Tropical Animal Health and Production (2018) 50:1279–1285
https://doi.org/10.1007/s11250-018-1555-1

REGULAR ARTICLES

Investigation on pseudorabies prevalence in Chinese swine breeding farms in 2013–2016

Yingyi Liu[1] · Shuo Zhang[1] · Qi Xu[1] · Jiajun Wu[1] · Xinyan Zhai[1] · Shuo Li[1] · Jing Wang[1] · Jianqiang Ni[1] · Lin Yuan[1] · Xiaohui Song[1] · Bolin Zhao[1] · Zhi Zhou[1] · Chuanbin Wang[1] · Lin Yang[1]

Received: 3 December 2017 / Accepted: 19 February 2018 / Published online: 19 March 2018

Abstract
Pseudorabies (PR) has been prevalent in Chinese swine breeding farms since the outbreak at the end of 2011. For investigating current prevalence of PR, a nationwide surveillance has been performed in this study. The swine serum samples were collected from 93, 100, 92, and 91 swine farms in China during 2013–2016, respectively. Since the extensive use of gE-deleted pseudorabies virus (PRV) vaccine, we could apply the PRV-gE antibody for determining wild-type virus infection and the PRV-gB antibody for evaluating vaccine immunization. The results were concluded as follows: (1) Nationally, the positive rate of PRV-gB was maintained at a high level (> 90%), while the positive rate of PRV-gE continued to decrease (from 22.17 to 13.14%). (2) The positive rates of PRV-gE were greatly varied in different geographical regions and swine farms (0~100%), while the positive rate of PRV-gB was generally high (> 90%). (3) The number of imported PRV attenuated vaccines were about twice that of domestic PRV attenuated vaccines, while the positive rate of PRV-gB was not significantly different ($P > 0.05$). (4) The performance of PR eradication developing or developed farms was better than the performance of common farms, with higher positive rate of PRV-gB (> 90%) and much lower positive rate of PRV-gE (nearly 0%).

Keywords Pseudorabies (PR) · Pseudorabies virus (PRV) · Prevalence · China · Antibody · Eradication

Introduction

Porcine pseudorabies (PR) is a porcine acute infectious disease caused by pseudorabies virus (PRV). PRV can be vertically transmitted transplacentally, mainly in the last third of gestation. The virus can also spread via colostrum to suckling piglets (Beran 1993). It has been a major infectious disease threatened the global swine breeding industry, especially in the swine breeding farms. The continuous accumulation and proliferation of PRV would lead to continuously raised positive rate in population and offspring (Pomeranz et al. 2005; Mettenleiter 2000).

At the end of 2011, the PRV was outbroke in China. Yu et al. (2014) and co-workers reported that the abortion rate of infected sow was about 35% and the mortality rate of piglets with neurological symptoms was more than 20%. The positive rate of wild-type virus-infected pigs could be as high as 50%, even in farms immunized with PRV attenuated vaccine (Bartha strains) (Yu et al. 2014; Wu et al. 2013). An (2012) confirmed that the mutations in PRV strains have resulted in enhanced pathogenicity of PRV, whereas commercial Bartha vaccine strains only provide partial protection for epidemic strains (An et al. 2013). It brought about great challenges to PR prevention and control. At present, a series of measures have been taken to prevent and control PR in China, including strengthening the construction of biosecurity system and improving the vaccination efficacy (Freuling et al. 2017; Yuan et al. 2016).

Since the extensive use of gE-deleted pseudorabies virus (PRV) vaccine, we could apply the PRV-gE antibody (PRV-gE for short) for determining wild-type virus infection and the PRV-gB antibody (PRV-gB for short) for evaluating vaccine immunization. The PR eradication project has been promoted and developed since 2012, the positive rate and incidence of

Xinyan Zhai
zhaixy2010@sina.cn

Lin Yang
cadcyang@163.com

[1] OIE Porcine Reproductive and Respiratory Syndrome Reference Laboratory, China Animal Disease Control Center, No. 20 Maizidian Street, Chaoyang District, Beijing 100125, China

the disease is declining year by year (Yuan et al. 2016; Hu et al. 2015).

