Veterinary Microbiology 237 (2019) 108394

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Effect of porcine circovirus type 2 on the severity of lung and brain damage in piglets infected with porcine pseudorabies virus

Xue Zhang[1], Xianghua Shu[1], Huayi Bai, Wengui Li, Xin Li, Changyue Wu, Yunmei Gao, Yulei Wang, Kun Yang, Chunlian Song*

College of Veterinary Medicine of Yunnan Agricultural University, Kunming, Yunnan Province, 650201, China

ARTICLE INFO

Keywords:
Porcine circovirus type 2
Porcine pseudorabies virus
Co-infection
Piglets

ABSTRACT

Porcine circovirus type 2 (PCV2) is widespread throughout Chinese farms, and the infection rate of porcine pseudorabies virus (PRV) is very high. The emergence of mixed infection involving PCV2 and PRV has been difficult to prevent and control and has caused considerable economic loss. The present study investigated lung and brain damage caused by PRV in piglets with PCV2 infection. Twenty piglets were divided randomly into two experiment groups (PRV group and PRV + PCV2 group; n = 10 per group). The pigs were observed for clinical signs at specified times. At necropsy, lung and brain tissue samples were collected for histopathological examination, and tissue virus load was determined using quantitative polymerase chain reaction. Severe pathogenicity due to PRV was evident in two-month-old piglets. PCV2 and PRV co-infection led to more severe neurological and respiratory symptoms and a higher mortality rate in the piglets. In addition, the pathological damage to the lung and brain was also aggravated. The co-infection was associated with a significant increase in the content of PRV in the brain and lung tissue. In conclusion, PCV2 and PRV co-infection could cause severe and irreversible damage to piglets.

1. Introduction

Since the first description of porcine circovirus by Tischer et al. in 1982, porcine circovirus type 2 (PCV2) has become one of the most important pathogens affecting the swine industry worldwide (Chae, 2004;Chae, 2005). PCV2 is the causative agent of a number of disease syndromes that are collectively termed porcine circovirus diseases (PCVAD). Of the syndromes, post-weaning multi-systemic wasting syndrome (PMWS), is the most important, economically (Kim et al., 2003, 2011). Infection solely with PCV2 rarely results in clinical disease (Kim et al., 2011). In the majority of cases, pigs are sub-clinically infected.

Porcine pseudorabies is an acute infectious disease caused by pseudorabies virus (PRV). The disease is characterized by fever and encephalomyelitis. Newborn piglets mainly display neurological symptoms, and adult pigs mainly display recessive infection. Consequences of the infection in pregnant sows include abortion, stillbirth, and mummified fetus (Klupp et al., 2003; Szpara et al., 2011; Boadella et al., 2012). Signs in pigs over two months of age include neuropathic symptoms, tremors, ataxia, head-up posture, arched back, limb spasm, intermittent seizures, and occasionally death. The disease may also occur in other animals (Wang et al., 2015a, 2015b).

PRV infection is prevalent and sporadic in Yunnan province, China. The rate of PRV infection in pigs on farms in China has increased in recent years (Song et al., 2017). Combination of the infection with other pathogens could lead to immunosuppression, complicate the condition, increase the morbidity and mortality, and increase the death rate. The mixed infection with PCV2 and PRV has become more serious on some pig farms in Yunnan province, and the prevalence of the mixed infection has increased to an epidemic proportion (Fachinger et al., 2008; Lee et al., 2010). Hence, there is an urgent need to detect and prevent the mixed infection.

In the present study, we established an animal model of PCV2 and PRV co-infection. Characteristic clinical signs included stillbirth or the birth of weak piglets with neurological symptoms that ultimately lead to death. Moreover, the brains and lungs of the infected piglets were seriously damaged, and the tissue virus load was higher than that of a single infection. A 100% death rate was observed in the co-infection

* Corresponding author at: College of Veterinary Medicine of Yunnan Agricultural University, No. 452, Feng Yuan Street, Panlong District, Kunming, Yunnan Province, 650201, China.
E-mail address: Chunlian-Song@outlook.com (C. Song).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.vetmic.2019.108394
Received 26 April 2019; Received in revised form 15 August 2019; Accepted 18 August 2019

group on the 14th day. The results showed that co-infection could lead to the rapid death of piglets.

