Matrine exhibits antiviral activity in a PRRSV/PCV2 co-infected mouse model

Na Sun
Shanxi Agricultural University
Hua Zhang
Shanxi Agricultural University
Panpan Sun
Shanxi Agricultural University
Ajab Khan
Shanxi Agricultural University
Jianhua Guo
Texas A&M University
Xiaozhong Zheng
The University of Edinburgh
Yaogui Sun
Shanxi Agricultural University
Kuohai Fan
Shanxi Agricultural University
Wei Yin
Shanxi Agricultural University
Hongquan Li (livets@163.com)

Research

Keywords: Matrine, PRRSV/PCV2 co-infection, mouse model, interstitial pneumonia, immune function

Posted Date: May 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-25795/v1

Version of Record: A version of this preprint was published at Phytomedicine on October 1st, 2020. See the published version at https://doi.org/10.1016/j.phymed.2020.153289.

Abstract

Background

PRRSV and PCV2 co-infection is very common in swine industry which results in huge economic losses worldwide. Although vaccination is used to prevent viral diseases, immunosuppression induced by PRRSV and PCV2 leads to vaccine failure. Our previous results have demonstrated that Matrine possessed antiviral activities against PRRSV/PCV2 co-infection in vitro. To establish a PRRSV/PCV2 co-infected KM mouse model and evaluate the antiviral activities of Matrine against PRRSV/PCV2 co-infection. A total of 144 KM mice were randomly divided into six groups with 24 mice in each group, named as: normal control, PRRSV/PCV2 co-infected group (PRRSV/PCV2 group), Ribavirin treatment positive control (Ribavirin control) and Matrine treatment groups (Matrine 40 mg/kg, Matrine 20 mg/kg and Matrine 10 mg/kg). Except normal control group, all mice in other five groups were inoculated with PRRSV, followed by PCV2 at 2 h later. At 7 days post-infection (dpi), mice in the treatment groups were intraperitoneally administered with various doses of Matrine and Ribavirin, twice a day for 5 consecutive days.

Results

PRRSV N and PCV2 CAP genes were detected by PCR in multiple tissues including heart, liver, spleen, lungs, kidneys, thymus and inguinal lymph nodes. The viral load of PCV2 was the highest in liver followed by thymus and spleen. Although PRRSV were detected in most of the tissues, but the replication of PRRSV was not significantly increased, as shown by qPCR analysis. Comparing with PCV2 infection alone, PRRSV infection significantly elevated PCV2 replication and also exacerbated PCV2 induced interstitial pneumonia. qPCR analysis demonstrated that 40 mg/ml Matrine significantly attenuated PCV2 replication in liver and alleviated virus induced interstitial pneumonia, suggesting that Matrine could directly inhibit virus replication. In addition, Matrine treatment enhanced peritoneal macrophages phagocytosis at 13 and 16 dpi, and 40 mg/kg of Matrine increased the proliferation activity of lymphocytes. Body weight gain was continuously promoted by administrating Matrine at 10 mg/kg.

Conclusion

Matrine possessed antiviral activities via inhibiting virus replication and regulating immune functions in mice co-infected by PRRSV/PCV2. These data provide new insight into controlling PRRSV and PCV2 infection and support further the research for developing Matrine as a new possible veterinary medicine.

Background

Co-infections involving porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) are still common world-widely in pig industry and has resulted in enormously economic losses. The co-infection status of four important swine viruses (classical swine fever virus, PRRSV, PCV2 and PCV3) in 159 diseased and dead pigs in eight regions of China from 2016 to 2018 was

evaluated by Chen et al (2019), demonstrated that co-infection is a common phenomenon and the percentage of PRRSV and PCV2 co-infection is the highest among them [1]. Vaccination is usually used to prevent and control viral diseases. Although there are several vaccines available, vaccines are not highly effective against PRRSV/PCV2 co-infection due to high mutation rate of PRRSV and PCV2 viruses as well as the complicated co-infection status. A trivalent vaccine against M. hyopneumoniae, PCV2, and PRRSV, licensed in many Asian countries did not provide the same protection as each respective monovalent vaccine [2]. Vaccination with PRRS modified live virus vaccine followed by challenge with PRRSV and PCV2, provide protection for PRRSV but enhances PCV2 replication and pathogenesis [3]. Therefore, these problems highlight the need for alternative approaches to control this disease.

Screening of antiviral natural compounds extracted from traditional Chinese medicines is gaining attentions in the world. Many natural compounds have the antiviral activity against African swine fever virus [4], H1N1 [5], porcine parvovirus [6], infectious bursal disease virus [7] and Marek's disease virus [8, 9]. Ginsenoside Rg1 [10], Sodium tanshinone IIA sulfonate [11], tea seed saponins [12], Dipotassium Glycyrrhetate [13] and Xanthohumol [14] were selected as a potential drug candidate to treat PRRS. Polysaccharide of Sargassum weizhouense [15], Epigallocatechin Gallate [16], saponins [17] and Astragalus polysaccharides [18] inhibit PCV2 replication in vitro and in mice. Matrine, a compound extracted from sophora flavescens Ait., possess anti-PRRSV [19], anti-PCV2 [20] and anti-PPRSV/PCV2 co-infection activity in vitro, demonstrated in our previous studies [21].

Undoubtedly an appropriate laboratory animal model is important for drug development. Due to easy handling and low price, mouse model is a better choice for preliminary drug screening. It has been reported that PRRSV does not replicate in rodents [22], but PCV2 infection was successfully established by multiple routs including intranasal, intraperitoneal, oral and intramuscular injection [23–26]. Piglets have been used for PRRSV and PCV2 co-infection model [27, 28], but there are few literatures on the mouse model for PRRSV and PCV2 co-infection.