To further understand the domestic prevalence of PR, the nationwide surveillance of both PRV-gB and PRV-gE was developed from 2013 to 2016. More than 90% of the swine breeding regions were covered and the results were analyzed per seven geographical regions (North China, Northeast, East China, Central China, South China, Southwest, and Northwest). In this study, we first calculated the mean and differences of PRV-gB and PRV-gE in the nation and seven regions. Then, the efficacy of imported and domestic PRV vaccines was compared. Finally, the effects of PR eradication on positive rate of PRV-gB and PRV-gE were analyzed.

Materials and methods

During the surveillance in 2013–2016, 93, 100, 92, and 91 breeding farms were included, covering 28, 28, 28, and 26 provinces and autonomous regions (covering more than 90% of Chinese swine breeding areas) respectively.

In each province, 2–7 farms were selected per the distribution density of swine farms. The samples were collected between May and June in each year. Thirty-five to fifty blood samples were collected in each farm, including sow, gilts, and boars. The serum was obtained 24 h after blood sample collection and frozen at − 20 °C until assay. The information of sampled swine was collected with standard form, including age in days, vaccine status, and vaccine brand.

The sampling was finished within 2 weeks in same year and all the samples were determined with IDEXX PRV/ADV gB Ab Test and IDEXX PRV/ADV gI Ab Test of the same batch. The detection was performed, and the results were interpreted according to the kit instructions.

The data was processed and demonstrated as boxplots with EXCEL 2016 (Microsoft, USA). The boxplots were applied for analyzing and demonstrating the positive rate and distribution of PRV-gB (in white) and PRV-gE (in black). In the boxplot, the polyline (in gray) indicated the average positive rate; the column in the box plot indicated the distribution of positive rate (the upper limit of the column was the upper quartile of the positive rate and the lower limit was the lower quartile); scattered points indicated the abnormal value exceeded the limit. For the same region, the average positive rate indicated the overall situation; the distribution presented the differences across farms. The wide range of distribution represented great difference of positive rates among farms, indicating higher risk of across farm infection. The statistical analysis in this study was performed with *t* test embedded in the EXCEL 2016 (Microsoft, USA) and $P < 0.05$ was considered as significant.

The positive rates of different geographical regions were calculated. The seven regions were as follows: North China (Beijing, Tianjin, Hebei, Shanxi, and Inner Mongolia), Northeast (Liaoning, Jilin, and Heilongjiang), East China (Shanghai, Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, and Shandong), Central China (Henan, Hubei, and Hunan), South China (Guangdong, Guangxi, and Hainan), Southwest (Chongqing, Sichuan, Guizhou, Yunnan, and Tibet), and Northwest (Shaanxi, Qinghai, Gansu, Ningxia, Xinjiang, and Xinjiang Corp).

Results

The general information for the surveillance in 2013–2016

The general information for the surveillance in 2013–2016 was summarized (Table 1). In 2013–2016, the overall PRV-gB positive rate per year was 91.23, 93.43, 96.77, and 92.75% nationwide; the PRV-gE positive rate per year was 22.17, 23.26, 18.20, and 13.74%, respectively. The positive rate of PRV-gB maintained above 90%. The positive rate of PRV-gE was higher in 2013 and 2014, but significantly decreased in 2015 and 2016.

The positive rates and distribution in different geographical regions

The positive rates and distribution of PRV-gB and PRV-gE in different geographical regions were demonstrated with box plot (Fig. 1). In the box plot, the polyline indicated the average positive rate of different swine farms; the column in the box plot indicated the distribution of positive rate (the upper limit of the column was the upper quartile of the positive rate and the lower limit was the lower quartile); scattered points indicated the abnormal value exceeded the limit. Both the data of PRV-gB (in white) and PRV-gE (in black) were provided. For the same region, the average positive rate (in gray) indicated the overall situation; the distribution presented the differences across farms. The wide range of distribution represented great difference of positive rates among farms, indicating higher risk of across farm infection.

On a national scale, the average positive rate of PRV-gB was above 90%; the average positive rate of PRV-gE was decreased yearly from 2013 to 2016 (Table 1). In addition, the distribution range of positive rate was increasingly concentrated, indicating lower difference of PRV-gE positive rate. The PRV-gE positive rate was high in a small number of farms.