2. Materials and methods

2.1. Viruses and experimental animals

The PRV gD strain named YN1was previously isolated from the lungs of infected pigs in Yunnan Province (Song et al., 2017). The virus was propagated in Vero cells and titrated in PK-15 cells as previously described (Wang et al). The titers of PCV2 and PRV were $10^{6.5}TCID_{50}$/mL.

All piglets in this study were two-month-old male that were born from several unvaccinated with PRV and PCV2 sows and tested negative for PRV, PCV2, porcine reproductive and respiratory syndrome virus, classical swine fever virus, and porcine parvovirus using a polymerase chain reaction (PCR) method. They were determined to be free of antibodies against PRV prior to the study using enzyme-linked immunosorbent assay (ELISA) kits.

The temperature of piglets is closely related to the course of the disease. Observation of clinical symptoms is a preliminary means to judge animal diseases. During the challenge period, the rectal temperature of piglets was measured at 08:30 and 17:30, parameters like cougha, pruritus, anorexi,tremor and diarrhea were recorded daily after inoculation of the viruses every day to determine the occurrence and development of the disease and the prognosis of piglets.

2.2. Experimental design

After 1 week of acclimation, the piglets were randomly allocated into two groups. 10 piglets were administered 2 mL PRV suspension intramuscularly, and in the PCV2 + PRV group, 10 pigs were injected intramuscularly with 1 mL each of prepared suspensions of PRV and PCV2. Since the mixed infection with 2 mL PRV +1 mL PCV2 together made the piglets die quickly in the previous pre-experiment, the total amount of viruses in this study was set on half of the amount in pre-experiment study, which was also consistent in the two groups.

After challenge, on day 3, 7, 14, 21, and 35, two piglets in each experimental group were euthanized and complete necropsies were performed. The lung and brain tissue samples were collected for DNA extraction and quantitative PCR (qPCR) detection.

2.3. Animal ethics statement

All experiments performed in this study were approved by the International Animal Care and Use Committee of the Yunnan Agricultural University (permission code: YAUACUC06; date of publication: July 10, 2017). The study complied with the guidelines of the institutional administrative committee and ethics committee of laboratory animals.

2.4. Protocol of qPCR

For the primer design, Lasergene v7.1 (DNASTAR) was used to analyze the published gene sequences of PRV strains available at NCBI GenBank. Primer Premier 5.0, Oligo 6.0, and other molecular biology analysis software were used to design primers for the detection of PRV. The primers were synthesized by San Gong Biotech Company. The qPCR amplification of PRV was carried out in 20 μL reaction mixtures containing 10 μL SYBR II, 0.8 μM upstream primer (F), 0.8 μM downstream primer (R,) and 2.0 μL cDNA template. Diethyl pyrocarbonate was added to produce a final volume of 20 μL. The PCR conditions were 95℃ for 2 min; 35 cycles consisting of 95℃ for 15 s, 59℃ for 20 s, and 65℃ for 5 s; and primer annealing at 65℃ for 5 min. The qPCR amplification of PCV2 was carried out using the same reaction mixture and reaction conditions. The accuracy of qPCR was determined by gel electrophoresis of the PCR products and visualization of the resulting bands using a gel imaging system. After purification of the target fragment, vector ligation was performed. The recombinant plasmid was identified by plasmid extraction, enzyme digestion, and sequencing.

PRV was amplified by PCR, and the standard curve was established. A strong linear relationship was evident, with a coefficient (R^2) of 0.998. The amplification efficiency (E) was 103.8%. The linear relationship between Ct and the copy number was calculated as: Ct = -3.232 × 28.027. PCV2 was also amplified by PCR, and the standard curve was established. A strong linear relationship was also evident, with an R^2 of 1.000, and E was 92.7%. The linear relationship between Ct and the copy number was calculated as: Ct = -3.510 × 32.996.

2.5. Statistical analysis

Values are expressed as the mean + SD. One-way analysis of variance followed by Student-Newman-Keul's multiple comparisons test was used for the comparison of means. A probability of 0.01 was taken as being statistically significant. The analysis was carried out using the SYSTAT 9 software package (SPSS 20).