In this study, mouse model co-infected by PRRSV and PCV2 was firstly established, which was used to further evaluate the antiviral activities of Matrine against PRRSV and PCV2 co-infection.

Methods

Compounds, viruses and KM mice

Matrine was purchased from Nanjing Zelang Meditech Ltd, China and its purity was 98% by HPLC. Ribavirin solution for injection (1ml:0.1g) was purchased from Shanxi Taiyuan Pharmaceutical Co., Ltd, China. The titers of PRRSV and PCV2 were $10^{7.5}TCID_{50}$/ml and $10^{5.15}TCID_{50}$/ml, respectively by Immunofluorescence assay on PAM cells. $10^{6}TCID_{50}$/ml PRRSV and $10^{4}TCID_{50}$/ml PCV2 were used in this experiment [21]. All mice used in this study were purchased from the Experimental Animal Center, Academy of Military Medical Sciences, Beijing, China, and were humanely managed according to the established guidelines of ethics committee of Shanxi Agriculture University.

Establishment of KM mouse model co-infected by PRRSV/PCV2

6-week-old KM mice were randomly divided into three groups with 6 in each group. The group inoculated with normal saline was named as an uninfected control group, with PRRSV first followed by PCV2 2 h later as PRRSV/PCV2 co-infected group and PCV2 only as PCV2 infected group. The inoculation routes and dose of both PRRSV and PCV2 were as followed: each mouse was respectively inoculated 0.5 ml intraperitoneally, 0.3 ml subcutaneously on back and 0.05 ml intranasally. At 7 days post-infection (dpi), samples of heart, liver, spleen, lungs, kidneys, thymus and inguinal lymph nodes were respectively collected and frozen at -80°C for detecting PRRSV and PCV2 by PCR. The samples of lungs were also fixed in Bouin's solution for the histopathological observation.

Experimental design

144 Kunming mice were randomly divided into six groups with 24 mice in each group: normal control, PRRSV/PCV2 co-infected group (PRRSV/PCV2 group), Ribavirin treatment positive control (Ribavirin control), Matrine treatment groups (Matrine 40 mg/kg, Matrine 20 mg/kg and Matrine 10 mg/kg). Selected doses of Matrine (40, 20 and 10 mg/kg) used in these experiments were determined by Acute toxicity test (data were not shown). Except for normal control group, animals in all other groups were inoculated with PRRSV followed by PCV2 2 h later, as described in the section of model establishment. At 7 dpi, mice in the treatment groups were intraperitoneally administered with various doses of Matrine and Ribavirin, twice a day for 5 days at a dose of 0.2 ml Matrine or Ribavirin per 10 g body weight each time. Mice in the control group were administered with the same volume of normal saline at the same frequency and by the same route. The body weight of mice in each group before virus infection and at 10, 13, 16 and 19 dpi were recorded. Six mice of each group were sacrificed at 10, 13, 16 and 19 dpi, and the spleen and thymus were weighed and their immune organ indices were calculated according to the following formula: the index of organ (mg/g) = weight of organ / body weight.

Quantification of PCV2 DNA in liver tissues

At 10, 13, 16 and 19 dpi, liver of six mice in each group was collected and stored in liquid nitrogen. After grinding with liquid nitrogen, DNA of liver tissue was extracted using TIANamp Genomic DNA Kit (TIANGEN, China). The concentration of DNA was measured using NanoDrop 1000 spectophotometer (NanoDrop Technologies, Wilmington, DE, USA). A 148 bp fragments from the CAP gene of PCV2 was amplified with specific forward primer 5'-GTCTACATTTCCAGCAGTTTG-3' and reverse primer 5'-CTCCCGCCATACCATAA-3'. Real time PCR was performed using Applied Biosystems® 7500 Real-Time PCR system, and carried out with 2×SYBR Green qPCR Master Mix from Bimake according to the manufacturer’s instructions. A recombinant plasmid vector containing PCV2 CAP gene fragment was used to generate a standard curve.

Measurement of phagocytosis of peritoneal macrophages

Phagocytosis of peritoneal macrophages were measured as a cellular uptake of FITC-dextran and quantified by flow cytometry as described by Xiu et al [29]. Briefly, peritoneal macrophages were aseptically collected and resuspended in 1 ml RPMI 1640 medium. 0.2 mg/ml FITC-dextran was added into each tube and incubated for 30 min at 37°C. Cells added with the same amount of FITC-dextran and incubated at 4°C for 30 min were used as a baseline for macrophages phagocytosis assay. All cells were then collected and stained with APC-conjugated anti-F4/80 antibody and PE-conjugated anti-CD11b antibody for 20 min at room temperature in dark. Phagocytic ability was evaluated using Median fluorescence intensity (MFI) at a percentage of FITC-positive cells gated on $CD11b^+$ $F4/80^+$ peritoneal macrophage.

Lymphocytes proliferation assay using CFSE labeling

Murine splenic lymphocytes were collected using lymphocyte separation medium (TBD, China). Lymphocyte proliferation was determined using CFSE labeling by flow cytometry as described by Wang [30]. 500 μl CFSE with a concentration of 4 μM was added into the same amount of lymphocytes suspension in PBS. After incubation at 37°C for 10 min with slight shaking, 5 ml PBS supplemented with 10% fetal calf serum (FCS) was added and then incubated for 5 min on ice to stop the dying process. The CFSE-labelled cells were placed in 24-well plates with a density of 4×10^6/ml and incubated in the culture medium supplemented with ConA (the final concentration was 5 μg/ml) or LPS (the final concentration was 20 μg/ml). The cells were cultured for 48 h at 37°C and then analyzed by flow cytometry.