The positive rates and distribution were also analyzed in different geographical regions (Fig. 1). In North China, the distribution range of PRV-gE positive rate was wide and discrete, indicating great differences among farms and high risk of inter-farm transmission. In the Northeast and Central

Table 1 The sampling source, number, positive rate of PRV-gB and PRV-gE in 2013–2016

Year	2013		2014		2015		2016	
	PRV-gB	PRV-gE	PRV-gB	PRV-gE	PRV-gB	PRV-gE	PRV-gB	PRV-gE
No. of included swine farms	93	93	100	100	92	92	91	91
No. of included provinces	28	28	28	28	28	28	26	26
No. of total samples	2783	2783	3990	3990	3720	3720	3654	3654
No. of positive samples	2539	617	3728	928	3600	677	3389	502
Positive rate (%)	91.23	22.17	93.43	23.26	96.77	18.20	92.75	13.74

China, both the average positive rates and distribution range of PRV-gE were increased, indicating increased overall risk. In the East and Northwest China, the PRV-gB positive rate remained high; both the average and distribution of PRV-gE positive rate were significantly reduced, indicating overall decreased risk.

In the South and Southwest China, the PRV-gB positive rate remained high (close to 100%); the average PRV-gE

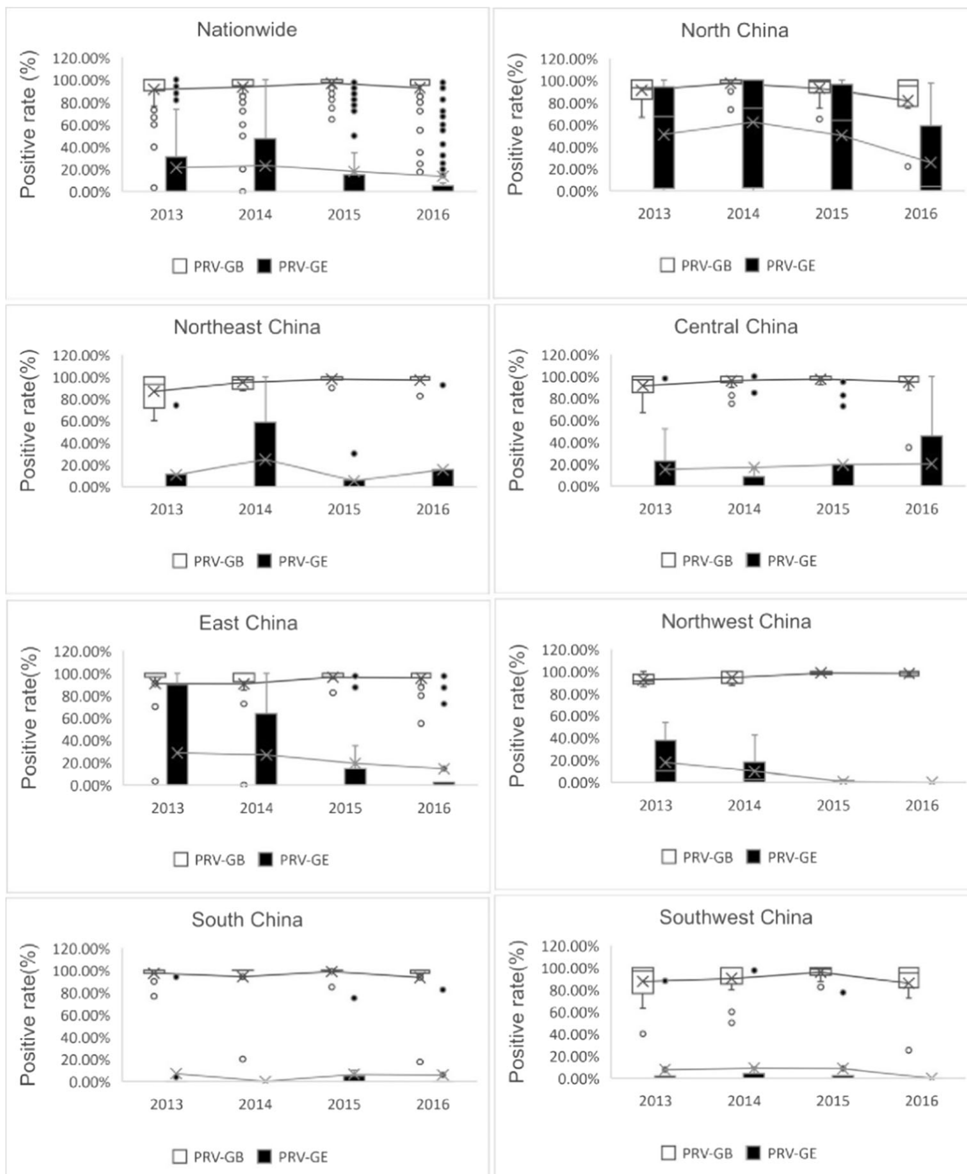

Fig. 1 The positive rates and distribution in different geographical regions. The box plot was applied to demonstrate positive rates and distribution of PRV-gB (in white) and PRV-gE (in black) in different geographical regions, and the polyline (in gray) indicated the average positive rate

positive rate remained at low level with minimum difference among farms, indicating minimum risk.