3. Results

3.1. PCV2 and PRV co-infection is lethal for piglets

Clinical symptoms are a fundamental method for the diagnosis of diseases. To preliminarily make sure that which one is more harmful for piglets with co-infection or PRV single infection, we examined the body temperature of co-infected or single infected piglets.

The body temperature of co-infected piglets increased rapidly at

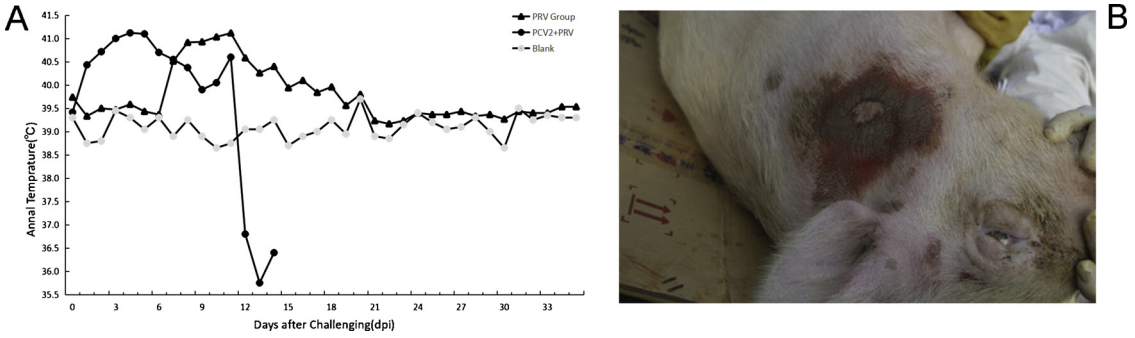


Fig. 1. Body temperature of piglets infected with PRV, PCV2 alone, or both PRV and PCV2 (A) and characteristic symptoms (B). A: The body temperature of the piglets increased significantly (41.0℃) on day 6 after PRV infection, began to decrease after 10 days, and then returned to normal after 14 days. In a contrast, piglets infected with both PCV2 and PRV (PCV2 + PRV) started to show fever (41.0℃) at 12 h post-infection, and the fever began to decrease after 10 days to below normal body temperature (35.8℃). B: One of the typical mental depression characteristics of leaving a wound after scratching.

12 h, reached the peak value of 41.8℃ on the 7th day, and decreased rapidly to 35.8℃ on the 8th day, which was below the normal body temperature suggested that the prognosis is poor (Fig. 1A). The body temperature of the pigs in the PRV group increased significantly on day 6 and returned to normal on day 20.

Piglets in the two experimental groups developed clinical signs characterized by typical neurological and respiratory symptoms. Piglets co-infected with PCV2 and PRV showed neurological symptoms such as swaying walk, head-back posture, circling, uncoordinated exercise, dog-sitting, limb-paddling, and mental depression (Fig. 1B). Respiratory symptoms included cough, asthma, and dyspnea. Interstitial pneumonia and severe lymphoplasmacytic and histiocytic bronchointerstitial pneumonia are characteristic symptoms. The clinical symptoms of PRV infection alone are less severe than those of co-infection, which are characterized by itching, depression, accelerated breathing, cough, and runny nose. Two piglets in the PCV2 + PRV group were dead by day 8, and all the piglets in this group had died by day 14. There was no piglet death due to disease in the PRV group.

3.2. PCV2 and PRV co-infection results in more severe brain and lung damage in piglets than PRV infection alone

The observations of severe neurological symptoms and dyspnea in piglets prompted a more detailed examination of clinical symptoms, pathological anatomy, and histological observations.

PRV infection resulted in damage to the lung and brain tissue, such as hemorrhage. These tissue injuries were exacerbated by co-infection with PCV2 and PRV, with increased bleeding and congestion in the lungs. The lung lesions were characterized by serous filled pulmonary alveoli, which may have been the cause of death. Nucleolysis in the brain tissue was also more prevalent in the co-infection group compared to that in the group infected with PRV alone, which may be the reason why the piglets in the co-infection group showed such severe neurological symptoms (Fig. 2).