Statistical analyses

All the data were expressed as mean ± standard errors of the mean (SEM) and the statistical analysis were performed using SPSS version 21.0 software (SPSS, Chicago, IL, USA). A one-way analysis of variance (ANOVA) followed by LSD was used to analyze the results. When the Test of Homogeneity of Variances was lower than 0.05, Mann-Whitney U-test (nonparametric test) was used for statistical analysis. Histograms were obtained from GraphPad Prism version 5 (GraphPad software, San Diego, CA). * means $P < 0.05$, * *means $P < 0.01$, *** means $P < 0.001$.

Results

Distribution of PRRSV and PCV2 in different organs

At 7 dpi of PRRSV and PCV2, tissues of inguinal lymph nodes, spleen, liver, heart, lungs, kidneys and thymus were collected to determine the distribution of PRRSV and PCV2 by PCR to amplify a 196 bp fragment of PRRSV N gene and 148 bp fragment of PCV2 CAP. Both PRRSV N gene (Fig. 1A) and PCV2 CAP gene (Fig. 1B) were detected in all tissues collected above. As shown in Table 1, only one in five mice was positive for PRRSV in inguinal lymph node while PRRSV was detected in all other tissues collected from 5 mice. For PCV2 CAP gene, at least three mice were positive in the tissues collected from 6 mice. According to the PCR results, both PRRSV and PCV2 transmitted to most of the collected mice tissues.

Table 1
The tissue distribution of PRRSV and PCV2 in co-infected mice by PCR

	#of positive mice	#of positive tissues						
		Inguinal lymph nodes	Lung	Liver	Spleen	Kidney	Heart	Thymus
PRRSV	5/5	1/5	5/5	5/5	5/5	5/5	5/5	5/5
PCV2	6/6	5/6	5/6	6/6	5/6	3/6	6/6	5/6

PRRSV infection enhanced PCV2 replication and induced more severe lung lesions

At 7 dpi, PCV2 CAP gene copy numbers were the highest in liver, followed by thymus and spleen as shown in Fig. 1C. These results were similar both in PRRSV/PCV2 co-infected and PCV2 infected groups, suggesting that PCV2 has tissue tropism in mouse model. Furthermore, the results showed that PCV2 CAP gene copies in liver ($p < 0.05$), thymus ($p > 0.05$) and spleen ($p < 0.01$) of PRRSV/PCV2 co-infected mice were significantly higher than PCV2 infected mice, indicating PRRSV infection enhanced PCV2 infection in mouse model. In addition, compared with normal control group, obvious lung lesions were observed in both PRRSV/PCV2 co-infected and PCV2 infected mice (Fig. 2A). Particularly, the hemorrhagic area was larger in PRRSV/PCV2 co-infected mice than PCV2 infected mice. H&E staining of lungs (Fig. 2B) revealed that both PRRSV/PCV2 co-infected and PCV2 infected mice displayed typical characteristics of interstitial pneumonia including thickened alveoli septa and decreased alveolar area. Taken all these histopathological observations, the lesions of lungs were more severe in PRRSV/PCV2 co-infected mice, suggesting that PRRSV infection enhanced PCV2 induced lungs lesions.

Matrine restrained PCV2 replication in liver and alleviated lungs lesions induced by virus infection

PCV2 CAP gene copy numbers in liver were measured by qPCR as shown in Fig. 3A. At 10 dpi, compared with virus control group, the CAP gene copies were significantly decreased in Matrine 40 mg/kg treatment group ($p < 0.01$). The CAP gene in Ribavirin treatment group was lower but no significant difference was observed ($p > 0.05$). At 13 dpi, CAP gene copies in Ribavirin ($p < 0.01$), Matrine 40 mg/kg ($p < 0.05$) and 20 mg/kg ($p < 0.05$) treatment groups were significantly lowered than that in virus control. Compared with virus control at 16 and 19 dpi, CAP gene copies were decreased in drug treatment groups but without statistical difference ($p > 0.05$). Compared with normal control, the lungs of PRRSV/PCV2 co-infected mice displayed typical characteristics of interstitial pneumonia including thickened alveoli septa and decreased alveolar area. However, both Ribavirin and Matrine treatments (especially 40 and 20 mg/kg) could significantly alleviated these pathological changes as shown in Fig. 3B.

Measurement of body weight gain, spleen and thymus indices in different treatments

Compared with normal control, the body weight gain in PRRSV/PCV2 group was significantly declined at 19 dpi ($p < 0.01$), while increased with varying degrees in Ribavirin and Matrine treatments, especially Matrine 10 mg/kg treatment group ($p < 0.001$) as shown in Fig. 4A. Moreover, the body weight gain in

Matrine 10 mg/kg was higher at each indicated time point: 10 ($p > 0.05$), 13 ($p < 0.01$) and 16 dpi ($p < 0.05$), suggesting that Matrine has an effect of gaining body weight with the dose of 10 mg/kg. The spleen index in PRRSV/PCV2 group was much higher than that in normal control at 10 ($p < 0.01$), 13 ($p < 0.05$), 16 ($p = 0.067$) and 19 ($p < 0.05$) dpi. However, at 13 dpi, Matrine with the dose of 40 ($p < 0.05$) and 10 ($p < 0.05$) mg/kg significantly decreased the spleen index induced by virus infection (Fig. 4B). As shown in Fig. 4C, compared with normal control, the thymus index in PRRSV/PCV2 group was significantly higher both at 10 ($p < 0.01$) and 13 ($p < 0.01$) dpi and there were no remarkable changes at 16 and 19 dpi. Meanwhile, both Ribavirin and Martine declined the increasing trend with varying degrees.