The selection of pseudorabies vaccine in 2013–2016

Pseudorabies vaccine was one of the most commonly used vaccines. In China, the gene-deleted attenuated vaccine derived from BarthaK61 strain was widely applied and a small number of farms applied HB-98 and EA strains. The number of farms used imported PRV vaccines was 63 in 2013, 68 in 2014, 63 in 2015, and 62 in 2016, while the number of farms used domestic PRV vaccine was 25 in 2013, 29 in 2014, 20 in 2015, and 26 in 2016. The number of farms used imported PRV was twice of that used domestic PRV vaccines (Fig. 2a).

The box plot was applied for demonstrating the positive rate of PRV-gB and PRV-gE in farms with imported and domestic PRV vaccines (Fig. 2b, c). The positive rate of both PRV-gB and PRV-gE differences in farms with imported and domestic PRV vaccines were tested with *t* test. For the positive rate of PRV-gB, there was a statistically significant difference

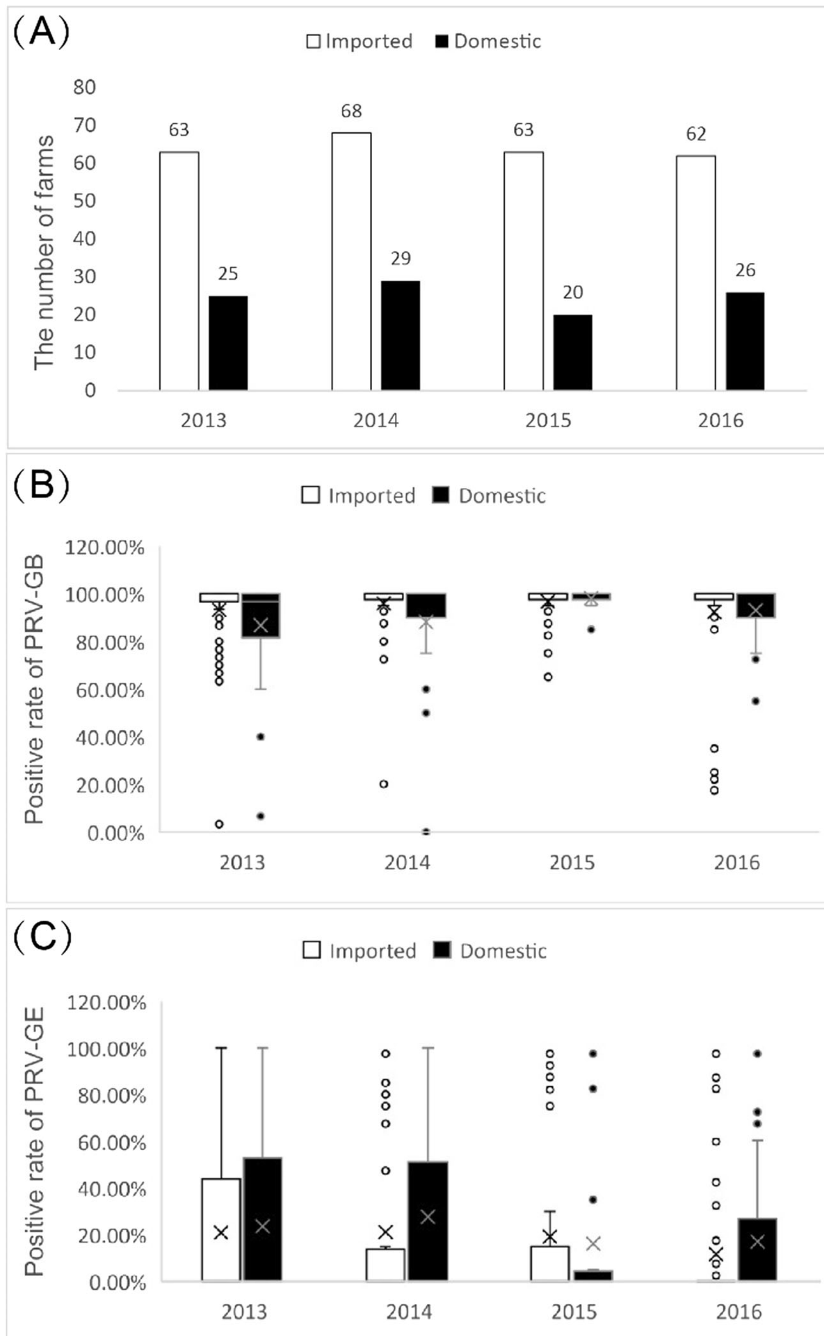

Fig. 2 The efficacy of imported and domestic vaccines. **a** The number of farms using imported and domestic vaccines in 2013–2016. **b** The PRV-gB positive rate distribution in farms with imported and domestic vaccines. **c** The PRV-gE positive rate distribution of imported and domestic vaccines

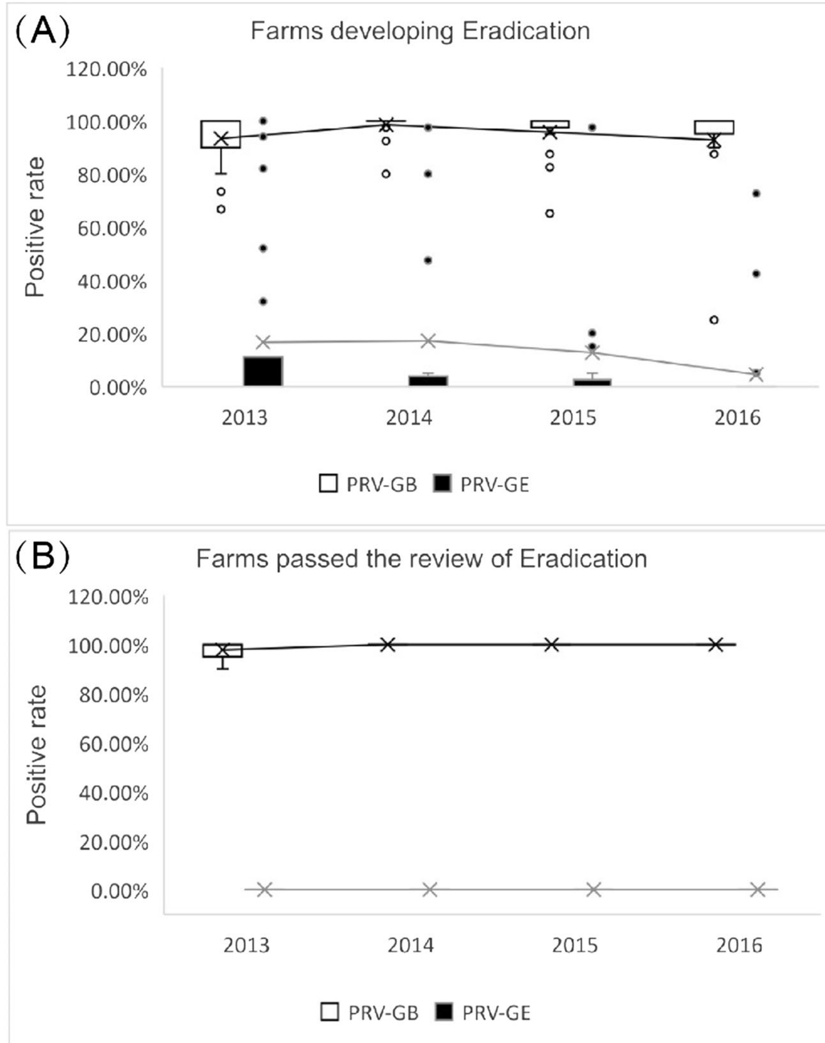

Fig. 3 The PRV-gB and PRV-gE positive rate in 2013 and 2016 for eradication developing farms (**a**) and eradication developed farms (**b**). PR indicated pseudorabies

between the two vaccines ($P < 0.05$) only in 2014. For the positive rate of PRV-gE, no significant difference was observed ($P > 0.05$).