3.3. Concurrent infection with PCV2 and PRV can significantly increase PRV content in lung and brain tissues

There is a correlation between pathological injury and viral load, and in order to study the effect of PCV2 on the content of PRV in the lungs and brains of the piglets, qPCR was utilized. In the co-infected group, the viral content continued to increase up until day 14. By that time, all the piglets had died (Fig. 3A). The viral levels in the PCV2 + PRV group were lower in the early stage than those in piglets infected solely with PRV. However, by day 14, the PRV levels in the lungs of the co-infected group were about 200 times higher than those of the PRV-alone infection group (Fig. 3B). PRV content in the brains of co-infected piglets was also higher than that in the PRV group. The viral content in the brain and lung tissue in the PRV group peaked at day 7 and decreased thereafter. Concurrently, the PCV2 content in the lung and brain samples was very low. The findings indicated that PCV2 may enhance the content of PRV in the lung and brain, leading to more severe pathological injury.

4. Discussion

Several reports demonstrated that co-infection with PCV2 and PRV is pervasive in piglets and has become a major threat to the global pig industry (Solano et al., 1997; Cai et al., 2005; Afolabi and Benson, 2017; Shuqing et al., 2017). PCV2 alone cannot cause extensive damage to the host (Sara and Valerie, 2018), but PCV2 infection can result in the elimination of B cells and impair PCV2 antibody production and immunosuppression. Sara and Valerie (2018) found that PRV infection is age-dependent; there were no obvious clinical symptoms in 15-week-old piglets, and the infection was not lethal at the age of two months. In this study, the Yunnan strain of PRV seriously harmed the respiratory and nervous systems of piglets. Symptoms including cough, dyspnea, scratching, rotation, tremor, ataxia, head-up posture, arched back, and limb spasm appeared 3–7 days after challenge. The presently observed clinical signs were similar to those described in a previous study that used 10^7 $TCID_{50}$ PRV HN1201 inoculation via the nose (Qing et al., 2016). In the present study, piglets inoculated with PRV developed a high fever and similar neurological symptoms. Their body temperatures began to return to normal at 14 days post-injection of the virus. More severe respiratory difficulties and neurological symptoms were observed in the PCV2 + PRV group. A sharp drop in body temperature at 10–14 days indicated a poor prognosis of piglets. The observations suggest that PRV re-infection could cause serious injuries and even death in piglets infected with PCV2. Therefore, even PRV, which is an age-dependent virus, can cause more significant damage when co-infected with PCV2, thus causing great economic losses.