Matrine Regulate Peritoneal Macrophages Phagocytosis

Phagocytic ability of peritoneal macrophages was evaluated using MFI at the percentage of FITC-positive cells gated on $F4/80^+/CD11b^+$ cells. As shown in Fig. 5A and B, at 10 dpi, the MFI and the ratio of $F4/80^+/CD11b^+$ in PRRSV/PCV2 group was significantly higher than that of normal control ($p < 0.01$). These results indicated that virus infection promoted an increase in macrophages and enhanced their phagocytic ability. Compared with PRRSV/PCV2 group, the percentage of $F4/80^+/CD11b^+$ in Ribavirin and Matrine treatment groups were not changed significantly, while the MFI in these groups were remarkable decreased ($p < 0.01$). In addition, at 13 and 16 dpi, the MFI in PRRSV/PCV2 group was much lowered than that in normal control which means that there were no remarkable changes in macrophages, indicating that persistent infection of virus has leaded to the decrease of the phagocytic ability of macrophages. However, Matrine with the dose of 40 and 20 mg/kg has promoted the phagocytic ability of macrophages compared with PRRSV/PCV2 group.

Effects Of Matrine On The Proliferation Of Splenic Lymphocytes

At 10 dpi, the proliferation index of splenic lymphocytes with and without mitogen stimulus in PRRSV/PCV2 group was remarkably higher compared with the normal control, while it decreased in Ribavirin both with and without LPS stimulus compared with PRRSV/PCV2 group ($p < 0.05$). Moreover, the proliferation indices in Matrine 40 and 10 mg/kg treatment groups stimulated with both ConA and LPS were much lowered than those in PRRSV/PCV2 group as shown in Fig. 6. In addition, at 16 dpi, Matrine with a dose of 40 mg/kg enhanced the proliferation index with and without mitogen stimulus compared with PRRSV/PCV2 group. The proliferation index showed no-significant changes among all the groups at 19 dpi.

Discussion

Fan et al have clarified the synergistic effects of PRRSV and PCV2 on pathogenesis in the infected pigs. PRRSV infection followed by PCV2 enhances the replication of viruses in piglets and results in more severe clinical signs and lesions [28]. In this study, PRRSV/PCV2 co-infected mouse model was established by inoculating PRRSV first, followed by PCV2 2 h later through multiple routes (intraperitoneal, subcutaneous on back and intranasal injections). The results showed that both PRRSV and PCV2 could be detected in inguinal lymph nodes, spleen, liver, heart, lungs, kidneys and thymus at 7

days of infection, indicating that both PRRSV and PCV2 could infect and spread in mice. qPCR was further used to determine whether the viruses have tissue specificity. The copy number of PRRSV N gene was less than 10, indicating that PRRSV could not replicate well in mice. PCV2 Cap gene copy numbers were highest in liver, followed by thymus and spleen both in PRRSV/PCV2 co-infected and PCV2 infected mice. Moreover, Cap gene copy number in PRRSV/PCV2 co-infected group was higher than that in PCV2 alone group. In addition, PRRSV/PCV2 co-infection induced sever lung lesion with more pulmonary hemorrhages, thickened alveoli septa and decreased alveolar space. These results indicated that although PRRSV did not replicate well in mice, could not only promote PCV2 replication, but also aggravate lung lesions in mice. Similar results have been reported in PRRSV/PCV2 co-infected piglets. Co-infection of PRRSV and PCV2 enhanced both PRRSV and PCV2 replication which induce more serious interstitial pneumonia [27, 28]. Therefore, the PRRSV/PCV2 co-infection mouse model established in this study could be preliminary used to study the mechanism of PRRSV/PCV2 co-infection as well as evaluating the antiviral activities of a drug.

Co-infection of PRRSV and PCV2 increased the number of peritoneal macrophages and phagocytic function at 10 days post-infection, which may be related to the route of virus inoculation. Different inoculation routes may induce different immune responses. In this study, mice were inoculated with viruses partly by intraperitoneal injection, so a large number of macrophages were gathered to remove these viruses. In addition, virus co-infection also increased the spleen and thymus index, and spleen lymphocyte proliferation which indicated that co-infection activated the immune response. However, co-infection prolonged both the body weight gain (19 dpi) and the phagocytic capacity of peritoneal macrophages which continued to decrease at 13 dpi and 16 dpi, indicating that the virus co-exist has eventually interfere with the body function. Pigs are less able to resist and eliminate secondary infectious agents due to the effect of PRRSV on the thymus [31]. The co-infection of PRRSV and PCV2 may aggravate this phenomenon.

