Prevalence of Porcine Pseudorabies Virus and Its Coinfection Rate in Heilongjiang Province in China from 2013 to 2018

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Abstract

Pseudorabies (PR) is a highly contagious disease affecting a wide range of animals, which annually causes great economic losses in China. In this study, a total number of 18,815 serum samples and 1,589 tissue samples were collected from 311 intensive pig farms (≥ 350 sows) located in eight cities in Heilongjiang province, and tested by ELISA and PCR. Overall, the serum positive rates of gE and gB protein were 16.3% and 84.5%, respectively. The PCR-positive rate of PR virus (PRV) in tissue samples was 17.8%. The coinfection rates of PRV with porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), and classical swine fever virus (CSFV) were also measured. The rate of PRV coinfecting with PRRSV was 36.0% followed by 12.9% with PCV2 and 1.8% with CSFV, respectively. These results clearly demonstrate PRV prevalence and its coinfection rate in Heilongjiang province, indicating high PR endemic in pig farms in this region. This study provides data for further epidemiological investigations and a reference for developing PRV control strategies in this region and in China.

Keywords: pseudorabies virus, veterinary epidemiology, coinfection rate, Heilongjiang province

Introduction

PSEUDORABIES (PR), ALSO known as Aujeszky's disease, is an economically crucial viral disease of pigs and other animals worldwide. It is caused by the pseudorabies virus (PRV), which is a member of genus *Varicellovirus* of the subfamily *alpha herpesviridae* within the family *Herpesviridae* (6). While PRV can infect numerous species of animals, including ruminants and rodents (9,11), pigs are recognized as the primary host as well as act as the only reservoir and latent carrier of PRV (3,5). PRV-infected pigs are commonly characterized by fatal infections in piglets, growth retardation in growing pigs, and respiratory disorders, encephalomyelitis, and reproductive failure in sows (1,4).

PRV was first discovered in 1902. The first report of PRV infection in China was back to the 1950s (2). From 1990s to late 2011, PR was well controlled, resulting from the adaptation of the Hungary-origin gpl-negative vaccine strain

Bartha-K61 in most of pig farms in China. Lower level of morbidity and mortality was observed in newborn piglets. It was recorded that PRV has been eradicated from domestic pigs in many western countries. gE-deleted vaccine plays a pivotal role in combating PRV in these free countries, particularly in terms of differentiating infected animals from vaccinated counterparts (10,13).

However, in late 2011, a PR outbreak characterized by neurological disorders and high mortality in piglets was identified in many of Bartha-K61-vaccinated pig farms, which rapidly spread to most regions in China (2,16). This reemergence indicated that the Bartha-K61 vaccine may not be able to provide effective protection against the variant PRV (vPRV) infection. Data on epidemiology of PRV and its coinfection rate with other major swine diseases may be of great importance for prevention and control of vPRV, particularly in regions with dense pig population.

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Heilongjiang is a province located in the northeast of China, sharing the border with Russia, which has massive pig farms and a large amount of pig population (18). To date, the total population of pigs and sows was estimated to be ~28 million and 3 million in Heilongjiang in 2017. Although PRV outbreaks have been reported in most provinces in China, no systematic studies have been carried out to investigate the epidemiology of the disease in intensive pig farms in Heilongjiang province on an annual basis. The goal of this study was to understand PRV prevalence and its coinfection rates by investigating 18,815 serum samples and 1,589 tissue samples collected from 311 pig farms in eight cities in Heilongjiang province from 2013 to 2018. PRV gB and gE antibody level, positive rate of PRV, and coinfection rates of PRV with other major swine viral diseases were analyzed. While concrete data are required to estimate the economic loss PRV led to, our study showed that PRV is highly prevalent in Heilongjiang province, should be considered one of the most critical viral pathogens to pig breeding industry.

Materials and Methods

Sampling strategy

A cross-sectional study was carried out in 311 intensive pig farms from eight cities in Heilongjiang province. A total number of 18,815 samples were collected to determine the seroprevalence of PRV. In this study, blood samples were collected from the precaval vein of pigs from January 2013 to August 2018. In this study, all pigs were released after sampling. Serum samples were categorized by sow, boar, fattening pig, replacement pig, and piglet. Based on the number of seropositive samples in each investigated city, 1,589 tissue samples were randomly selected to detect positive rate of PRV and its coinfection rate with PRRSV, CSF, and porcine circovirus type 2 (PCV2), respectively, by using PCR.

Sample collection and ELISA

A total number of 18,815 serum samples were collected from eight main geographic cities of Heilongjiang province, which are Harbin, Jiamusi, Qiqihar, Suihua, Hegang, Daqing, Mudanjiang, and Jixi. Anti-gB and anti-gE antibody levels of investigated samples were determined by using a commercial ELISA kit (IDEXX Laboratories, Westbrook, ME) according to the manufacturer's instructions to differentiate the vaccine strain from field strains.

