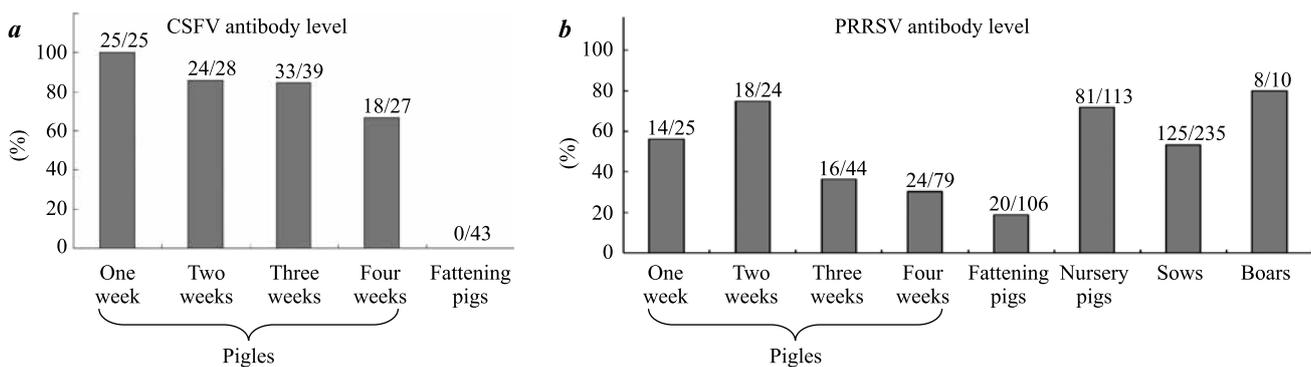

Fig. 2. Rate of CSFV/PRRSV antibody positivity in unvaccinated pigs at different growth stages.
The Y-axis represents the positive rate in different groups, and the ratio above each column is the number of positive samples divided by the number of test samples. (a) For detecting CSFV antibody, the positivity rates from left to right were as follows: Piglets (one-week-old: 100%; two-week-old: 85.7%; three week-old: 82.9%; four-week-old: 69.6%) and fattening pigs (0). (b) For detecting PRRSV antibody, the positivity rates from left to right were as follows: Piglets (one-week-old: 56.0%; two-week-old: 75.0%; three-week-old: 35.0%; four-week-old: 30.4%), fattening pigs (18.9%), nursery pigs (71.3%), sows (53.2%) and boars (80.0%).

(n=140) vaccinated for CSFV from herds of different sizes sampled from 2017-2019 (Fig. 1). For PRRSV antibody testing, 1562 blood samples were obtained from pigs that were (n=935) or were not (n=627) vaccinated for PRRSV from the same herds (Fig. 1).

Seroprevalence rates in unvaccinated pigs.

A total of 140 unvaccinated pigs from four regions were sampled for CSFV antibody tests, and 58.6% (82/140) were positive for CSFV (Table 1). Furthermore, the Yongzhou and Changde regions showed the minimum and maximum positive rates for CSFV, namely, 0 (0/27) and 90% (54/60), respectively. Notably, the rates of CSFV seropositivity were signifi-

cantly different among these regions (Hengyang and Changde/Shaoyang, Yongzhou and Changde/Shaoyang, Hengyang and Yongzhou) (p<0.05). When all these samples are grouped according to age, the results showed no CSFV infection in fattening pigs (0/43), while all the positive CSFV samples were from the piglets. It is worth noting that the CSFV antibody level in the piglets declined gradually with age (Fig. 2a).

For PRRSV antibody detection, 627 samples obtained from pigs that were not vaccinated against PRRSV were examined by ELISA, and the average rate of PRRSV-specific antibody positivity was 47.8% (Table 2). According to the results, the highest positive rate was 94.3% in Yiyang, whereas the lowest was 37.4% in Changde (Table 2). The rate of PRRSV

Table 2. Seroprevalence-specific rates of PRRSV in pigs by sampling location.

Regions	PRRSV antibody-positive sera from unvaccinated pigs (%)			PRRSV antibody-positive sera from immunized pigs (%)		
	Total samples evaluated (n)	Percentage of positive (%)	95% CI	Total samples evaluated (n)	Percentage of positive (%)	95% CI
Changsha	n/a	n/a	n/a	126	88.1 [A]	82.4-93.8
Hengyang	151	50.3 [A, B]	42.3-58.4	23	73.9	54.5-93.3
Shaoyang	93	51.6 [A, B]	41.3-62.0	73	93.2 [A]	87.2-99.1
Yueyang	n/a	n/a	n/a	270	84.4 [A]	80.1-88.8
Changde	310	37.4 [B]	32.0-42.8	320	79.7 [A]	75.3-84.1
Yiyang	35	94.3 [C]	86.2-102.4	24	95.8	87.2-104.5
Chenzhou	38	71.1 [A, C]	55.9-86.2	59	89.8 [A]	81.9-97.8
Yongzhou	n/a	n/a	n/a	40	95.0 [A]	87.9-102.1
Total	627	47.8	43.9-51.8	935	84.8	82.5-87.1