The positive rates of PRV-gB and PRV-gE in PR eradication developing or developed farms

Some swine farms have developed PR eradication program and passed the review of national eradication criteria in 2015–2016. In our study, there were six farms have passed the national PR eradication criteria (eradication developed farms) and 29 farms have developing the eradication projects (eradication developing farms).

For further exploring the effects of eradication, the results of these farms in 2013–2016 were selected for analyzing. In the eradication developing farms, the PRV-gB positive rate was maintained at a high level, and the difference in field was decreased year by year (Fig. 3a). The positive rate of PRV-gE was high in 2013 (16.49%) (number of gE positive samples/total number of samples), while it was decreased year by year. Until 2016, the positive rate of PRV-gE was below 5% with small difference among farms. For the eradication developed farms, PRV-gB positive rate was close to 100% in all swine farms in 2013–2016, with minimum differences. The positive rate of PRV-gE was 0% (Fig. 3b).

Discussion

At the end of 2011, there was a nationwide outbreak of porcine pseudorabies prevalence in China. The positive rate of PRV-gE in swine farms of North China have exceeded 50% and up to 90% (Wu et al. 2013). PR has been listed as one of the priority swine diseases to be controlled in “Mid- and Long-term Animal Disease Prevention and Control Program in China (2012–2020).” The program has been carried out since 2012. One of the tasks of the program is to eradicate PR in pig breeding farms in China by the end of 2020 (The State Council of the People’s Republic of China 2012) (Yuan et al. 2016; Hu et al. 2015). The results obtained in our study were consistent with the tendency of eradication; the positive rate of PRV-gE was 22.17% (2013), 23.26% (2014), 18.20% (2015), and 13.74% (2016). The positive rate of PRV-gE was continuously decreased, indicating that PRV was effectively controlled. We believed that there were two reasons contributed to the effective control of the disease: the improved immune quality of PRV and the development of PR eradication.

The immune quality of PRV has been improved (including the vaccine quality and vaccination density), thereby increasing the herd resistance to the disease. After 2012, both the immunization rate of pigs and the positive rate of PRV-gB were improved (Yuan et al. 2016; Hu et al. 2015). In this study, the immunization rate in swine breeding farms was almost 100% and the positive rate of PRV-gB was above 90%, while the positive rate of gE was decreased year by year. It suggested that the improved immunization density and antibody level would be effective factors for controlling PRV prevalence.

The development of PR eradication program has reduced the prevalence of wild-type PRV in China, since it started in 2012, PR eradication criteria have been issued and the eradication guidance has been published. Eradication was reported to make effects in Germany, Netherlands, and other countries. The positive rate of wild-type virus infection has been reduced below 5% and the application of attenuated vaccine has been stopped (Stegeman 1997; Müller et al. 2003; Vannier et al. 2000). The swine farms were encouraged to start the eradication program and participate in the review of national PR eradication criteria. The swine farms could develop eradication plans according to the eradication guidance, as well as sufficiently considering their own conditions, including biosecurity, animal monitoring and elimination, immunization, and so on.

In our study, specific study has been performed on the swine farms developed or developing eradication. It was observed that the positive rate of immunization antibody was very high with minimum difference, while the positive rate of wild-type virus infection was close to 0, indicating the feasibility of domestic purification program and the availability of purification technology. Therefore, the eradication technology and experience should be promoted and the scope of the eradication farms should be expanded.

There were some differences in the prevention and control of the disease in different regions. To prevent and control pseudorabies in a comprehensive and effective manner, it was advisable to adopt a regional prevention strategy in different regions. Firstly, the positive rate of wild virus infection and differences among farms should be explored through the surveillance of PRV-gE. Secondly, the corresponding prevention strategy should be developed. For the farms with high positive rate, the biosecurity should be enhanced, and the immunization plan should be optimized. The wild-type virus-infected sows should be eliminated and cleared from the herds, preventing from intra-farm and inter-farm prevalence. For the farms with great difference of positive rates, more strict prevention strategy should be applied in farms with high positive rate, preventing the prevalence of wild-type virus towards other farms. The difference should be minimized then the regional positive rate would be gradually reduced.