Matrine with a dose of 40 mg/kg significantly decreased PCV2 Cap gene expression in liver both at 10 and 13 dpi, indicating that Matrine could inhibit PCV2 replication in mice. However, at 16 and 19 dpi, Matrine inhibited the Cap gene expression in varying degrees, but with no significant difference. The above results demonstrated that Matrine interfere the virus replication in the early stage of infection. During the study, it was found that 10 mg/kg Martine promote the continuous body weight gain. 40 mg/kg Martine increased lymphocytic proliferation index and enhanced the phagocytic ability of macrophages. These results indicated that Matrine could reduce the virus-induced lesions by enhancing the immune function. It has been reported that Astragalus polysaccharides inhibited PCV2 replication by inhibiting oxidative stress and blocking NF-κB pathway [18]. Our previous results showed that Matrine and scutellarin inhibited PCV2 infection in vitro [20]. Other researches also demonstrated that carboxymethylpachymaran, Glutamine and selenium yeast reduced the inflammatory response and pathological phenomenon induced by PCV2 though regulating the immune response, but had no effect on PCV2 replication [32–34].

Conclusions

PRRSV/PCV2 co-infection mouse model was established by inoculating PRRSV followed by PCV2 2 h later through multiple routes including the intraperitoneal, subcutaneous on back and intranasal injections. Both PRRSV and PCV2 spread in mice, but only PCV2 replicates more efficiently. PRRSV infection promoted PCV2 replication and aggravated lungs lesions in mice. Matrine in a dose-dependent manner inhibited PCV2 replication in liver of mice co-infected by PRRSV/PCV2. 40 mg/kg Martine increased lymphocytic proliferation and enhanced the phagocytic ability of macrophages through regulating the immune function to reduce the virus-induced lesions.

Abbreviations

PRRSV
porcine reproductive and respiratory syndrome virus
PCV2
porcine circovirus type 2
Dpi
days post-infection
MFI
Median fluorescence intensity
FCS
fetal calf serum
SEM
standard errors of the mean

Declarations

Ethics approval and consent to participate

The experimental protocol was approved by the Bioethical Committee of the University of Shanxi Agricultural University.

Consent for publication

All authors critically revised the manuscript for important intellectual contents and approved the final manuscript.

Availability of data and material

The datasets analyzed in the present study are available from the corresponding author on reasonable request.

Conflict of interest statement

The authors declare no competing financial interests.

Funding

This work was supported by National Key R&D Program of China (Grant No. 2017YFD0501500), National Natural Science Foundation of China (Grant No. 31702285) and Shanxi Province Science Foundation for Excellent Youths (Grant No. 201801D211003).

Author contributions

Na Sun： Conceptualization，Methodology，Investigation，Data curation，Formal analysis，Writing - original draft，Funding acquisition

Hua Zhang，Investigation，Data curation，Formal analysis，Visualization，Writing - original draft

Panpan Sun，Investigation，Data curation，Formal analysis

Ajab Khan: Writing - review & editing，Visualization

Jianhua Guo，Writing - review & editing，Visualization

Xiaozhong Zheng，Writing - review & editing，Visualization

Yaogui Sun，Validation，Resources，Supervision

Kuohai Fan，Resources，Supervision

Wei Yin，Resources，Supervision

Hongquan Li，Conceptualization，Supervision，Project administration，Funding acquisition

Acknowledgements

We thank Ping Jiang and Shaobo Xiao for their skillful technical support.

References

1. Chen NH, Huang YC, Ye MX, Li S, Xiao YZ, Cui BL, et al. Co-infection status of classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circoviruses (PCV2 and PCV3) in eight regions of China from 2016 to 2018. Infect Genet Evol. 2019;68:127–35.
2. Oh T, Park KH, Yang S, Jeong J, Kang I, Park C, et al. Evaluation of the efficacy of a trivalent vaccine mixture against a triple challenge with Mycoplasma hyopneumoniae, PCV2, and PRRSV and the efficacy comparison of the respective monovalent vaccines against a single challenge. BMC Vet Res. 2019;15:342.

3. Niederwerder MC, Bawa B, Serão NVL, Trible BR, Kerrigan MA, Lunney JK, et al. Vaccination with a porcine reproductive and respiratory syndrome (PRRS) modified live virus vaccine followed by challenge with PRRS virus and porcine circovirus type 2 (PCV2) protects against PRRS but enhances PCV2 replication and pathogenesis compared to results for nonvaccinated cochallenged controls. Clin Vaccine Immunol. 2015;22:1244–54.
4. Hakobyan A, Arabyan E, Avetisyan A, Abroyan L, Hakobyan L, Zakaryan H. Apigenin inhibits African swine fever virus infection in vitro. Arch Virol. 2016;161:3445–53.
5. Jie C, Luo Z, Chen H, Wang M, Yan C, Mao ZF, et al. Indirubin, a bisindole alkaloid from Isatis indigotica, reduces H1N1 susceptibility in stressed mice by regulating MAVS signaling. Oncotarget. 2017;8:105615–29.
6. Ma X, Guo Z, Zhang Z, Li X, Wang X, Liu Y, et al. Ferulic acid isolated from propolis inhibits porcine parvovirus replication potentially through Bid-mediate apoptosis. Int Immunopharmacol. 2020;83:106379.
7. Sun N, Sun PP, Xie N, Khan A, Sun YG, Fan KH, et al. Antiviral and immunomodulatory effects of Dipotassium Glycyrrhizinate in chicks artificially infected with Infectious Bursal Disease Virus. Pak Vet J. 2019;39:43–8.
8. Wang J, Sun PP, Feng YN, Guo JH, Sun YG, Lei HM, et al. Sodium tanshinone IIA sulfonate affects Marek's disease virus replication by inhibiting gB expression. Pharm Biol. 2016;54:701–4.
9. Zhang JQ, Gu YL, Wu CH, Ma HL, He JP, Bai YS, et al. Effects of sodium tanshinone IIA sulfonate against Marek’s diseasevirus in experimentally infected chickens. Int J Biol Macromol. 2013;58:258–62.
10. Yu ZQ, Yi HY, Ma J, Wei YF, Cai MK, Li Q, et al. Ginsenoside Rg1 Suppresses Type 2 PRRSV Infection via NF-κB Signaling Pathway In Vitro, and Provides Partial Protection against HP-PRRSV in Piglet. Viruses. 2019;11:1045.
11. Sun N, Sun PP, Yao MJ, Khan A, Sun YG, Fan KH, et al. Autophagy involved in anti-viral activity of Sodium tanshinone IIA sulfonate against porcine reproductive and respiratory syndrome virus (PRRSV) infection in vitro. Antivir Ther. 2019;24:27–33.
12. Li E, Sun N, Zhao JX, Sun YG, Huang JG, Lei HM, et al. In vitro evaluation of antiviral activity of tea seed saponins against porcine reproductive and respiratory syndrome virus. Antivir Ther. 2015;20:743–52.
13. Wang ZW, Sun N, Wu CH, Jiang JB, Bai YS, Li HQ. In vitro antiviral activity of Dipotassium Glycyrrhetate against porcine reproductive and respiratory syndrome virus. Antivir Ther. 2013;18:997–1004.
14. Liu XW, Bai J, Jiang CL, Song ZB, Zhao YX, Nauwynck H, et al. Therapeutic effect of Xanthohumol against highly pathogenic porcine reproductive and respiratory syndrome viruses. Vet Microbiol. 2019;238:108431.
15. Chen HL, Tan HL, Yang J, Song ML, Feng HY, Kuang N, et al. Inhibitory effect of polysaccharide of Sargassum weizhouense on PCV2 induced inflammation in mice by suppressing histone acetylation.

