Seroprevalences of classical swine fever virus and porcine reproductive and respiratory syndrome virus in pigs in Hunan Province, China

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Research article

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Abstract

Background: 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 will be critical to maintain the health status of sow farms in Hunan Province, which is one of the top pig production provinces in China.

The objective of our study was to investigate the seroprevalence of CSF virus (CSFV) and PRRS virus (PRRSV) in Hunan Province from 2017 to 2019 using ELISA. The cohort pig serum samples were collected from vaccinated and unvaccinated sow farms in eight cities in Hunan Province.

Results: CSFV and PRRSV seropositivity showed significant differences among parts of Hunan Province in China. Interestingly, our findings show that the level of CSFV antibody in piglet sera decreases gradually with increasing piglet age, whereas PRRSV-specific antibodies may appear in various growth stages in unvaccinated animals. Meanwhile, the average rate of CSFV antibody seropositivity was 82.2%, while the rate of PRRSV antibody seropositivity was slightly higher (84.4%) in the immunized group.

Conclusions: The results from this study suggest that a serological investigation of these two major infectious diseases (CSF and PRRS) among pigs at various growth stages will provide a basis for the further development of more effective prevention and control measures.

Background

Classical swine fever (CSF) is listed as a major reportable disease by the World Organization for Animal Health (OIE) and listed as a Class A animal epidemic disease in China [1]. CSF virus (CSFV) is a small RNA virus with an envelope in the *Flaviviridae* family, and the genome is approximately 12.3 kb in length [2]. Importantly, CSF is a highly infectious disease that occurs in both piglets and adult pigs by contact and causes persistent infections, as CSFV can evade host immune surveillance [3]. CSF has been endemic in China since the early 20th century, and the prevention and control of this disease mainly depends on prophylactic vaccination, such as by the Chinese lapinized vaccine [4]. However, compared to the classic strain, some newly emerging CSFV isolates could also be epidemic in many immunized pig farms or sporadically isolated from immunized pig farms and have shown distinct virulence and clinical manifestations [5, 6].

Porcine reproductive and respiratory syndrome virus (PRRSV), an enveloped and single positive-stranded RNA virus, belongs to a member of the family *Arteriviridae* [7]. It has been determined to be the key etiologic agent responsible for porcine reproductive and respiratory syndrome (PRRS) since the early 1990s [8]. PRRSV has been epidemic for more than 20 years in China, and the administration of PRRSV vaccines has provided efficient protection from the virus by reducing clinical signs, decreasing viremia and shortening the duration of viral shedding [9]. To date, the existing commercial vaccines, mostly consisting of modified live attenuated viruses, provide the greatest levels of protection [10]. However, attenuated vaccines fall short in a variety of areas [11]. 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.

In order to accelerate the eradication of CSFV and PRRSV, surveillance serology for both virus infections in the context of the current immunization programmes should be performed, and vaccine administrations also need to be further evaluated to efficiently manage these virus-associated diseases on farms.

To our knowledge, few studies in Hunan Province in China have examined the serological prevalence of these two critical pathogens simultaneously. 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 serological prevalence of both viruses.

Results

Seroprevalence rates in unvaccinated pigs.

A total of 129 unvaccinated pigs from eight regions were sampled for CSFV antibody testing, and 77.5% (100/129) were positive for CSFV (Table 1). Furthermore, the Chenzhou and Yueyang regions showed the minimum and maximum positive rates for CSFV, i.e., 0 (0/10) and 100% (4/4), respectively, and the rate of CSFV seropositivity showed a significant difference among the sample locations ($n=8$) according to the chi-square test ($\chi^2 = 43.648$, $df = 7$, $p < 0.001$). The results showed no CSFV infection in fattening pigs (0/10); all the positive CSFV samples were from the piglets (100/100). It is worth noting that the CSFV antibody level in piglets decreased gradually with increasing piglet age (Fig. 2A).

For detecting PRRSV antibody, 636 serum samples from pigs not vaccinated against PRRSV were examined by ELISA, and the average rate of PRRSV-specific antibody positivity was 48.1% (Table 1). PRRSV appeared in the six regions of Hunan Province, the highest positive rate was 94.3% in Yiyang, whereas the lowest was 37.4% in Changde (Table 1). The rate of PRRSV seropositivity showed a significant difference among the sample locations ($n=6$) according to the chi-square test ($\chi^2 = 54.095$, $df = 5$, $p < 0.001$). Moreover, the highest positive rate among the different growth stages was 77.8% in boars, and the lowest was 18.9% in fattening pigs (Fig. 2B). Unlike for CSFV, the PRRSV antibody level in piglets first increased from one week old to two weeks old and then decreased gradually with increasing piglet age (Fig. 2B).