Real-time PCR

One thousand six hundred thirty-eight tissue samples from different pig farms were selected. Tissue samples (lymph node, kidney, spleen, and brain) were determined for PRV, PRRSV, PCV2, and CSF by using commercial real-time polymerase chain reaction (RT-PCR) kits (Beijing Anheal Laboratories, Beijing China) according to the manufacturer's instructions. If the CT value ≤ 30 , the tested sample would be considered positive.

Ethics statements

The animal protocol for this research was reviewed and approved by the Animal Care and Use Committee of Northeast Agricultural University. Blood collection from pigs was performed in accordance with animal ethics guidelines and approved protocols.

Results

Seroprevalence rate of PRV gB and PRV gE

In this study, 18,815 serum samples of pigs in different production phases were collected from pig farms in eight investigated cities in Heilongjiang province. Samples were subsequently examined by ELISA on an annual basis to investigate gB- and gE-specific antibody level and seroprevalence rate (SPR). More than 80% of pigs were vaccinated in the investigated farms in Heilongjiang province, according to the higher level of PRV gB SPR rates described in Table 1 from 2013 to 2018. Moreover, as shown in Supplementary Table S1, S/N ratio of different investigated herds showed lower than 0.6, ranging from 0.17 to 0.54, indicating that high gB antibody levels were observed in different herds in the region.

As shown in Table 2, independent of investigated herds, the average SPR of PRV-gE of pigs was 16.3% and showed a decrease from 20.9% in 2013 to 11.6% in 2018. There is a similar trend, gradual decrease, of SPR of PRV-gE observed in different investigated herds throughout the investigation period, with the exception of year 2015 showing higher SPR of PRV-gE in fattening pigs, replacement pigs, and piglets compared to that of in 2014.

Positive rate of PRV and its coinfection

One thousand five hundred eighty-nine tissue samples were detected by RT-PCR. Results in Table 3 showed that the average positive rate of PRV was 14.1% (225/1589) in Heilongjiang province during the investigation period. The overall positive rate of PRV was the highest at 17.8% in 2013 (39/209) and gradually decreased at 11.5% in year 2018 with approximate rate at 14.1% (Table 3).

Regarding the coinfection rates of PRV with PCV2, PRRSV, and CSFV, all 225 positive samples were tested by RT-PCR. Results are shown in Table 4, categorized by investigated cities. In this study, 36.0% (81/225) of PRV-

TABLE 1. SEROPREVALENCE RATE OF PSEUDORABIES VIRUS gB IN HEILONGJIANG PROVINCE FROM 2013 TO 2018

Year	Sample numbers	Positive numbers	SPR
2013	2968	2480	83.6%
2014	2880	2410	83.7%
2015	3402	3008	88.4%
2016	3086	2599	84.2%
2017	3748	3232	86.2%
2018	2731	2200	80.6%
Total	18815	15929	84.5%

SPR, seroprevalence rate.

TABLE 2. SEROPREVALENCE RATE OF PSEUDORABIES VIRUS gE IN DIFFERENT HERDS IN HEILONGJIANG PROVINCE FROM 2013 TO 2018

Year	Herds					Sample numbers	Positive numbers	SPR
	Sows	Boars	Fattening pigs	Replacement pigs	Young piglets			
2013	23.8%	20.6%	23.2%	18.5%	15.5%	2968	619	20.9%
	354/1489	66/323	34/146	53/289	112/721			
2014	22.9%	17.1%	20.2%	16.1%	9.7%	2880	519	18.0%
	305/1334	64/374	30/149	53/328	67/695			
2015	20.2%	15.5%	21.2%	17.6%	11.5%	3402	591	17.4%
	337/1668	37/241	36/172	85/485	96/836			
2016	18.7%	13.9%	21.4%	11.8%	9.4%	3086	474	15.4%
	293/1567	38/272	33/156	38/325	72/766			
2017	18.3%	11.3%	18.7%	10.7%	8.8%	3748	539	14.4%
	311/1697	51/447	46/246	51/472	80/886			
2018	13.9%	10.9%	15.4%	7.6%	8.9%	2731	318	11.6%
	172/1237	35/325	28/180	26/344	57/645			
Total	19.7%	14.6%	19.7%	13.6%	10.6%	18815	3060	16.3%
	1772/8992	291/1982	207/1049	306/2243	484/4559			

positive pigs were coinfecting by PRRSV, followed by 12.9% (29/225) by PCV2 and 1.8% (4/225) by CSF. The coinfection rates of PRV and PRRSV were over 40% in Harbin (18/34) and Jiamusi (14/35).

Discussion

PR was recognized as a major threat to the swine industry in China. It was listed in “Mid- and Long-term Animal Disease Prevention and Control Program in China,” targeting on eradicating PR in pig farms in China by the end of 2020 (7,15). Since 2011, a PRV eradication program has been launched at the national level, which is being implemented in most pig farms, especially intensive farms, in Heilongjiang Province. Previous studies have indicated that the positive rate of PRV-gE in breeding farms in different regions of China have outweighed over 50% and up to 90% (8,20). However, there is limited information on PRV prevalence in Heilongjiang province. This study therefore was designed to investigate PRV prevalence and its coinfection rates with other major swine viral diseases in Heilongjiang province. To the best of our knowledge, this is the first study reporting pig farm PRV prevalence and its coinfection rate in the region.