Biomed Pharmacother. 2019;112:108741.
16. Li JR, Song DF, Wang SN, Dai YD, Zhou JY, Gu JY. Antiviral Effect of Epigallocatechin Gallate via Impairing Porcine Circovirus Type 2 Attachment to Host Cell Receptor. Viruses. 2020;12:176.
17. Yang HF, Chen XL, Jiang CM, He KW, Hu YY. In vivo Antiviral and Immunoregulatory Role Against PCV2 of Chinese Herbal Medicinal Ingredients. J Vet Res. 2017;61:405–10.
18. Xue H, Gan F, Zhang Z, Hu J, Chen X, Huang K. Astragalus polysaccharides inhibits PCV2 replication by inhibiting oxidative stress and blocking NF-κB pathway. Int J Biol Macromol. 2015;81:22–30.
19. Sun N, Wang ZW, Wu CH, Li E, He JP, Wang SY, et al. Antiviral activity and underlying molecular mechanisms of Matrine against porcine reproductive and respiratory syndrome virus in vitro. Res Vet Sci. 2014;96:323–7.
20. Sun N, Yu T, Zhao JX, Sun YG, Jiang JB, Duan ZB, et al. Antiviral activities of natural compounds derived from traditional Chinese medicines against Porcine Circovirus Type 2 (PCV2). Biotechnol Bioproc E. 2015;20:180–7.
21. Sun N, Sun PP, Lv HP, Sun YG, Guo JH, Wang ZR, et al. Matrine displayed antiviral activity in porcine alveolar macrophages co-infected by porcine reproductive and respiratory syndrome virus and porcine circovirus type 2. Sci Rep-UK. 2016;6:24401.
22. Rosenfeld P, Turner PV, Maclnnes JI, Nagy Éva Y, Dongwan. Evaluation of porcine reproductive and respiratory syndrome virus replication in laboratory rodents. Can J Vet Res. 2009;73:313–8.
23. Kiupel M, Stevenson GW, Choi J, Latimer KS, Kanitz CL, Mittal SK. Viral replication and lesions in BALB/c mice experimentally inoculated with porcine circovirus isolated from a pig with postweaning multisystemic wasting disease. Vet Pathol. 2001;38:74–82.
24. Cságola A, Cadar D, Tuboly T. Replication and transmission of porcine circovirus type 2 in mice. Acta Vet Hung. 2008;56:421–7.
25. Li J, Yuan XY, Zhang CF, Miao L, Wu J, Shi J, et al. A mouse model to study infection against porcine circovirus type 2: viral distribution and lesions in mouse. Virol J. 2010;7:158.
26. Deng ZB, Wang ND, Xu DJ, Yan AW, Ge M, Luo W, et al. Viral distribution and lesions in Kunming mice experimentally infected with porcine circovirus type 2b. Vet Res Commun. 2011;35:181–92.
27. Rovira A, Balasch M, Segalés J, García L, Plana-Durán J, Rosell C, et al. Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. J Virol. 2002;76:3232–9.
28. Fan PH, Wei YW, Guo HL, Wu H, Huang L, Liu J, et al. Synergistic effects of sequential infection with highly pathogenic porcine reproductive and respiratory syndrome virus and porcine circovirus type 2. Virol J. 2013;10:265.
29. Xiu FM, Stanojcic M, Jeschke MG. Norepinephrine inhibits macrophage migration by decreasing CCR2 expression. Plos One. 2013;8:e69167.
30. Wang XQ, Duan XM, Liu LH, Fang YQ, Tan Y. Carboxyfluorescein diacetate succinimidyl ester fluorescent dye for cell labeling. Acta Biochim Biophys Sin (Shanghai). 2005;37:379–85.