Seroprevalence rates in immunized pigs.

The efficacy of vaccination against CSFV was studied in Hunan Province, and the rate of CSFV antibody positivity was determined to be 82.2% by analysing 1320 serum samples from Hunan Province (Table 1). Analysing regional differences showed that the vaccination efficacy was the best in Yongzhou, with a seropositivity rate of 96.9%, while the positivity rates in four regions (Changsha, Shaoyang, Changde, and Chenzhou) were below the average (Table 1). On further statistical analysis, the rate of CSFV

seropositivity showed a significant difference among the sample locations ($n=8$) according to the chi-square test ($\chi^2 = 17.074$, $df = 7$, $p = 0.017$). The vaccination efficacy was not uniform at different growth stages, and the highest to lowest positivity rates were as follows: boars (92.6%), sows (90%), piglets (75.9%), fattening pigs (69.3%), and nursery pigs (50.3%) (Table 2).

The seroprevalence of PRRSV-specific antibodies in immunized pigs was also studied in Hunan Province, and the rate of PRRSV antibody positivity in sera was found to be 84.4% by analysing 935 serum samples (Table 1). Regarding regional differences, the highest positivity rate was 95.8% in Yiyang, and the lowest was 74% in Hengyang (Table 1). The rate of PRRSV seropositivity showed a significant difference among the sample locations ($n=8$) according to the chi-square test ($\chi^2 = 20.309$, $df = 7$, $p = 0.005$). Among the various growth stages, sows showed the highest seropositivity rate (88.1%), while piglet showed the lowest (28.6%) (Table 3).

Discussion

The transportation of people and pigs is different now from that in the past, and this has led to an increasing concern about viruses, such as CSFV or PRRSV, spreading to pathogen-free areas, leading not only to animal infections but also to economic loss in the pig industry [12]. 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 whole breeding industry.

The serological prevalence of 77.5% is an indication that CSF may exist in this study area without the use of a CSFV vaccine. However, Chen et al. similarly reported that only 4 pigs were solely infected with CSFV among 159 diseased pigs in China from 2016 to 2018 [13]. Thus, our prevalence rate (100/129, 77.5%), determined by ELISA, is significantly higher than the reported rate of CSFV positivity (4/159, 2.52%) determined by real-time PCR. It is possible that all of the positive CSFV samples were from unvaccinated piglets while their mothers were immunized against CSFV; therefore, the serological CSFV antibody level in the nonvaccinated pigs could originate from maternally derived antibodies (MDAs) and thus decrease with increasing piglet age. It is worth noting that CSFV vaccination should be performed when the residual MDA level is minimal and before pigs naturally become infected, avoiding potential interference from MDAs at the time of vaccination [14].

What distinguishes CSFV from PRRSV? The answer is that the PRRSV antibody level in unvaccinated piglets (whose mothers were immunized against PRRSV) increases first and then decreases with age. Furthermore, more unvaccinated pigs from different growth stages (nursery pigs, sows, and boars) were used to detect the PRRSV antibody level, which can better reflect the prevalence of PRRSV in the corresponding region. Each growth stage (except piglets) presented obviously different positivity rates: 71.7% for nursery pigs, 18.9% for fattening pigs, 53.4% for sows, and 77.8% for boars. The high positivity of boars in this study, may explain why PRRSV is widespread in pig farms.

The efficacy of vaccination against CSFV was studied in Hunan Province. Only the rate of CSFV antibody seropositivity in Yongzhou was above 90%, showing an ideal vaccination efficacy; however, 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, while that in others (piglets, fattening pigs, and nursery pigs) was not yet satisfactory.

Combined with clinical experience, both the rate of PRRSV antibody positivity in immunized pigs and the related data of S/P values can indirectly reflect the serological status of a farm. Our study showed that most sera of PRRSV-immunized pigs from areas in Hunan Province under investigation were positive (84.4%), and the rate of positivity was higher than that in the unvaccinated group (48.1%). 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 less than the rate in others (nursery pigs, fattening pigs, sows and boars). This low percentage suggest that the antibodies in piglets could be affected by the presence of MDAs. Importantly, the nucleocapsid-based antibodies detected by ELISAs are non-neutralizing, and further suitable assays for evaluating vaccine efficacy against PRRSV are warranted.