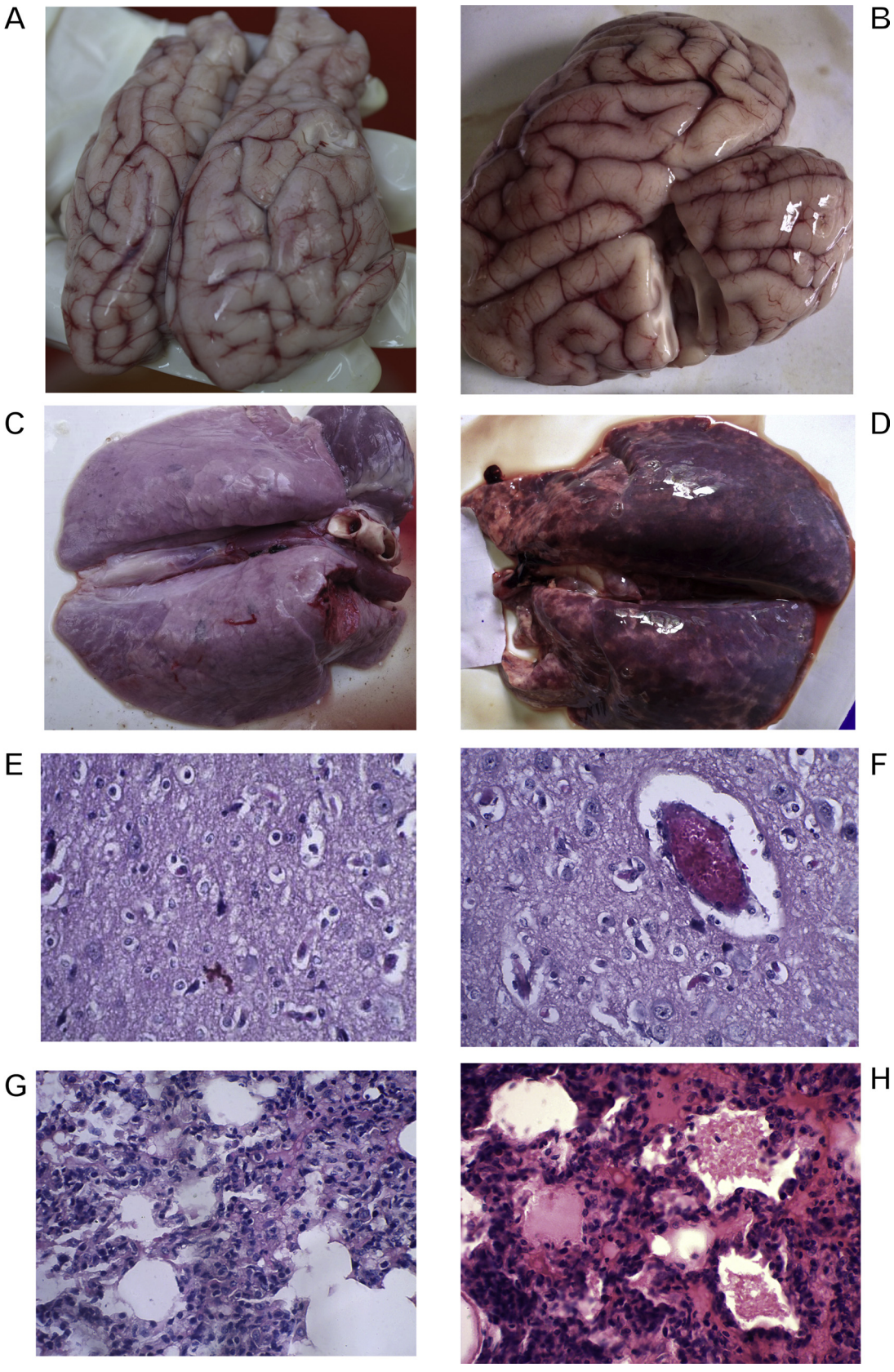
The PRV infection rate is increasing, and protection from airborne infection is becoming more difficult. This is a major problem for livestock farms and veterinarians (Kim et al., 2002).

PRV has a pantropic effect on the respiratory and nervous systems of pigs. Virus particles enter the termini of sensory nerves and stimulate the infected mucosal epithelium. The virus propagates abundantly in the nasal cavity mucous membrane. Pathogenic foci form after the first round of virus replication and subsequently pass through the trigeminal, olfactory, and glossopharyngeal nerve endings. Ultimately, the virus enters the olfactory bulb and the trigeminal nerve ganglion. It takes 2–4 weeks for this process to occur in the form of a core-capped shell after replication (Nauwynck et al., 2007; Sarah et al., 2011; Sara et al., 2017). The damage to the nervous system caused by PCV2 infection has not been previously reported (Segalés, 2012). In pigs, PRV strain HeN1 can cause lymphocyte infiltration around the small blood vessels in the brain cortex (Tong et al., 2013). Presently, PRV infection alone resulted in similar symptoms, and neurological symptoms were more severe in the pigs challenged with PCV2 and PRV. In addition, there was more surface bleeding and more eosinophilic inclusion-bodies in the brain, with the dissolution of neuronal cell bodies being prevalent. The results support the view that PCV2 can aggravate brain tissue damage in piglets infected with PRV.

Both PCV2 and PRV can cause lung damage in piglets. Interstitial pneumonia and severe lymphoplasmacytic and histiocytic bronchointerstitial pneumonia are characteristic symptoms that occur in piglets after PCV2 infection (Jizong et al., 2015). Pulmonary consolidation edema and lung hemorrhage could be caused by PRV. In this study, pulmonary hemorrhage was observed in piglets inoculated with PRV, with more severe congestion and bleeding as well as pulmonary consolidation in the whole lungs observed in piglets of the PCV2 + PRV group. Histopathological lung lesions were characterized by serious bleeding, and the alveoli were filled with serous fluid, which may have been the cause of death in piglets with dyspnea. The findings suggest that co-infection with PCV2 and PRV can cause lung injury and more severe damage compared with PRV infection alone.