Note: 95% CI means confidence interval; n/a means not available, different superscripts within a column indicate significantly different group mean percentage of positive antibodies ($P<0.05$) while same superscripts within a column indicate no significant difference.
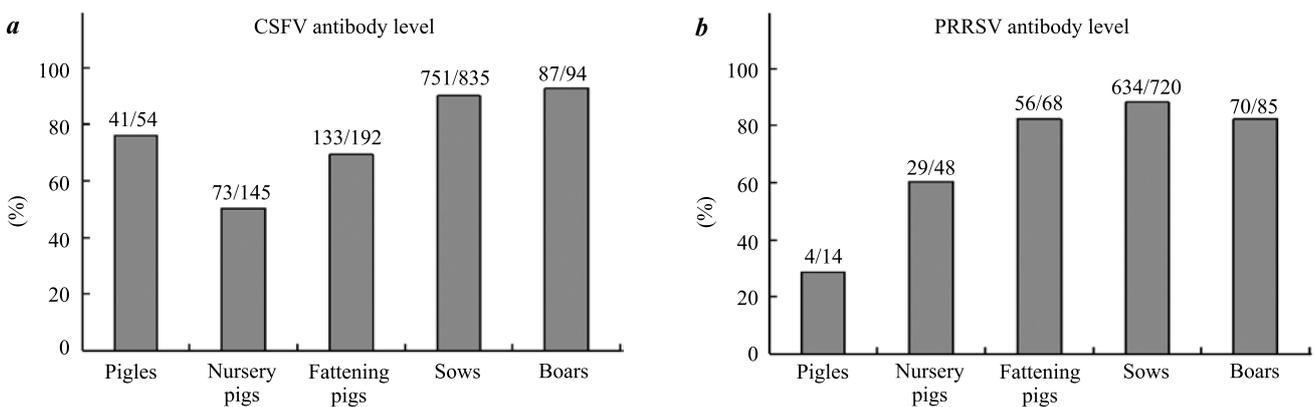
Fig. 3. Rate of CSFV/PRRSV antibody positivity in immune pigs at different growth stages.
The Y-axis represents the positive rate in different groups, and the ratio above each column is the number of positive samples divided by the number of test samples. (a) For detecting CSFV antibody, the positivity rates from left to right were as follows: Piglets (75.9%), nursery pigs (50.3%), fattening pigs (69.3%), sows (89.9%) and boars (92.6%). (b) For detecting PRRSV antibody, the positivity rates from left to right were as follows: Piglets (28.6%), nursery pigs (60.4%), fattening pigs (82.4%), sows (88.1%) and boars (82.4%).

seropositivity showed a significant difference between these two regions (Yiyang and Changde/Hengyang//Shaoyang, Chenzhou and Changde) (p<0.05). Moreover, the highest positive rate among the different growth stages was 80.0% in boars, and the lowest was 18.9% in fattening pigs (Fig. 2b). Unlike that for CSFV, the antibody level for PRRSV in piglets firstly increased from one to two weeks of age and then declined gradually with age (Fig. 2b).

Seroprevalence rates in immunized pigs.

Analysis of 1320 serum samples from Hunan Province showed the efficacy of vaccination against CSFV in Hunan Province, and the rate of CSFV antibody positivity was determined to be 82.2% (Table 1).

Furthermore, analysis of regional differences showed that the vaccination efficacy was best in Yongzhou, with a seropositivity rate of 96.9%, while the positivity rates in four regions (from high to low: Changde, Shaoyang, Changsha and Chenzhou) were below average (Table 1). Importantly, in contrast to the unvaccinated group, the rate of CSFV seropositivity showed no significant difference among the eight regions by further statistical analysis. The vaccination efficacy was not uniform at different growth stages. The highest to lowest positivity rates were as follows: boars (92.6%), sows (89.9%), piglets (75.9%), fattening pigs (69.3%), and nursery pigs (50.3%) (Fig. 3a).

Analysis of 935 serum samples showed that the seropositivity of PRRSV-specific antibodies in immunized pigs in Hunan Province, and the rate of serum

PRRSV antibody positivity was 84.8% (Table 2). Regarding regional differences, the highest positivity rate was 95.8% in Yiyang, the lowest was 73.9% in Hengyang, and the positivity rates in the other two regions (Changde and Yueyang) were below average (Table 2). Additionally, in contrast to those of the unvaccinated group, the rates of PRRSV seropositivity were not significantly different among the cities (Changsha, Shaoyang, Yueyang, Changde, Chenzhou, and Yongzhou). Among the various growth stages, sows had the highest seropositivity rate (88.1%), while piglets had the lowest (28.6%) (Fig 3b).