Conclusion

This study determined the serological statistics of CSFV and PRRSV antibodies from immunized and unvaccinated pigs in Hunan Province in China from 2017 to 2019. Continuous monitoring of these viral antibodies among pigs in China is hereby recommended, as well as improved immune procedures with intensified awareness campaigns.

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 is consisted of 8 herds and the herds were located in the northeastern (Changsha and Yueyang), northern (Changde and Yiyang), southeastern (Chenzhou), and southern (Hengyang, Shaoyang, Yongzhou) parts of Hunan Province (Fig. 1). The number of pigs to be sampled per herd was based on the availability of pigs and willingness of the farmers to allow their pigs to be sampled. Furthermore, face-to-face interviews were carried out with farmers (> 18 years old) who were mostly involved in swine breeding and management and completed the registration form covering information related to the samples, such as the growth stage, age, and immunity condition of the pigs (Additional file 1).

For detecting CSFV antibodies, a total of 1449 blood samples from pigs not vaccinated (n=129) and vaccinated (n=1320) for CSFV from herds of different sizes sampled during 2017-2019 were used (Fig.

1). Additionally, 1571 blood samples from pigs not vaccination or vaccinated for PRRSV from the same herds were collected for PRRSV antibody testing (Fig. 1).

Three millilitres of blood was collected from each pig by using a 5-ml syringe and corresponding needle. The blood was then gently transferred into a 5-ml sterile tube, labelled appropriately and allowed to stand overnight. Then, the blood was centrifuged at $3000 \times g$ for 10 min to separate the serum from the clotted blood. 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, i.e., the IDEXX PRRS X3 Ab Test (indirect for PRRS) and the IDEXX CSFV Ab Test (E2 competitive for CSF) from IDEXX Laboratories, Inc. (Westbrook, ME, USA) were used to test for the presence of PRRSV and CSFV antibodies in sera, respectively. The manufacturer's instructions were strictly followed, and the results were read and interpreted using an ELISA reader at a wavelength of 450 or 650 nm. For PRRSV, samples were considered positive if the S/P ratio was ≥ 0.4 and negative if the ratio was < 0.4 , where $S/P = (\text{sample}_{\text{OD}650} - \text{negative control}_{\text{OD}650}) / (\text{positive control}_{\text{OD}650} - \text{negative control}_{\text{OD}650})$. For CSFV, samples were considered positive if the blocking % was ≥ 0.4 and negative if it was ≤ 0.3 , where $\text{blocking \%} = (\text{negative control}_{\text{OD}450} - \text{sample}_{\text{OD}450}) / \text{negative control}_{\text{OD}450} \times 100$.

Data analysis

All statistical analyses were performed using SPSS software (version 20, Chicago, USA).

Abbreviations

CSFV: Classical swine fever virus; PRRSV: Porcine reproductive and respiratory syndrome virus; ELISA: Enzyme-linked immunosorbent assay; OIE: World Organization for Animal Health

Declarations

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Authors' contributions

Conceived and designed the study: ZY, DZB, WND, and WGP. Performed the experiments and analysed data: YHY, ZLH, CYF, and PT. Wrote the paper: YHY, ZLH, and ZY. Revised the manuscript: LL and WND. All authors have read and approved the final manuscript.

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Availability of data and materials

All datasets supporting our findings are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All experiments were approved and reviewed by the Ethics Committee for Biomedical Research of Hunan Agricultural University, Changsha, China, following national legislation regarding animal welfare. Furthermore, the owners of the pig farms were asked for verbal permission to conduct this study using samples from their pigs.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest related to this work.

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Figures

Figure 1

Geographic distribution of the swine herds in this study Map (enlarged area), with the location of Hunan Province in China highlighted in red and the 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 (A) or PRRSV(B), respectively. A filled triangle (▲) or square(■) represents pigs immunized for CSFV (A) or PRRSV(B). The number of samples in each group is shown with the corresponding shape. Acknowledgement: The geographical maps used in this figure are from the network material (website: <http://www.51yuansu.com/>); the geographical maps are

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Figure 2

Rate of CSFV/PRRSV antibody positivity in unvaccinated pigs at different growth stages Note: The ratio above each column is the number of positive samples divided by the number of test samples.

Supplementary Files

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