Pigs infected with PCV2 continue to carry the virus and show subclinical symptoms. PCV2 is linked with a variety of clinical disease manifestations collectively referred to PCVAD (Harding and Clark, 1997), and co-infection heavily influences the severity and outcome of PCVAD. In pigs, the co-infections involved include PCV2, porcine parvovirus, porcine reproductive and respiratory syndrome virus, *Haemophilus parasuis* serovar 4, and classical swine fever virus (Ellis et al., 2000; 2004; Shuqing et al., 2017). In these cases, some co-infections increase the pathogenicity of PCV2 or other pathogens. Therefore, mixed infection with PCV2 and PRV enhances the pathogenicity of both, leading to severe disease and even death of pigs.

In this study, PCV2 infection also led to the increase in PRV in the brain tissue of piglets. The amount of PRV in pigs infected with PCV2 + PRV was half of that in pigs infected with PRV alone, and the PRV load in the brain tissue increased to the highest level on day 14. The detection of a large amount of PCV2 in the brains of piglets



(caption on next page)

suggested that PCV2 aggravated the brain tissue damage of piglets due to PRV. This may be related to the restriction of the immune response by PCV2, which could lead to the proliferation of PRV in the lungs. When PCV2 levels in the lungs are still well below the PRV levels, we suspect that PCV2 plays a role in the acceleration of PRV reproduction. Therefore, the damage to the lung and brain caused by PRV is

Fig. 2. Pathological lesions of piglets infected with PRV alone (A, C, E, G) or with PRV and PCV2 (B, D, F, H). A & B: Brain from a PRV-infected piglet showed swollen and congested blood vessels on the brain surface. Blood filaments were observed on the surface of the gyrus and the choroid plexus (A). In comparison, a piglet infected with both PCV2 and PRV showed more serious damage and congestion, and more blood filaments on the surface of the gyrus in the brain than that of the PRV group (B). C & D: A small amount of bleeding and interstitial enlargement was observed in the lung of a piglet from the PRV group (C), while more severe injury than that of the PRV group was observed in the PRV + PCV2 group, with massive congestion and hemorrhage, increased weight and hardness of the lung, gray-red, local atrophy of the lung, emphysema, widening of the pulmonary lobule, consolidation of local lung parenchyma, and shrimp meat changes (D). E & F: Histopathological changes in the brain of a piglet from the PRV group. Contraction of neuronal bodies in the brain. (×400) (E). Neuronal shrinkage and dissolution were observed (×400) in all piglets from the PCV2 + PRV group. In addition, eosinophilia inclusion bodies were found in the nuclei of neurons and glial cells in the brain. There were glial cells and proliferating vascular outer membrane cells in and outside the perivascular space to form the vascular sheath (F). G & H: There was serous exudation between alveolar wall cells, and a few dust cells appeared (×400) (G). In addition, there was serous exudation between alveolar wall cells, the alveoli were filled with serous, and the alveolar wall cells fell off. The degree of injury was greater than that of the PRV group (×400) (H). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