TABLE 3. POSITIVE RATE OF PSEUDORABIES VIRUS IN HEILONGJIANG PROVINCE FROM 2013 TO 2018

Year	Sample numbers	Positive numbers	Positive rate
2013	219	39	17.8%
2014	221	36	16.3%
2015	236	38	16.1%
2016	235	32	13.6%
2017	287	35	12.2%
2018	391	45	11.5%
Total	1589	225	14.1%

In this study, 18,815 serum samples of pigs in different production phases were collected from pig farms in eight investigated cities in Heilongjiang province, and were subsequently examined by ELISA on an annual basis to investigate gB- and gE-specific antibody level and SPR. Consistent with results of previous publications (7,14,19,21), >80% of pigs were vaccinated in the investigated farms in Heilongjiang province, according to the higher level of PRV gB SPR rates described in Table 1 from 2013 to 2018. Moreover, S/N ratio of different investigated herds showed lower than 0.6, ranging from 0.17 to 0.54. Results indicated that the immunization density of PRV and gB antibody level were considerably high in Heilongjiang province, which was expected to provide effective prevention and control against PRV infection.

The SPR of gE in all investigated herds of sows, boars, fattening pigs, replacement pigs, and young piglets showed similar changing trend, a gradual decrease from 2013 to

TABLE 4. POSITIVE RATE OF PSEUDORABIES VIRUS AND ITS COINFECTION RATE IN DIFFERENT REGIONS IN HEILONGJIANG PROVINCE FROM 2013 TO 2018

City	City No./ Total No.		PRV+ PRRSV	PRV+ PCV2	PRV+ CSF
Jiamusi	35/225	15.6%	14	3	1
Harbin	34/225	15.1%	18	5	0
Qiqihar	31/225	13.8%	11	6	2
Suihua	29/225	12.9%	7	3	1
Hegang	28/225	12.4%	8	4	0
Daqing	26/225	11.6%	9	4	0
Mudanjiang	23/225	10.2%	9	2	0
Jixi	19/225	8.4%	5	2	0
Total	225		81/225 36.0%	29/225 12.9%	4/225 1.8%

CSF, classical swine fever; PCV2, porcine circovirus type 2; PRRSV, porcine reproductive and respiratory syndrome virus; PRV, pseudorabies virus;

2018. There was an exception, a slight increase of SPR of gE observed, in fattening pigs, replacement pigs, and young piglets in 2015 compared to that of 2014, probably due to local temperature variation. Consistent with results of PRV-gE, the overall positive rate of PRV showed a trend of gradual decrease from 2013 to 2018 (Table 3). Previous studies conducted in Shandong province in China have shown that a high proportion of pigs were latently infected by PRV in the investigated region (4,17), which was recognized to be the most critical source of infection when the latent viral genome is reactivated (2,12). Heilongjiang province has recorded significant lower temperature compared with Shandong province, which may contribute to PRV prevention and control and its further spread in the region. Our results demonstrated a trend of decline of gE SPR- and PRV-positive rate during the period of investigation, from 2013 to 2018, resulting from the development of vaccines based on the epidemic PRV strains, the scale of selected pig farms, and a geographical factor. It therefore can be safely concluded that vaccination is by no means unimportant for PRV prevention and control; current vaccines applied in the field in the region may provide effective protection against PRV infection. It is also necessary to take other forceful measures (8) to provide further control of PRV, including strengthening biosecurity management, animal monitoring, identification and removal of the latent carriers, and so forth.

It is generally acknowledged that, under current intensive swine breeding system, concurrent infection with more than one pathogen was commonly observed in pigs independent of age, breed, and herds. It has been demonstrated in previous studies that coinfection of PRV with other pathogens in pig herds exists, such as PCV2, PRRSV, CSF, and *Streptococcus* spp. (9,15). Our study showed that 12.9%, 36.0%, and 1.8% of PRV-positive pigs were coinfecting with PCV2, PRRSV, and CSF, respectively (Table 4). There has also been a report indicating higher rate of concurrence of PRRS in winter (1), which coincides with our results, suggesting the seasonal factor in Heilongjiang as a possible reason for higher co-infection rate of PRV and PRRSV. This research reveals co-infection status of PRV associated with other pathogens in pig herds of Heilongjiang province.

Limitation of this study may skew the results, requiring cautions during interpretation. First, the investigated pig farms are only intensive farms in eight major cities in the region, so the results of this study could not be used to draw a comprehensive conclusion on the PRV situation of the whole province. Second, the majority of samples were not equally collected in each month of the investigated 5 years due to logistic constraints; seasonal variation therefore may slightly influence the prevalence and coinfection rate reported.

In conclusion, our study clearly demonstrates that, in Heilongjiang province, PRV was highly prevalent in intensive pig farms. A notable coinfection rate of PRRSV was observed in PRV-infected pigs in this region, which

should be considered an essential factor in terms of the design of PRV disease control programs and its further eradication.

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Supplementary Material

Supplementary Table S1

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