Discussion

The current methods of transportation of people and pigs are different from previous ones. This has led to an increasing concern about viruses, such as CSFV and PRRSV, spreading to pathogen-free areas, leading not only to animal infections but also to economic loss in the pig industry (VanderWaal and Deen 2018). Hunan is the third-largest swine-raising province in China, and the serological statistics of CSFV and PRRSV antibodies in Hunan Province have certain effects on the breeding industry. The moderately serological positivity of CSF (58.6%) in unvaccinated piglets may be caused by either CSF infections or maternally derived antibodies (MDAs), since their mothers were routinely immunized against CSFV. Because these suspected serum samples were collected from healthy piglets and the positive rates of CSFV-specific antibodies declined with age (Fig. 2a), there is a strong possibility that the anti-CSFV antibodies present in these serum samples originated from MDAs. A previous study reported that the presence of MDAs in piglets negatively influences the efficacy of a marker vaccine candidate (CP7_E2alf) (Suradhat et al. 2007, Farsang et al. 2017). Thus, our results together with those of the previous study suggest that timing is an important factor for using CSFV vaccine since the residual MDA may have a negative effect on the vaccine.

What distinguishes CSFV from PRRSV is the initial increase and subsequent decline that in the PRRSV antibody level with age in unvaccinated piglets (whose mothers were immunized against PRRSV). Furthermore, more unvaccinated pigs from different growth stages (fattening pigs, nursery pigs, sows, and boars) were used to detect the PRRSV antibody level. Each growth stage (except the piglet stage) had a significantly different positivity rate: 18.9% for fattening pigs, 71.3% for nursery pigs, 53.2% for sows and 80.0% for boars (Fig. 2b). The high positivity of unvaccinated boars in this study may explain why PRRSV is widespread in pig farms.

To reduce the infection rate and prevalence of viral diseases, there is an urgent need for annual vaccinations, also in the pregnant sow. The efficacy of vaccination against CSFV was studied in Hunan Province. Compared to the unvaccinated group, routine immunization with the CSFV attenuated vaccine in the vaccinated group reduced the difference in CSFV seropositivity between regions (Table 1). However, only the rate of CSFV antibody seropositivity (>90%) in Yongzhou showed an ideal vaccination efficacy. Hence, enhanced vaccination efficacy is needed in other areas in Hunan Province. Regarding the different growth stages of CSFV-immune pigs, a high level of vaccination efficacy was achieved in sows and boars due to the immune protocol, while that in others (piglets, fattening pigs, and nursery pigs) was not yet satisfactory (Fig. 3a). The low level of CSFV specific antibodies indicates a partial vaccine failure that is probably due to improper vaccination, poor quality of the vaccine antigens (e.g., low virus titer and failure in cold chain maintenance), co-circulation of another viral infection, or immunosuppressive conditions in the pigs (Suradhat et al. 2007, Deka et al. 2021). To study the effectiveness of vaccination, continuous routine monitoring of CSFV antibodies in the vaccinated herd will be crucial.

Combined with clinical experience, both the rate of PRRSV antibody positivity in immunized pigs and the related data for S/P values can indirectly reflect the serological status of a farm. Our study showed that most serum samples from PRRSV-immunized pigs were positive (84.8%), and the rate of positivity was higher than that in the unvaccinated group (47.8%). Notably, routine immunization with PRRSV attenuated vaccine reduced the differences in PRRSV seropositivity among regions (Table 2). Since all serum samples were collected from healthy pigs under proper vaccination, it is possible that the vaccine significantly increased the antibody levels in immunized pigs without natural infection. However, the positive rate in piglets was 28.6%, which was lower than that in other pigs (nursery pigs, fattening pigs, sows and boars) (Fig. 3b). This low percentage suggests that the antibodies in piglets could be affected by the presence of MDAs leading to extensive vaccine failure. These findings revealed that ELISA was a suitable method for the evaluation of antibody response to the attenuated vaccine and for guiding vaccination strategies. Importantly, the nucleocapsid-based antibodies detected by ELISAs are non-neutralizing, and further suitable assays for evaluating vaccine efficacy against PRRSV are warranted.

Conclusions

This study has determined serological statistics for CSFV and PRRSV antibody levels in immunized and unvaccinated pigs from Hunan Province of China from 2017 to 2019. Continuous monitoring of these viral antibodies among pigs in China is recommended, and improved immune procedures with intensified awareness campaigns are advised.

Acknowledgements

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