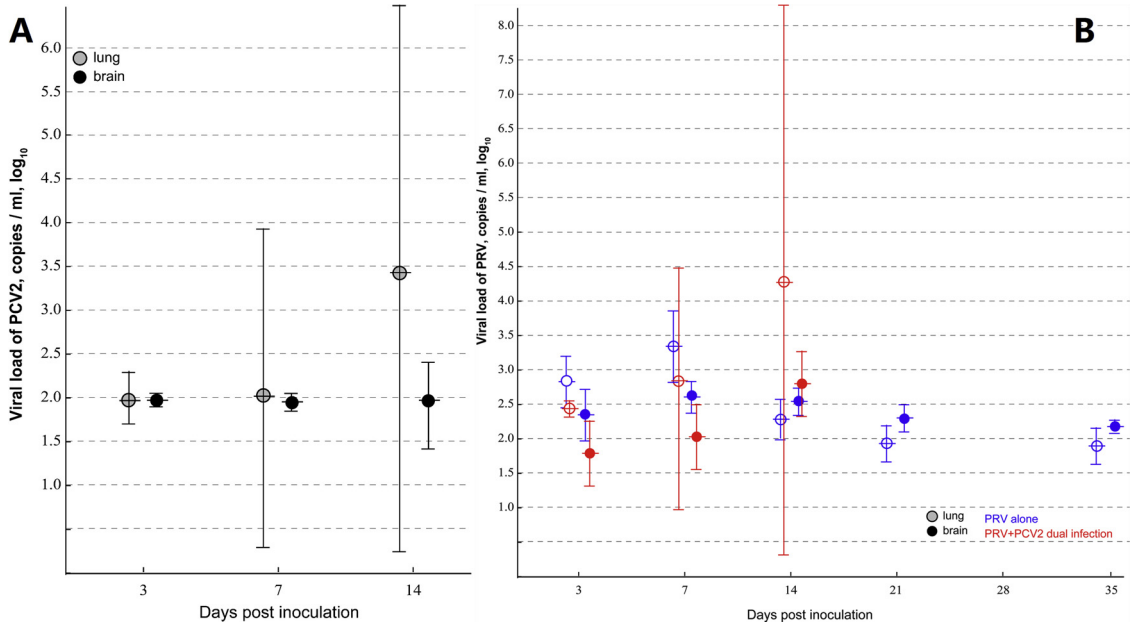


Fig. 3. PCV2 burden of piglets infected with PCV2 + PRV (A), Change of PRV in the lungs and brains of piglets infected with PRV alone and PRV with PCV2 (B). Quantitative PCR was used to measure the PCV2 copy number on days 3, 7, and 14 after infection. The copy number of PCV2 in the lungs of infected piglets was 2518.78 copies/μL, which was significantly higher on day 14 than on days 3 and 7. However, the copy number of PCV2 did not differ significantly in brain tissue examined at days 3, 7, and 14. The viral copy number was logarithmically normalized for statistical comparison(A).Open and filled circles denote viral burden in the lung and brain tissue, respectively. The blue symbol represents piglets infected with PRV alone. The red font represents piglets infected with both PRV2 and PCV2. The PRV content in the lungs of the PCV + PRV group was significantly greater on day 14 (19910.53 copies/μL) than that in the other groups. The regulation of PRV content displayed two patterns in the PCV2 + PRV group. One pattern was that the content of PRV in the brain tissue remained fairly constant. In other words, the increment of change was lower than that in the lung, and the attenuation rate was slower than that in the lung. In the second pattern, the content of PRV in the lung and brain tissues in the single-infection group increased to a maximum on day 7 and was higher than that in the mixed infection group before 7 days. Piglets infected with PCV2 + PRV showed elevated levels and a maximum at 14 days (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

aggravated by PCV2.

Funding

None.

Declaration of Competing Interest

None.

Acknowledgement

Thank Professor Gefen Yin of the Animal Infectious Diseases Laboratory of Yunnan Agricultural University for providing the PRV.

References

Afolabi, K.O., Benson, C., 2017. Global status of porcine circovirus type 2 and its associated diseases in Sub-Saharan Africa. Adv. Virol., 6807964.

Boadella, M., Gortázar, C., Vicente, J., Ruiz-Fons, F., 2012. Wild boar: an increasing concern for Aujeszky's disease control in pigs? BMC Vet. Res. 8, 7.

Cai, X., Chen, H., Blackall, P.J., et al., 2005. Serological characterization of *Haemophilus parasuis* isolates from China. Vet. Microbiol. 111, 231–236.

Chae, C., 2004. Postweaning multisystemic wasting syndrome: a review of aetiology, diagnosis and pathology. Vet. J. 168, 41–49.

Chae, C., 2005. A review of porcine circovirus 2-associated syndromes and diseases. Vet. J. 169, 326–336.

Ellis, J., et al., 2004. Porcine circovirus-2 and concurrent infections in the field. Vet. Microbiol. 98, 159–163.

Ellis, J.A., et al., 2000. Coinfection by porcine circoviruses and porcine parvovirus in pigs with naturally acquired postweaning multisystemic wasting syndrome. J. Vet. Diagn. Invest. 12, 21–27.

Fachinger, V., Bischoff, R., Jedidia, S.B., Saalmuller, A., Elbers, K., 2008. The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. Vaccine 26, 1488–1499.

Harding, J., Clark, E., 1997. Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). Swine Health Prod. 5, 201–203.