31. Wang G, Yu Y, Cai XH, Zhou EM, Zimmerman JJ. Effects of PRRSV infection on the porcine thymus. Trends Microbiol. 2020;28:212–23.
32. Wei YY, Hu TJ, Su ZJ, Zeng Y, Wei XJ, Zhang SX. Immunomodulatory and antioxidant effects of carboxymethylpachymaran on the mice infected with PCV2. Int J Biol Macromol. 2012;50:713–9.
33. Ren WK, Li YH, Yu XL, Luo W, Liu G, Shao H, et al. Glutamine modifies immune responses of mice infected with porcine circovirus type 2. Brit J Nutr. 2013;110:1053–60.
34. Liu G, Yang G, Guan GP, Zhang Y, Ren W, Yin J, et al. Effect of dietary selenium yeast supplementation on porcine circovirus Type 2 (PCV2) infections in mice. PLoS One. 2015;10:e0115833.

Figures

Fig. 1

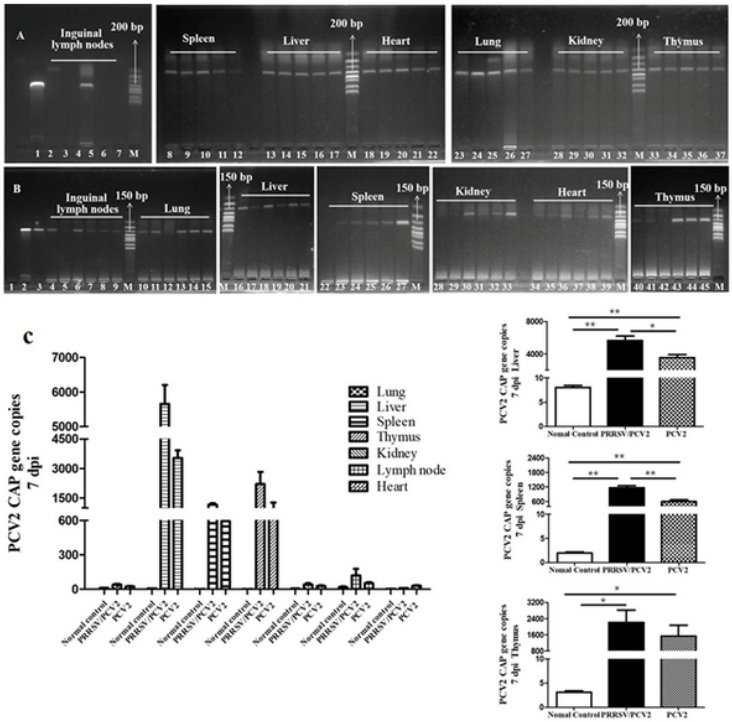

Figure 1

Agarose gel electrophoresis of PCR products of PRRSV N gene (A) and PCV2 CAP gene (B) PCR products in tissues from PRRSV/PCV2 co-infected mice. The products size of PRRSV N gene and PCV2 CAP gene were 196 bp and 148 bp respectively. M means Marker; 1: negative control; 2 (A and B): positive control; 3 (B): positive control. C: The distribution of PCV2 in both PRRSV/PCV2 co-infected mice and PCV2 alone infected mice at 7 dpi by qPCR assay. Data were expressed as mean±SEM. *means $p<0.05$, ** means $p<0.01$, *** means $p<0.001$.

Fig. 2

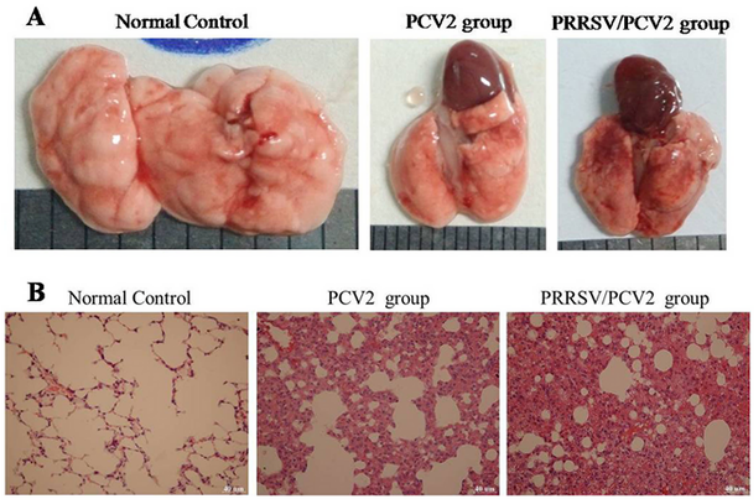

Figure 2

Lung morphological observation and H&E histology in normal and infected mice. No lung lesions in normal control group. Obvious lesions were observed in lungs of both PRRSV/PCV2 co-infected and PCV2 infected mice (A). Hemorrhages and typical characteristics of interstitial pneumonia including thickened alveoli septa and decreased alveolar spaces were displayed both in PRRSV/PCV2 co-infected and PCV2 infected mice from the results of H&E staining (B). Compared with PCV2 infected group, the lungs lesions were much severe in PRRSV/PCV2 co-infected group.