The effects of imported and domestic vaccines were also compared. From the positive rate of PRV-gB, no significant differences were observed ($P > 0.05$), indicating that no significant difference in antibody production between domestic and imported vaccines. Protection could be provided with produced antibody while it was not directly related to the positive rate of wild-type infection rate. Here we just compared the positive rates of PRV-gB and PRV-gE. More assessment should be performed from the aspects of protection, safety, adverse reactions, and so on (Tong et al. 2016; Dong et al. 2017; Freuling et al. 2017).

In general, in 2013–2016, the nationwide positive rate of immunized antibody remained at a high level (above 90%), while the wild-type virus infection rate continued to be decreased (from 22.17 to 13.14%). The differences of positive rates could be applied for indicating the risk of pseudorabies prevalence. The farms developed or developing PR eradication performed better than that of general farms. We proposed to maintain and expand the scope of the eradication, controlling the pseudorabies prevalence by integrating eradication with immunization.

Acknowledgements The authors thank for the financial support from Beijing Agriculture Innovation Consortium (BAIC-02).

Compliance with ethical standard

The sampling process was in line with animal welfare requirements.

Conflict of interest statement The authors declare that they have no conflict of interest.

References

An, T.Q., Peng, J.M., Tian, Z.J., Zhao, H.Y., Li, N., Liu, Y.M., Chen, J.Z., Leng, C.L., Sun, Y., Chang, D., Tong, G.Z., 2012. Pseudorabies virus variant in bartha-k61–vaccinated pigs, china, 2012. Emerging Infectious Diseases, 19, 1749–1755.

Beran, G.W., 1993. Understanding the transmission of PRV. Veterinary Medicine. 88, 70–79.

Dong, J., Bai, J., Sun, T., Gu, Z., Wang, J., Sun, H., Jiang, P., 2017. Comparative pathogenicity and immunogenicity of triple and double gene-deletion pseudorabies virus vaccine candidates. Research in Veterinary Science, 115, 17–23.

Freuling, C.M., Müller, T.F., Mettenleiter, T.C., 2017. Vaccines against pseudorabies virus (PrV). Veterinary Microbiology, https://doi.org/10.1016/j.vetmic.2016.11.019.

Hu, D., Lv, L., Zhang, Z., Xiao, Y., Liu, S., 2015, Seroprevalence and associated risk factors of pseudorabies in Shandong province of China. Journal of Veterinary Science, 17, 361–368.

Mettenleiter, T.C., 2000. Aujeszky's disease (pseudorabies) virus: the virus and molecular pathogenesis-state of the art, June 1999. Veterinary research, 31, 99–115.

Müller, T., Bätza, H.J., Schlüter, H., Conraths, F.J., Mettenleiter, T.C., 2003. Eradication of aujeszky's disease in Germany. Journal of Veterinary Medicine B Infectious Diseases & Veterinary Public Health, 50, 207–13.

Pomeranz, L.E., Reynolds, A.E., Hengartner, C.J., 2005. Molecular biology of pseudorabies virus: impact on neurovirology and veterinary medicine. Microbiology and molecular biology reviews, 69, 462–500.

Stegeman, A., 1997. Aujeszky's disease (pseudorabies) virus eradication campaign in the Netherlands. Veterinary Microbiology, 55, 175–180.

Tong, W., Li, G., Liang, C., Liu, F., Tian, Q., Cao, Y., Li, L., Zheng, X., Zheng, H., Tong, G., 2016. A live, attenuated pseudorabies virus strain JS-2012 deleted for gE/gI protects against both classical and emerging strains. Antiviral research, 130, 110–117.

Vannier, P., Vedeau, F., Allemeersch, C., 2000. Eradication and control programmes against Aujeszky's disease (pseudorabies) in France. Veterinary Microbiology, 15, 519–536.

Wu, R., Bai, C.Y., Sun, J.Z., Chang, S.K., Zhang, X., 2013, Emergence of virulent pseudorabies virus infection in Northern China. Journal of veterinary science, 14, 363–365.

Yu, X., Zhou, Z., Hu, D., Zhang, Q., Han, T., Li, X., Gu, X., Yuan, L., Zhang, S., Wang, B., Qu, P., Liu, J., Zhai, X., Tian, K., 2014. Pathogenic pseudorabies virus, China,2012. Emerging infectious diseases, 20, 102–104.

Yuan, S., Luo, Y., Wang, C.H., Yuan, J., Li, N., Song, K, Qiu, H. J., 2016, Control of swine pseudorabies in China: Opportunities and limitations. Veterinary Microbiology, 183, 119–124.