Jizong, L., Tianqi, Y., Xiaobo, W., 2015. Comparative efficacy of experimental inactivated and live-attenuated chimeric porcine circovirus (PCV) 1-2b vaccines derived from PCV1 and PCV2b isolates originated in China. Virol. J. 12, 113.

Kim, J., Chung, H.K., Chae, C., 2003. Association of porcine circovirus 2 with porcine respiratory disease complex. Vet. J. 166, 251–256.

Kim, D., Ha, Y., Oh, Y., Chae, C., 2011. Prevalence of porcine circovirus types 2a and b in

pigs with and without post-weaning multi-systemic wasting syndrome. Vet. J. 188, 115–117.
Kim, J., Chung, H.K., Jung, T., 2002. Postweaning multisystemic wasting syndrome of pigs in Korea: prevalence, microscopic lesions and coexisting microorganisms. J. Vet. Med. Sci. 64 (1), 57–62.
Klupp, B.G., Hengartner, C.J., Mettenleiter, T.C., Enquist, L.W., 2003. Complete, annotated sequence of the pseudorabies virus genome. J. Virol. 78, 424–440.
Lee, C., Kim, H., Kang, B., Yeom, M., Han, S., Moon, H., Park, S., Kim, H., Song, D., Park, B., 2010. Prevalence and phylogenetic analysis of the isolated type I porcine reproductive and respiratory syndrome virus from 2007 to 2008 in Korea. Virus Genes 40, 225–230.
Nauwynck, H., Glorieux, S., Favoreel, H., Pensaert, M., 2007. Cell biological and molecular characteristics of pseudorabies virus infections in cell cultures and in pigs with emphasis on the respiratory tract. Vet. Res. 38 (2), 229–241.
Qing, Y., Zhe, S., FeiFei, T., et al., 2016. Pathogenicity of a currently circulating Chinese variant pseudorabies virus in pigs. World J. Virol. 5 (1), 23–30.
Sara, V., Valerie, R., 2018. Reduced virulence of a pseudorabies virus isolate from wild boar origin in domestic pigs correlates with hampered visceral spread and age-dependent reduced neuroinvasive capacity. Virulence 9 (1), 149–162.
Sara, V., Brigitte, C., Herman, F., et al., 2017. Age-dependent differences in pseudorabies virus neuropathogenesis and associated cytokine expression. J. Virol. 91 (2), e02058–16.
Sarah, G., Herman, W.F., Lennert, S., et al., 2011. A trypsin-like serine protease is involved in pseudorabies virus invasion through the basement membrane barrier of porcine nasal respiratory mucosa. Vet. Res. 42 (1), 58.
Segalés, J., 2012. Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis. Virus Res. 164, 10–19.
Shuqing, L., Wentao, L., Yang, W., 2017. Coinfection with *Haemophilus parasuis* serovar 4 increases the virulence of porcine circovirus type 2 in piglets. Virol. J. 14, 227.
Solano, G.I., Segales, J., Collins, J.E., 1997. Porcine reproductive and respiratory syndrome virus (PRRSv) interaction with Haemophilus parasuis. Vet. Microbiol. 55, 247–257.
Song, C., Gao, L., Bai, W., 2017. Molecular epidemiology of pseudorabies virus in Yunnan and the sequence analysis of its gD gene. Virus Genes 53 (3), 392–399.
Szpara, M.L., Tafuri, Y.R., Parsons, L., et al., 2011. A wide extent of inter-strain diversity in virulent and vaccine strains of alphaherpes-viruses. PLoS Pathog. 7, e1002282.
Tong, Q.A., JinMei, P., ZhiJun, T., 2013. Pseudorabies Virus Variant in Bartha-K61–Vaccinated Pigs, China, 2012. Emerg Infect Dis. 19 (11), 1749–1755.
Wang, Y., Qiao, S., Li, X., et al., 2015a. Molecular epidemiology of outbreak-associated pseudorabies virus (PRV) strains in central China. Virus Genes 50 (3), 401–409.
Wang, Y., Qiao, S., Li, X., et al., 2015b. Molecular epidemiology of outbreak-associated pseudorabies virus (PRV) strains in central China. Virus Genes 50 (3), 401–409.