Fig. 3

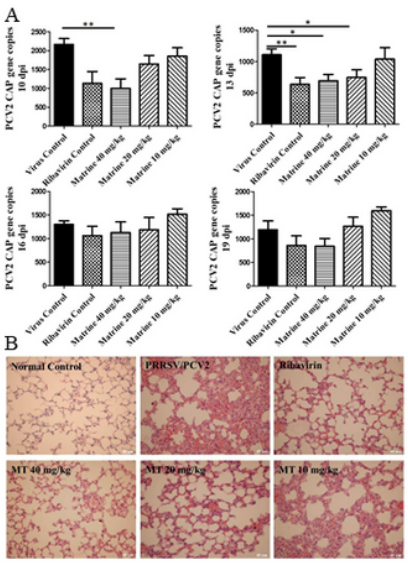

Figure 3

Matrine restrained PCV2 replication in liver (A) and alleviation of virus-induced lung lesions (B). A: Effect of Matrine on PCV2 CAP gene expression in liver at 10, 13, 16 and 19 dpi. qPCR results demonstrated that Matrine significantly inhibited PCV2 CAP gene expression both at 10 and 13 dpi. Data were expressed as mean ± standard errors mean (SEM). * means $p < 0.05$, ** indicates $p < 0.01$, *** means $p < 0.001$. B: H&E staining demonstrated that compared with normal control, PRRSV/PCV2 group displayed severe thickened alveoli septa and decreased alveolar space. While both Ribavirin treatment and Matrine treatments (40 mg/kg and 20 mg/kg) could significantly alleviate these pathological changes. Scale bar = 40 μm.

Fig. 4

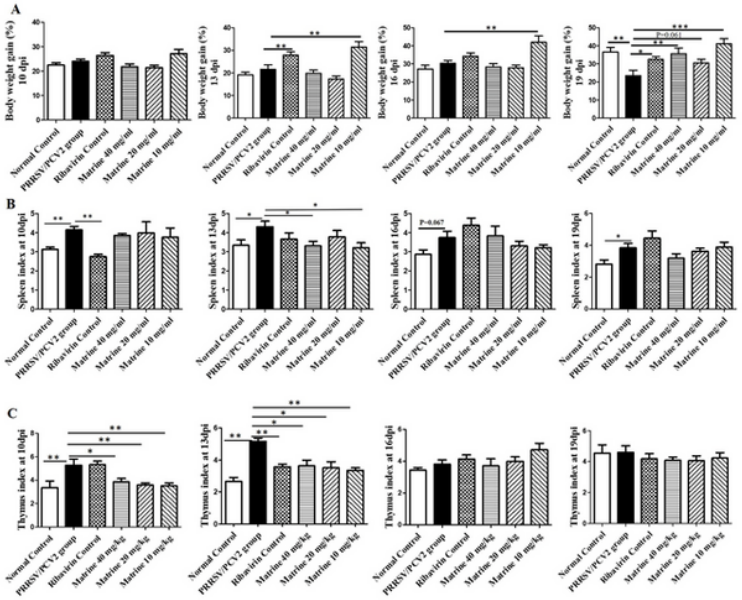

Figure 4

Effect of Matrine on body weight gain, spleen and thymus indices at 10, 13, 16 and 19 d post-infection. Data were expressed as mean±standard errors mean (SEM). * means p <0.05, ** indicates p <0.01, *** means p <0.001.

Fig. 5

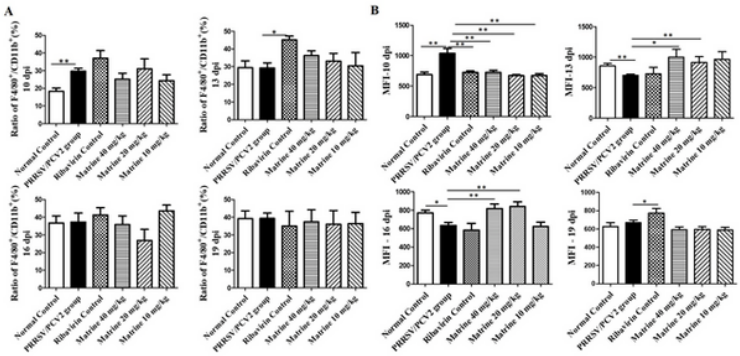

Figure 5

Effect of Matrine on the percentage of peritoneal macrophages and its phagocytic capacity in all the groups at 10, 13, 16 and 19 dpi. The macrophages were labeled by both anti-F4/80 antibody and anti-CD11b antibody. The ratio of F4/80+/CD11b+ was counted by flow cytometry. The capacity of phagocytosis was expressed by median fluorescence intensity (MFI). Data were expressed as mean ± standard errors mean (SEM). * means $p < 0.05$, ** indicates $p < 0.01$, *** means $p < 0.001$.

Fig. 6

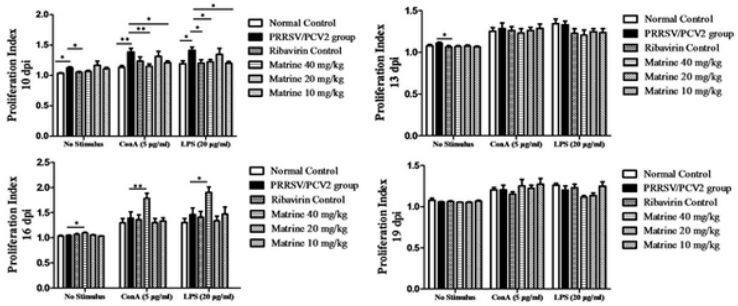

Figure 6

Effect of Matrine on proliferation of splenic lymphocytes at 10, 13, 16 and 19 dpi. After labeled by CFSE, Proliferation index of different treatment groups wererespectively detected by flow cytometry without or with ConA and LPS treatment. Data were expressed as mean±standard errors mean (SEM). * means $p < 0.05$, ** indicates $p < 0.01$, *** means $p < 0.001$.